B E S
The Bioelectrochemical Society

ABSTRACT BOOK

XXVI TH
International Symposium on Bioelectrochemistry and Bioenergetics

MAY 9-13, 2021
CLUJ-NAPOCA

BES2021.ORG

UMF
UNIVERSITATEA DE MEDICINĂ ȘI FARMACIE
IULIU HAȚIEGANU
CLUJ-NAPOCA

Program of the

XXVIth International Symposium on Bioelectrochemistry and Bioenergetics of the Bioelectrochemical Society

Online

9-13 May, 2021

Cluj-Napoca, Romania

The Bioelectrochemical Society
Chemin du Closelet 2
1006 Lausanne
Switzerland

Printed in Romania

Organising Committee

Chair: **Robert Săndulescu**, Iuliu Haţieganu University, Cluj-Napoca, Romania
Lo Gorton, Lund University, Sweden
Elisabeth Lojou, Marseille University, France
Camelia Bala, University of Bucharest, Romania
Cecilia Cristea, Iuliu Haţieganu University, Cluj-Napoca, Romania
Victor Diculescu, National Institute of Materials Physics, Bucharest
Graziella Turdean, Babeș-Bolyai University, Cluj-Napoca, Romania
Eleonora-Mihaela Ungureanu, Politehnica University Bucharest, Romania

Local Organising Committee

Ede Bodoki, Iuliu Hatieganu University Cluj-Napoca, Romania
Mihaela Tertiș, Iuliu Hatieganu University Cluj-Napoca
Oana Hosu, Iuliu Hatieganu University Cluj-Napoca
Bogdan Feier, Iuliu Hatieganu University Cluj-Napoca
Radu Oprean, Iuliu Haţieganu University Cluj-Napoca
Andreea Cernat, Iuliu Hatieganu University Cluj-Napoca
Bogdan Iacob, Iuliu Haţieganu University Cluj-Napoca
Stela Pruneanu, Isotopic and Molecular Technology Institute Cluj-Napoca
Liana Mureșan, Babes-Bolyai University Cluj-Napoca

Sponsors

International Society of Electrochemistry

UMF
UNIVERSITATEA DE MEDICINĂ ŞI FARMACIE
IULIU HAŢIEGANU
CLUJ-NAPOCA

ELSEVIER

Metrohm
Electrochemistry

biosensors
an open access journal by MDPI

Program

Table of Contents

Online Special Meeting Program……...6
Oral presentation program.....7
Poster presentation program.....31
Abstracts of Giulio Milazzo Prize, Luigi Galvani Prize, Plenary, Keynote and Invited Lectures…….....43
Abstracts of Oral presentation.....67
Abstracts of Poster presentation.....149
Authors Index.....224

Online Special Meetings Program

Please be aware that the schedule (dates and hours) involves Central European Time (CET) and not Romania's time zone!

Sunday, 9 May 2021

13:30 – 15:00
BES Council, Room 1

17:30 – 18:00
Opening Ceremony, Room 1

18:00 – 18:50
Giulio Milazzo Prize Lecture, Room 1

19:00 – 19:30
Luigi Galvani Prize Lecture, Room 1

Monday, 10 May 2021

17:20 – 19:30
S1 Poster Exhibition and Networking

Tuesday, 11 May 2021

12:20 – 13:20
BES General Assembly, Room 1

14:30 – 16:00
Flash Poster Session 1, Room 1
Flash Poster Session 2, Room 2

16:20 – 19:30
S2 Poster Exhibition and Networking

Wednesday, 12 May 2021

16:40 – 19:30
S3, S4, S5, and S6 Poster Exhibition and Networking

Thursday, 13 May 2021

13:00 – 13:20
Closing Ceremony, Room 1

Program of the Online XXVI[th] International Symposium on Bioelectrochemistry and Bioenergetics

Please be aware that the schedule (dates and hours) involves Central European Time (CET) and not Romania's time zone!

Sunday, 9 May 2021 - Afternoon

17:30 – 18:00

Opening Ceremony, **Room 1**

Giulio Milazzo Prize Lecture

Room 1

Chaired by: Lo Gorton

18:00 to 18:50

Lluis Mir (METSY, UMR 9018 CNRS - Université Paris-Saclay, France)

Electroporation, from Fundamentals to Medical Applications

Luigi Galvani Prize Lecture

Room 1

Chaired by: Lo Gorton

19:00 to 19:30

Francesco Ricci (Laboratory of Biosensors and Nanomachines, Chemistry Department, University of Rome, Tor Vergata, Italy)

DNA-based Nanodevices for Sensing Applications

Monday, 10 May 2021 - Morning

Plenary Lecture

Room 1

Chaired by: Lo Gorton

09:00 to 10:00 PL-01

Guillermo Bazan (University of Singapore)

Living Bioelectrochemical Composites

S1 Smart Materials for Bioelectrochemistry

Room 1

Chaired by: Wolfgang Schuhmann and Ilaria Palchetti

10:00 to 10:20 S1-O-01

Bogdan C. Iacob (Analytical Chemistry Department, Iuliu Hatieganu University, Cluj-Napoca, Romania) Ioan Adrian Stoian, João P. Prates Ramalho, Iuliu O. Marian, Radu Oprean, Ede Bodoki

Chiral Interactions of Propranolol Enantiomers at the Surface of Cysteine Modified Gold Nanoparticles

10:20 to 10:40 S1-O-02

Katarzyna Szot-Karpińska (Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw, Poland) Justyna Borkowska, Patryk Kudła, Sławomir Filipek, Magdalena Narajczyk, Joanna Niedziółka-Jönsson

Peptide-based Materials for Studying Interactions with CRP Protein - Development and Application

10:40 to 11:00 S1-O-03

Panpan Wang (Center for Electrochemical Sciences, Ruhr University Bochum, Germany) Anna Frank, Fangyuan Zhao, Marc M. Nowaczyk, Adrian Ruff, Felipe Conzuelo, Wolfgang Schuhmann

A Mixed Monolayer Comprised of Trimeric and Monomeric Photosystem I Enables Improved Anisotropic Electron Flow in Biophotovoltaic Devices

11:00 to 11:30

Coffee Break and Posters

11:30 to 12:00 Keynote S1-KL-01

Paolo Actis (School of Electronic and Electrical Engineering, University of Leeds, United Kingdom) Mukhil Raveendran, Andrew J. Lee, Christoph Wälti

DNA Nanostructures for Single-Molecule Biosensing

12:00 to 12:20 **S1-O-04**

Sergey Shleev (Biomedical Science and Biofilms-Research Center for Biointerfaces, Malmö University, Sweden) Olga Aleksejeva, Chiara di Bari, Elena Gonzalez-Arribas, Antonio L. de Lacey, Marcos Pita, Roland Ludwig, Victor Andoralov

Membrane and Mediator Free High Voltage Enzymatic Supercapacitors

12:20 to 12:40 **S1-O-05**

Alaa Oughli (Professur für Elektrobiotechnologie, TU München, Germany) Steffen Hardt, Olaf Rüdiger, James Birrell, Nicolas Plumeré

Reactivation of Sulfide-Protected [FeFe] Hydrogenase in a Redox-active Hydrogel

12:40 to 13:00 **S1-O-06**

Giulia Selvolini ("Ugo Schiff" Chemistry Department, University of Florence, Italy) Giovanna Marrazza

Towards Electrochemical Detection of Deoxynivalenol by Exploiting Molecular Docking Simulations

13:00 to 13:20 **S1-O-07**

Paolo Bollela (Department of Chemistry and Biomolecular Science, Clarkson University, Potsdam, USA) Zhong Guo, Selvakumar Edwardraja, Vasantha Krishna Kadambar, Kirill Alexandrov, Artem Melman, Evgeny Katz

Self-Powered Molecule Release Systems Activated with Chemical Signals Processed Through Reconfigurable Implication or Inhibition Boolean Logic Gates

---

S2 Electrochemical Sensors for Diagnostics and Therapy Monitoring

---

Room 2

Chaired by: Włodzimierz Kutner and Miroslav Fojta

10:00 to 10:20 **S2-O-01**

Christopher Brett (Department of Chemistry, University of Coimbra, Portugal) Wanderson Da Silva

Electrochemical Enzyme Biosensor Platforms with Iron Oxide Nanoparticles and Polyphenazines Prepared in Ethaline Deep Eutectic Solvent

10:20 to 10:40 **S2-O-02**

Adrian Blidar (Analytical Chemistry Department, Iuliu Hatieganu University, Cluj-Napoca, Romania) Oana Hosu, Geanina Ştefan, Diana Bogdan, Karolien de Wael, Cecilia Cristea

The Development of an Aptasensor for Oxytetracycline using Au-based Nanostructured Platforms

10:40 to 11:00 **S2-O-03**

Mihaela Puiu (LaborQ, University of Bucharest) Lucian-Gabriel Zamfir, George Mădălin Dănila, Camelia Bala

Detection of Allosteric Modulators of Growth Hormone Secretagogue Receptor According to their Affinity Profiles in Competitive Assays

11:00 to 11:30

Coffee Break and Posters

11:30 to 12:00 Keynote **S2-KL-01**

Francesco Paolucci (Dipartimento di Chimica Giacomo Ciamician, Università di Bologna, Italy) Giovanni Valenti, Sara Rebeccani, Massimo Marcaccio

Insight into the Mechanism of Coreactant Electrochemiluminiscence Empower its Analytical Strenght

12:00 to 12:20 **S2-O-04**

Ana Maria Oliveira-Brett (Department of Chemistry, University of Coimbra, Portugal) W.B.S. Machini

Electrochemical Evaluation of Bacteria *Xylella Fastidiosa* DNA-Copper(II) Interaction Using DNA-Electrochemical Biosensors

12:20 to 12:40 **S2-O-05**

Fernando Otero (Department of Chemical Sciences and Bernal Institute, University of Limerick, Ireland) Urszula Salaj-Kosla, Tofail Syed, Edmond Magner

Electrochemical Detection of DNA-conjugated Methylene Blue: Optimization of DNA Probe Surface Coverage, Hybridization Time and Length of Target DNA Sequences

12:40 to 13:00 **S2-O-06**

Simona de Zio (Dipartimento di Chimica Giacomo Ciamician, Università di Bologna, Italy) Marco Malferrari, Stefania Rapino

Micrometric Electrochemical Biosensors for Spatially Resolved Metabolites Studies

S6 Electron Transport in Biological Systems - Theory and Experiment

Room 3

Chaired by: Kylie Vincent and Pau Gorostiza

10:00 to 10:20 **S6-O-01**

Elisabeth Lojou (Bioénergétique et Ingénierie des Protéines, CNRS, Aix-Marseille Université, Marseille, France) Romain Clément, Xie Wang, Frédéric Biaso, Marianne Ilbert, Ievgen Mazurenko

Copper Activation of *Thermus thermophilus* Laccase: Which Structural Features are involved?

10:20 to 10:40 **S6-O-02**

Anna Lagunas (Biomedical Research Networking Center in Bioengineering, Biomaterials, and Nanomedicine Madrid, Spain) Christine Belloir, Loïc Briand, Pau Gorostiza, Josep Samitier

Ligand Binding Induces a Shift in the Open-Circuit Voltage of Olfactory Receptor 1A1

10:40 to 11:00 **S6-O-03**

Jiaao Wei (Department of Chemistry, University of Oxford, United Kingdom) Miguel Ramirez, Stephen Carr, Patricia Rodriguez-Macia, Rhiannon Evans, Philip Ash, Kylie Vincent

Probing Iron-Sulfur Cluster Redox States by Incorporating an Unnatural Amino Acid

11:00 to 11:30

Coffee Break and Posters

11:30 to 12:00 Keynote **S6-KL-01**

Phil N. Bartlett (School of Chemistry, University of Southampton, UK)

Mediated and Direct Electron Transfer

12:00 to 12:20 Invited **S6-IL-01**

Marco Rolandi (Department of Electrical and Computer Engineering, University of California, Santa Cruz, USA)

Closed Loop Control of Biological Processes Using Bioelectronics

12:20 to 12:40 **S6-O-04**

Kylie Vincent (Department of Chemistry, University of Oxford, United Kingdom) Rhiannon Evans, Philip Ash, Stephen Carr, Wangzhe Li

Electrochemical Control over Single Hydrogenase Protein Crystals Coupled with IR Microspectroscopy

12:40 to 13:00 **S6-O-05**

Manuel López-Ortiz (Institute for Bioengineering of Catalonia, Barcelona, Spain) Ricardo Zamora-Brito, Marina Inés Giannotti, Nuria Camarero, Chen Hu, Roberta Croce, Pau Gorostiza

Distance- and Potential-dependent Charge Exchange Through Oriented Single Photosystem I Complexes

13:00 to 13:20 **S6-O-06**

Ross Milton (Department of Inorganic and Analytical Chemistry, University of Geneva, Switzerland) Jaloliddin Khushvakov, Robin Nussbaum, Cécile Cadoux, Jifu Duan, Sven Strip

Following Electroenzymatic Hydrogen Production with Rotating Ring Disk Electrochemistry and Mass Spectrometry

Monday, 10 May 2021 - Afternoon

S1 Smart Materials for Bioelectrochemistry

Room 1

Chaired by: Camelia Bala

14:30 to 15:00 Keynote **S1-KL-02**

Petra Hellwig (Laboratoire de Bioélectrochimie et Spectroscopie, Université de Strasbourg, France) Iryna Makarchuk, Anton Nikolaev, Schara Safarian, Alexander Thesseling, Thorsten Friedrich, Hartmut Michel, Tomoichirou Kusumoto, Junshi Sakamoto, Frederic Melin

Electrocatalytic studies on membrane proteins from bacterial respiratory chains

15:00 to 15:20 Invited Lecture **S1-IL-01**

Mădălina Maria Bârsan, (National Institute of Materials Physics, Măgurele, Romania) Caroline G. Sanz, Ariana Șerban, Alexandru Evanghelidis, Ionuț Enculescu, Victor C. Diculescu

3D Flexible Electrodes for *In Vivo* Measurements in Cell Cultures Based on Conductive Electrospun Polymeric Fibers

15:20 to 15:40 **S1-O-08**

Ales Danhel (Institute of Biophysics of the Czech Academy of Sciences, Brno, Czech Republic) Lukas Fojt, Miroslav Fojta

Electrochemical Pre-treatment of Pyrolytic Graphite Electrode and Its Negative Effect on Voltammetric Detection of DNA

15:40 to 16:00 **S1-O-09**

Barbara Jachimska (Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences, Cracow, Poland) Magdalena Szota, Kamil Rakowski

Corona Formation and Conformational Changes in Proteins on Dendrimers

16:00 to 16:20

Coffee Break and Posters

S2 Electrochemical Sensors for Diagnostics and Therapy Monitoring

---

Room 2

Chaired by: Ana Maria Oliveira-Brett

14:30 to 15:00 Keynote **S2-KL-02**

Tautgirdas Ruzgas (Department of Biomedical Science, Malmö University, Sweden)

Franz Cell Setup for Evaluation of Epirermal Biosensing Approaches

15:00 to 15:20 **S2-O-07**

Estelle Lebègue (Université de Nantes, CNRS, CEISAM UMR 6230, F-44000 Nantes, France) Frédéric Barrière, Allen J. Bard

Single Electrochemical Nano-impacts of Synthetic Redox Phospholipid Liposomes

15:20 to 15:40 **S2-O-08**

Ana-Maria Chiorcea-Paquim (Department of Chemistry, University of Coimbra, Portugal) Isabel Garrido Fernandes, Ana Maria Oliveira-Brett

Calcium Channel Blocker Lercanidipine Electrochemistry at a Carbon Black Modified Glassy Carbon Electrode

15:40 to 16:00 **S2-O-09**

Stela-Maria Pruneanu (National Institute for Research and Development of Isotopic and Molecular Technologies, Cluj-Napoca, Romania) Florina Pogăcean, Codruţa Varodi, Maria Coros, Alexandra Ciorita, Valentin Mirel

Graphene-Modified Electrodes used for the Enhanced Detection of Biomolecules

16:00 to 16:20

Coffee Break and Posters

S6 Electron Transport in Biological Systems - Theory and Experiment

Room 3

Chaired by: Elisabeth Lojou

14:30 to 15:00 Keynote S6-KL-02

David H. Waldeck (Department of Chemistry, University of Pittsburgh, USA) Jimeng Wei, Caleb Clever, Jose Rivas, Brian Bloom

Electron Transfer and Spin Selectivity in Biomolecules

15:00 to 15:20 S6-O-07

Simon Guette-Marquet (Laboratoire de Génie Chimique, Université de Toulouse, France) Christine Roques, Alain Bergel

First Evidence of Electron Transfers Between Animal Cells and Electrodes

15:20 to 15:40 S6-O-08

Patricia Rodriguez-Macia (University of Oxford, Department of Chemistry, Inorganic Chemistry Laboratory, UK) Jifu Duan, Amy Lian, Simone Morra, Martin Winkler, Thomas Happe, Kylie Vincent

Digging at the Catalytic Cycle of [FeFe] Hydrogenase by Single Crystal Electrochemical IR Microspectroscopy

15:40 to 16:00 S6-O-09

Andrew Marcus (Biodesign Swette Center for Environmental Biotechnology, Arizona State University, Tempe, AZ, USA)

Mathematical Modeling of Multiple Extracellular Electron Transfer Pathways

16:00 to 16:20

Coffee Break

Plenary Lecture

Room 1

Chaired by: Renata Bilewicz

16:20 to 17:20 PL-02

David Cahen (Weizmann Inst. of Science, Israel)

Proteins as Bio-Electronic Materials

17:20 to 19:30

Poster Exhibition and Networking

S1 Smart Materials for Bioelectrochemistry

The Poster presenting authors are kindly asked to be connected online in order to answer to the questions addressed by participants.

Tuesday, 11 May 2021 - Morning

Plenary Lecture

Room 1

Chaired by: Robert Săndulescu

09:00 to 10:00 PL-03

Serge Cosnier (University of Grenoble Alpes, France)

Toolbox based on Nanoobjects for the Design of Bioelectrochemical Devices

---

S2 Electrochemical sensors for diagnostics and therapy monitoring

---

Room 1

Chaired by: Giovanna Marrazza and Renata Bilewicz

10:00 to 10:20 S2-O-10

Miroslav Fojta (Institute of Biophysics of the CAS, Brno, Czech Republic) Ales Danhel, Daniel Dobrovodsky, Stanislav Hason, Hana Pivonkova, Zuzana Soldanova, Martina Outla, Veronika Ostatna

New Findings in Label-Free Nucleic Acid Electrochemistry: Effects Homonucleotide Blocks and Catalytic Hydrogen Evolution

10:20 to 10:40 S2-O-11

Felipe Conzuelo (Center for Electrochemical Sciences, Faculty of Chemistry and Biochemistry, Ruhr University Bochum, Germany) Marc Riedel, Adrian Ruff, Fred Lisdat, Wolfgang Schuhmann

Multiplexed Readout of Enzymatic Redox Reactions Triggered by Light

10:40 to 11:00 S2-O-12

Yi-Tao Long (School of Chemistry and Chemical Engineering, Nanjing University, P. R. China)

Single Molecular Sensing by the Nanopore-Based Single-Biomolecule Interface

11:00 to 11:30

Coffee Break and Posters

11:30 to 11:50 **S2-O-13**

Pankaj Vadgama (School of Engineering and Materials Science, Queen Mary University of London, UK) Anna Spehar-Délèze, Salzitsa Anastasova

Lactate Sensor Use in Tissue, Saliva and Sweat: Physiological Uncertainties of Meaningful Measurement

---

S3 Pulsed electric and magnetic fields in biology, medicine and biotechnology

---

Room 2

Chaired by: Michal Cifra and Erika Kis

10:00 to 10:20 **S3-O-01**

Fernanda Leomil (Biophysics Department, Federal University of São Paulo, Brazil; Max Planck Institute of Colloids and Interfaces, Potsdam, Germany) Rafael Lira, Marcelo Zoccoler, Karin Riske, Rumiana Dimova

The Role of Charges on Membrane Stability upon Electroporation

10:20 to 10:40 **S3-O-02**

Dóra Agoston (University of Szeged, Department of Dermatology and Allergology, Szeged, Hungary) Sándor Rátkai, Eszter Baltás, Judit Oláh, Lajos Kemény, Erika Kis

Evaluation of Calcium Electroporation for the Treatment of Cutaneous Metastases: a Double Blinded Randomized Controlled Phase II Trial

10:40 to 11:00 **S3-O-03**

Mina Aleksanyan (Max Planck Institute of Colloids and Interfaces, Potsdam, Germany) Rafael Lira, Jan Steinkühler, Rumiana Dimova

GM1 Leaflet Asymmetry Stabilizes Membrane Pores

11:00 to 11:30

Coffee Break and Posters

11:30 to 12:00 Keynote **S3-KL-01**

Antoni Ivorra (Department of Information and Communication Technologies, Universitat Pompeu Fabra Barcelona, Spain)

Injectable Wireless Microstimulators based on Electronic Rectification of Volume Conducted Currents

S1 Smart Materials for Bioelectrochemistry

Room 3

Chaired by: Sergey Shleev and Nicolas Plumeré

10:00 to 10:20 **S1-O-10**

Fred Lisdat (Biosystems Technology, Institute of Life Sciences and Biomedical Technologies, Technical University Wildau, Germany) Dmitri Ciornii, Marc Riedel, Athina Zouni

Scalable 3D Metal Oxide Electrodes with Improved Transmission for Constructing Photo-Active Electrodes

10:20 to 10:40 **S1-O-11**

Andreia D. Veloso (CQ-VR e Departamento de Química, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal) Maria C. Oliveira, Romeu A. Videira

Electrochemical Characterization of Hydrophilic Carbon Nanomaterials Generated in Carboxylic Buffers

10:40 to 11:00 **S1-O-12**

Gheorghe Melinte (Analytical Chemistry, Faculty of Pharmacy, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania) Oana Hosu, Andreea Cernat, Geanina Ștefan, Giovanna Marrazza, Cecilia Cristea

Polymer and Gold Structured Electrochemical Platforms for Biomedical and Environmental Applications

11:00 to 11:30

Coffee Break and Posters

11:30 to 12:00 Keynote **S5-KL-01**

Uwe Schroeder (Institute of Environmental and Sustainable Chemistry, Technische Universität Braunschweig, Germany)

From Surface Structuring to Bio-Inorganic Hybrid Systems: How Can We Push the Limits of Microbial Biofilm Electrodes?

12:20 to 13:20

BES General Assembly, Room 1

Tuesday, 11 May 2021 - Afternoon

Flash Poster Session 1

Room 1

Chaired by: Fred Lisdat and Renata Bilewicz

14:30 to 16:00

14.30-14.34	**Ricardo Leote**, A. Aldea, E. Matei, V.C. Diculescu	Flexible Electrodes as Platforms for Bio(sensors) for Biomarker Monitoring in Sweat
14.34-14.38	**Laurentiu Spiridon**, L. Manoliu, E. Martin, A. Milac	Using Robot Mechanics for Virutal Screening of Apelin Receptor as an Alzheimer Target
14.38-14.42	**Francisco Prieto-Dapena**, Z. Su, E. Drago, J. Alvarez-Malmagro, M. Rueda, J. Lipkowski	Molecular Recognition of Guanine with Mixed Monolayers of a Nucleolipid and a Phospholipid Supported on Gold (111) Electrodes
14.42-14.46	**Marta Jarczewska**, W. Bojarski, M. Mieczkowska, A. Majewska, E. Malinowska	Electrochemical Detection of miRNA
14.46-14.50	**Denise Demurtas**, E. Magner	An Os polymer and Galactose Oxidase Modified Mesoporous Gold Biosensor for the Determination of Galactose
14.50-14.54	**Varvara Pagali**, D. Soulis, E. Stavra, A. Economou	Fabrication of an Electrochemical Enzymatic Biosensor for Glycose using a Dual Pen-on-Paper Approach
14.54-14.58	**Justyna Borkowska**, S. Oloketuyi, R. Bernedo, A. Christmann, G. Cazzaniga, H.W. Schuchmann, H. Kolmar, A. de Marco, J. Niedziółka-Jönsson, K. Szot-Karpińska	Novel Nanobodies for the Differentiation of Viral from Bacterial Infection
14.58-15.02	**Marcin Jaskółowski**, A. Więckowska	Enhancement of the tetracycline detection process by using an aptasensor modified with gold clusters
15.02-15.06	**Iulia Rus,** A. Pusta, M. Tertiș, R. Săndulescu, C. Cristea	Gemcitabine Electrochemical Direct Detection from Serum and Pharmaceutical Formulations using Boron Doped Diamond Electrode
15.06-15.10	**Ovidiu-Teodor Matica**, E.M. Ungureanu, M. Cristea, L. Pintilie, A. Ștefaniu	Estimation of Chemical Reactivity Parameters through DFT Investigations on 2-thioxo-thiazolidin-4-one Derivatives
15.10-15.14	**Xiaomei Yan**, J. Tang, S. Ma, D. Tanner, R. Ludwig, J. Ulstrup, X. Xia	Enhanced Direct Electron Transfer of Cellobiose Dehydrogenase on Three-Dimensional Graphene Modified Carbon Electrodes
15.14-15.18	**Huaiguang Li**, U. Münchberg, A. Oughli, D. Buesen, W. Lubitz, E. Freier, N. Plumeré	Implementation of Oxygen-Sensitive Catalysts in Fuel-cells
15.18-15.22	**Bruno Roberto Rossi**, G. C. Sedenho, Wilson T. L. da Silva, F. N. Crespilho	Microbial Electrogenicity Evaluation in Domestic Wastewater
15.22-15.26	**Emmanuel Nwanebu**, A. Gomez Vidales, S. Omanovic, B. Tartakovsky	CO_2 Conversion to CH_4 and Acetate in a Microbial Electrosynthesis Cell with Conductive Polymer Cathode Enhanced by Electrodeposition of Ni-based Alloys
15.26-15.30	**Marianne Haberbauer**, S. Spiess, A. Sasiain Conde, S. Thallner, N. Waldmann, E. Neuhauser, A.P. Loibner, N. Kieberger	Bioelectrochemical Methanation of CO_2 from Untreated Steel Mill Gas

Flash Poster Session 2

Room 2

Chaired by: Wolfgang Schuhmann and Ede Bodoki

14:30 to 16:00

14.30-14.34	**Elizaveta Shcherbacheva**, E. Karpova, A. Karyakin	Nanozymes based on Stabilized Prussian Blue Nanoparticles as Substitute for Natural Peroxidase
14.34-14.38	**Ali Jafarov**, A. Elsakova, M. Merzlikin	3D printed electrochemical cells for biosensing on flexible carbon electrodes
14.38-14.42	**Eleni Anna Economou**	Electrochemical Sensing of Organohalide Pollutants Using Heme Functionalized SnO_2 Films on Flexible Plastic Substrates
14.42-14.46	**Aleksandra Buta**, E. Nazaruk, B. Kulawiak, P. Koprowski, A. Szewczyk, Renata Bilewicz	Tracing Potassium Ion Transport through Model Lipid Membranes with Reconstituted Membrane Proteins
14.46-14.50	**Lucian-Gabriel Zamfir**, M. Puiu, G. Madalin Danila, C. Bala	Novel Conductive Peptide Molecular Materials for Electrochemical Sensing of Biomarkers
14.50-14.54	**Sascha Morlock**, M. Riedel, S. Höfs, F. Lisdat	A Bio-hybrid Tandem: Coupling Two Photobioelectrodes for High Voltage Energetics
14.54-14.58	**Eithne Dempsey**	Enzymatic Polymerisation of 1,10 phenanthroline-5,6 dione in Conducting Biocompatible Ink as a Redox Mediator for Glutamate and Glucose Biosensing
14.58-15.02	**Ana-Maria Drăgan**, F. Truţă, M. Tertiş, A. Florea, J. Schram, A. Cernat, B. Feier, K. de Wael, C. Cristea, R. Oprean	Electrochemical Fingerprinting of MDMA for Fast Analysis in Street and Water Samples Using a Graphene-Based Sensor
15.02-15.06	**Kwankao Karnpakdee**, L. Schwaiger, D. Kracher, R. Ludwig	Electron Transfer in Cellobiose Dehydrogenase Multilayers on Electrodes
15.06-15.10	**Vassiliki Katseli**, M. Angelopoulou, C. Kokkinos,	3D-Printed Electrochemical Microwells for Quantum Dot-Based Bioassays
15.10-15.14	**P. Thomas Vernier**, E. B. Sözer	Intracellular distribution of dihydroethidium oxidation products after nanosecond electrical stimulation
15.14-15.18	**Artsiom Klimko**, C. O.Matei, M.G. Moisescu, T. Savopol, M.B. Matei	Evaluation of Electrochemotherapy Efficacy on a 3D Spheroid Neuroblastoma/Monocyte Co-Culture Model
15.18-15.22	**Anna Szewczyk**, N. Rembialkowska, A. Choromanska, K. Biezunska-Kusiak, J. Saczko, J. Kulbacka	Calcium Electroporation Stimulates ROS Release and Alternates ASPH Expression in Human Colon Cancer
15.22-15.26	**Joshua M. Lawrence**, Eleanor R. Clifford, Robert W. Bradley, Laura T. Wey,, X. Chen, C.J. Howe, J.Z. Zhang	Phenazines as low-midpoint potential electron shuttles for photosynthetic bioelectrochemical systems
15.26-15.30	**Giovana Rossi Mendes**, I. Modenez, F.N. Crespilho, G.C. Sedenho	Extracellular Electron Transfer in Saccharomyces Cerevisiae: The Origin of the Bioelectricity

16:00 to 16:20

Coffee Break

16:20 to 19:30

Poster Exhibition and Networking

S2. Electrochemical sensors for diagnostics and therapy monitoring

The Poster presenting authors are kindly asked to be connected online in order to answer to the questions addressed by participants.

Wednesday, 12 May 2021 - Morning

Plenary Lecture

Room 1

Chaired by: Cecilia Cristea

09:00 to 10:00 PL-04

Hubert Girault (Laboratory of Physical and Analytical Electrochemistry, Ecole Polytechnique Fédérale de Lausanne, Switzerland) Sorour Darvishi, Wanderson da Silva Oliveira, Bhawna Nagar, Yingdi Zhu

From Printed Electrodes to Scanning Electrochemistry Microscopy for Bacteria and Biofilms Monitoring

---

S2 Electrochemical sensors for diagnostics and therapy monitoring

---

Room 1

Chaired by: Arkady Karyakin and Serge Cosnier

10:00 to 10:20 S2-O-14

Wlodzimierz Kutner (Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw, Poland) Patrycja Lach, Maciej Cieplak, Marta Majewska, Krzysztof R. Noworyta, Piyush Sindhu Sharma

p-Synephrine Electrochemical Selective Sensing with a Molecularly Imprinted Polymer and a Redox Probe Engaged in a "Gate Effect"

10:20 to 10:40 S2-O-15

Constantin Apetrei, (Chemistry Physics & Environment Department, "Dunărea de Jos" University, Galaţi, Romania) Irina Mirela Apetrei

Detection of Olive Oil Adulteration Using Electrochemical Sensors and Biosensors

10:40 to 11:00 S2-O-16

Clara Abardía-Serrano (Dpto. Química Física y Analítica, Facultad de Química, Universidad de Oviedo, Spain) Rebeca Miranda-Castro, Noemí de-los-Santos-Álvarez, María Jesús Lobo-Castañón

Aptamer-Based Assays for Diagnosis and Management of Celiac Disease

11:00 to 11:30

Coffee Break and Posters

11:30 to 12:00 Keynote **S2-KL-03**

Arkady A. Karyakin (Faculty of Chemistry, M.V. Lomonosov Moscow State University, Russia)

Electrochemical Sensors for Non-invasive Monitoring

12:00 to 12:20 **S2-O-17**

Renata Bilewicz (Faculty of Chemistry, Biological and Chemical Research Centre, University of Warsaw, Poland) Michalina Zaborowska, Damian Dziubak, Dorota Matyszewska, Aleksandra Bartkowiak

How Drugs Decreasing Cholesterol Affect Structure and Electrical Properties of Model Lipid Membranes

12:20 to 12:40 **S2-O-18**

Florence Lagarde (Université Claude Bernard Lyon 1, Institut des Sciences Analytiques, Villeurbanne, France) Manon Mornay, Catherine Jose, Joelle Saulnier

3D-printing of Polylactic Acid Based Interdigitated Electrodes for Greener and Low-Cost Biosensing

12:40 to 13:00 **S2-O-19**

Elena Suprun (Institute of Biomedical Chemistry, Moscow, Russia) Svetlana Khmeleva, Gulnaz Kutdusova, Konstantin Ptitsyn, Viktoriya Kuznetsova, Sergey Lapa, Alexander Chudinov, Sergey Radko

Deoxyuridine Triphosphates Modified with Tyrosine Aromatic Groups for Direct Electrochemical Detection of Double-Stranded DNA Produced by Polymerase Chain Reaction or Isothermal Amplification

13:00 to 13:20 **S2-O-20**

Szilveszter Gáspár (International Centre of Biodynamics, Bucharest, Romania) Raluca-Elena Munteanu, Luciana Stanică, Mihaela Gheorghiu

The Use of Microelectrochemistry to Investigate pH Regulation in Cultured Cancer Cells

---

S4 Bioenergetics and biosynthesis

Room 2

Chaired by: Nicolas Plumeré and Roland Ludwig

10:00 to 10:20 **S4-O-01**

Stéphane Arbault (University of Bordeaux, Pessac, France) Emmanuel Suraniti, Camille Colin, Yumeng Ma, Neso Sojic, Philippe Diolez

Electrochemical Monitoring in Real-Time of The Redox State of Mitochondria

10:20 to 10:40 **S4-O-02**

Anna Lielpetere (Analytical Chemistry - Center for Electrochemical Sciences, Faculty of Chemistry and Biochemistry, Ruhr-University Bochum, Germany) Jana Becker, Julian Szczesny, Felipe Conzuelo, Adrian Ruff, James Birrell, Wolfgang Lubitz, Wolfgang Schuhmann

High Viologen Loading Polymer for Highly Efficient Wiring of Hydrogenases

10:40 to 11:00 **S4-O-03**

Leonardo Castañeda-Losada (Center for Electrochemical Sciences, Ruhr-Universität Bochum; Fraunhofer IGB; Germany) David Adam, Nicole Paczia, Darren Buesen, Fabian Steffler, Volker Sieber, Tobias Erb, Michael Richter, Nicolas Plumeré

CO_2-fixating Bioelectrocatalytic Cascades in Redox-Active Hydrogel for Stereoselective C-C Bond Formation *via* Reductive Carboxylation

11:00 to 11:30

Coffee Break and Posters

11:30 to 12:00 Keynote **S4-KL-01**

Jenny Zhang (University of Cambridge, Department of Chemistry, UK)

Photosynthesis on an Electrode

12:00 to 12:20 **S4-O-04**

Vincent Fourmond (CNRS, Aix-Marseille Université, Marseille, France) Marta Meneghello, Ana R. Oliveira, Aurore Jacq-Bailly, Inês A.C. Pereira, Christophe Léger

Protein Film Electrochemistry Studies of CO_2-reducing Enzymes

12:20 to 12:40 **S4-O-05**

Wassim El Housseini (Laboratoire de Chimie Physique et Microbiologie pour les Matériaux et l'Environnement, Villers-lès-Nancy, France) Alain Walcarius, Neus Vilà, François Lapicque, Elisabeth Lojou, Mathieu Etienne

Efficient Electrochemical Regeneration of the NAD(P)H cofactor

12:40 to 13:00 **S4-O-06**

Steffen Hardt (Center for Electrochemical Sciences, Faculty of Chemistry and Biochemistry, Ruhr University Bochum, Germany) Stefanie Stapf, Dawit Tedros Filmon, James A. Birrell, Olaf Rüdiger, Vincent Fourmond, Christophe Léger, Nicolas Plumeré

Reversible Catalysis for H_2 Oxidation and Evolution by a [FeFe] Hydrogenase in a Viologen Modified Film

13:00 to 13:20 **S4-O-07**

Paolo Bombelli (Environmental sciences and Politics, University of Milan, Italy; Department of Biochemistry, University of Cambridge, UK) Christopher J. Howe

Bio Photo Voltaic (BPV): from Fundamental Principles to Practical Applications

S1 Smart Materials for Bioelectrochemistry

Room 3

Chaired by: Maria Jesús Lobo-Castañón

10:00 to 10:20 S1-O-13

Izabella Brand (Department of Chemistry, University of Oldenburg, Germany) Paulina Komorek, Barbara Jachimska

Effect of the Surface Charge Density of a Gold Electrode on The Amount and Conformation of Adsorbing Lysozyme

10:20 to 10:40 S1-O-14

Andrew Gross (Département de Chimie Moléculaire, Univ. Grenoble Alpes, Grenoble, France) Serge Cosnier, Luminița Fritea, Shunya Tanaka, Clara Colomies, Fabien Giroud, Yuta Nishina, Seiya Tsujimura, Michael Holzinger

Covalent vs. Non-Covalent Surface Modification with Phenazines and Phenothiazines for the Electrical Wiring Of FAD-Glucose Dehydrogenase at Carbon Nanotube Electrodes

10:40 to 11:00 S1-O-15

Jing Tang (College of Environmental Science and Engineering, Hunan University, Changsha, China; Department of Chemistry, Technical University of Denmark, Kongens Lyngby, Denmark) Xiaomei Yan, Wei Huang, Christian Engelbrekt, Jens Øllgaard Duus, Jens Ulstrup, Xinxin Xiao, Jingdong Zhang

Bilirubin Oxidase Oriented on Novel Type Three-Dimensional Biocathodes with Reduced Graphene Aggregation for Biocathode

11:00 to 11:30

Coffee Break and Posters

S5 Microbial Films and Biocorrosion

Chaired by: Pankaj Vadgama

11:30 to 11:50 S5-O-01

Carlo Santoro (University of Milano-Bicocca, Dept. of Material Science, Milano, Italy) Kateryna Arthyushkova, Plamen Atanassov, Sofia Babanova, Alain Bergel, Orianna Bretschger, Robert Brown, Kayla Carpenter, Alessandra Colombo, Rachel Cortese, Pierangela Cristiani, Benjamin Erable, Falk Harnisch, Mounika Kodali, Sujal Phadke, Sebastian Riedle, Luis Rosa, Uwe Schröder

How Comparable Are Microbial Electrochemical Systems Around the Globe? An Electrochemical and Microbiological Cross-Laboratory Study

11:50 to 12:10 **S5-O-02**

Matteo Grattieri (Dipartimento di Chimica, Università degli Studi di Bari "Aldo Moro", Italy) Gabriella Buscemi, Rossella Labarile, Pinalysa Cosma, Angela Agostiano, Massimo Trotta, Gianluca M. Farinola

Sticky Like a Mussel: Polydopamine for Purple Bacteria Biohybrid Photoanodes

12:10 to 12:30 **S5-O-03**

Lucila Martinez Ostormujof (Laboratoire de Génie Chimique, Université de Toulouse, France) Sébastien Teychené, Benjamin Erable

Unravelling Performance Limitations of Microbial Anodes: A Real-time Microscale Approach

12:30 to 12:50 **S5-O-04**

Annemiek Ter Heijne (Environmental Technology, Wageningen University & Research, The Netherlands) Casper Borsje, Tom Sleutels, Cees Buisman

Capacitive Bioanodes in Moving Bed Reactors

12:50 to 13:10 **S5-O-05**

Yolina Hubenova (Institute of Electrochemistry and Energy Systems "Acad. E. Budevski", Bulgarian Academy of Sciences, Sofia, Bulgaria) Galin Borisov, Evelina Slavcheva, Mario Mitov

Gram-positive Bacteria Covered Bioanode in a Membrane Electrode Assembly of Bioelectrochemical Systems

Wednesday, 12 May 2021 - Afternoon

S2 Electrochemical Sensors for Diagnostics and Therapy Monitoring

---

Room 1

Chaired by: Christopher Brett

14:30 to 15:00 Keynote **S2-KL-04**

Maria Jesús Lobo-Castañón (Departamento de Química Física y Analítica, Universidad de Oviedo, Spain) Ramón Lorenzo-Gómez, Ana Díaz-Fernández, Rebeca Miranda-Castro, Noemí de-los-Santos Álvarez

Electrochemical Aptasensors for Cancer-Related Biomarkers - Moving Toward a More Specific Diagnosis

15:00 to 15:20 **S2-O-21**

Itay Algov (Departments of Life Sciences, Chemistry; Ilse Katz Institute for Nanoscale Science and Technology; Ben-Gurion University of the Negev, Beer-Sheva, Israel) Lital Alfonta, Aviv Feiertag

Site-specific Wiring of Glucose Dehydrogenase for Controlled Orientation using Unnatural Amino acid

15:20 to 15:40 **S2-O-22**

Caroline G. Sanz (National Institute of Materials Physics, Măgurele, Romania) Ricardo J. B. Leote, Victor C. Diculescu

Evaluation of Anti-Cancer Drug Shikonin Interaction with dsDNA in Incubated Solutions and *in situ* Sensing at DNA Biosensors

15:40 to 16:00 **S2-O-23**

Isabela Mattioli (São Carlos Institute of Chemistry, University of São Paulo, São Carlos, Brasil) Ayaz Hassan, Natalia Sanches, Nirton Vieira, Frank Crespilho

Graphene Electrical-Electrochemical Hybrid Devices for Zepto-Molar DNA Detections

16:00 to 16:40

Coffee Break and Posters

S4 Bioenergetics and biosynthesis

Room 2

Chaired by: Jenny Zhang

14:30 to 15:00 Keynote **S4-KL-02**

Nicolas Plumeré (Campus Straubing for Biotechnology and Sustainability, TU Munich, Germany)

Protecting Hydrogenases for Energy Conversion

15:00 to 15:20 **S4-O-08**

Laura Wey (Department of Biochemistry, University of Cambridge) Jenny Zhang, Christopher Howe

The Cell-Electrode Interface in Cyanobacterial Exoelectrogenesis

15:20 to 15:40 **S4-O-09**

Sascha Morlock (Biosystems Technology, Institute of Life Sciences and Biomedical Technologies; Technical University Wildau, Germany) Senthil Kumar Subramanian, Athina Zouni, Fred Lisdat

Photosystem I Integrated in 3D Structured Reduced Graphene Oxide for Scalable Biophotovoltaics

15:40 to 16:00 **S4-O-10**

Abraham Gomez Vidales (National Research Council of Canada, Montreal, Canada) Hongbo Li, Sasha Omanovic, Boris Tartakovsky

Evaluation of Biocathode Materials for CO_2 Bioelectroconversion in Microbial Electrosynthesis Cells

16:00 to 16:20 **S4-O-11**

Thiago Bertaglia (São Carlos Institute of Chemistry, University of São Paulo, São Carlos, Brasil) Graziela C. Sedenho, Emily F. Kerr, Roy G. Gordon, Michael J. Aziz, Frank N. Crespilho

A Bioinspired Organic Microbattery Using a Self-Gelling Anthraquinone derivative

16:20 to 16:40

Coffee Break and Posters

16:40 to 19:30

Poster Exhibition and Networking

S3 Pulsed electric and magnetic fields in biology, medicine and biotechnology;

S4 Bioenergetics and biosynthesis;

S5 Microbial Films and Biocorrosion, and

S6 Electron Transport in Biological Systems - Theory and Experiment

The Poster presenting authors are kindly asked to be connected online in order to answer to the questions addressed by participants.

Thursday, 13 May 2021 - Morning

Plenary Lecture

Room 1

Chaired by: Marie Pierre-Rols

09:00 to 10:00 PL-05

Damijan Miklavčič (Laboratory of Biocybernetics, Faculty of Electrical Engineering, University of Ljubljana, Slovenia)

Electroporation based Technologies and Treatment

S3 Pulsed electric and magnetic fields in biology, medicine and biotechnology

Room 1

Chaired by: Damijan Miklavcic and Mihaela Moisescu

10:00 to 10:20 S3-O-04

Mihaela Georgeta Moisescu (Biophysics and Cellular Biotechnology Deptartment, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania) Violeta Liuba Călin, Mona Mihăilescu, Nicolae Tarba, Ana Maria Sandu, Eugen Scarlat, Tudor Savopol

Evaluation of Dynamic Cell Response to Electroporation by Means of Digital Holographic Microscopy

10:20 to 10:40 S3-O-05

Michal Cifra (Institute of Photonics and Electronics of the Czech Academy of Sciences, Prague, Czech Republik) Jiří Průša, Ahmed Taha Ayoub, Djamel Eddine Chafai, Daniel Havelka

All-atom Molecular Dynamics Simulation of Strong Electric Field Effect on Microtubules

10:40 to 11:00 S3-O-06

Sara Gouarderes (IMRCP CNRS UMR 5623, Université Toulouse III - Paul Sabatier, France) Camille Ober, Layal Doumard, Jany Dandurand, Alexander Golberg, Valérie Samouillan, Laure Gibot

Pulsed Electric Fields as Physical Tool to Indirectly Remodel Dermal Extracellular Matrix

11:00 to 11:30

Coffee Break and Posters

11:30 to 12:00 Keynote S3-KL-02

Julita Kulbacka (Department of Molecular and Cellular Biology, Faculty of Pharmacy, Wroclaw Medical University, Poland) Nina Rembiałkowska, Joanna Rossowska, Julia Rudno-Rudzińska, Anna Szewczyk, Anna Choromańska, Olga Michel, Agnieszka Chwiłkowska, Jolanta Saczko, Vitalij Novickij

Milli-, Micro and Nanosecond PEF in Gastrointestinal Related Cancers - *in vitro* and *in vivo* Model

12:00 to 12:20 Invited Lecture S3-IL-01

Felix Sima (CETAL, National Institute for Lasers, Plasma and Radiation Physics, Măgurele, Romania) S. Orobeti, C. Butnaru, F. Jipa, E. Axente, H. Kawano, A. Miyawaki, K. Obata, D. Serien, K. Sugioka

Ultrafast Laser Processing of Glass Microfluidic Systems: Application to Cancer Research

12:20 to 12:40 S3-O-07

Aurel Ottlakan (University of Szeged, Department of Surgery, Hungary) Gyorgy Lazar, Judit Olah, Renata Koszo, Katalin Hideghety, Erika Gabriella Kis

Bleomycin based Electrochemotherapy for Deep-Seated Soft Tissue Sarcomas- Initial Results in Szeged, Hungary

12:40 to 13:00 S3-O-08

Ioan Tivig Biophysics and Cellular Biotechnology Deptartment, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania) Mihaela G Moisescu, Eugenia Kovacs, Tudor Savopol

Changes in the Packing of Bilayer Lipids triggered by Electroporation

---

S2 Electrochemical Sensors for Diagnostics and Therapy Monitoring

---

Room 2

Chaired by: Karolien de Wael and Tautgirdas Ruzgas

10:00 to 10:20 S2-O-24

Tibor Hianik, (Faculty of Mathematics, Physics and Informatics, Comenius University, Bratislava, Slovakia)

Comparative Analysis of the Detection of Leukemia Cells by Electrochemical and Acoustics Aptasensors

10:20 to 10:40 S2-O-25

E.V. Karpova (M.V. Lomonosov Moscow State University, Moscow, Russia) E.V. Shcherbacheva, D.V. Tikhonov, A.A. Karyakin

Transition Metal Hexacyanoferrates Based (Bio)Sensors for Medical Diagnostics

10:40 to 11:00 **S2-O-26**

Hucheng Chang (Food Science and Technology Dept., University of Natural Resources and Life Sciences, Vienna, Austria) Lena Wohlschlager, Adrian Ruff, Wolfgang Schuhmann, Florian Csarman, Stefan Scheiblbrandner, Roland Ludwig

Real-time Determination of Cellobiose and Glucose Formation During Enzymatic Biomass Hydrolysis

11:00 to 11:30

Coffee Break and Posters

11:30 to 11:50 **S2-O-27**

Sorin Sebastian Gheorghe (Faculty of Applied Chemistry and Material Science, University Politehnica of Bucharest, Romania) Ruxandra-Maria Ilie-Mihai, Raluca-Ioana Ștefan-van Staden, Marius Bădulescu

Disposable Stochastic Sensor Based on Deposition of a Nanolayer of Silver on Silk for Molecular Recognition of CA 19-9, CEA, and p53

11:50 to 12:10 **S2-O-28**

Jyoti (Institute of Physical Chemistry, Polish Academy of Sciences,Warsaw, Poland) Carlo Gonzato, Karsten Haupt, Teresa Żołek, Dorota Maciejewska, Andrzej Kutner, Piyush Sindhu Sharma, Krzysztof Noworyta, Włodzimierz Kutner

Acrylate Derivatives Based Molecularly Imprinted Polymer Nanoparticles for the Fabrication of Cilostazol Electrochemical Sensor

12:10 to 12:30 **S2-O-29**

Giada Caniglia (Institute of Analytical and Bioanalytical Chemistry, Ulm University, Germany) Anna Heinzmann, Maria Chiara Sportelli, Antonio Valentini, Nicola Cioffi, Christine Kranz

Antimcrobial Activity of Silver Nanoparticle-Based Films Studied by Scanning Probe Microscopy

12:30 to 12:50 **S2-O-30**

Sevinc Kurbanoglu (Department of Analytical Chemistry, Faculty of Pharmacy, Ankara University, Turkey) Cem Erkmen, Bengi Uslu

Detection of Catechol and Azinphos Methyl Using Poly(3,4-Ethylenedioxythiophene)-Iridium Oxide Nanocomposite Based Tyrosinase Biosensor

S4 Bioenergetics and biosynthesis

Room 3

Chaired by: Lo Gorton

10:00 to 10:20 **S4-O-12**

Krzysztof Stolarczyk (Faculty of Chemistry, University of Warsaw. Poland) Michal Kizling, Maciej Dzwonek, Anna Nowak, Lukasz Tymecki, Agnieszka Wieckowska, Renata Bilewicz

Multi - Enzyme Anode based Biosupercapacitor Operating in Sucrose Solution

10:20 to 10:40 **S4-O-13**

Xinxin Xiao (Department of Chemistry, Technical University of Denmark, Kongens Lyngby, Denmark)

Revisit Mediated Bioelectrodes: How Open-Circuit Potential Is Determined by Enzymatic Kinetics?

10:40 to 11:00 **S4-O-14**

Marcos Pita (Instituto de Catálisis y Petroleoquímica, CSIC, Madrid, Spain) Julia Alvarez-Malmagro, Ana R. Oliveira, Cristina Gutierrez-Sanchez, Beatriz Villajos, Inês A C Pereira, Marisela Velez, Antonio L De Lacey

Bioelectrocatalytic Activity of W-Formate Dehydrogenase Covalently Immobilized on Functionalized Gold and Graphite Electrodes

13:00 – 13:20

Closing Ceremony, Room 1

Poster Presentations

The abstracts marked with (*) are selected for Flash Poster presentation

S1 Smart Materials for Bioelectrochemistry

S1-P-01 *

Vassiliki Katseli (Laboratory of Analytical Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, Greece) Michailia Angelopoulou, Christos Kokkinos

3D-Printed Electrochemical Microwells for Quantum Dot-Based Bioassays

S1-P-02 *

Kwankao Karnpakdee Department of Food Science and Technology, University of Natural Resources and Life Sciences, Vienna, Austria) Lorenz Schwaiger, Daniel Kracher, Roland Ludwig

Electron Transfer in Cellobiose Dehydrogenase Multilayers on ElectrodesS1-P-03

Katarzyna Krukiewicz (Department of Physical Chemistry and Technology of Polymers, Silesian University of Technology, Gliwice, Poland) Dominika Czerwinska-Glówka, Malgorzata Skorupa

Cell Adhesive, Bacteriostatic and Bactericidal Conducting Polymer Coatings for Neural Regeneration

S1-P-04

Florina Maria Truţă (Analytical Chemistry Department,"Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania) Ana-Maria Drăgan, Mihaela Tertiş, Anca Florea, Jonas Schram, Andreea Cernat, Bogdan Feier, Karolien De Wael, Cecilia Cristea, Radu Oprean

Electrochemical Fingerprinting of Cocaine in Street and Water Samples Using a Multi-Walled Carbon Nanotubes-Based Sensor

S1-P-05 *

Ana-Maria Drăgan (Analytical Chemistry Department,"Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania) Florina Maria Truţă, Mihaela Tertiş, Anca Florea, Jonas Schram, Andreea Cernat, Bogdan Feier, Karolien De Wael, Cecilia Cristea, Radu Oprean

Electrochemical Fingerprinting of MDMA for Fast Analysis in Street and Water Samples Using a Graphene-Based Sensor

S1-P-06 *

Eithne Dempsey (Department of Chemistry, Kathleen Lonsdale Institute for Human Health, Maynooth University, Maynooth Co. Kildare, Ireland)

Enzymatic Polymerisation of 1,10 phenanthroline-5,6 dione in Conducting Biocompatible Ink as a Redox Mediator for Glutamate and Glucose Biosensing

S1-P-07

Maria Coros (National Institute for Research and Development of Isotopic and Molecular Technologies, Cluj-Napoca, Romania) Florina Pogăcean, Codruţa Varodi, Alexandru Turza, Emese Gal, Alexandra Ciorita, Stela Pruneanu

Nitrogen, Sulfur co-doped Graphene: Smart Materials for Electrochemical Applications

S1-P-08 *

Sascha Morlock (Biosystems Technology, Institute of Life Sciences and Biomedical Technologies, Technical University Wildau, Germany) Marc Riedel, Soraya Höfs, Fred Lisdat

A Bio-hybrid Tandem: Coupling Two Photobioelectrodes for High Voltage Energetics

S1-P-09 *

Lucian-Gabriel Zamfir (LaborQ, University of Bucharest, Romania), Mihaela Puiu, George Mădălin Dănilă, Camelia Bala

Novel Conductive Peptide Molecular Materials for Electrochemical Sensing of Biomarkers

S1-P-10 *

Aleksandra Buta (Faculty of Chemistry, University of Warsaw; Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology Polish Academy of Science, Warsaw, Poland) Ewa Nazaruk, Bogusz Kulawiak, Piotr Koprowski, Adam Szewczyk, Renata Bilewicz

Tracing Potassium Ion Transport through Model Lipid Membranes with Reconstituted Membrane Proteins

S1-P-11

Adrian Enache (National Institute of Materials Physics, Măgurele, Romania) Melania Onea, Mihaela Bacalum

Cells under Irradiation – Electrochemical Evaluation

S1-P-12

Dominika Czerwinska-Glowka (Department of Physical Chemistry and Technology of Polymers, Silesian University of Technology, Gliwice, Poland) Magdalena Skonieczna, Sebastian Student, Wioletta Przystas, Ewa Zablocka-Godlewska, Beata Cwalina, Katarzyna Krukiewicz

Antimicrobial and Neuroprotective Tetracycline-Loaded Poly(3,4-Ethylenedioxypyrrole) Matrix for Biomedical Applications

S1-P-13 *

Eleni Anna Economou (Department of Materials Science, University of Patras, Greece)

Electrochemical Sensing of Organohalide Pollutants Using Heme Functionalized SnO_2 Films on Flexible Plastic Substrates

S1-P-14

Katarzyna Lesniak-Ziolkowska (Faculty of Chemistry, Silesian University of Technology, Gliwice, Poland) Kasjana Brodacz, Alicja Kazek-Kesik, Wojciech Simka

Bacteriostatic properties of Mg-containing oxide coatings formed on Ti surface via plasma electrolytic oxidation

S1-P-15

Alexandra Elsakova (Institute of Technology, University of Tartu, Estonia) Mark Merzlikin, Ali Jafarov, Ausra Baradoke

Examination of carbon fibre microcylinder as an electrode for electrochemical sensing

S1-P-16 *

Ali Jafarov (Institute of Technology, University of Tartu, Estonia) Alexandra Elsakova, Mark Merzlikin, Ausra Baradoke

3D printed electrochemical cells for biosensing on flexible carbon electrodes

S1-P-17

Katherine D. Pershina (Vernadsky Institute of General and Inorganic Chemistry NAS of Ukraine, Kiev, Ukraine) M.O. Khodykina

Carrier impact on the properties of the organo-mineral heterostructures based on vegetable extracts

S1-P-18 *

Elizaveta Shcherbacheva (Faculty of Chemistry, Lomonosov Moscow State University, Moscow, Russia) Elena Karpova, Arkady Karyakin

Nanozymes Based on Stabilized Prussian Blue Nanoparticles as Substitute for Natural Peroxidase

S1-P-19

Marina D. Zavolskova (Faculty of Chemistry, Lomonosov Moscow State University, Moscow, Russia) V. N. Nikitina, A.A.Karyakin

Synthesis and Catalytic Properties of Ultrasmall Prussian Blue Nanoparticles with Peroxidase Activity

S1-P-20

Yoshua Moore (TUM Campus Straubing for Biotechnology and Sustainability, Straubing, Germany) Darren Buesen, Huaiguang Li, Xialong Chen, Jenny Zhang, Nicolas Plumeré

An Electroanalytical Approach to Measure the Pore Size Distribution of Inverse Opal Electrodes

S1-P-21

Patrick Severin Sfragano (Dipartimento di Chimica "Ugo Schiff", Università degli Studi di Firenze, Sesto Fiorentino, Italy) Francesca Bettazzi, Chiara Ingrosso, Valentina Pifferi, Luigi Falciola, M. Lucia Curri, Ilaria Palchetti

Gold Nanoparticles Decorated Reduced Graphene Oxide for Highly Sensitive Electrochemical Monitoring of Fortified Infant Food and Formulae

S1-P-22

Patrick Severin Sfragano (Dipartimento di Chimica "Ugo Schiff", Università degli Studi di Firenze, Sesto Fiorentino, Italy) Giulia Moro, Alessandro Angelini, Federico Polo, Ilaria Palchetti

A Novel Bicyclic Peptide as Bioreceptor in Electrochemical Biosensing of Clinical Markers

S1-P-23

Maria Kuznowicz (Chemical Technology and Engineering Institute, Faculty of Chemical Technology, Poznan University of Technology, Poznan, Poland) Artur Jędrzak, Teofil Jesionowski

Poly(caffeic acid)@Carbon Nanotube Decorated with CuO for Glucose Detection

S2 Electrochemical Sensors for Diagnostics and Therapy Monitoring

S2-P-01 *

Varvara Pagali (Department of Chemistry, National and Kapodistrian University of Athens, Greece) Dionysios Soulis, Eleftheria Stavra, Anastasios Economou

Fabrication of an Electrochemical Enzymatic Biosensor for Glycose using a Dual Pen-on-Paper Approach

S2-P-02 *

Francisco Prieto-Dapena (Department of Physical Chemistry, University of Seville, Spain) ZhangFei Su, Esrella Drago, Julia Alvarez-Malmagro, Manuela Rueda, Jacek Lipkowski

Molecular Recognition of Guanine with Mixed Monolayers of a Nucleolipid and a Phospholipid Supported on Gold (111) Electrodes

S2-P-03

Franziska Schachinger (Biocatalysis and Biosensing Laboratory, Department of Food Science and Technology, University of Natural Resources and Life Sciences, Vienna, Austria) Stefan Scheiblbrandner, Su Ma, Roland Ludwig

Cytochrome-enzyme Chimeric Proteins for Direct Electron Transfer-Based Glucose Biosensors

S2-P-04

Lorenz Schwaiger (Biocatalysis and Biosensing Laboratory, Department of Food Science and Technology, University of Natural Resources and Life Sciences, Vienna, Austria) Hucheng Chang, Stefan Scheiblbrandner, Roland Ludwig

Measuring Lytic Polysaccharide Monooxygenase Activity on Solid Substrates

S2-P-05

Florina Pogăcean (National Institute for Research and Development of Isotopic and Molecular Technologies, Cluj-Napoca, Romania) Codruţa Varodi, Maria Coros, Alexandra Ciorita, Valentin Mirel

Electrochemical detection of L-Tryptophan with Graphene-Modified Electrode

S2-P-06

Codruţa Varodi (National Institute for Research and Development of Isotopic and Molecular Technologies, Cluj-Napoca, Romania) Maria Coros, Florina Pogăcean, Alexandra Ciorita, Stela Pruneanu

Amperometric Detection of L-Cysteine with N and S co-doped Graphene-Modified Electrodes

S2-P-07

Denisa Căpăţînă (Analytical Chemistry Department,"Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania) Cecilia Cristea, Bogdan Feier

Electrochemical Sensor Based on Molecularly Imprinted Polymer for the Detection of Bacterial Quorum Sensing Molecules

S2-P-08

Alexandra Pusta (Analytical Chemistry Department,"Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania) Denisa Căpătînă, Adrian Blidar, Bogdan Feier, Cecilia Cristea

The Development of an Electrochemical Method for the Detection of Gentamicin in Biological Samples

S2-P-09

Anca Aldea (National Institute of Materials Physics, Măgurele, Romania) Elena Matei, Ionuţ Enculescu, Victor C. Diculescu

Applications of Conductive Electrospun Polymeric Fibers in DNA Biosensing

S2-P-10

Caroline G. Sanz (National Institute of Materials Physics, Măgurele, Romania) Anca Aldea, Ricardo J.B. Leote, Mădălina M. Bârsan

Disposable SOD Biosensors Based on Metallized Electrospun Polymeric Fibers for the Detection of Superoxide in Cell Culture Media

S2-P-11

Geanina Maria Ştefan (Analytical Chemistry Department,"Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania) Oana Hosu, María Jesús Lobo-Castañón, Noemí de-los-Santos-Álvarez, Cecilia Cristea

Aptamer Selection for Vancomycin Using Magnetic Beads-Based SELEX Technology

S2-P-12

Alexandra Canciu (Analytical Chemistry Department,"Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania) Andreea Cernat, Mihaela Tertiş, Cecilia Cristea

Electrochemical Detection of Enterobactin as a Biomarker for *Escherichia Coli* with a Hydrogel and Nanoparticle Layer-based Sensor

S2-P-13

Simin Arshi (Department of Chemical Sciences and Bernal Institute, University of Limerick, Ireland) Edmond Magner

Controlled Immobilization of Enzymes in Biocatalytic Reactors

S2-P-14 *

Iulia Rus (Analytical Chemistry Department,"Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania) Alexandra Pusta, Mihaela Tertiș, Robert Săndulescu, Cecilia Cristea

Gemcitabine Electrochemical Direct Detection from Serum and Pharmaceutical Formulations using Boron Doped Diamond Electrode

S2-P-15 *

Denise Demurtas (Department of Chemical Sciences and Bernal Institute, University of Limerick, Ireland) Edmond Magner

An Os polymer and Galactose Oxidase Modified Mesoporous Gold Biosensor for the Determination of Galactose

S2-P-16 *

Ricardo Leote (National Institute of Materials Physics; Faculty of Physics, University of Bucharest, Măgurele, Romania) Anca Aldea, Elena Matei, Victor C. Diculescu

Flexible Electrodes as Platforms for Bio(sensors) for Biomarker Monitoring in Sweat

S2-P-17

Alina Alexandra Vasile (Corbei) ("Politehnica" University of Bucharest, Romania) Veronica Anastasoaie, Mihaela Cristea, Eleonora-Mihaela Ungureanu, Amalia Ştefaniu

Theoretical Studies Using Quantum Mechanical Calculations for 1,3,4-Thiadiazole Derivatives with Electrochemical Applications

S2-P-18

Marie-Christin Viehauser (Biocatalysis and Biosensing Laboratory, Department of Food Science and Technology, University of Natural Resources and Life Sciences, Vienna, Austria) Roland Ludwig

Generation of a Chimeric Oxidoreductase Capable of Direct Electron Transfer

S2-P-19 *

Ovidiu-Teodor Matica ("Politehnica" University of Bucharest, Romania) Eleonora-Mihaela Ungureanu, Mihaela Cristea, Lucia Pintilie, Amalia Ştefaniu

Estimation of Chemical Reactivity Parameters through DFT Investigations on 2-thioxo-thiazolidin-4-one Derivatives

S2-P-20

Krzysztof Stolarczyk (University of Warsaw, Faculty of Chemistry, Warsaw, Poland) Elzbieta U. Stolarczyk, Andrzej Les, Marta Laszcz, Marek Kubiszewski, Katarzyna Sidoryk

New Perspective of Biologically Active Compounds and Thiocompounds Conjugated with Gold Nanoparticles for Nanotechnology Applications

S2-P-21

Maria Madej (Jagiellonian University, Faculty of Chemistry, Kraków, Poland) Dariusz Matoga, Klaudia Skaźnik, Radosław Porada, Bogusław Baś, Jolanta Kochana

Sensitive Determination of Antidepressant Citalopram with MOF-Based Voltammetric Sensor

S2-P-22 *

Laurențiu Spiridon (Institute of Biochemistry of the Romanian Academy, Bucharest, Romania) Laura Manoliu, Eliza Martin, Adina Milac

Using Robot Mechanics for Virutal Screening of Apelin Receptor as an Alzheimer Target

S2-P-23 *

Marta Jarczewska (Medical Biotechnology Chair, Faculty of Chemistry, Warsaw University of Technology, Poland) Wiktor Bojarski, Magdalena Mieczkowska, Aleksandra Majewska, Elżbieta Malinowska

Electrochemical Detection of miRNA

S2-P-24 *

Marcin Jaskółowski (University of Warsaw, Faculty of Chemistry, Warsaw, Poland) Agnieszka Więckowska

Enhancement of the Tetracycline Detection Process by using an Aptasensor Modified with Gold Clusters

S2-P-25 *

Justyna Borkowska (Institute of Physical Chemistry Polish Academy of Sciences, Warsaw, Poland) Sandra Oloketuyi, Roberto Bernedo, Andreas Christmann, Giulia Cazzaniga, Horst Wilhelm Schuchmann, Harald Kolmar, Ario de Marco, Joanna Niedziółka-Jönsson, Katarzyna Szot-Karpińska

Novel Nanobodies for the Differentiation of Viral from Bacterial Infection

S2-P-26

Ekaterina D. Maksimova (Department of Chemistry, M. V. Lomonosov Moscow State University, Moscow, Russia) V.N. Nikitina, A.A. Karyakin

Flow Injection Amperometry as an Alternative to Potentiometry for Solid Contact Ion-Selective Membrane-Based Electrodes

S2-P-27

Agnieszka Więckowska (University of Warsaw, Faculty of Chemistry, Warsaw, Poland) Marcin Jaskółowski

Oligonucleotides monolayers – comparison of the method of attachment to the substrate

S2-P-28

Artur Jędrzak (Chemical Technology and Engineering Institute, Faculty of Chemical Technology, Poznan University of Technology, Poznan, Poland) Maria Kuznowicz, Teofil Jesionowski

Accurate and Rapid Sensor based on Polydopamine Nanomaterial

S3 Pulsed electric and magnetic fields in biology, medicine and biotechnology

---

S3-P-01 *

Anna Szewczyk (Department of Molecular and Cellular Biology, Faculty of Pharmacy, Wroclaw Medical University, Poland) Nina Rembialkowska, Anna Choromanska, Katarzyna Biezunska-Kusiak, Jolanta Saczko, Julita Kulbacka

Calcium Electroporation Stimulates ROS Release and Alternates ASPH Expression in Human Colon Cancer

S3-P-02 *

Artsiom Klimko (Faculty of Medicine, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania) Christien Oktaviani Matei, Mihaela G. Moisescu, Tudor Savopol, Mircea Bogdan Matei

Evaluation of Electrochemotherapy Efficacy on a 3D Spheroid Neuroblastoma/ Monocyte Co-Culture Model

S3-P-03 *

P. Thomas Vernier (Frank Reidy Research Center for Bioelectrics, Old Dominion University Norfolk, VA, USA) Esin B. Sözer

Intracellular Distribution of Dihydroethidium Oxidation Products after Nanosecond Electrical Stimulation

S3-P-04

Julita Kulbacka (Department of Molecular and Cellular Biology, Faculty of Pharmacy, Wroclaw Medical University, Poland) Olga Michel, Wojciech Szlasa, Jolanta Saczko, Mounir Tarek

Can the Green Tea Catechin Play a Role In Enhancing the Efficacy of Electroporation in Pancreatic Cancer Cells?

S3-P-05

Petra Rózsa (Department of Dermatology and Allergology, University of Szeged, Hungary) Dóra Ágoston, Krisztina Bottyán, Edit Szederkényi, Anita Varga, Henriette Ócsai, Eszter Baltás, Lajos Kemény, Judit Oláh, Erika Kis

Electrochemotherapy Treatment of Multiple Non-Melanoma Skin Tumors in a Renal Transplant Patient

S3-P-06

Anna Szewczyk (Department of Molecular and Cellular Biology, Faculty of Pharmacy, Wroclaw Medical University, Poland) Zofia Łapińska, Urszula Szwedowicz, Agnieszka Gajewska-Naryniecka Jolanta Saczko

Calcium Electroporation (CaEP) Fused with 17β-estradiol in Ovarian Cancer Treatment *in vitro*

S3-P-07

Dahae Kim (Division of Animal, Horticultural and Food Sciences; Brain Korea 21 Center for Bio-Health Industry, Chungbuk National University, Cheongju, Republic of Korea) Sora Lee, Myung-Min Oh

Growth Response of Tomato under Different Direction of External Electric Field

S4 Bioenergetics and biosynthesis

S4-P-01 *

Emmanuel Nwanebu (National Research Council of Canada, Montreal, QC, Canada) Abraham Gomez Vidales, Sasha Omanovic, Boris Tartakovsky

CO_2 Conversion to CH_4 and Acetate in a Microbial Electrosynthesis Cell with Conductive Polymer Cathode Enhanced by Electrodeposition of Ni-based Alloys

S4-P-02

Marianne Haberbauer (K1-MET GmbH, Stahlstraße 14, 4020 Linz, Austria) Klemens Kremser, Georg M. Gübitz, Sabine Spiess, Sophie Thallner, Jiri Kucera, Martin Mandl

Bioelectrochemical Recovery of Metals from Ashes and Slags

S4-P-03 *

Xiaomei Yan (Department of Chemistry, Technical University of Denmark, Kgs Lyngby, Denmark) Jing Tang, Su Ma, David Tanner, Roland Ludwig, Jens Ulstrup, Xinxin Xia

Enhanced Direct Electron Transfer of Cellobiose Dehydrogenase on Three-Dimensional Graphene Modified Carbon Electrodes

S4-P-04

Luana Cristina Italiano Faria (São Carlos Institute of Chemistry, University of São Paulo, Brasil) Graziela Cristina Sedenho, Frank Nelson Crespilho

Carbon-based Electrodes for Application in Bio-inspired Organic Batteries

S4-P-05

José Eduardo Clarindo (São Carlos Institute of Chemistry, University of São Paulo, Brasil) Lucyano Macedo, Graziela Sedenho, Frank Crespilho

In-situ FTIR and UV-Vis for the Mapping of Redox Reactions of Symmetrical Quinones

S4-P-06 *

Bruno Roberto Rossi (São Carlos Institute of Chemistry, University of São Paulo, Brasil) Graziela Cristina Sedenho, Wilson T. L. da Silva, Frank Nelson Crespilho

Microbial Electrogenicity Evaluation in Domestic Wastewater

S4-P-07

Paulo Henrique Maciel Buzzetti (Université Grenoble Alpes, DCM UMR 5250, Grenoble, France) Marie Carrière, Karine Gorgy, Fabien Giroud, Mumtaz Muhammad, Redouane Borsali, Serge Cosnier

Polystyrene-β-cyclodextrin nanoparticles for Oriented Electro Connection of Enzyme

S4-P-08 *

Huaiguang Li (Campus Straubing for Biotechnology and Sustainability, Technical University Munich, Straubing, Germany) Ute Münchberg, Alaa A. Oughli, Darren Buesen, Wolfgang Lubitz, Erik Freier, Nicolas Plumeré

Implementation of Oxygen-Sensitive Catalysts in Fuel-cells

S5 Microbial Films and Biocorrosion

S5-P-01 *

Marianne Haberbauer (K1-MET GmbH, Stahlstraße 14, 4020 Linz, Austria) Sabine Spiess, Amaia Sasiain Conde, Sophie Thallner, Niels Waldmann, Eva Neuhauser, Andreas Paul Loibner, Nina Kieberger

Bioelectrochemical Methanation of CO_2 from Untreated Steel Mill Gas

S5-P-02

Mario Mitov (Innovative Center for Eco Energy Technologies; Department of Chemistry, South-West University "Neofit Rilski", Blagoevgrad, Bulgaria) Elitsa Chorbadziyska, Ivo Bardarov, Krassimir L. Kostov, Yolina Hubenova

Precious Metals Recovery by Microbial Electrochemical Snorkel

S6 Electron Transport in Biological Systems - Theory and Experiment

S6-P-01

Dimitrios Zouraris (Laboratory of Physical Chemistry and Applied Electrochemistry, School of Chemical Engineering, National Technical University of Athens, Greece) Anthi Karnaouri, Pavlos Pandis, Christos Argirusis, Evangelos Topakas, Antonis Karantonis

Voltammetric Study of the Binding of Phosphoric Acid-Swollen Cellulose with Immobilized Lytic Polysaccharide Monooxygenases

S6-P-02 *

Giovana Rossi Mendes (São Carlos Institute of Chemistry, University of São Paulo, Brasil) Iago Modenez, Frank N. Crespilho, Graziela C. Sedenho

Extracellular Electron Transfer in *Saccharomyces Cerevisiae*: The Origin of the Bioelectricity

S6-P-03

Christine Lewis (Biodesign Institute, Arizona State University, Tempe Arizona, USA) Justin Flory, Sidney Hecht, Thomas Moore, Ana Moore, Bruce Rittmann, César Torres, Petra Fromme

Unlocking Efficiency: Electro-Molecular Investigations of Photosynthetic Energy Flow with Microbial Electro Photosynthetic System

S6-P-04 *

Joshua M. Lawrence (Department of Biochemistry, University of Cambridge, UK) Eleanor R. Clifford, Robert W. Bradley, Laura T. Wey, Xiaolong Chen, Christopher J. Howe, Jenny Z. Zhang

Phenazines as low-midpoint potential electron shuttles for photosynthetic bioelectrochemical systems

S6-P-05

Tin Lai (University of Oxford, Department of Chemistry, Inorganic Chemistry Laboratory, Oxford, UK) Patricia Rodriguez-Macia, Simone Morra, Miguel Ramirez-Hernandez, Kylie Vincent

Recombinant Expression and Artificial Maturation of *C. acetobutylicum* [FeFe] Hydrogenase: Electrochemical and Spectroscopic Characterization

S6-P-06

Ethan Howley (Arizona State University, Tempe Arizona, USA) Rosa Krajmalnik-Brown, César Torres

Correlating Cyclic Voltammetry and mRNA transcriptomics in *G. sulfurreducens*

Abstracts of
Giulio Milazzo Prize,
Luigi Galvani Prize,
Plenary, Keynote and
Invited Lectures

Giulio Milazzo Prize

Electroporation, from Fundamentals to Medical Applications

Lluis M. MIR
METSY, UMR 9018 CNRS - Université Paris-Saclay,
Gustave Roussy PR2, 114 r. E. Vaillant, 94805 Villejuif, France
luis.mir@cnrs.fr

Antitumor electrochemotherapy is the cancer treatment that I developed from the inception to the clinics. It is based on the electroporation, also termed electropermeabilisation, one of the areas of interest of the BES. Elaborating the combination of an « old » anticancer drug and the electric pulses (EP) delivery from the bench to the animal housing facilities and later to the clinics, both human and veterinarian, has been an incredible personal and collective adventure, shared with many different teams. Electrochemotherapy in humans is still developing today, but it starts to be the protocol to apply in several complicated oncological situations. In veterinary medicine, there is a very rapid growth of its use, with several producers and hundreds of devices in use around the world.

Electrochemotherapy is based on the reversible electroporation of the cells. The EP do not kill the cells by themselves, as they are employed to allow anticancer drugs to penetrate the cells. This principle and its consequences explain efficacy and safety. Consequences of the EP delivery (namely, antivascular effects and cellular reactions mimicking an immunogenic cell death) also contribute synergistically to the electrochemotherapy safety and efficacy.

Electric pulses, through the so-called irreversible electroporation can kill the cells. I contributed to establish the principle of an ablative method based on the EP delivery alone (no combination with a drug), and published the first treatment of mice tumors under conditions leading to the complete regression (cure) of most of the treated tumors. This method is also in the clinics, and is extended nowadays in cardiology, to treat cardiac arrhythmias.

I also tried to better understand the phenomena occurring at the membranes exposed to the EP, not only from the electrical point of view but also from a chemical point of view. The pores, as hydrophilic conduits in the membrane created by an increase of the transmembrane potential difference (TMV), seem to vanish almost immediately when the high TMV dissipates. However, oxidations and peroxidations occur at the time of the EP delivery and they can explain why the transport of molecules across the membrane allowed by the hydrophilic pores (during the electroporated state of the membrane) can continue for long periods after the EP delivery (during the electropermeabilized state of the membrane). However, the chemical modifications affect not only the membrane lipids but also the proteins and new models can be proposed.

Luigi Galvani Prize

DNA-based Nanodevices for Sensing Applications

Francesco Ricci
Chemistry Department, University of Rome, Tor Vergata, Rome, Italy
francesco.ricci@uniroma2.it

DNA nanotechnology uses synthetic DNA (or nucleic acids) sequences as versatile and programmable material to rationally engineer tools and molecular devices at the nanoscale.

During this lecture I will provide an overview of the most representative examples developed in our lab where we exploited the "designability" of synthetic DNA to fabricate nature-inspired DNA-based nanoswitches and nanodevices that are specifically designed to undergo a conformational change (switch) upon binding to a specific input (target).

This input-triggered conformational change mechanism can be easily coupled to an electrochemical output through the expedient of conjugating an electrochemical label on the responsive DNA device and immobilizing it on an electrode surface. In our research group we have demonstrated in the past years that this approach can be successfully applied to detect a wide range of molecular targets including antibodies, DNA sequences, metal ions and proteins with excellent performances in terms of sensitivity and selectivity.

PL-01

Living Bioelectrochemical Composites

Guillermo C. Bazan
Departments of Chemistry and Chemical and Biomolecular Engineering
National University of Singapore
chmbgs@nus.edu.sg

Composites, in which two or more material elements are combined to provide properties unattainable by single components, have a historical record dating to ancient times. Few include a living microbial community as a key design element. A logical basis for enabling bioelectronic composites stems from the phenomenon that certain microorganisms transfer electrons to external surfaces, such as an electrode. A bioelectronic composite which allows one to address cells beyond the confines of an electrode surface can impact bioelectrochemical technologies, including microbial fuel cells for power production and bioelectrosynthesis platforms where microbes produce desired chemicals. In this presentation we show that the conjugated polyelectrolyte CPE-K functions as a conductive matrix to electronically connect a three-dimensional network of *Shewanella oneidensis* MR-1 to a gold electrode, thereby increasing biocurrent ~150-fold over control biofilms. These biocomposites spontaneously assemble from solution into an intricate arrangement of cells within a conductive polymer matrix. While increased biocurrent is due to more cells in communication with the electrode, current extracted per cell is also enhanced indicating efficient long-range electron transport. Further, biocomposites show almost an order-of-magnitude lower charge transfer resistance than CPE-K alone, supporting that the electroactive bacteria and the conjugated polyelectrolyte work synergistically towards an effective bioelectronic composite.

PL-02

Proteins as Bio-Electronic Materials

David Cahen*

Department of Materials and Interfaces, Weizmann Institute of Science, Israel

david.cahen@weizmann.ac.il

Solid state Electron Transport (ETp), *electronic* conduction, across junctions with an ultra-thin protein film as active layer, can be surprisingly efficient. Length-normalized, their ETp efficiency can be similar or even exceed that of conjugated molecules; on top of that, ETp can be temperature-independent down to 4K. That is amazing as nature does not seem to need these features for room temperature electron transfer, ET, which involves proteins in solution and/or membranes as a central, ion transport coupled process. If contacts do not limit ETp, i.e., intra-protein transport dominates, we cannot really measure a transport barrier. I'll show how understanding ETp may be important also for ET, in which coupling to the contacts is replaced by electron injection/extraction. I will rely on experimental data (ours and of others)[1,2,3], incl. recent ones[4] that bear on (at least are consistent with) quantum mechanical tunnelling. This, though, is hard to swallow for > 5 nm proteins, a puzzle, which we want to solve.

* work done with **Mordechai Sheves & Israel Pecht**, *at the Weizmann Inst. of Science, Rehovot, Israel (DC is also at Bar-Ilan University, Israel).*

References:

[1] C. Bostick et al. *Rep. Prog. Phys.*, 81 (2018) 026601.
[2] N. Amdursky et al., *Adv. Mater.* 42 (2014) 7142.
[3] S. Mukhapadhyay et al., *iScience*, 23 (2020) 101099.
[4] J. Fereiro et al., *PNAS*, 115 (2018) E4577; *JACS*, 140 (2018) 3317; 142 (2020) 19217; *Angew.Chem.*, 58 (2019)11852; *Small* (2021) in press 3-2021.

PL-03

Toolbox Based on Nanoobjects for the Design of Bioelectrochemical Devices

Serge Cosnier
Université Grenoble-Alpes University - CNRS
Département de Chimie Moléculaire UMR CNRS 5250, 570 rue de la Chimie,
Université Grenoble-Alpes, CS 40700, 38058 Grenoble cedex 9, France
Serge.Cosnier@univ-grenoble-alpes.fr

For four decades, the development of biointerfaces has been the subject of increasing research efforts in the field of analytical chemistry and energy conversion. In particular, the functionalization of electrodes by biomaterials based on electrogenerated polymers, carbon nanotubes and / or nano-objects, is widely used for the design of biosensors and biofuel cells [1,2]. Some new approaches for developing nanostructured biomaterials based on functionalized carbon or tungsten nanotubes, glyconanoparticles and compressions of carbon nanotubes will be illustrated with enzymes as biosensing element.
In particular, the anchoring of biological macromolecules to the surface of electrodes has been widely undertaken by chemically or photochemically postfunctionalizable electrogenated polymers. The self-assembly of carbon nanotubes via crosslinking polymers in the form of buckypapers has been used to generate electrodes exhibiting chemical or affinity functions allowing grafting of proteins and / or redox mediators. Biosourced block polymers have also been exploited to create water-stable nanoparticles by self-assembly. These particles constitute a multivalent platform for the anchoring of redox mediators and modified enzymes by inclusion reaction. This innovative approach will be applied to the elaboration of solubilized enzymatic fuel cell or biosensors. An innovative concept of reagentless biosensors based on enzymatic inhibition was developed by trapping and releasing the enzyme substrate from the structure of the biosensor. A polyurethane support comprising perforated microcapsules filled with catechol and covered with a conductive deposit of multiwalled carbon nanotubes, modified by laponite clay and tyrosinase enzyme will be described and applied to the detection of benzoic acid [3].

1. X. Chen, L. Yin, J. Lv, A. J. Gross, M. Le, N. G. Gutierrez, Y. Li, I. Jeerapan, F. Giroud, A. Berezovska, R. K. O'Reilly, S. Xu, S. Cosnier, J. Wang. Adv. Funct. Mater., Adv. Funct. Mater., 29 (2019) 1905785.
2. A. Berezovska, Y. Nedellec, F. Giroud, A. Gross, S. Cosnier. Electrochem. Commun., /doi.org/10.1016/j.elecom.2020.106895
3. Y. Nedellec, C. Gondran, K. Gorgy, S. MC Murtry, O. El Mazria, P. Agostini, S. Cosnier. Submitted.

PL-04

From Printed Electrodes to Scanning Electrochemistry Microscopy for Bacteria and Biofilms Monitoring

S. Darvishi, W. Da Silva Oliveira, B. Nagar, Y. Zhu and Hubert H. Girault,
Laboratory of Physical and Analytical Electrochemistry
Ecole Polytechnique Fédérale de Lausanne, Switzerland
Hubert.girault@epfl.ch

In this period of viral pandemic, it is important to remember that other threats for humanity may be impending due to bacterial infection. It is never too late or rather imperative to develop methodologies to facilitate a rapid and accurate bacterial detection and antimicrobial susceptibility testing.
Currently, most detection systems are based on the cell culture approach. Here, I shall present amperometric approaches based on the use of redox mediators to monitor the presence of living bacteria.

First, I shall discuss disposable electrode fabrication techniques, which combines screen printing and inkjet printing to produce arrays of electrodes for bacterial detection and antibiotic assays. Different formats of electrochemical sensors have thus been developed, like the (1 x 8) 3-electrode cell arrays to carry out susceptibility tests on a given bacteria sample with a given antibiotic and the (16 x 16) 2-electrode cell multiplexer array to carry out antibiotic screening tests. Prior to the amperometric measurements, bacterial cells are enriched and isolated from patient's body fluids using affinity probes, either specifically or non-specifically and then the changes in current signals corresponding to the type or concentration of living cells is measured using these fabricated electrodes.

In the second part, I shall present our work on biofilm imaging by scanning electrochemical microscopy (SECM) that uses an adhesive sampling approach and a soft-probe imaging sensor. With the capability to monitor the metabolic activity of living bacterial cells, this imaging procedure allows the investigation of structure, activity and response-to-antibiotics of a biofilm.

PL-05

Electroporation based Technologies and Treatments

Damijan Miklavčič
Laboratory of Biocybernetics, Faculty of Electrical Engineering,
University of Ljubljana, Trzaska 25, 1000 Ljubljana, Slovenia
damijan.miklavcic@fe.uni-lj.si

We are witnessing increasing interest in electroporation as the basis for various applications in food processing, biotechnology, and biomedicine. However, our understanding of the electroporation phenomenon continues to be obscured. In this talk I will describe the events observed on the membrane, cell, and tissue level in the light of accumulated evidence and I will reconcile this evidence with naturally evolved cell defense mechanisms. Starting from the assumption that there are no special/specific mechanisms by which a cell responds to electroporation, we can benefit from existing knowledge in other fields of science. When we look at electroporation simply as a cell membrane or tissue damage, which cells try to repair with their natural repair mechanisms, we can explain a cascade of events observed in various electroporation-based applications. Nevertheless, we still lack understanding when it comes to the mechanisms of molecular transmembrane transport and the "point of no return" for cell death. Quantification of the latter will allow us to improve electroporation-based treatments to facilitate food, biotechnology and biomedical applications. CAR T based treatment, DNA and RNA vaccination, tissue/cell cryopreservation, and cardiac tissue ablation are just some of the most exciting therapies that are emerging and can benefit from developing a mechanistic understanding of electroporation.

S1-KL-01

DNA Nanostructures for Single-Molecule Biosensing

Mukhil Raveendran, Andrew J. Lee, Christoph Wälti, Paolo Actis
School of Electronic and Electrical Engineering, University of Leeds
Woodhouse Lane, Leeds, LS2 9 JT, UK
p.actis@leeds.ac.uk

Early and accurate diagnosis significantly improves patient outcomes. This ambition, however, presents a major technological challenge because detecting specific host-response biomarkers requires the identification of low numbers of molecules in small volumes of complex biological fluids, ideally within minutes.

We will present how to fingerprint the nanopore translocation signals of individual biomarkers while simultaneously enhancing the performance of their detection within biological fluids[1]. Our technique employs DNA origami nanostructures as carriers able to bind the biomarker of interest and hence improve its detection with the nanopore platform. DNA origami is a technique that considers DNA molecules as a building material and folds them into a desired geometry, similar to the Japanese art of paper origami.

We have designed picture frame-like DNA origami objects capable of capturing an inflammation biomarker, C-reactive protein (CRP), with in a central void. Upon translocation through a nanopore, this complex generates a distinctive signal which is disrupted upon capture of the biomarker, enabling us to directly count the binding events.

As our approach is sensitive to individual molecules passing through the nanopore, it conveys a distinct advantage over competing technologies that often require the detection of trillions of analytes before a signal can be processed.
We will further discuss our plans to develop simultaneous multiplexed biomarker detection with the same single molecule resolution and rapid speed on an expanded range of analytes.

1. Raveendran et al, Rational design of DNA nanostructures for single molecule biosensing, *Nature Commun*, 11, 4384 (**2020**)

S1-KL-02

Electrocatalytic studies on membrane proteins from bacterial respiratory chains

Iryna Makarchuk[a], Anton Nikolaev[a], Schara Safarian[b], Alexander Thesseling[c], Thorsten Friedrich[c], Hartmut Michel[b], Tomoichirou Kusumoto[d], Junshi Sakamoto[d], Frederic Melin[a], Petra Hellwig[a]

[a] Laboratoire de Bioélectrochimie et Spectroscopie, UMR 7140, Chimie de la Matière Complexe, Université de Strasbourg – CNRS 4, rue Blaise Pascal, 67081 Strasbourg, France ; hellwig@unistra.fr; [b] Max Planck Institute of Biophysics, Frankfurt am Main, Germany ; [c] Institut für Biochemie, Albert-Ludwigs-Universität Freiburg, Freiburg, Germany; [d] Department of Bioscience and Bioinformatics Kyushu Institute of Technology, 680-4 Kawazu, Fukuoka Japan

Although the architectures of several membrane proteins in respiration as well as the basic chemical reactions have been described, the interactions on molecular level, the diversity and efficiency of the reaction mechanisms in bacterial systems, are under discussion. Electrochemical and spectroscopic experiments are developed to study the coupled electron and proton reactions, the reactivity towards small molecules and, importantly, correlate it with the microenvironment of the cofactors. Respiratory chain enzymes are membrane proteins and direct electrochemical studies of these proteins require the adaptation of the electrode surface, for example with different types of thiols, the optimization of the lipid and detergent content.(1)

First an overview on the immobilization techniques on membrane proteins for direct electrocatalytic studies will be given. Then we will focus on cytochrome *bd* oxidases. These enzymes catalyze the reduction of oxygen in the respiratory chains of bacteria, including several pathogens. They play a crucial role in protection against oxidative stress, in virulence, adaptability and antibiotics resistance. No homologues are found in eukaryotes.

This electrocatalytic study of the cytochrome *bd* oxidases from *Escherichia coli*, *Corynebacterium glutamicum* and *Geobacillus thermodenitrificans* gives evidence for a different reactivity towards oxygen. An inversion of the redox potential values of the two of the hemes was found when comparing the enzymes.(2) This inversion may be correlated with different glutamic acids with a pK higher than 9 as evidenced by reaction induced FTIR spectroscopy.

(1) A. Nikolaev, I. Makarchuk, A. Thesseling, J. Hoeser, T. Friedrich, F. Melin, P. Hellwig, Molecules 25 (2020) 3240.

(2) S. Safarian, A. Hahn, D.J. Mills, M. Radloff, M.L. Eisinger, A. Nikolaev, J. Meier-Credo, F. Melin, H. Miyoshi, R.B. Gennis, J. Sakamoto, J.D. Langer, P. Hellwig, W. Kühlbrandt, H. Michel, Science 366 (2019) 100-104.

S2-KL-01

Insights into the Mechanism of Coreactant Electrochemiluminescence Empower its Analytical Strength

Giovanni Valenti, Sara Rebeccani, Massimo Marcaccio, Francesco Paolucci

Dipartimento di Chimica Giacomo Ciamician, Alma Mater Studiorum - Università di Bologna Via Selmi 2, 40126 Bologna, Italy
Francesco.paolucci@unibo.it

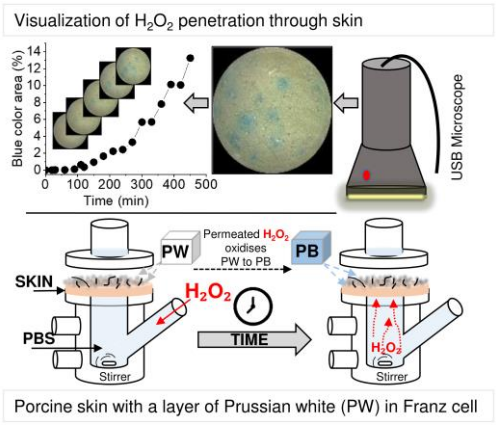
Electrochemiluminescence (ECL) is a powerful transduction technique with a leading role in the biosensing field due to its high sensitivity and low background signal. Although the intrinsic analytical strength of ECL depends critically on the overall efficiency of the mechanisms of its generation, studies aimed at enhancing the ECL signal have mostly focused on the investigation of materials, either luminophores or coreactants. Unravelling the intimate mechanisms which govern the light generation is however mandatory for the design of ultrasensitive devices. Using an innovative combination of ECL imaging techniques and electrochemical mapping of radical generation, we recently discovered an unexpected but highly efficient mechanistic path for ECL generation close to the electrode surface, also supported by quantum chemical calculations and spin trapping methods. This led to the identification of a family of alternative branched amine coreactants, which raises the analytical strength of ECL well beyond that of present state-of-the-art immunoassays.

S2-KL-02

Franz Cell Setup for Evaluation of Epidermal Biosensing Approaches

Tautgirdas Ruzgas
Prof. Biomedical Technology
Department of Biomedical Science, Malmö University, 205 06 Malmö, Sweden
Biofilms – research center for biointerfaces, Malmö University, Malmö, Sweden
tautgirdas.ruzgas@mau.se

In 2015 more than 192 million animals were used in biomedical research. This is 37% increase since 2005. Increasing use of animals can be seen in exploding research on wearables, smart textiles, wireless biosensors, epidermal electronics and epidermal sensing. The trend is logical since the sensors and sensor-driven drug delivery must function in intimate contact with biological barrier, e.g., skin.



It is easy to note that wearable and epidermal biosensors have considerable similarity to topical and transdermal drug delivery; the area where the research has been under high pressure to reduce animal tests. To meet the demand, the development of topical and transdermal drug delivery systematically rely on in vitro tests. The same demands, to reduce the use of animals for testing biosensors, should be of high concern. In this context, we adopted a well-accepted in vitro system, a Franz cell setup, to study epidermal sensing of H_2O_2 on skin surface.

In this presentation, epidermal sensing of H_2O_2 based on Prussian Blue and horseradish peroxidase modified e-textile will be described. Optical and wireless approaches will be compared and discussed.

Jankovskaja et al. Visualisation of H_2O_2 penetration through skin indicates importance to develop pathway-specific epidermal sensing. *Microchim Acta* 2020, 187, 656.

Hoang et al. Franz cells for facile biosensor evaluation: A case of SWCNT-based hydrogen peroxide detection via amperometric and wireless passive RFID tag. Manuscript.

S2-KL-03

Electrochemical Sensors for Non-invasive Monitoring

Arkady A. Karyakin
Chemistry faculty of M.V. Lomonosov Moscow State University
Lenin Hills, 1/3, Moscow,119991, Russsia
aak@analyt.chem.msu.ru

Non-invasive methods, which exclude not only injury to blood vessels, but also damage to the skin surface, are preferred for diagnostics: such methods are painless and avoid potential infection and trauma to patients. Noninvasively collected sweat has already found use in clinical diagnostics of mucoviscidosis (cystic fibrosis) through its conductivity.

A sufficient requirement for noninvasive diagnostics would be a correlation in variation rates between metabolite concentrations in the excreted liquid and the corresponding values in blood [1-4]. Pearson correlation coefficient for variation rates of glucose content reaches the value of 0.75 [2]; the intercept of the regression line is close to zero, and its slope is close to unity. For comparison, the variation rates for lactate content in capillary blood and in vein blood display worse correlation.

The only solution for real-time monitoring of sweat metabolites is first introduced by us the flow-through biosensor [1]. In such setup secreted sweat reaches the biosensor surface and wastes through the open outlet. The readout of the biosensor is in a good agreement with the sweat metabolite content measured independently by sampling it from the monitor outlet: Pearson correlation coefficient $r > 0.98$ [2]. Operational lifetime of this type of biosensor is expected to be at the level of that for existing low-invasive commercial devices.

The possibility of diabetes monitoring on the basis of flow-through biosensor through continuous analysis of sweat has been shown [2]. The dynamics of blood glucose concentration is in a good accordance with the readout of the biosensor. With this example, we thus clearly show that humans (diabetic patients, individuals subjected to glucose tolerance test, etc.) can actually be monitored reliably via non-invasive approach.

References.

1. M.M. Pribil, G.U. Laptev, E.E. Karyakina, A.A. Karyakin. *Anal. Chem.* **86** (2014) 5215.
2. E.V. Karpova, E.V. Shcherbacheva, A.A. Galushin, D.V. Vokhmyanina, E.E. Karyakina, A.A. Karyakin. *Anal. Chem*. **91** (2019) 3778.
3. E.V. Karpova, E.E. Karyakina, A.A. Karyakin. *Talanta* **215** (2020) 120922.
4. E.V. Karpova, A.A. Karyakin. *Curr. Opin. Electrochem.* **23** (2020) 16.

Acknowledgements. The financial support through Russian Science Foundation grant 19-13-00131 is greatly acknowledged.

S2-KL-04

Electrochemical aptasensors for cancer-related biomarkers: moving toward a more specific diagnosis

María Jesús Lobo-Castañón, Ramón Lorenzo-Gómez, Ana Díaz-Fernández, Rebeca Miranda-Castro, Noemí de-los-Santos Álvarez
Departamento de Química Física y Analítica, Universidad de Oviedo, Av. Julián Clavería 8, 33006 Oviedo (Spain)
Instituto de Investigación Sanitaria del Principado de Asturias, Avenida de Roma, 33011 Oviedo (Spain)
mjlc@uniovi.es

A mode of cancer prevention involves the use of screening and early detection tools, leading to the diagnosis of the disease at the earliest possible stage, when it is most likely to be successfully treated. Substantial research effort is being devoted to deciphering the molecular events that contribute to cancer initiation and progression, which leads to the identification of more specific biomarkers, including glycoproteins suffering aberrant glycosylation or extracellular matrix (ECM) components that are altered during tumorigenesis. The use of these new biomarkers for early cancer detection is promising, but there are still several limitations that must be overcome before they could be translated to our healthcare systems. One of the most important is the limited sensitivity and selectivity of the analytical methods available for the detection of these biomarkers, a challenging task due to their low abundance and the subtle changes they present with respect to other molecules produced by normal cells.

Aptamers are especially suitable for developing sensitive and selective sensors for glycoproteins and ECM components. It is possible to obtain aptamers with high affinity toward a specific molecule. In addition, their selection can be properly directed to target a small region within this molecule thus achieving high selectivity. This communication describes the development of aptamer-based electrochemical sensors for the rapid and convenient detection of prostate specific antigen (PSA) and ECM components, from the selection of the aptamers to their integration into the electrochemical transducer. Their analytical performance is presented, not only in aqueous buffer but also in clinical samples, analyzing their clinical sensitivity and specificity.

Acknowledgements
The work has been financially supported by Spanish Government (project RTI-2018-095756-B-I00) and Principado de Asturias Government (Project IDI/2018/000217, co-financed by FEDER funds).

S3-KL-01

Injectable Wireless Microstimulators Based on Electronic Rectification of Volume Conducted Currents

Antoni Ivorra
Department of Information and Communication Technologies
Serra Húnter Fellow Programme
Universitat Pompeu Fabra
Roc Boronat, 138, 08018 Barcelona, Spain
antoni.ivorra@upf.edu

Implantation of most electrical stimulation systems requires complex surgeries which hamper their use for the development of neuroprostheses. Previously developed systems based on central stimulation units are not adequate for applications in which many sites must be individually stimulated over large and mobile body parts, thus hindering practical neuroprosthetic solutions for patients suffering paralysis due to spinal cord injury or other neurological disorders. It has been determined that a solution to these challenges could consist in developing addressable single-channel wireless microstimulators which could be implanted with simple procedures such as injection. However, past attempts in this direction were not successful because the developed implants were stiff and too large. Further miniaturization was prevented because of the use of inductive coupling and batteries as energy sources. Here I will present some results from the eAXON and the EXTEND projects which are funded by the European Commission. These projects are aimed at exploring an innovative method for performing electrical stimulation in which the implanted microstimulators operate as rectifiers of bursts of innocuous high frequency current supplied through skin electrodes shaped as garments. This approach will allow the development of injectable stimulators with a diameter of less than 1 mm. Most of the implants' volume will consists of materials whose density and flexibility match those of neighboring living tissues for minimizing invasiveness. In fact, implants based on the proposed method will look like short pieces of flexible thread.

S3-KL-02

Milli-, Micro, and Nanosecond PEF in Gastrointestinal Related Cancers - *in vitro* and *in vivo* Models

Julita Kulbacka[1], Nina Rembiałkowska[1], Joanna Rossowska[2], Julia Rudno-Rudzińska[3], Anna Szewczyk[1], Anna Choromańska[1], Olga Michel[1], Agnieszka Chwiłkowska[1], Jolanta Saczko[1] & Vitalij Novickij[4]

Department of Molecular and Cellular Biology, Faculty of Pharmacy, Wroclaw Medical University, Wroclaw, Poland; [2]Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland [3]Department of General and Oncological Surgery, Medical University Hospital, Wroclaw, Poland; [4]Institute of High Magnetic Fields, Vilnius Gediminas Technical University, Vilnius, Lithuania;
julita.kulbacka@umed.wroc.pl

Gastrointestinal cancers still are poorly diagnosed, thus their therapy brings not satisfactory outcomes. The main problem is the primary and acquired resistance, which is characteristic for these types of cancers. As chemotherapy is not as effective alone, pulsed electric fields (PEFs) seems to be a promising support in the conventional anticancer methods. Despite enhanced drug delivery, varied parameters of PEF such as duration and number of pulses and the intensity of electric field can be responsible for the regulation of specific cellular responses. It has been demonstrated that nsPEF may induce oxidative stress in cells by stimulating ROS production and disrupting the balance of oxygenases and antioxidant enzymes, which in turn cause cell damage with increased oxidative markers in membrane lipid peroxidation [1]. Our results proved that PEFs protocols stimulated lipid peroxidation, protein damage, changes in the cytoskeleton, and alternations in proteasome activity. There was also observed that multidrug resistant protein expression or function can be modulated. In the present study, we summarize our results concerning PEF application in gastric, colon, and pancreatic cancers *in vitro* and *in vivo*. We have verified milli-, micro-, and nanosecond pulses alone, and in combination with chemotherapeutics, photosensitizing agents, and calcium ions. Our results indicate various cell responses depending on the type, size, and level of resistance of cancer cells.

The application of nsPEF seems to be a very promising method for the efficacious cancer elimination and significantly facilitates the action of conventional cytotoxic pharmacological agents.

Financing: This work was supported by the Polish National Science Centre project SONATA BIS 6 no.: 2016/22/E/NZ5/00671 (PI: J. Kulbacka), PRELUDIUM 14 no.: 2017/27/N/NZ3/01110 (PI: O. Michel), and project no.: 2020/ABM/01/00098, financed by the Medical Research Agency, Poland.

References

[1] Maccarrone M et.al., *Biochem Biophys Res Commun.* **1995**; 209(2):417-25. [2] Beebe SJ. *Bioelectrochem*. **2015**; 103: 52-59.

S4-KL-01

Photosynthesis on an Electrode

Jenny Zhang
University of Cambridge
Department of Chemistry, Lensfield Road, Cambridge, CB21EW
jz366@cam.ac.uk

Photosynthesis is the primary route from which solar energy is harnessed to fuel life on Earth. Underlying this important process are impressive machineries that convert Earth-abundant resources (sunlight, water and carbon dioxide) into complex chemicals. We now have the capability to electrochemically tap into the photosynthetic electron transport chain *in vivo* and harness its electrons for alternative energy conversion technologies.[1] However, enormous hurdles remain when wiring living photosynthetic cells to electrodes compared to the wiring of molecular catalysts or enzymes. Mainly, cells are much larger in dimensions and higher in structural complexity (the catalysts are enclosed within membranes) and therefor also give rise to much lower photocurrent densities. Endogenous mediators are typically used to boost photocurrent output, but their use comes with an energetic cost, as well as a host of other problems such as cell toxicity. The electrode structure itself also require careful consideration to ensure optimal cell loading and interactions.

Here, I will talk about our latest efforts to establish design principles for i) diffusional electron mediators and ii) electrode architecture for the wiring of photosynthetic cells onto electrodes. We examined phenazines as novel low-midpoint potential molecules for wiring Synechocystis sp. PCC 6803 to electrodes and found a trade-off between energetic gain and cytotoxicity.[2] We also explored 3D-printing as a new method of producing benchmark hierarchically structured electrodes for photosynthetic materials.

1. Zhang, J. Z.; Reisner, E., Advancing photosystem II photoelectrochemistry for semi-artificial photosynthesis. *Nature Rev. Chem.* **2020,** *4* (1), 6-21.
2. Clifford, E. R.; Bradley, R. W.; Wey, L. T.; Lawrence, J. M.; Chen, X.; Howe, C. J.; Zhang, J. Z., Phenazines as model low-midpoint potential electron shuttles for photosynthetic bioelectrochemical systems. *Chem. Sci*. **2021**, 3328 - 3338.

S4-KL-02

Protecting Hydrogenases for Energy Conversion

Nicolas Plumeré
Campus Straubing for Biotechnology and Sustainability, TU Munich, Germany
nicolas.plumere@tum.de

Widespread implementation of hydrogenases in energy conversion cannot proceed without solutions that mitigate their intrinsic fragility. Protection strategies using a redox matrix can effectively stabilize the hydrogenase and significantly increase its operational life-time (1, 2). However, mass transport and electron transfer limitations emerge as trade-offs when increasing film thickness for protection considerations (3). Novel methods for formation of homogeneous thin films (4, 5) that enable high catalyst utilization can theoretically provide O_2 resistance for non-limiting periods of time even when using highly fragile hydrogenases (6). Different protection mechanisms can be exploited depending on matrix dimensions and intrinsic catalyst properties. Experimentally, the lifetimes of hydrogenases under constant aerobic turnover can reach up to one week at the condition that protection also targets reactive oxygen species (7). The use of hydrogenase maturation (8) or hydrogenase reactivation (9) also simplify the preparation of the catalytic films, possibly bypassing entirely the need for anaerobic conditions. Finally, engineering reversibility into the redox-active films embedding the hydrogenase enables H_2 oxidation and H_2 evolution at minimal overpotential, possibly enabling a plethora of practical applications for both energy conversion and electrosynthesis (10).

(1) Plumeré, Rüdiger, Oughli, Williams, Vivekananthan, Pöller, Schuhmann, Lubitz, *Nature Chemistry*, **2014**, *6*, 822–827.
(2) Oughli, Conzuelo, Winkler, Happe, Lubitz, Schuhmann, Rüdiger, Plumeré, *Angew. Chem. Int. Ed*., **2015**, 54, 12329 –12333.
(3) Fourmond, Stapf, Li, Buesen, Birrell, Rüdiger, Lubitz, Schuhmann, Plumeré, Léger, *JACS*, **2015**, *137*, 5494-5505.
(4) Li, Buesen, Williams, Henig, Stapf, Mukherjee, Freier, Lubitz, Winkler, Happe, Plumeré, *Chemical Science*, **2018**, 9, 7596-7605.
(5) Buesen, Li, Plumeré *Chemical Science*, **2020**, *11*, 937 – 946
(6) Li, Buesen, Démentin, Léger, Fourmond, Plumeré *JACS.* **2019**, *141*, 16734
(7) Li, Münchberg, Alsheikh Oughli, Buesen, Lubitz, Freier, Plumeré *Nature Communications*, **2020**, *11*, 920
(8) Oughli, Hardt, Rüdiger, Birrell, Plumeré, *ChemComm.* **2020**, 56, 9958
(9) Felbek, Hardt, Papini, Pramanik, Artero, Fontecave, Fourmond, Plumeré, Léger, *ChemComm* **2021**, 57, 1750-1753
(10) Hardt, Stapf, Filmon, Birrell, Rüdiger, Fourmond, Léger, Plumeré, *Nature Catalysis*, **2021**, 10.1038/s41929-021-00586-

S5-KL-01

From Surface Structuring to Bio-inorganic Hybrid Systems: How Can We Push the Limits of Microbial Biofilm Electrodes?

Uwe Schröder
Institute of Environmental and Sustainable Chemistry, Technische Universität, Braunschweig, Germany
uwe.schroeder@tu-bs.de

Biofilm electrodes are the living heart of the great majority of bioelectrochemical systems. Despite the recent progress, the further increase of the biofilm electrode performance is still a key issue for the future success of the deriving microbial electrochemical technologies. The current density of high-performing anodic microbial biofilms at flat, macroscopically smooth, electrode surfaces (e.g., at smooth polycrystalline graphite) reaches about 1 mA cm^{-2}, a further increase appearing hampered. Is this final limit in the performance of biofilm electrodes? Different approaches have been proposed to increase the electrode performance. They reach from surface structuring on a nanometer scale to a wealth of 3D electrode structures. How effective are such approaches? This presentation shows a systematic approach to the role of structural electrode dimensions on the short-term and long-term performance of biofilm electrodes. It also discusses opportunities by using promoting effects of unusual electrode materials, such as copper or silver.

References

Schröder U., Harnisch F., Angenent L. (2015). ***Energy and Environm.Science***, 8: 513-519
Baudler A., Riedl S., Schröder U. (2014). ***Frontiers in Energy Research,*** doi: 10.3389/fenrg.2014.00030
Baudler A., Schmidt I., Langner M., Greiner A., Schröder U. (2015). ***Energy and Environmental Science***, 8: 2048-2055
Moß C., Patil S.A., Schröder U. (2019) ***Frontiers in Energy Research* 7;** doi: 10.3389/fenrg.2019.00018
Moß C., Behrens A., Schröder U. (2020) ***ChemSusChem* 13** (2020) 582-589
Beuth L., Pfeiffer C.P., Schröder U. (2020) ***Energy and Environm.Science***, 13: 3102-3109

S6-KL-01

Mediated and Direct Electron Transfer

P. N. Bartlett
School of Chemistry, University of Southampton
Southampton, SO17 1BJ, UK
pnb@soton.ac.uk

Glucose oxidase (EC 1.1.3.4) and cellobiose dehydrogenase (EC 1.1.99.18) are both flavin containing enzymes and both can be used to catalyse the electrochemical oxidation of glucose to gluconolactone.
In the case of glucose oxidase, a homodimer, the two flavin redox centres and buried deep within the protein and direct electron transfer (DET) to electrode surfaces is not expected on grounds of the large distance between the active site and the surface of the native protein[1]. Despite this there are many papers in the literature that claim DET for glucose oxidase at nanostructured electrodes. Cellobiose dehydrogenase, has a flavin domain with a separate haem domain linked by a flexible peptide chain such that internal electron transfer can occur between the two.
In this lecture I will review results for the study of the two enzymes. In the case of glucose oxidase I will show that there is no evidence for DET for the native enzyme despite the many published claims of DET [2]. For cellobiose dehydrogenase I will present data for the immobilization of the enzymes in different orientations at the electrode surface [3,4]. The immobilized enzyme is found to be very stable and this allows us to study in detail, and to compare, the kinetics for DET and mediated electron transfer (MET) for the enzyme in different orientations.

References

1. G. S. Wilson, "Native glucose oxidase does not undergo direct electron transfer", *Biosens. Bioelectron.*, **82** (2016), pp. vi-vii.
2. P. N. Bartlett and F. Al-Lolage, "There is no evidence to support literature claims of direct electron transfer (DET) for native glucose oxidase (GOx) at carbon nanotubes or grapheme", *J. Electroanal. Chem*., 819(2018)26-37.
3. F. A. Al-Lolage, M. Meneghello, S. Ma, R. Ludwig and P. N. Bartlett,
"A flexible method for the stable, covalent immobilization of enzymes at electrode surfaces", *ChemElectroChem*, **4** (2017)1528-1534.
4. S. Ma, C. V. F. P Laurent, M. Meneghello, Marta, J. Tuoriniemi, C. Oostenbrink, L. Gorton, P. N. Bartlett and R., Ludwig, "Direct electron-transfer anisotropy of a site-specifically immobilized cellobiose dehydrogenase", *ACS Cat*., **9**(2019)7607-7615.

S6-KL-02

Electron Transfer and Spin Selectivity in Biomolecules

David H. Waldeck, Jimeng Wei, Caleb Clever, Jose Rivas, and Brian Bloom
Department of Chemistry, University of Pittsburgh,
219 Parkman Avenue, Pittsburgh PA 15260 USA
dave@pitt.edu

We discuss how the properties of biomolecules (nucleic acids. peptides, and proteins) affect their electron transfer and electron spin filtering properties. We present experimental results on the single molecule conductance through nucleic acid duplexes as a function of their length, sequence, and duplex flexibility, and we discuss the relationship between molecular conductance and electron transfer. We present experimental results on electrode measurement that probes the spin polarization of chiral molecules by the magnetization that they generate on an electrode surface, and we examine the effect of spin selectivity on electron transfer with peptides and proteins.

S1-IL-01

3D Flexible Electrodes for *in vivo* Measurements in Cell Cultures based on Conductive Electrospun Polymeric Fibers

Mădălina M. Bârsan, Caroline G. Sanz, Ariana Șerban, Alexandru Evanghelidis,
Ionuț Enculescu, Victor C. Diculescu
National Institute of Materials Physics
Atomiștilor Str 405A, PO Box MG7, Măgurele, 077125 Ilfov, Romania
madalina.barsan@gmail.com

3D flexible electrodes can be prepared from electrospun polymeric fibers, with different morphology and structure, easily controlled during the fabrication process. Such electrodes have large surface areas and porosity, and their coverage with metals makes them attractive tools in electroanalysis[1]. 3D flexible electrodes have been successfully used for the development of pH sensors [1], O_2 sensor [2] and are suitable for various enzyme immobilization.
Biocompatible polymers allow the fabrication of flexible meshes with broad applicability in the medical area, being promising candidates in tissue engineering. On the other hand, monitoring O_2 levels in 3D tissue constructs is crucial for many tissue engineering applications, since the delivery of sufficient O_2 supply is the key for the survival of cells within the scaffold. Therefore, we propose an electrochemical sensor made of electrospun polycaprolactone (PCL) fibers covered with Au as a new sensing platform for O_2 detection via electrochemical methods. The PCL/Au scaffold enabled cells to grow, making possible the monitoring of the O_2 level in the cellular layer grown directly on the sensor surface. Another application that is being exploited is the use of the electrospun polymeric fibers as substrate for the immobilization of enzymes. One in particular has been the focus of our recent research, the enzyme superoxide dismutase[3], since it is an important antioxidant defense to fight against the toxic effect of the superoxide radical on cells. Different enzyme immobilization procedures are being investigated to develop an optimum biosensor architecture for *in vivo* continuous monitoring of $O_2^{\bullet-}$ released from cell cultures, grown directly on the electrochemical biosensor.

Acknowledgements: Financial support from UEFISCDI, PN-III-P1-1.2-PCCDI-2017-0697, PN-III-P1-1.1-TE-2019-0387, POC NANOBIOSURF-SMIS 103528.

References

[1] V. C. Diculescu, M. Beregoi, A. Evanghelidis, R. F. Negrea, N. G. Apostol, I. Enculescu, *Sci. Rep.* **2019**, *9*, 8902.
[2] A. Serban, A. Evanghelidis, M. Onea, V. Diculescu, I. Enculescu, M. M. Barsan, *Electrochem. commun.* **2020**, *111*, 106662.
[3] M. Braik, M. M. Barsan, C. Dridi, M. Ben Ali, C. M. A. Brett, *Sensors Actuators, B Chem.* **2016**, *236*, 574.

S3-IL-01

Ultrafast Laser Processing of Glass Microfluidic Systems: Application to Cancer Research

F. Sima[1], S. Orobeti[1], C. Butnaru[1], F. Jipa[1], E. Axente[1],
H. Kawano[2], A. Miyawaki[2], K. Obata[3], D. Serien[3], K. Sugioka[3]

[1]CETAL, National Institute for Lasers, Plasma and Radiation Physics, Măgurele, Ilfov, 00175, Romania
[2]BSI, RIKEN, 2-1 Hirosawa, Wako, Saitama, 351-0198, Japan
[3]RIKEN Center for Advanced Photonics, 2-1 Hirosawa, Wako, Saitama, 351-0198, Japan
felix.sima@inflpr.ro

Three-dimensional (3D) microfluidic biosystems that mimic *in vivo* environment and allow live observation of cells with a high microscopic resolution over long time periods are of great interest for cancer research. Specifically, transparent glass biochips that can be optically interrogated by imaging techniques for sub-cellular characteristics are essential for understanding mechanism of cancer cell migration, in particular in confining environments. Femtosecond laser assisted chemical etching (FLAE) and picosecond laser assisted chemical etching (PLAE) are subtractive processing technologies used to develop 3D microfluidics embedded in glass microchips. We report herein on the fabrication of new glass microfluidic biochips with complex hierarchical geometries by FLAE and PLAE. Relevant glass model platforms that mimic intravasation-extravasation processes were further produced. They proved capable to offer both observation of collective cancer cells migration over long time periods and individual visualization at unicellular and subcellular levels on the target cell. It could be then demonstrated that cancer cells were capable of migrating though narrow submicrometric confining spaces while retaining viability. The cells were able to further proliferate while probability of division was retained after the migration. This confirms the dynamic adaptability of cancer cells when they were passing even through sub-micrometric spaces.

S6-IL-01

Closed Loop Control of Biological Processes Using Bioelectronics

Marco Rolandi

Department of Electrical and Computer Engineering, University of California, Santa Cruz, CA 95064, USA
mrolandi@ucsc.edu

Closed loop control of biological processes based on feedback is widespread in nature and essential to life itself. Examples include cardiac rhythms, homeostasis, and thermoregulation. Bioelectronic devices are now able to sense and actuate physiological processes by translating biological signals in the form of ions and small molecules into electronic signals that can be analyzed and processed by information technologies. I will present our work that merges AI based closed loop with bioelectronic sensors and actuators to direct physiological processes. I will focus on using fluorescent reporters and ion pumps that can change pH to dial in desired values of membrane potential on stem cells. Membrane potential is a key variable that drives cell function and with this bioelectronic toolbox we can open up opportunities to dial in cellular function.

This research is sponsored by the Defense Advanced Research Projects Agency (DARPA) through Cooperative Agreement D20AC00003 awarded by the U.S. Department of the Interior (DOI), Interior Business Center. The content of the information does not necessarily reflect the position or the policy of the Government, and no official endorsement should be inferred.

Abstracts of
Oral communications

S1 Smart Materials for Bioelectrochemistry

S1-O-01

Chiral Interactions of Propranolol Enantiomers at the Surface of Cysteine Modified Gold Nanoparticles

Bogdan-Cezar Iacob[1], Ioan-Adrian Stoian[1], João P. Prates Ramalho[2], Iuliu O. Marian[3], Radu Oprean[1], Ede Bodoki[1]
[1] *Analytical Chemistry Department, "Iuliu Haţieganu" University of Medicine and Pharmacy, 4, Louis Pasteur St., 400349, Cluj-Napoca, Romania*
[2] *Departamento de Química, Centro de Química de Évora e Laboratório Hercules, Escola de Ciências e Tecnologia, Universidade de Évora, Portugal*
[3] *Research Center in Electrochemistry and Non-conventional Materials, Faculty of chemistry and chemical engineering, Babeş-Bolyai University*
iacob.cezar@umfcluj.ro

Gold nanoparticles (AuNPs) continue to attract considerable interest in the design of chemo/biosensors offering unique optoelectronic and catalytic properties. Furthermore, pursuing different analytical objectives, L- and D-cysteine (Cys) have been conveniently used for the functionalization of AuNPs. Besides forming a self-assembled chiral monolayer on the Au surface, D/L-Cys also enables a more controlled electrodeposition of AuNPs onto the electrode's surface.
Besides chromatographic techniques deployed as chiral identification tests of enantiopure drug products, more and more often various electroanalytical strategies are being proposed as environmentally-friendly, cost- or time effective alternatives.
An in-depth investigation of the enantioselective interaction of a model chiral drug, propranolol (PRNL) at the surface of D- and L-Cys decorated AuNPs using electrochemical techniques and computational modeling has been carried out.
Cys-modified AuNPs were electrodeposited on the surface of a glassy carbon electrode (-0.4V vs. Ag/AgCl), followed by the spontaneous adsorption of propranolol's enantiomers on the Au surface. Particularities in the voltammetric behavior of the two physisorbed enantiomers were investigated in 0.1M phosphate buffer (pH 7.0). Molecular dynamics simulations served for the identification of the main attractive forces responsible of the chiral molecular interactions between the chiral modifier and the enantiomer adsorbates. It was shown that the R(+)-PRNL is involved in a significantly higher number of H-bonding than the S(-)-antipode, being responsible of the recorded enantiospecific electrochemical signal.
Correlating electrochemical data with computational modeling allowed a deeper understanding of fundamental molecular interactions occurring at the electrified solid-liquid interface, which may enable a more rational design of future chiral electrochemical sensing platforms.
Acknowledgement: This work was supported by a grant of the Romanian Ministry of Education and Research, CCCDI - UEFISCDI, project number PN-III-P2-2.1-PED-2019-5473, within PNCDI III.

S1-O-02

Peptide-based Materials for Studying Interactions with CRP Protein – Development and Application

Justyna Borkowska [1], Patryk Kudła [1], Sławomir Filipek [2], Magdalena Narajczyk [3], Joanna Niedziółka-Jönsson [1], Katarzyna Szot-Karpińska [1]
[1] Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland
[2] Faculty of Chemistry & Biological and Chemical Research Centre, University of Warsaw, Warsaw 02-093, Poland
[3] Department of Electron Microscopy, Faculty of Biology, University of Gdansk, Wita Stwosza 59, 80-308 Gdansk, Poland
kszot@ichf.edu.pl

Over the last years, peptides have gained ground for developing new sensing platforms. They are cheaper, easier in production, more stable in harsh environments and more resistant to degradation than antibodies [1]. Their sensitivity is also comparable to that of antibodies [2].
Herein, using the phage display technology, novel peptides that bind CRP protein - markers of inflammatory processes in the human body - have been identified, synthesized, characterized and compared with our previous studies [3]. The binding efficiency of the peptides' bearing phages towards the bait protein was demonstrated via biological methods (ELISA and plaque tests). Biomolecular interactions between the cysteine labelled peptide-modified electrodes and CRP have been evaluated using electrochemical studies as well as optical methods, including microscopic and spectroscopic analyses. Moreover, computational modelling analysis has shown how the selected peptides interact with CRP. These studies have also revealed that one of the investigated peptides exhibits the highest binding energy towards CRP yet measured [4].

[1] V.A. Petrenko, V.J. Vodyanoy, *J. Microbiol. Methods*. 53 (2003) 253.
[2] V. Vanova, K. Mitrevska, V. Milosavljevic, D. Hyneka, L. Richtera, V. Adam, *Biosens. Bioelectr*. 180 (2021) 113087.
[3] K. Szot-Karpińska, P. Kudła, A. Szarota, M. Narajczyk, F. Marken, J. Niedziółka-Jönsson, *Bioelectrochem.*, 136 (2020) 107629.
[4] J. Borkowska, P. Kudła, S. Filipek, M. Narajczyk, J. Niedziółka-Jönsson, K. Szot-Karpińska, manuscript in preparation 2021.

Acknowledgements
This work was funded by the Polish National Science Centre under the SONATA 13 grant UMO 2017/26/D/ST5/00980 to Dr. Katarzyna Szot-Karpińska. Financial support from the project 3/DOT/2016 funded by the City of Gdynia, Poland is also acknowledged.

S1-O-03

A Mixed Monolayer Comprised of Trimeric and Monomeric Photosystem I Enables Improved Anisotropic Electron Flow in Biophotovoltaic Devices

Panpan Wang[1], Anna Frank[2], Fangyuan Zhao[1], Marc M. Nowaczyk[2], Adrian Ruff[1], Felipe Conzuelo[1], and Wolfgang Schuhmann[1]

[1] Analytical Chemistry - Center for Electrochemical Sciences (CES)
Faculty of Chemistry and Biochemistry, Ruhr University Bochum, Germany
[2] Plant Biochemistry, Faculty of Biology and Biotechnology
Ruhr University Bochum, Germany
panpan.wang@rub.de

The use of photosynthetic protein complexes for the fabrication of solar energy conversion devices is a promising strategy due to their abundant existence and high quantum efficiency. In particular, photosystem I (PSI), one of the main protein complexes driving photosynthesis, is able to convert visible light into high energy electrons. Upon light-induced charge separation and internal electron transfer, a final state is obtained consisting of two redox centers with a potential difference of about 1 V. However, this large potential difference also translates into a significant driving force for charge recombination processes. One of the challenges in the fabrication of PSI biodevices is to overcome the related low efficiency in the extraction of high energy electrons. Therefore, controlled immobilization strategies are important, allowing anisotropic electron flow. Taking advantage of the inherent amphiphilicity of PSI, we have shown the possibility for a successful immobilization of a Langmuir-Blodgett (LB) film of PSI protein complexes with a preferential anisotropic orientation over the electrode surface. The estimated surface coverage of the LB film constituted by PSI trimers was about 61%.[1] Although the fabricated monolayer contributed to a substantial decrease of charge recombination processes, the remaining gaps between PSI trimeric structures may still contribute to a limited performance of the biophotovoltaic device. In order to achieve an improved PSI LB film, a mixed monolayer comprised of trimeric and monomeric PSI was investigated. The integration of smaller PSI monomers enables to fill the residual gaps between PSI trimers, which also translates in an increased surface coverage and an overall improved performance.

Reference

[1] F. Zhao, P. Wang, A. Ruff, V. Hartmann, S. Zacarias, I. A. C. Pereira, M. M. Nowaczyk, M. Rögner, F. Conzuelo, W. Schuhmann, *Energy Environ. Sci.* **2019**, *12*, 3133–3143.

S1-O-04

Membrane and Mediator Free High Voltage Enzymatic Supercapacitors

Sergey Shleev[a], Olga Aleksejeva[a], Chiara Di Bari[b], Elena Gonzalez-Arribas[a], Antonio L. De Lacey[b], Marcos Pita[b], Roland Ludwig[c], Victor Andoralov[d]

[a]*Biomedical Science and Biofilms-Research Center for Biointerfaces, Malmö University, 20560 Malmö, Sweden*
[b]*Instituto de Catálisis y Petroleoquímica, 28049 Madrid, Spain*
[c]*Bicatalysis and Biosensing Laboratory, BOKU−University of Natural Resources and Life Sciences, A-1190 Wien, Austria*
[d]*KEMET Electronics AB, 563 31 Gränna, Sweden*

Address: Jan Waldenströms gata 25, SE-214 28 Malmö, Sweden
sergey.shleev@mau.se

Research and development regarding bioelectrochemical power sources for portable and implantable electronic devices have intensified in the last decade. In contrast to conventional biological fuel cells, which deliver low but continuous currents, hybrid devices in general, and self-charging biosupercapacitors in particular can provide high currents in pulsed mode. However, widespread application of biological electrochemical power sources has been thwarted by the low operating voltage, typically less than 0.5 V. Here, we report on a multi-cell, single-electrolyte glucose/oxygen enzymatic supercapacitor with adjustable open-circuit and operating voltage. Remarkably, voltages do not depend on the difference in equilibrium redox potentials of the two redox couples, gluconolactone/glucose and oxygen/water (1.18 V), but on the number of half-cells connected in series. With seven biosupercapacitors connected in series in a common electrolyte, an open-circuit voltage of about 3 V is achieved, *i.e.*, exceeding the thermodynamic limit by more than twofold. We anticipate that this work will serve as the starting point for new designs of simple, low-cost biological power sources that can reach operating voltages and power outputs required to power portable electronic devices without the need for step-up converters or energy managing systems. Given the huge market size of 3 V electronic devices, this could truly lead to significant advancements in the ongoing endeavors to provide emission-less energy conversion and access renewable energy sources.

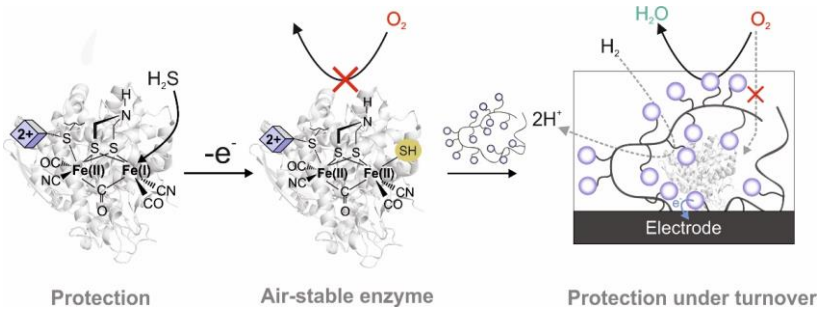
Acknowledgement
This work was financially supported by the Swedish Knowledge Foundation (project number 20170168).

S1-O-05

Reactivation of Sulfide-Protected [FeFe] Hydrogenase in a Redox-active Hydrogel

Alaa A. Oughli [a], Steffen Hardt [a], Olaf Rüdiger [b], James A. Birrell [b] and Nicolas Plumeré [a]
a. Professur für Elektrobiotechnologie, TU München, Schulgasse 16, 94315 Straubing (Germany)
nicolas.plumere@tum.de; alaa.oughli@tum.de
b. Max Planck Institute for Chemical Energy Conversion, Stiftstrasse 34–36, 45470 Mülheim an der Ruhr (Germany)
james.birrell@cec.mpg.de

Hydrogenases are biocatalysts that catalyse reversible hydrogen oxidation and production at high efficiencies. However, their sensitivity from O_2 and high potential hinders their use in technological devices. Redox hydrogels have been used for protecting hydrogenases from oxidative damage under turnover conditions. However, the preparation of electrodes in air is not possible due to extreme enzyme sensitivity to O_2 which is impractical for applications. Sulfide coordination at the active center of [FeFe] hydrogenase from *Desulfovibrio desulfuricans* enables protection of the enzyme under non-turnover conditions. Here, we show that sulfide-protected hydrogenase can be reactivated in a redox hydrogel enabling practical use of this highly O_2 sensitive enzyme without the need for anaerobic conditions (1).



Reference

(1) Oughli, Hardt, Rüdiger, Birrell, Plumeré, *ChemComm.* **2020**, 56, 9958.

S1-O-06

Towards Electrochemical Detection of Deoxynivalenol by Exploiting Molecular Docking Simulations

Giulia Selvolini, Giovanna Marrazza
"Ugo Schiff" Chemistry Department, University of Florence
Via della Lastruccia 3, 50019 Sesto Fiorentino (FI), Italy
giulia.selvolini@unifi.it

Mycotoxins pose serious threats to animal and human health, because of their highly toxic and carcinogenic effects [1]. Food safety is progressively becoming a global issue in the intensive agriculture and food industry growth scenario; the health reliability of food products must be determined in a quick, cost-effective and precise way [2]. This work proposes an enzyme-labeled voltammetric aptasensor to detect deoxynivalenol (DON) mycotoxin based on a competitive format. The development steps of the aptasensor were partnered for the first time to a computational study, to gain insights onto the molecular mechanisms involved into the exploited competition: the molecular interaction between a thiol-tethered DNA aptamer (80mer-SH) and DON was investigated by a docking study, which allows to find the binding region of the oligonucleotide sequence and to determine DON preferred orientation in the binding event. A biotinylated complementary oligonucleotide sequence (20mer-BIO) of the aptamer sequence binding with DON was chosen to carry out a competitive format. Disposable graphite screen-printed electrodes (GSPEs) were electrochemically modified with polyaniline and gold nanoparticles (AuNPs@PANI) by means of cyclic voltammetry (CV) [3] and worked as a scaffold for the immobilization of the DNA aptamer. Solutions containing increasing concentrations of DON and a fixed amount of 20mer-BIO were dropped onto the aptasensor surface: the resulting hybrids were labeled with an alkaline phosphatase (ALP) conjugate to hydrolyze 1-naphthyl phosphate (1-NPP) substrate into 1-naphthol product, detected by differential pulse voltammetry (DPV). The aptasensor response was signal-off, in accordance to the competitive format applied. Under optimized experimental conditions, a dose-response curve was obtained between 5.0 $ng \cdot mL^{-1}$ and 30.0 $ng \cdot mL^{-1}$ DON concentration range and a limit of detection (LOD) of 3.2 $ng \cdot mL^{-1}$ was achieved within a 1-hour detection time. Finally, preliminary experiments in maize flour samples spiked with DON standard solutions were also performed, retrieving good recovery values.

References

[1] T. Wei, P. Ren, L. Huang et al., *Food Chem.* **2019**, *300*, 125176 – 125182.
[2] G. Bülbül, A. Hayat, S. Andreescu, *Sensors* **2015**, *15*, 30736 – 30758.
[3] G. Selvolini, C. Lazzarini, G. Marrazza, *Sensors* **2019**, *19*, 3097 – 3106.

S1-O-07

Self-powered Molecule Release Systems Activated with Chemical Signals Processed Through Reconfigurable Implication or Inhibition Boolean Logic Gates

Paolo Bollella[a,d], Zhong Guo[b], Selvakumar Edwardraja[c], Vasantha Krishna Kadambar[a], Kirill Alexandrov[c], Artem Melman[a], Evgeny Katz[a]

[a]*Department of Chemistry and Biomolecular Science, Clarkson University, 8 Clarkson Ave., Potsdam, NY 13699, USA*
[b]*CSIRO-QUT Synthetic Biology Alliance, ARC Centre of Excellence in Synthetic Biology Centre for Agriculture and the Bioeconomy, School of Biology and Environmental Science, Queensland University of Technology, Brisbane 4001, QLD, Australia*
[c]*Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane 4072, QLD, Australia*
[d]*Department of Chemistry, Università degli Studi di Bari A. Moro 4, Via E. Orabona, 70125, Bari, Italy*
pbollella@clarkson.edu

Enzyme-based systems have been successfully used for mimicking almost all Boolean logic operations (e.g., YES, NOT, OR, NOR, XOR, NXOR, AND, NAND, INHIB, etc.). While some Boolean logic gates (particularly OR, AND) have been mimicked with various (bio)chemical systems very often, others received much less attention, probably because they are less popular in the chemical community and more difficult for realization.
The Implication (IMPLY) and Inhibition (INHIB) Boolean logic gates were realized using switchable chimeric pyrroloquinoline quinone-dependent glucose dehydrogenase (PQQ-GDH-Clamp) containing a fused affinity clamp unit recognizing a signal-peptide. The second component of the logic gate was the wild-type PQQ-glucose dehydrogenase working cooperatively with the PQQ-GDH-Clamp enzyme. The IMPLY and INHIB gates were realized using the same enzyme composition activated with differently defined input signals, thus representing reconfigurable logic systems. The logic gates were first tested while operating in a solution with optical analysis of the output signals. Then, the enzymes were immobilized on a buckypaper electrode for electrochemical transduction of the output signals. The switchable electrodes were integrated in a biofuel cell as a self-powered device triggering molecule release function controlled by the logically processed molecule signals. [1]

[1] P. Bollella et al., *Bioelectrochemistry*, **2021**, art. No. 107735.

S1-O-08

Electrochemical Pre-treatment of Pyrolytic Graphite Electrode and Its Negative Effect on Voltammetric Detection of DNA

Ales Danhel, Lukas Fojt, Miroslav Fojta
Institute of Biophysics of the Czech Academy of Sciences
Kralovopolska 135, CZ-61265 Brno, Czech Republic
danhel@ibp.cz

Electrochemical activity of DNA at mercury was discovered by E. Palecek in 1959. [1] First reports on electrochemical oxidizability of high-molecular-weight nucleic acids at carbon electrodes were published by V. Brabec and G. Dryhurts in 1978. [2] Up to now, other surfaces based on carbon/graphite, metals, or metal oxides have been studied and utilized in various applications devoted to DNA damage, hybridization, interaction with various species, etc. [3–5] Recently, an innovative approach using basal plane pyrolytic graphite electrode (bPGE) was successfully utilized in the detection of all DNA bases (including 5-methylcytosine) and products of their electrochemical transformation using broad applicable cathodic and anodic potential range (−2.0 and +1.6 V). [6]

In this comparative study, effects of the PGE electrochemical pre-treatment (pre-anodization to +2.0 V or pre-cathodization to −2.0 V by linear scan voltammetry in the Britton-Robinson buffer pH 7.0) on its surface morphology and electrochemical behavior towards DNA electroanalysis were studied by Raman spectroscopy, X-ray photoelectron spectroscopy, scanning electron microscopy, and by cyclic voltammetry of $[Ru(NH_2)_6]Cl$ or $K_3[Fe(CN)_6]$ at the pre-treated bPGE surfaces with or without an adsorbed layer of plasmid DNA. The electrochemical pre-treatment increased the abundance of oxygenous functional groups on the bPGE surface, which negatively influenced electrochemical responses of the DNA. Untreated bPGE was found to be the most suitable for electroanalysis of the polymeric DNA.

Acknowledgement: This work has been supported by the SYMBIT project reg. no. CZ.02.1.01/0.0/0.0/15_003/0000477 financed from the ERDF.

[1] E. Paleček, *Nature* **1960**, *188*, 656–657.
[2] V. Brabec, *Bioelectrochemistry Bioenerg.* **1981**, *8*, 437–449.
[3] E. Paleček, M. Bartošík, *Chem. Rev.* **2012**, *112*, 3427–3481.
[4] M. Fojta, etal, *Trends Anal. Chem.* **2016**, *79*, 160–167.
[5] J. M. George, etal., *Microchim. Acta* **2018**, *185*, 358–383.
[6] J. Špaček, etal., *Electrochem. Commun.* **2017**, *82*, 34–38.

S1-O-09

Corona Formation and Conformational Changes in Proteins on Dendrimers

Barbara Jachimska, Magdalena Szota, Kamil Rakowski

Jerzy Haber Institute of Catalysis and Surface Chemistry Polish Academy of Sciences, Niezapominajek 8, 30-239 Cracow, Poland
ncjachim@cyf-kr.edu.pl

The physicochemical properties of a nanocarrier are essential parameters in drug delivery system design (DDS). This work addresses how PAMAM dendrimers form complexes with bovine serum albumin (BSA) and fibrinogen (Fib). Analytical techniques, such as UV-vis spectrophotometry, dynamic light scattering (DLS), electrophoretic mobility, quartz crystal microbalance with dissipation monitoring (QCM-D), circular dichroism (CD), and contact angle were used to analyze the properties of the dendrimers-protein systems. The protein binding to dendrimers can alter the dendrimer's structure, mobility, conformation, and functional activity. Measurements of electrophoretic mobility allow tracking changes during the formation of dendrimer-protein complexes depending on environmental conditions. The compensation of functional groups charge in the dendrimer molecule by protein immobilization affects the complexes' effective charge. The results show that proteins' interactions with PAMAM dendrimer carriers are driven both by electrostatic and hydrophobic forces. The protein corona formed on the carrier's surface is very stable, as evidenced by the QCM-D measurements.

On the other hand, the CD spectra indicate a change in the secondary structure of the protein. The size of the change is highly dependent on the protein to dendrimer ratio. Molecular dynamics (MD) clearly shows that the heterogeneous charge distribution on the surface of proteins plays an essential role in their behavior in the context of dendrimer systems. It enables identifying crucial amino acids involved in the formation of the dendrimer-protein complex and the observation of conformational changes in the system, and the participation of hydrophobic forces in this process.

Protein corona formation on the carrier surface is advantageous for the potential use of DDS as it inhibits the adsorption of plasma proteins to the dendrimer surface, reduces dendrimer toxicity, and prevents rapid clearance from the blood increasing circulation time. The biological properties and bioavailability are also improved when a stable protein-dendrimer complex is formed.

Acknowledgments. This work was partially supported by Grant NCN OPUS 2016/23/B/ST5/02788

Reference

J. M. Rae, B. Jachimska, Nanoscale, 2021, 13, 2703–2713

S1-O-10

Scalable 3D Metal Oxide Electrodes with Improved Transmission for Constructing Photo-active Electrodes

D. Ciornii[1], M. Riedel[1], A. Zouni[2], F. Lisdat[1]
1 Biosystems Technology, Institute of Life Sciences and Biomedical Technologies, Technical University Wildau, Germany
2 Biophysics of Photosynthesis, Institute for Biology, Humboldt University Berlin, flisdat@th-wildau.de

The application of three-dimensional electrode structures holds great promise in photobiovoltaics since large amounts of active proteins can be coupled with a transducing electrode. Template-based approaches have developed significantly allowing different materials to be prepared in a 3D fashion. Often the preparation starts from nanoparticles which are sintered together during the temperature step. This has already been used for enzyme electrodes, but also exploited for photobioelectrodes. However, transparency is limited to rather thin electrodes although the technique allows in principle to prepare rather thick structures due to the spin coating applied for layer build-up. We have modified this procedure by starting from a liquid precursor and the template particles. This can be exemplified with TiO_2 and ITO as materials. We can demonstrate that defined 3D structures can be prepared with increased transmission [1, 2].
For the TiO_2 material we have used this basic electrode to deposit PbS quantum dots within the structure. The contact TiO_2/PbS is applied to introduce light sensitivity and has then been coupled to an enzymatic reaction. Scalability of electrode response to the enzyme substrate can be demonstrated for electrodes with different thicknesses.
Furthermore, a biological photoactive protein complex – photosystem I - has been coupled to 3D ITO electrodes which have been prepared using indium iso-propoxide and tin-isopropoxide as precursors. PS I can stably be immobilised within the constructed electrodes. As direct electron transfer has been found rather limited, contact is improved by exploiting another protein – cyt. c – acting as a shuttle molecule. Thus, well defined photocurrents have been obtained upon illumination. The influence of the template particle size used during the fabrication has been studied. Finally, the scalability has been evaluated. Here 3-15 spin coating steps have been applied during fabrication and thicknesses up to 17µm have been achieved. We can clearly show that the proteins can access the prepared surface and that the electrode performance is scalable within this thickness range. Thus, a 15-layer electrode gives raise for a photocurrent of about 270µA/cm^2.

[1] M. Riedel, W. Parak, A. Ruff, W. Schuhmann, F. Lisdat, ACSCatalysis, 2018, 8, 5212
[2] D. Ciornii, A. Kolsch, A. Zouni, F. Lisdat, Nanoscale, 2019, 11(34), 15862

S1-O-11

Electrochemical Characterization of Hydrophilic Carbon Nanomaterials Generated in Carboxylic Buffers

Andreia D. Veloso[a], Romeu A. Videira[b], Maria C. Oliveira[a]
[a]*CQ-VR e Departamento de Química, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal*
[b]*REQUIMTE/LAQV, Laboratório de Farmacognosia, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal*
andreiaveloso8@hotmail.com

Electron-transfer reactions play a key role in many biochemical pathways and signaling cascades that support physiological functions. Since cellular redox status is under homeostatic control, changes in the electron flow that exceeds the buffering redox capacity of cells lead to oxidative stress, a pathological hallmark of diverse human diseases. Intrinsic redox-active nanomaterials are a promising tool for biomedical applications as therapeutic/preventive agents to preserve and/or restore the cellular redox balance. Hydrophilic carbon nanomaterials generated in citric acid buffer with outstanding electron-donor ability, have high radical scavenging activity and capability to protect cells against oxidative damage and toxicity promoted by external toxic stimuli. These properties give them the potential to be used as therapeutic/preventive agents of oxidative stress-related diseases. However, the elucidation of the electron transfer mechanism underlying hydrophilic carbon nanomaterial electroactivity is a prerequisite for the conception of well-succeed biomedical applications. In this context, six carboxylic acid buffers with similar properties (similar pka, and similarities in the molecular structure), including citric acid were used to generate the carbon nanomaterials. Each nanomaterials solution was submitted to a detailed voltammetric characterization by cyclic voltammetry using a glassy carbon working electrode. The results showed that the electrochemical behavior of the generated nanomaterials is dependent on the chemical structure of the carboxylic acid used as electrolyte, leading to the generation of carbon nanomaterials with different redox behaviors (electron-acceptors or electron-donors, or non-electroactive). The underlying mechanism responsible for the different electroactivities of the carbon nanomaterials is proposed. This work opens new perspectives to the efficient design of redox-active carbon-based nanomaterials for nanomedicine purposes.

Acknowledgments
The authors thank FCT (Portugal's Foundation for Science and Technology) for financial support through the CQ-VR Research Unit (UIDB/00616/2020,

S1-O-12

Polymer and Gold Structured Electrochemical Platforms for Biomedical and Environmental Applications

Gheorghe Melinte[1,2], Oana Hosu[1], Andreea Cernat[1], Geanina Ștefan[1,3], Giovanna Marrazza[2], Cecilia Cristea[1]
[1]*Department of Analytical Chemistry, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, 4 Pasteur St, 400349 Cluj-Napoca, Romania*
[2]*Department of Chemistry "Ugo Schiff", University of Florence, Via della Lastruccia 3, 50019 Sesto Fiorentino (FI), Italy*
[3]*Robert Bosch SRL, Physical and Chemical Analysis Department (RBRO/EQV-A), Tetarom 3 Industrial Park, Jucu Herghelie 407352, Cluj, Romania*
melinte.gheorghe@umfcluj.ro

Polymers and gold nanoparticles have attracted a lot of attention in biosensors field thanks to their ability to increase the catalytic effect and electroactive area of electrochemical platforms and to potentiate their properties when in combination.
Three strategies are presented based on (1) cone-like shapes of poly(carboxylic pyrrole) modified screen printed electrodes for direct electrochemical detection of folic acid, (2) gold nanovoids platform obtained at glassy carbon electrodes for the development of a sensitive aptasensor for tetracycline and (3) the combination of a polymeric film (Poly-L-Lysine) and gold nanosized geometrical structures for the elaboration of a sandwich type aptasensor for Lysozyme.
The electrochemical characterization of the platforms was realised by cyclic voltammetry and electrochemical impedance spectroscopy, and the surface morphologies of the nanosized architectures were examined by scanning electron microscopy and atomic force microscopy.
All sensors were successfully tested in real samples such as pharmaceutical, environmental and biological samples.

Acknowledgments
Funds received from the Italian Government in the framework of PRIN 2017, Prot. 2017YER72 K_005, the Romanian Ministry of Education and Research, CNCS - UEFISCDI, project number PN-III-P1-1.1-PD-2019-0631, within PNCDI III and UMF "Iuliu Haţieganu" internal grant no. 1034/2/13.01.2021 are acknowledged.

S1-O-13

Effect of the Surface Charge Density of a Gold Electrode on the Amount and Conformation of Sdsorbing Lysozyme

Izabella Brand[1], Paulina Komorek[2] and Barbara Jachimska[2]
[1] *Department of Chemistry, University of Oldenburg, 26111 Oldenburg, Germany*
[2] *Jerzy Haber Institute of Catalysis and Surface Chemistry Polish Academy of Sciences, Niezapominajek 8,30-239 Cracow, Poland*
izabella.brand@uni-oldenburg.de

A number of factors determine the behavior of protein systems at the interface. Among the most frequently studied are the type of solvent and the type of adsorption surface. One of the interesting but not fully understood factors influencing proteins' behavior during the adsorption process is the electric potential at the interface. This is of particular importance in materials science for the design of biosensors, drug delivery systems, and anti-fouling coatings.
In our studies, lysozyme, which is characterized by high conformational stability, was used. The pH of the electrolyte solution and the surface charge determine the amount, conformation, and structure of lysozyme adsorbing on a gold electrode surface. Independent of the potential applied to the Au electrode, the amount of the adsorbed lysozyme increased with an increase in the electrolyte solution's pH. Electrostatic interactions, and thus the surface charge density on the metal surface further affected the amount of adsorbing protein. In protein films formed at net positive surface charge densities ($E_{ads} > E_{pzc}$), the specific adsorption of anions screens the positive surface charge of the lysozyme molecules. In consequence, large number of the protein adsorbs on the Au surface. At net negative surface charge densities ($E_{ads} < E_{pzc}$), no anions were adsorbed on the Au surface, and therefore the number of adsorbed protein molecules was lower.
In situ spectroelectrochemical studies indicated that the process of conformational changes in lysozyme involved two parallel processes. One process comprised changes in the hydration/hydrogen bond network at helices, leading to diverse helical structures:α, 3_{10} and /or π helices. In the second process, other structural elements:β turns, β sheets and random coils displayed an ability to form aggregated β sheet structures. Our results indicate that two parallel processes occurring at helices and other structural elements contribute to structural changes in lysozyme films on the Au surface. We shined more light into understanding electric potential-dependent changes involved in the protein missfolding process in this work.

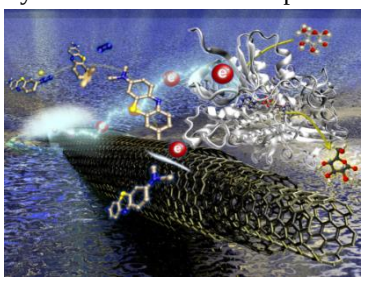
Acknowledgments This work was partially supported by Grants: DAAD PPP 57449009,

S1-O-14

Covalent *vs.* Non-covalent Surface Modification with Phenazines and Phenothiazines for the Electrical Wiring of FAD-Glucose Dehydrogenase at Carbon Nanotube Electrodes

Andrew J. Gross[a], Shunya Tanaka[b], Luminita Fritea[c], Fabien Giroud[a], Serge Cosnier[a], Seiya Tsujimura[b], Michael Holzinger[c],
[a] *Département de Chimie Moléculaire, Univ. Grenoble Alpes, CNRS, 38000 Grenoble, France*
andrew.gross@univ-grenoble-alpes.fr
[b] *Faculty of Pure and Applied Science, University of Tsukuba, 1-1-1, Tennodai, Tsukuba, Ibaraki, 305-5358 Japan*
[c] *Medicine and Pharmacy, University of Oradea, 410073 Oradea, Romania*

The development of bioelectrodes based on carbon nanotubes (CNTs) with immobilised oxidoreductases is of great interest for the development of enzymatic biofuel cells to power low-power electronics. Fungal-based FAD-glucose dehydrogenase (FAD-GDH) has emerged as arguably the most promising enzyme for bioanode construction; however, major limitations in terms of stability are consistently encountered, for example, with catalytic activity dropping to ≤ 50% after a week of storage stability tests in phosphate buffer[1,2]. This talk will explore the use of phenazines and phenothiazines for the electrical wiring of FAD-GDH at multiwalled-walled carbon nanotube electrodes for glucose oxidation. Comparative data will be presented for phenazine (Azure A)-modified electrodes prepared by diazonium salt electrografting *vs.* classical physical adsorption, and prepared using different CNT mass loadings[3,4]. An alternative strategy based on covalent tethering of a related phenothiazine (thionine) to NHS functionalised pyrene or polynorbonene will also be presented[5].



Diazonium modification of carbon nanotubes with Azure A via diazonium chemistry for the wiring of FAD-GDH for glucose oxidation.

[1] A. J. Gross, X. Chen, F. Giroud, C. Abreu, A. Le Goff, M. Holzinger, S. Cosnier. A High Power Buckypaper Biofuel Cell: Exploiting 1,10-Phenanthroline-5,6-Dione with FAD-Dependent Dehydrogenase for Catalytically-Powerful Glucose Oxidation, *ACS Catalysis*, **2017**, 7, 4408
[2] M. Zafar, N. Beden, D. Leech, C. Sygmund, R. Ludwig, Lo Gorton. Characterization of different FAD-dependent glucose dehydrogenases for possible use in glucose-based biosensors and biofuel cells. *Anal Bioanal Chem*, **2012**, 402, 2069
[3] A. J. Gross, S. Tanaka, C. Colomies, F. Giroud, Y. Nishina, S. Cosnier, S. Tsujimura, M. Holzinger. Diazonium Electrografting vs. Physical Adsorption of Azure A at Carbon Nanotubes for Mediated Glucose Oxidation with FAD-GDH. *ChemElectroChem*, **2020**, 7, 22, 4543
[4] S. Tsujimura, S. Tanaka, A. J. Gross, M. Holzinger. Electrochemical modification at multiwalled carbon nanotube electrodes with Azure A for FAD-glucose dehydrogenase wiring: Structural optimization to enhance catalytic activity and stability. J. Phys Energy, **in press** https://doi.org/10.1088/2515-7655/abd298
[5] L. Fritea, A. J. Gross, K. Gorgy, R. O'Reilly, A. Le Goff, S. Cosnier. A bifunctional triblock polynorbornene/carbon nanotube buckypaper bioelectrode for low-potential/high-current thionine-mediated glucose oxidation by FAD-GDH, **2019**, J. Mater. Chem. A, **2019**, 7, 1447

S1-O-15

Bilirubin Oxidase Oriented on Novel Type Three-Dimensional Biocathodes with Reduced Graphene Aggregation for Biocathode

Jing Tang[a,b], Xiaomei Yan[b], Wei Huang[b], Christian Engelbrekt[b], Jens Øllgaard Duus[b], Jens Ulstrup[b], Xinxin Xiao[*,b], Jingdong Zhang[b]
[a]*College of Environmental Science and Engineering, Hunan University, Changsha, 410082, China*
[b]*Department of Chemistry, Technical University of Denmark, Kongens Lyngby 2800, Denmark*
tangjing_12@163.com (J. Tang); xixiao@kemi.dtu.dk (X. Xiao)

Aggregation of reduced graphene oxide (RGO) due to π-π stacking is a recurrent problem in graphene-based electrochemistry, decreasing the effective working area and therefore the performance. Dispersing RGO on three-dimensional (3D) carbon paper electrode is one strategy towards overcoming this, which partially relieves aggregation[1, 2].
In this report[3], we describe the graft of negatively charged 4-aminobenzoic acid (4-ABA) onto a graphene functionalized carbon paper electrode surface. 4-ABA functionalization induces separation of the RGO layers, at the same time leading to favorable orientation of the blue multi-copper enzyme *Myrothecium verrucaria* bilirubin oxidase (*Mv*BOD) for direct electron transfer (DET) in the dioxygen reduction reaction (ORR) at neutral pH. Simultaneous electroreduction of graphene oxide to RGO and covalent attachment of 4-ABA are achieved by applying alternating cathodic and anodic electrochemical potential pulses, leading to a very high catalytic current density (Δj_{cat}:193 ± 4 μA cm^{-2}) under static conditions. Electrochemically grafted 4-ABA not only leads to a favorable orientation of BOD as validated by fitting a kinetic model to the electrocatalytic data, but also acts to alleviate RGO aggregation as disclosed by scanning electron microscopy, most likely due to the electrostatic repulsion between 4-ABA-grafted graphene layers. With a half-lifetime of 55 h, the bioelectrode also shows the highest operational stability for DET-type *Mv*BOD-based bioelectrodes reported to date.

References

1 J. Tang, R. M. L. Werchmeister, L. Preda, W. Huang, Z. Zheng, S. Leimkühler, U. Wollenberger, X. Xiao, C. Engelbrekt, J. Ulstrup and J. Zhang, *ACS Catalysis*, 2019, **9**, 6543-6554.
2 R. M. L. Werchmeister, J. Tang, X. Xiao, U. Wollenberger, H. A. Hjuler, J. Ulstrup and J. Zhang, *J. Electrochem. Soc.*, 2019, **166**, G170-G177.
3 J. Tang, X. Yan, W. Huang, C. Engelbrekt, J. Ø. Duus, J. Ulstrup, X. Xiao and J. Zhang, *Biosens. Bioelectron.*, 2020, **167**, 112500.

S2 Electrochemical Sensors for Diagnostics and Therapy Monitoring

S2-O-01

Electrochemical Enzyme Biosensor Platforms with Iron Oxide Nanoparticles and Polyphenazines Prepared in Ethaline Deep Eutectic Solvent

Christopher M.A. Brett, Wanderson da Silva
University of Coimbra, CEMMPRE, Department of Chemistry, 3004-535 Coimbra, Portugal
cbrett@ci.uc.pt

The sensitivity and selectivity of electrochemical sensing and biosensing is particularly influenced by the type and nanostructure of electrode modifier materials. It has been found that films of redox polymers prepared from phenazine or triarylmethane dyes, acting as redox mediators, lead to enhancement of the analytical performance [1]. We have recently shown that polymerization of these dyes in acid-doped ethaline deep eutectic solvent (DES), increases the polymer surface uniformity and leads to more robust sensing capabilities, promoting better electrocatalytic effects and stability than films formed in aqueous electrolyte [2]. Nanocomposites of poly(phenazine) films and iron oxide nanoparticles (Fe_2O_3), instead of carbon nanotubes, is a new nanomaterial combination that should have improved conducting and sensing performance. They offer a suitable microenvironment for biomolecule immobilization, leading to enhanced biosensing characteristics [3,4].
The formation and characteristics of poly(phenazine) films on Fe_2O_3 nanoparticle modified electrodes, for sensing and biosensing, is described. These include poly(neutral red) (PNR), poly(methylene green) (PMG), and poly(Nile blue) (PNB). The poly(phenazine) films were formed in acid-doped ethaline (1 choline chloride: 2 ethylene glycol molar ratio) DES. Characterization of the platforms was by voltammetric, electrochemical impedance and surface analysis techniques. Electrocatalysis and analytical performance for biological molecules, such as the antibiotic dapsone, and as enzyme biosensors will be illustrated. Enzyme immobilisation was done with BSA as carrier protein and glutaraldehyde cross-linking agent. Catalase and acetylcholinesterase enzyme biosensors were prepared, with excellent analytical parameters in relation to the literature, that will be discussed.

[1] M.M. Barsan, M.E. Ghica, C.M.A. Brett, *Anal. Chim. Acta* 881 (2015) 1-23.
[2] C.M.A. Brett, *Curr. Opin. Electrochem.* 10 (2018) 143-148.
[3] W. Da Silva, A.C. Queiroz, C.M.A. Brett, *Electrochim. Acta* 347 (2020) 136284.
[4] W. Da Silva, C.M.A. Brett, *J. Electroanal. Chem.* 872 (2020) 114050.

S2-O-02

The Development of an Aptasensor for Oxytetracycline using Au-based Nanostructured Platforms

Adrian Blidar[1], Oana Hosu[1], Geanina Ștefan[1,2], Diana Bogdan[3], Karolien De Wael[4], Cecilia Cristea[1]

[1]*"Iuliu Haţieganu" University of Medicine and Pharmacy, Analytical Chemistry Department, 4 Pasteur Street, 400349 Cluj-Napoca, Romania*
[2]*Robert Bosch SRL, Physical and Chemical Analysis Department (RBRO/EQV-A), Tetarom 3 Industrial Park, Jucu Herghelie 407352, Cluj, Romania*
[3]*National Institute for Research and Development of Isotopic and Molecular Technologies, 67-103 Donath Street, 400293, Cluj-Napoca, Romania*
[4]*AXES Research Group, Department of BioEngineering, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium*
blidar.adrian@umfcluj.ro

Oxytetracycline (OXT) is an important with widespread use antibiotic, which overuse fuels the rise of the problem of antibiotic resistance. A way to combat this problem is by monitoring the antibiotic concentrations in the environment, which in turn highlights the need for new analytical methods capable of performing in field analysis [1].
In this context, the aim of our work was the developement of an aptasensor for OXT, using as a starting platform carbon-based screen printed electrodes (C-SPE), modified with Au-based nanostructures (AuNSs).
The obtained C-SPE modified with AuNSs platforms (C-SPE|AuNSs) were modified with a thiolated deoxyribonucleic acid (DNA) aptamer, labelled with ferrocene. The morphologies of the AuNSs were investigated using scanning electron microscopy and atomic force microscopy; the influence of these arhitectures was evaluated based on the redox signal of Fc-Aptamer.
Using the optimum C-SPE|AuNSs platform, an innovative and sensitive "signal on" aptasensor was developed for OXT. The response depended linearly to the concentration of OXT in the range 0.05−1.2 μM with an estimated limit of detection of 9 nM.

Acknowledgements. This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CCCDI-UEFISCDI, project number ERANET-RUS-PLUS-PLASMON-ELECTROLIGHT/46/2018, within PNCDI III.

[1]. Y. Li *et al.*, *Sensors Actuators, B Chem.*, 240 **(2017)** 785–792, doi: 10.1016/j.snb.2016.09.042.

S2-O-03

Detection of Allosteric Modulators of Growth Hormone Secretagogue Receptor According to their Affinity Profiles in Competitive Assays

Mihaela Puiu, Lucian-Gabriel Zamfir, George Madalin Danila, Camelia Bala
LaborQ, University of Bucharest
4-12 Regina Elisabeta Blvd., 030018 Bucharest, Romania
e-mail: elenamihaela.puiu@g.unibuc.ro

The growth hormone secretagogue receptor (GHS-R1a) belongs to the G protein-coupled receptor (GPCR) class, which is one of the largest integral membrane protein families, with crucial role in signaling and transducing the extracellular information [1]. GHS-R1a is an essential drug target due to its involvement in the regulation of metabolic processes. The endogenous ligand of GHS-R1a (ghrelin, GHR) and many other receptor-targeted drugs bind the orthosteric site of the receptor, activating it (agonists, partial agonists) or inhibiting it (antagonists) [2]. As an alternative strategy for GHS-R1a-targeting drugs, allosteric modulation is a novel approach, where ligands are designed to bind not the orthosteric site, but the allosteric one [3, 4]. In this work we provide the proof-of-concept of an affinity format for the detection of GHS-R1a modulators based on their affinity profile in competitive assays with labelled ghrelin. It was found that peptidic or non-peptidic ligands such as [D-Lys^{3}]-GHRP-6 (DLS), [D-Arg^{1},D-Phe^{5},D-Trp^{7,9},Leu^{11}]-Substance P and YIL-781 ($C_{24}H_{28}FN_3O_2$) promote the GHR/GHS-R1a interaction at low molar ratios GHR/modulator but prevent it at higher values. We developed a sequence of coupled equilibriums able to sustain the bell-shaped pattern of the labelled GHR/GHS-R1a binding curves in the presence of modulators. Thus, the allosteric modulators were detected in the nanomolar range by exploiting the linear parts of their bell-shaped "signature" in colorimetric and electrochemical assays.

This work was supported by a grant of the Romanian Ministry of Education and Research, CNCS - UEFISCDI, project number PN-III-P4-ID-PCE-2020-0998, within PNCDI III

[1] E.A. Wold, J. Zhou, Curr Top Med Chem, 18 (2018) 2002-2006.
[2] V. Casadó-Anguera, E. Moreno, J. Mallol, S. Ferré, E.I. Canela, A. Cortés, V. Casadó, Pharmacol. Res., 139 (2019) 337-347.
[3] P.J. Conn, A. Christopoulos, C.W. Lindsley, , Nat Rev Drug Discov, 8 (2009) 41-54.
[4] G.M. Danila, M. Puiu, L.-G. Zamfir, C. Bala, Analytical Chemistry, 91 (2019) 14812-14817.

S2-O-04

Electrochemical Evaluation of Bacteria *Xylella Fastidiosa* DNA-Copper(II) Interaction Using DNA-Electrochemical Biosensors

W. B. S. Machini and A. M. Oliveira-Brett
University of Coimbra, Centre for Mechanical Engineering, Materials and Processes (CEMMPRE), Department of Chemistry, 3004-535, Coimbra, Portugal
brett@ci.uc.pt

New emerging bacterial plant diseases are spreading fast in different geographical regions, and effective safety measures have not been found. *Xylella fastidiosa* (*Xf*) is a Gram-negative bacterial plant pathogen classified as a priority quarantine pest, which has affected economically important agricultural crops.
Copper-based compounds have been widely used as antimicrobial in plant diseases control. Although there is no treatment for diseases caused by *Xf*, these compounds are widely applied to its hosts.
The electrochemical investigation of the interaction between bacteria *Xf*-dsDNA and Cu(II) using dsDNA-electrochemical biosensors, was performed. The interactions of Cu(II) with calf thymus dsDNA, polyguanosine and polyadenosine, were also investigated using electrochemical biosensors, in order to better understand the dsDNA-Cu(II) interaction.
The effect of the Cu(II)-*Xf*-dsDNA interaction was electrochemically followed comparing the changes in the oxidation peaks of guanosine and adenosine residues, in the absence and presence of Cu(II), and monitoring the occurrence of free guanine and free adenine, and the purine biomarkers: 8-oxoguanine, and 2,8-dihydroxyadenine.
The Cu(II)-*Xf*-dsDNA interaction occurs by the binding of Cu(II) in different sites, independent of the bacterial DNA sequence, leading to the condensation/aggregation of *Xf*-DNA strands, due to the formation of a rigid Cu(II)-*Xf*-dsDNA complex structure. The peak attributed to the oxidation of 8-oxoGua and/or 2,8-oxoAde was not electrochemically detected, suggesting that, under the experimental conditions used, Cu(II) did not induce oxidative DNA damage. Also, a resistance of the bacterial *Xf*-dsDNA to Cu(II) was observed.

Acknowledgements Projects FCT (UID/EMS/00285/2020) and PhD Grant 232296/2014-6 (W.B.S. Machini) from CNPq, Brazil.

S2-O-05

Electrochemical Detection of DNA-conjugated Methylene Blue: Optimization of DNA Probe Surface Coverage, Hybridization Time and Length of Target DNA Sequences

Fernando Otero [a], Urszula Salaj-Kosla[a], Tofail Syed [a] and Edmond Magner [a]
[a] Department of Chemical Sciences and Bernal Institute, University of Limerick, Limerick (Ireland)
fernando.otero.diez@ul.ie

The vast majority of electrochemical sensing of DNA are based on the hybridization of strands of DNA. These approaches offer high selectivity for the target DNA (tDNA) strand and low limits of detection (LOD) that can enable the detection of diseases at early stages. The detection of the TP53 cancer biomarker has been investigated through the electrochemical measurement of changes in DNA structures before and after the hybridization process with a maximum signal gain upon hybridization of 63.1 nA at a surface coverage of 3.9 pmol/cm^2 (Figure.1). The biosensor is capable of discriminating between the healthy sequence and "short" cDNA strands, with a maximum discrimination factor of 3. In contrast, the biosensor is not capable of discriminating between the healthy sequence and "long" complementary DNA. To achieve the maximum analytical performance, DNA probe surface coverage, the optimal length of the target DNA strand and hybridization times were investigated.

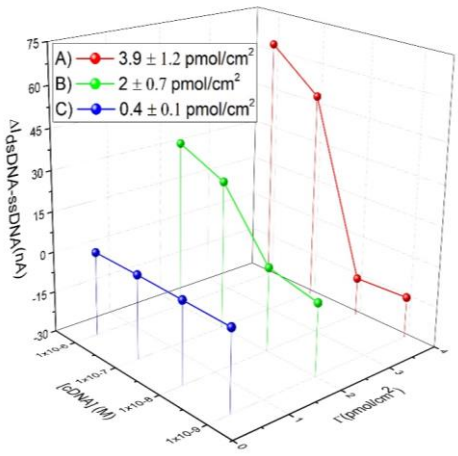


Figure.1 Influence of the probe surface coverage and concentration of "short" cDNA.

S2-O-06

Micrometric Electrochemical Biosensors for Spatially Resolved Metabolites Studies

Simona De Zio, Marco Malferrari, Stefania Rapino

Department of Chemistry "Giacomo Ciamician", University of Bologna, via F. Selmi 2, 40126, Italy.
simona.dezio2@unibo.it

The mechanisms underlying cancer development and proliferation are at the center of cancer research. The characterization of the tumor microenvironment and the development of new devices and analytical techniques are therefore fundamental to obtain important information, to predict the development of the disease and to intervene adequately. Scanning electrochemical microscopy (SECM) is a powerful technique able to investigate cells and their surroundings with high spatial and temporal resolution using ultramicroelectrodes as probes to obtain information about cell metabolic activity.[1]
Glucose microbiosensors[2] can be used as probes of SECM to measure, with high sensitivity, glucose concentration, a central molecule in the cell metabolism. The rate of glucose consumption is often altered in tumor metabolism, in *ex vivo* tissues.
We developed a functionalization route to efficiently incorporate the enzyme Glucose Oxidase on platinum ultramicroelectrodes. The obtained biosensor can be used to measure the local glucose concentration in hypoxic microenvironments. This condition often affects solid tumor tissues. The response of the glucose biosensor, its selectivity and sensitivity, was tested at different levels of oxygen.

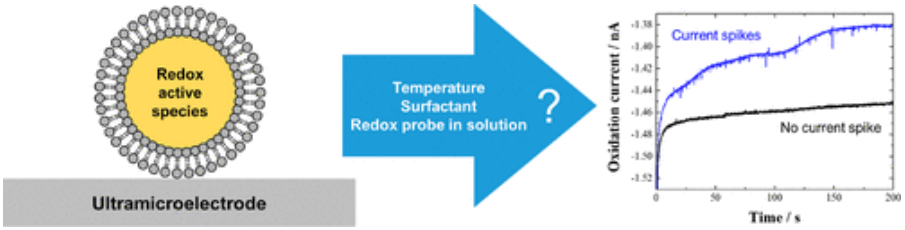
[1] T.-E. Lin, S. Rapino, H. H. Girault and A. Lesch; Chem. Sci., 2018, 9,4546-4554.
[2] A. Soldà, G. Valenti, M. Marcaccio, M. Giorgio, P. G. Pelicci, F. Paolucci and S. Rapino; ACS Sens., 2017; 2; 1310-131.

S2-O-07

Single Electrochemical Nano-impacts of Synthetic Redox Phospholipid Liposomes

Estelle Lebègue,[1] Frédéric Barrière,[2] Allen J. Bard[3]
[1]*Université de Nantes, CNRS, CEISAM UMR 6230, F-44000 Nantes, France*
[2]*Univ Rennes, CNRS, Institut des Sciences Chimiques de Rennes - UMR 6226, F-35000 Rennes, France*
[3]*Center for Electrochemistry, Department of Chemistry, The University of Texas at Austin, Austin, Texas 78712, United States*
estelle.lebegue@univ-nantes.fr

The electrochemical detection of synthetic redox DMPC (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine) liposomes by single collisions at carbon and Pt ultramicroelectrodes (UMEs) is reported. To study the parameters influencing the lipid membrane opening/permeability, the electrochemical detection of single redox DMPC liposome collisions at polarized UMEs was investigated under different experimental conditions (addition of surfactant, temperature). The electrochemical responses recorded showed that the permeability of the DMPC lipid membrane (tuned by addition of Triton X-100 surfactant or by the increase of the solution temperature) is a key parameter for the liposome membrane electroporation process and hence for the release and oxidation of its redox content during the collision onto UMEs. This electrochemical technique is a promising strategy to detect toxins produced by pathogenic bacteria and interacting with the liposomes lipid bilayer membrane like a biosurfactant.



Schematic representation of the redox DMPC liposome collision experiments at a polarized UME in aqueous solution and typical electrochemical responses observed in the chronoamperometry measurements.

[1] Lebègue, E.; Barrière, F.; Bard, A. J. *Anal. Chem.* **2020**, *92* (3), 2401.
[2] Lebègue, E.; Farre, C.; Jose, C.; Saulnier, J.; Lagarde, F.; Chevalier, Y.; Chaix, C.; Jaffrezic-Renault, N. *Sensors* **2018**, *18* (2), 599.
[3] Lebègue, E.; Anderson, C. M.; Dick, J. E.; Webb, L. J.; Bard, A. J. *Langmuir* **2015**, *31* (42), 11734.

S2-O-08

Calcium Channel Blocker Lercanidipine Electrochemistry at a Carbon Black Modified Glassy Carbon Electrode

Ana-Maria Chiorcea-Paquim, Isabel Garrido Fernandes and
Ana Maria Oliveira-Brett
University of Coimbra, CEMMPRE, Department of Chemistry,
3004-535 Coimbra, Portugal
anachior@ipn.pt

Dihydropyridine calcium channel blockers (DHP-CCBs) have an established role in antihypertensive therapy, being effective both as monotherapy and in combination with other drugs. Lercanidipine is a lipophilic DHP-CCB that presents high vascular selectivity and persistence in the smooth muscle cell membranes. Comparing to the first and second generation of DHP-CCBs, it dilates both the afferent and the efferent glomerular arteries, while maintaining the intraglomerular pressure, and presents anti-inflammatory, antioxidant, and anti-atherogenic properties. Lercanidipine efficacy to treat hypertension has been demonstrated in either elderly or young patients, and also in the presence of other risk factors, such as diabetes and renal impairment. Drug testing methods are nowadays laborious and time-consuming; hence, the development of rapid, sensitive, selective, low-cost electrochemical methods of detection and quantification of pharmaceutical compounds in pharmacological compositions received increased attention. The carbon black (CB) nanomaterial was used in many electrochemical studies, due to its high conductivity, chemical stability, large surface area, and very low cost.

The electrochemical behaviour of lercanidipine at glassy carbon electrode (GCE), boron doped diamond electrode (BDDE), and GCE and BDDE modified with a CB nanoparticles embedded within a dihexadecylphosphate (DHP) nanostructured film (CB–DHP/GCE and CB–DHP/BDDE) was investigated by cyclic, square-wave and differential pulse voltammetry [1]. In the pH interval 3.4–9.5, the lercanidipine undergoes a pH-dependent, diffusion-controlled, irreversible oxidation process, that takes place at the N1 and C4 positions from the 1,4-dihydropyridine ring, in two consecutive each involving the transfer of one electron and one proton. For pH > 9.5, both oxidation processes are pH-independent and a pKa 9.40 was determined. The lercanidipine reduction is irreversible, and the lercanidipine reduction products are also electroactive, following a reversible electron transfer process. Based on the lercanidipine oxidation, and with no need for N2 purging, the lercanidipine electroanalytical determination at a nanostructured CB–DHP/GCE was achieved, showing a limit of detection of 0.058 μM (3.58×10^{-5} g L^{-1}) and a limit of quantification of 0.176 μM (1.08×10^{-4} g L^{-1}), more than ten times lower than previous described.

S2-O-09

Graphene-Modified Electrodes used for the Enhanced Detection of Biomolecules

Stela Pruneanu, Florina Pogăcean, Codruţa Varodi, Maria Coroş,
Alexandra Cioriţă, Valentin Mirel

National Institute for Research and Development of Isotopic and Molecular Technologies,
Donath street, no. 67-103, 400293
Cluj-Napoca, Romania;
stela.pruneanu@itim-cj.ro

The electrochemical synthesis of graphene using different electrolytes (e.g. ammonium sulfate or a mixture of ammonium sulfate, boric acid, and sodium chloride) was investigated. The obtained materials were morphologically and structurally characterized by TEM/SEM, XRD, XPS and elemental analysis. The synthesis method was developed in our group and was based on the exfoliation of graphite rods by pulses of currents. The pulse duration was set at 0.8 s and the pause between two pulses was 0.2 s. During electrochemical exfoliation the applied voltage was 10 V and the generated current was around 0.5 A.

The X-ray powder diffraction (XRD) technique revealed that the graphene samples generally consisted in a mixture of few-layer graphene (FLG), multi-layer graphene (MLG) and a small amount of graphene oxide (GO). XPS analysis proved that the samples were either co-doped with hetero atoms such as nitrogen and sulfur or triple-doped with nitrogen, sulfur, and boron. Each sample was dispersed in an organic solvent (DMF) and then deposited onto the surface of glassy carbon electrodes (GC). The graphene-modified electrodes were next tested in standard laboratory solutions towards the detection of various biomolecules (dopamine, 8-hydroxy-2′-deoxyguanosine, L-tryptophan) proving their enhanced electrochemical response.

Acknowledgement

This work was supported by grant of Romanian Ministry of Research and Innovation, CNCS-UEFISCDI, project number PN-III-P4-ID-PCCF-2016-0006, within PNCDI III.

S2-O-10

New Findings in Label-Free Nucleic Acid Electrochemistry: Effects Homonucleotide Blocks and Catalytic Hydrogen Evolution

Miroslav Fojta, Ales Danhel, Daniel Dobrovodsky, Stanislav Hason, Hana Pivonkova, Zuzana Soldanova, Martin Outla, Veronika Ostatna
Institute of Biophysics of the CAS, Brno, Czech Republic.
fojta@ibp.cz

Nucleic acids are electrochemically active due to cathodic reducibility or anodic oxidizability of particular nucleobase residues. Electrochemical signals of DNA are influenced by its structure, which has been explained in terms of differences of the accessibility of the nucleobase residues involved in structured, base-paired DNA segments *versus* those located in unstructured (unpaired, denatured) regions. Our recent findings suggest that this scheme rather simplistic. (a) Quite recently, A and C (but not G an T) or their reduction products have been shown to exhibit catalytic hydrogen evolution (CHE) at the mercury electrodes. Earlier we proposed that reduction of G includes a chemical step, probably the CHE observed in the presence of platinum complexes. Recently we have observed a strong positive effects of electrode processes involving A and/or C residues on the G reduction. In the absence of A and C, the G reduction was inefficient. (b) Homo-oligonucleotides exhibit characteristic behavior at the mercury electrode surface. Homopyrimidine stretches undergo (in contrast to homopurine blocks) 2D condensation processes at the negatively charged electrode surface and are more firmly adsorbed at the electrode surface. (c) Reduction of cytosine in homo-C blocks and required lower acidity of the medium than reduction of isolated C residues, suggestion which correlated with proclivity of the longer homo-C blocks to form cytosine i-motifs involving hemiprotonated C^+.C pairs. Taken together, these observations suggest that not only secondary structure, but also base composition (and/or sequence) of DNA strongly influences electrochemical responses of DNA via mutual influence of individual nucleobases.

Acknowledgements *This research was supported by the Czech Science Foundation (project No. 18-01710S) and by the SYMBIT project reg. no. CZ.02.1.01/0.0/0.0/15_003/0000477 financed from the ERDF.*

S2-O-11

Multiplexed Readout of Enzymatic Redox Reactions Triggered by Light

Felipe Conzuelo[1], Marc Riedel[2], Adrian Ruff[1], Fred Lisdat[2], Wolfgang Schuhmann[1]
[1] Analytical Chemistry – Center for Electrochemical Sciences (CES), Faculty of Chemistry and Biochemistry, Ruhr University Bochum, Universitätsstr. 150, D-44780 Bochum, Germany
[2] Biosystems Technology, Institute for Life Sciences and Biomedical Technologies, Technical University Wildau, Hochschulring 1, D-15745 Wildau, Germany
felipe.conzuelo@rub.de

Enzymatic redox reactions are the basis for numerous biotechnological and analytical devices. The possibility for an on-demand control of the transfer of electrons over a modified electrode provides additional capabilities for the implementation of electrochemical platforms enabling a multiplexed detection of analytes. For this, photoelectrochemical devices can be rationally designed and used in combination with light as the trigger for allowing a directed stimulation of redox processes. Here, the integration of redox enzymes with quantum dot (QD)-sensitized electrode architectures [1] is used as the basis for a spatially resolved photoelectrochemical detection of two model analytes over a single electrode surface [2]. The implemented platform consists of a 3D inverse opal-TiO_2 structure modified with PbS QDs using a successive ionic layer adsorption reaction approach. Furthermore, the enzymes lactate oxidase and flavin adenine dinucleotide-dependent glucose dehydrogenase are used for the catalytic conversion of lactate and glucose, respectively. The enzymes are immobilized and electrically wired by integration within an Os-complex modified redox polymer (P-Os). Illumination of the assembly translates in the generation of electron-hole pairs at the QDs leading to the oxidation of polymer-bound Os-complexes by the generated holes and the concomitant injection of high energy electrons into the conduction band of TiO_2, resulting in the occurrence of an anodic photocurrent. Enzymatic turnover enables amplification of the photocurrent response by the transfer of electrons to P-Os. As a result, the enzyme substrate can be locally detected and quantified.

References

[1] M. Riedel, W. J. Parak, A. Ruff, W. Schuhmann, F. Lisdat, *ACS Catal.* **2018**, *8*, 5212 – 5220.
[2] M. Riedel, A. Ruff, W. Schuhmann, F. Lisdat, F. Conzuelo, *Chem. Commun.* **2020**, *56*, 5147 – 5150.

Acknowledgements
The work was supported by the Deutsche Forschungsgemeinschaft (German Research Foundation) within the framework of the Cluster of Excellence RESOLV (EXC-2033).

S2-O-12

Single Molecular Sensing by the Nanopore-Based Single-Biomolecule Interface

Yi-Tao Long*
School of Chemistry and Chemical Engineering, Nanjing University
Nanjing 210023, P. R. China
E-mail: yitaolong@nju.edu.cn

Single molecule sensing has greatly enhanced the precision and depth of our understanding of living system. Single membrane proteins provide a well-defined confined space for accommodating an individual single molecule, and thus it could be regarded as a single-biomolecule interface for capturing and identifying a single molecule from bulk solution. Herein, we focus on the nanopore-based single-biomolecule interface for single molecule sensing, which includes both the precise construction of the sensing interface and direct sensing of single biomolecules. Nanopore analysis involving massive stochastic information, big-data analysis and other methodologies can be used to obtain single molecule information from electrochemical analysis. The concept of a "single-molecule ionic spectrum" is proposed and discussed in detail, which may allow mapping of noncovalent interactions at an atomic level in the future.

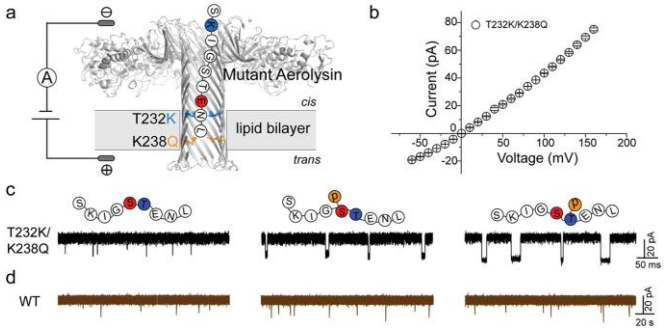


Fig. 1 Nanopore-based single-biomolecule interface for mapping adjacent phosphorylation sites of a single peptide.

References

[1] S. Li, X. Y. Wu, M. Y. Li, S. C. Liu, Y. L. Ying, Y. T. Long, *Small Methods*, **2020**, *4*, 2000014.
[2] M. Y. Li, Y. L. Ying, S. Li, Y. Q. Wang, X. Y. Wu, Y. T. Long, *ACS Nano*, **2020**, *14*, 12571–12578.
[3] Y. L. Ying and Y. T. Long, *J. Am. Chem. Soc.*, **2019**, *141*, 15720–15729.

S2-O-13

Lactate sensor use in tissue, saliva and sweat: physiological uncertainties of meaningful measurement

P Vadgama, A. Spehar-Délèze, S. Anastasova
School of Engineering and Materials Science, Queen Mary University of London, Mile End Road, London E1 4NS, UK
p.vadgama@qmul.ac.uk

Lactate is the end product of glucose utilization under hypoxia. Its measurement is of value in shock and in sports physiology. Classical lactate sensors based on lactate oxidase (*Pediococcus* sp) with H_2O_2 detection were used in needle format and as planar electrodes to variously measure lactate in tissue, saliva and sweat. The low K_m (0.5mM lactate) of the oxidase precluded its direct use, and measuring range needed to be extended through diffusion limiting membranes. Polyurethane laminates served as the external barrier, and an ionomeric polymer as inner barrier providing selectivity for H_2O_2. The constructs provided for a linear range to >20mM lactate. Linearity, surprisingly, could also be achieved with a lower permeability inner layer. A postulated mechanism for this is reduced H_2O_2 loss to the working electrode, retention within the enzyme layer and some degradative change leading to oxygen *in situ*. Despite membrane use, oxygen dependence was seen at pO_2 <70 mmHg. Such electrodes, however, allowed subcutaneous lactate monitoring in the pig during shock [1]. When lactate elevation was achieved instead by intravenous infusion in the rat, no subcutaneous tissue lactate change was observed. This would suggest a capillary barrier to lactate under the conditions employed, a contrast to glucose. Lactate changes seen during shock were possibly due to lactate generation outside the circulation and a reduced blood pH.

Salivary lactate measurement in subjects undergoing exercise showed lactate increase that tracked with blood without a lag period. Though concentrations in saliva were <50% of those in blood and there were individual and time dependent variations, trend monitoring in exercise would appear to be feasible. By contrast, lactate in sweat showed marked inter-individual difference, including a steep rise and fall despite with ongoing exercise. A meaningful correlation with blood would not seem feasible based on our experience. Sweat lactate excursions are primarily a construct of intrinsic sweat gland physiology, and not a consequence of blood efflux, simple or otherwise. If there is a correlation with exercise intensity that can be extracted in future, it is likely to be complex, multifactorial and driven more by thermoregulatory stimulus than an outcome of hypoxia.

[1] Rong, Z.; Leitao, E.; Popplewell, J. *et al IEEE Sensors* **2008**, 8, 113.

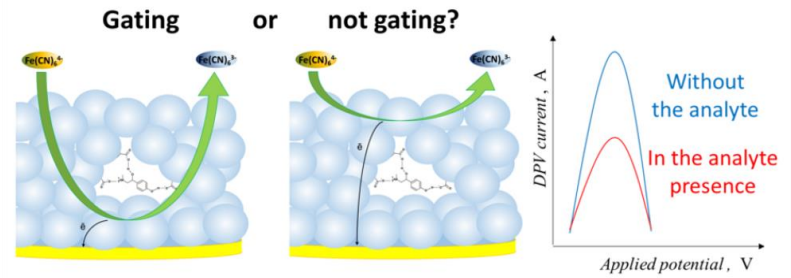
Generous support from the UK EPSRC (ESPRIT Project) is acknowledged.

S2-O-14

p-Synephrine Electrochemical Selective Sensing with a Molecularly Imprinted Polymer and a Redox Probe Engaged in a "Gate Effect"

Wlodzimierz Kutner,[1,2,*] Patrycja Lach,[1] Maciej Cieplak,[1,*] Marta Majewska,[1] Krzysztof R. Noworyta,[1] Piyush Sindhu Sharma[1]
[1] *Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland*
[2] *Faculty of Mathematics and Natural Sciences. School of Sciences, Cardinal Stefan Wyszynski University in Warsaw, Wóycickiego 1/3, 01-815 Warsaw, Poland*
wkutner@ichf.edu.pl

A "gating" mechanism [1] was engaged for *p*-synephrine determination, selective to creatinine, adrenalin, urea, and glucose interferences, using Pt electrodes coated with conductive molecularly imprinted polymer (MIP) films [2]. The in-situ AFM determined MIP-film thickness was similar in the *p*-synephrine in solution absence and presence. Hence, the decrease of the DPV peak current for the $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ redox probe with the increase of the *p*-synephrine concentration, measured at the polythiophene (*p*-synephrine)-templated MIP film-coated electrode, did not originate from MIP film swelling or shrinking, as it was postulated formerly, but from changes in the electrochemical process kinetics. *p*-Synephrine determination at the MIP film-coated electrode was examined with CV, DPV, EIS, and SPR. Moreover, doping of the MIP film was not affected by *p*-synephrine binding in MIP-film molecular cavities. Presumably, the "gate effect" originated from changes in radical cation mobility in the MIP film.



Scheme 1. Illustration of a "gate effect" in *p*-synephrine electrochemical determination using *p*-synephrine templated MIP film-coated Pt electrode.

References

[1] Sharma, P. S., et al., *Curr. Opin. Electrochem.* 2019, *16*, 50-56.
[2] Lach P. et al., *Anal. Chem.* 2019, *91*, 7546−7553.

S2-O-15

Detection of Olive Oil Adulteration Using Electrochemical Sensors and Biosensors

C. Apetrei[1], I.M. Apetrei[2]
[1]*Department of Chemistry, Physics and Environment, Faculty of Sciences and Environment*
[2]*Department of Pharmaceutical Sciences, Faculty of Medicine and Pharmacy*
"Dunărea de Jos" University of Galaţi 47 Domneasca Street, 800008 Galaţi, Romania
apetreic@ugal.ro

The study described the efficiency of electrochemical sensors and biosensors to detect extra virgin olive oil adulteration with different vegetable oils. Extra virgin olive oil is a natural product with a great economic impact and it is very appreciated by the consumers due to nutritional and health benefits. Thus, extra virgin olive oil adulteration with low-grade olive oils or other vegetable oils could be profitable for natural products suppliers or sellers. Prevention or highly sensitive detection of adulteration of extra virgin olive oil are important issue in the actuality being related to consumers' confidence, which involve different social, economic, and health aspects. The methods used for the analysis of compounds present in liquid fraction of extra virgin olive oils are mainly based on spectroscopy, chromatography, and isotopic analysis [1]. However, different electrochemical sensors and biosensors has been developed and used in the analysis of the extra virgin olive oils. They are based on different detection principles, mainly potentiometry, voltammetry and electrochemical impedance spectroscopy. The olive oils samples are minimally preprocessed in order to facilitate the measurements. The strategies used for the detection of the olive oil adulteration are mainly based on detection of biomarkers, on the detection of some compounds or characteristics and the coherences among them, or obtaining of chemical fingerprints and multivariate data analysis [2]. The results obtained with sensors and biosensors are promising and development of portable devices for the detection of extra virgin olive oil adulteration is of great interest.

References

[1] Meenu, M.; Cai, Q.; Xu, B. *Trends Food Sci Tech* **2019**, 91, 391–408.
[2] Jimenez-Lopez, C.; Carpena, M.; Lourenço-Lopes, C.; Gallardo-Gomez, M.; Lorenzo, J. M.; Barba, F. J.; Prieto, M. A.; Simal-Gandara, J. *Foods* **2020**, 9, 1014.

Acknowledgments

This work was supported by a grant of the Romanian Ministry of Education and Research, CNCS – UEFISCDI, project number PN-III-P4-ID-PCE-2020-0923, within PNCDI III.

S2-O-16

Aptamer-based Assays for Diagnosis and Management of Celiac Disease

Clara Abardía-Serrano, Rebeca Miranda-Castro, Noemí de-los-Santos-Álvarez, María Jesús Lobo-Castañón
Dpto. Química Física y Analítica, Facultad de Química, Universidad de Oviedo
Av. Julián Clavería, 8, 33006, Oviedo, Spain
Instituto de Investigación Sanitaria del Principado de Asturias
Av. de Roma, 33011, Oviedo, Spain
abardiaclara@uniovi.es

Celiac disease (CD) is an immune-mediated systemic disorder affecting genetically predisposed individuals. It is triggered by the exposure to gluten, a group of proteins found in certain cereals such as wheat, barley, rye or some varieties of oats [1]. So far, CD is a chronic disease that cannot be cured but can be controlled with a strict gluten-free diet for the whole life that alleviates symptoms. Genetic testing for human leukocyte antigens (HLA-DQ2 and HLA-DQ8), serological tests for celiac-specific antibodies, the biopsy of the intestine, and finally the response to the gluten-free diet complete the current protocol to diagnose the disease, although the result is not always conclusive. There is therefore a need for alternative or complementary assays to avoid the invasive biopsy both in the diagnosis of CD and in its follow-up.

Thus motivated, we have designed a simple, fast, non-invasive, and self-reporting electrochemical biosensor for assisting in CD diagnosis. It involves a nucleic acid aptamer as recognition element, which was evolved against the hydrophobic immunodominant peptide from α2-gliadin, the so-called 33-mer, by means of a SELEX process carried out in our research group [2]. The aptamer is linked at the 3' end to the redox-active molecule methylene blue, while the 5' end is chemisorbed to a gold surface via a thiol group. The target recognition induces a structure switching of the aptamer thus altering the distance or dynamics of methylene blue with respect to the gold surface [3]. Based on this binding-induced conformational change, we have designed different self-reporting strategies, which are compared in terms of sensitivity and reproducibility, as well as their feasibility in clinical specimens.

[1] Cereal Chemistry. 2008, 85 (1), 1-13
[2] Anal. Chem. 2014, 86, 5, 2733–2739
[3] ACS Sens. 2019, 4, 2832-2837

S2-O-17

How drugs decreasing cholesterol affect structure and electrical properties of model lipid membranes

Michalina Zaborowska [1,2], Damian Dziubak[2], Dorota Matyszewska[2], Aleksandra Bartkowiak[1,2], Renata Bilewicz[1,2]

[1]*Faculty of Chemistry, University of Warsaw, Pasteura 1, 02-093 Warsaw, Poland*
[2]*Faculty of Chemistry, Biological and Chemical Research Centre, University of Warsaw, Żwirki i Wigury 101, 02-089 Warsaw, Poland*
bilewicz@chem.uw.edu.pl

Statins are the most common hypolipidemic drugs which act as competitive inhibitors of 3-hydroxy-3-methyl-glutaryl-Coenzyme A (HMG-CoA) reductase (HMG-CoA reductase), the enzyme which catalyzes deacylation of HMG-CoA to CoA and mevalonate in the cholesterol biosynthetic pathway. A negative side of employing these effective drugs as inhibitors is that they interact not only with their therapeutic target protein but also cause several side effects including modification of cell membranes properties and structure. This would in turn cause alterations in the function of membrane proteins by changing e.g. packing of the lipid molecules around the proteins. An important goal of research on statins is, therefore, to identify the changes that occur in the lipid membrane structure under influence of statins, and factors that control the membrane penetration by this drug. This encouraged us to study the incorporation of statins into model lipid membranes: Langmuir monolayers and supported black lipid membranes (s-BLMs) [1]. The model membranes were formed at the air-water interface and by combination of Langmuir-Blodgett and Langmuir-Schaefer methods were transferred onto gold substrate to form supported lipid bilayers (s-BLMs). The architecture of the bilayer resembled that of natural raft membranes. Application of electrochemical techniques ACV and EIS provides information on the changes of electrical properties, organization and homogeneity of the supported model membranes under influence of statins. We also show why simvastatin complexation by (2-hydroxypropyl)-β-cyclodextrin facilitates smooth delivery of this highly lipophilic drug through the model lipid membranes composed of DMPC and Cholesterol (7:3). An improved understanding of the interactions of different statins with lipid membranes will help in the development of preparation methods for *in vivo* applications.

1. M. Zaborowska, M. Broniatowski, P. Wydro, D. Matyszewska, R. Bilewicz, *J. Molec. Liq.*, 2020,313, 113570.

S2-O-18

3D-printing of Polylactic Acid based Interdigitated Electrodes for Greener and Low-Cost Biosensing

Florence Lagarde, Manon Mornay, Catherine Jose, Joelle Saulnier
Univ Lyon, CNRS, Université Claude Bernard Lyon 1, Institut des Sciences Analytiques
5 Rue la Doua, F-69100 Villeurbanne
florence.lagarde@univ-lyon1.fr

Additive manufacturing, commonly referred to as 3D printing, is an emerging and rapidly expanding bottom-up manufacturing technology with the potential to achieve rapid prototyping of complex geometries. Due to the many advantages it offers, 3D printing technology has become very popular in many fields of application, among which analytical and bioanalytical sciences[1]. In this area, examples include the use of 3D printing for the fabrication of microfluidic devices, packaging structures for integrated devices, smartphone interfaces, and even more recently sensors and biosensors[2-4].

Here, we report the design and elaboration of interdigitated supported electrodes entirely printed using fused deposition modelling (FDM) of polylactic acid (PLA) based materials. Among the variety of 3D-printing techniques, FDM is probably the most widespread because of the low price of printer devices and cost per print. PLA is a biosourced polymer derived from the hydrolysis and fermentation of corn and rice starch. Filaments coils of conductive PLA (PLA loaded with carbon black or graphene) are commercially available and have been used in this work to produce interdigitated electrodes on PLA printed support.
The electrode design and treatment have first been optimized to achieve efficient electron transfer and the immobilization of different enzymes have been further considered in view of conductometric biosensors development. Optimization of the development steps and first analytical features of the enzyme biosensors will be presented.

References
(1) Gross, B. et al. *Anal. Chem.* **2016**, *89*, 57-70.
(2) Weisgrab, G. et al. *Adv. Mater. Technol* **2019**, 1900275.
(3) Manzanares Palenzuela, C. L. and Pumera, M. *Tr. Anal. Chem*. **2018**, *103*, 110
(4) Cardoso, R. M. et al. *Analytica Chim. Acta* **2020**, *1118*, 73e91

S2-O-19

Deoxyuridine Triphosphates Modified with Tyrosine Aromatic Groups for Direct Electrochemical Detection of Double-Stranded DNA Produced by Polymerase Chain Reaction or Isothermal Amplification

Elena V. Suprun[1], Svetlana A. Khmeleva[1], Gulnaz R. Kutdusova[2], Konstantin G. Ptitsyn[1], Viktoriya E. Kuznetsova[3], Sergey A. Lapa[3], Alexander V. Chudinov[3], Sergey P. Radko[1]
[1)]Institute of Biomedical Chemistry, Pogodinskaya Street, 10/8, Moscow, 119121 Russia
[2)]Chemistry Faculty of M.V. Lomonosov Moscow State University, Lenin Hills, 1/3, Moscow, 119991 Russia
[3)]Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Vavilov Street, 32, Moscow, 119991 Russia
lenasuprun@mail.ru

Direct electrochemical detection of double-stranded DNA (dsDNA) may provide a reliable and cost-effective approach to quantify DNA amplification products, with a great potential for coupling to isothermal amplification techniques [1]. The aim of this work was to combine a set of novel 2'-deoxyuridine-5'-triphosphate (dUTP) derivatives modified with Tyr or Trp aromatic groups with polymerase chain reaction (PCR) and isothermal amplification technique such as recombinase polymerase amplification (RPA). For this purpose, dUTP derivatives were tested as electroactive 'labels' by square wave voltammetry on carbon screen printed electrodes and as proper substrates for polymerase enzymes in PCR and RPA methods. The 'labeled' dUTP nucleotides have demonstrated their oxidation peaks at 0.5–0.7 V, similar to free Tyr or Trp, respectively [2]. Moreover, PCR generated dsDNA fragments with modified nucleotides showed similar oxidation signals at micromolar concentrations, while no peaks were observed for unmodified dsDNA at the same conditions [2]. However, among nucleotides under study, only 5-aminoallyl-dUTP derivative modified with 4-hydroxyphenylacetic acid revealed good compatibility with the RPA assay, probably due to a small size of the additional functional group and linker flexibility.

This work was financially supported by the Russian Science Foundation, grant 19-14-00247.

1. Y. Zhao, F. Chen, Q. Li, L. Wang, C. Fan, Chem. Rev. 115 (2015) 12491.
2. E. V. Suprun, S. A. Khmeleva, G. R. Kutdusova, I. F. Duskaev, V. E. Kuznetsova, S. A. Lapa, A. V. Chudinov, S. P. Radko, Electrochim. Acta 362 (2020) 137105.

S2-O-20

The Use of Microelectrochemistry to Investigate pH Regulation in Cultured Cancer Cells

Raluca-Elena Munteanu, Luciana Stănică, Mihaela Gheorghiu, Szilveszter Gáspár
International Centre of Biodynamics
1B Intrarea Portocalelor, 060101 – Bucharest, Romania
sgaspar@biodyn.ro

Unlike normal cells, cancer cells are able to proliferate even at pH as low as 6.7. This ability of the cancer cells is currently investigated with great interest as it could be exploited for cancer therapy. Consequently, we have developed two, microelectrochemistry-based methods which are able to provide new insights into the pH regulation of cultured cancer cells (see Fig. 1). For the first method, we built a voltametric pH microsensor by modifying a carbon fiber microelectrode (CFME; Ø = 37 µm) with graphene and syringaldazine. Subsequently, this voltametric pH microsensor was integrated into a Scanning Electrochemical Microscope (SECM), and the resulting system used to measure the extracellular pH of adherently growing cancer cells at ~ 25 µm above the cells. In the second method, we positioned the just mentioned CFME above cancer cells which were previously loaded with pH-sensitive fluorescent dye. Subsequently, water electrolysis was carried out on the CFME in order to induce a sudden pH change in the extracellular space of the cancer cells found in the close proximity of the CFME, and, simultaneously, an inverted microscope was used to monitor the intracellular pH of the very same cells. While the first method allowed revealing the heterogeneity of the investigated cancer cells in terms of extracellular pH, the second method allowed observing how fast a pH change induced in the extracellular space of cancer cells propagates into their intracellular space (i.e., allowed revealing the "buffering capacity" of the investigated cancer cells). Experimental details and results obtained with both cancer cells and normal cells will be presented.

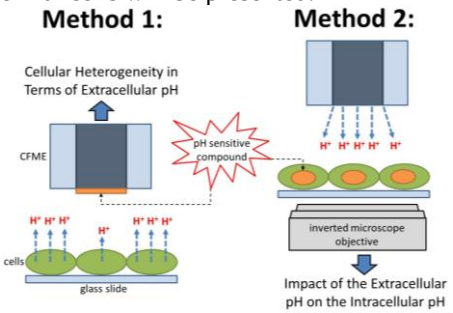


Figure 1. Schematic representation of our microelectrochemistry-based methods for gaining new insights into the pH regulation of cultured cancer cells.

S2-O-21

Site-specific Wiring of Glucose Dehydrogenase for Controlled Orientation using Unnatural Amino acid

Itay Algov, Aviv Feiertag, Lital Alfonta
Departments of Life Sciences, Chemistry and
Ilse Katz Institute for Nanoscale Science and Technology
Ben-Gurion University of the Negev, PoBox 653, Beer-Sheva 8410501, Israel
algov@post.bgu.ac.il

Enzymatic glucose biosensors are widely used for the measurement of blood glucose levels in diabetes patients. Wearable non-invasive devices have emerged in the past decade and allow the non-invasive measurement of glucose concentrations in other bodily fluids like sweat and tears. Glucose concentration in those samples, compared to blood samples, are ca. ten times lower and range from 0.1 to 0.6 mM. These low concentrations require a sensor with high sensitivity towards glucose. One way of getting highly sensitive sensors is by the use of direct electron transfer (DET) – ET from the enzyme to the electrode without a mediating molecule. As protein matrices are mostly insulators, controlling the enzyme orientation towards the electrode can improve ET efficiency. In our research, we have designed a Flavin adenine dinucleotide dependent glucose dehydrogenase fused to a minimal cytochrome domain (FAD-GDH-MCD) with a site-specifically incorporated unnatural amino acid (Algov et al., 2021). The unnatural amino acid that we used was propargyl-l-lysine (PrK), which has an alkyne side-chain that provides a unique "chemical handle" for the enzyme wiring to the electrode. We have rationally chosen two amino acid incorporation sites, in a way that will allow high proximity to one of the electroactive sites (either FAD or the heme domains) without interfering with the protein structure and function. By comparing the two different wiring sites to a non-specifically wired enzyme and to an FAD-GDH that lacks the cytochrome domain, we have shown the importance of controlled orientation, as well as the participation of the minimal cytochrome in the ET process. Site-specifically wired enzyme showed high currents in response to glucose with 15 times higher i_{max} and ca. 3 times higher electron transfer rate (k_{ET}), compared to the non-specifically wired protein. The high currents result in high resolution of glucose measurements with a linear range between 0.01 to 1 mM, which can fit the requirements of wearable glucose measurement devices. Application of this approach for enzyme wiring on other enzymes can improve present DET systems and allow more efficient ET.

Reference

Algov, I., Feiertag, A., Alfonta, L., 2021. Site-specifically wired and oriented glucose dehydrogenase fused to a minimal cytochrome with high glucose sensing sensitivity. Biosens. Bioelectron. 180, 113117.

S2-O-22

Evaluation of Anti-Cancer Drug Shikonin Interaction with dsDNA in Incubated Solutions and *in situ* Sensing at DNA Biosensors

Caroline G. Sanz[1], Ricardo J. B. Leote[1,2], Victor C. Diculescu[1]
[1]*National Institute of Materials Physics, Str. Atomistilor 405A, Măgurele, Romania*
[2]*Faculty of Physics, University of Bucharest, Atomistilor 405, Măgurele, Romania*
caroline.sanz@infim.ro

Shikonin is a 1,4-naphtoquinone derivative (found in the roots of *Alkanna Tinctoria* and *Lithospermum erythrorhizon)* with anti-inflammatory properties that have been exploited over several centuries. Recently, the anti-tumor activity of shikonin has been investigated *in vivo* revealing its interference in several signaling pathways of different cell tissues, which makes it a suitable candidate for the development of anti-cancer drugs [1]. In this work, the electrochemical oxidation mechanism of shikonin was evaluated using CV and DPV at bare glassy carbon electrodes (GCE), and three pH dependent oxidation process were observed in the positive potential range (E_{p1a} = 0.093 – 0.053 pH, E_{p2a} = 0.20 – 0.049 pH and E_{p3a} = 0.87 – 0.053 pH). The electro-oxidation mechanism was attributed to the oxidation of the hydroquinone moiety (E_{p1a} and E_{p2a}) and the oxidation of the hydroxyl group close to the aromatic ring (E_{p3a}). Moreover, the interaction mechanism of shikonin and dsDNA was investigated in incubated solutions and using a dsDNA biosensor allowing the characterization of the DNA conformational modifications and oxidative damages.

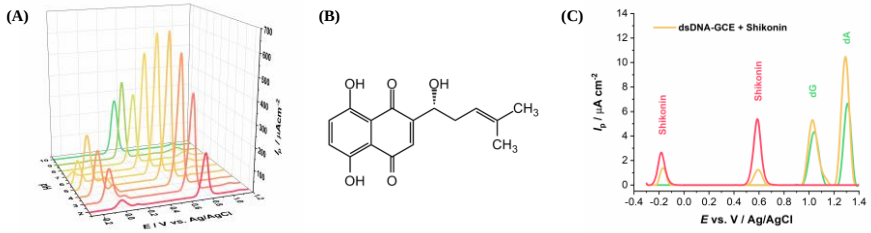


Fig. 1 (A) DP voltammograms at bare GCE in BR buffer containing 50 µM of shikonin, (B) Structure of shikonin and (C) DP voltammograms obtained at (—) bare GCE in 0.1M acetate buffer pH 4.5 containing 1.0 µM shikonin and at dsDNA-GCE biosensor (—) prior and (—) after 5 min of incubation in 10 µM shikonin solution.

References

[1] Cancer Letters, 2019, 459, 248–267.

Acknowledgements

Financial support from the Romanian Ministry of Research and Innovation through Operational Program Competitiveness 2014-2020, Project: NANO BIOSURF SMIS103528.

S2-O-23

Graphene Electrical-Electrochemical Hybrid Devices for Zepto-Molar DNA Detections

Isabela A. Mattioli[1], Ayaz Hassan[1], Natalia M. Sanches[1], Nirton C. S. Vieira[2], Frank N. Crespilho[1]

[1]*São Carlos Institute of Chemistry, University of São Paulo, Avenida Trabalhador São-Carlense, 400, CEP 13566-590 - São Carlos - SP – Brasil*

[2]*Institute of Science and Technology, Federal University of São Paulo, Rua Talim, 330, CEP 12231-280 - São José dos Campos - SP, Brazil*

isabela.mattioli@usp.br

Herein, an Electrical-Electrochemical Vertical Device (EEVD) based on monolayer graphene and ferrocene heterojunction (graphene-fc) was developed aiming on drop DNA detections.[1] To properly form the heterojunction, ferrocene was adsorbed onto graphene through van der Waals interactions. Raman Spectroscopy measurements enabled the estimative of charge carrier insertion and resulting Fermi level due to band gap opening.[2] The electrical-electrochemical hybrid operation mechanism at the EEVDs is established through the basal plane current flowing on the device between its terminals due to the high basal plane charge carrier mobility of monolayer graphene. The use of an $Ag/AgCl_{sat}$ electrode as a reference one enables the recording of I_{ds} *vs.* V_{ds} currents without its polarization, as well as the observation of vertical electron transfer processes on graphene-fc heterojunction. Therefore, interfacial OCP potential alterations due to ferrocene adsorption onto graphene, as well as DNA adsorption onto the heterojunction could be monitored. Electrochemical experiments, as Electrochemical Impedance Spectroscopy (EIS) and Cyclic Voltammetry (CV) also gave insights on these interfacial OCP potential alterations. Thus, highly sensitive DNA detections were made through OCP quantifications as a function of adsorbed DNA in different concentrations, in a label-free and ready-to-use detection assay, with an LOD of 5.3 10^{-21} mol L^{-1}.

References

(1) Mattioli, I. A.; Hassan, A.; Sanches, N. M.; Vieira, N. C. S.; Crespilho, F. N. *Biosens. Bioelectron.* **2021**, *175* (November), 112851.

(2) Zribi, B.; Haghiri-Gosnet, A. M.; Bendounan, A.; Ouerghi, A.; Korri-Youssoufi, H. *Carbon N. Y.* **2019**, *153*, 557–564.

S2-O-24

Comparative Analysis of the Detection of Leukemia Cells by Electrochemical and Acoustics Aptasensors

Tibor Hianik
Faculty of Mathematics, Physics and Informatics, Comenius University, Mlynska dolina F1, 842 48 Bratislava, Slovakia
tibor.hianik@fmph.uniba.sk

Oncological diseases belong to the most serious illnesses with high mortality. The most common cancer in children is acute lymphoblastic leukemia (ALL). It is important to develop diagnostic methods that will be able to detect this disease in early stage. One of the possible options can be non-invasive diagnostics using the biosensors based on nucleic acid aptamers. Aptamer recognizes the surface markers on the membrane of cancer cells with the high binding affinity. Biosensors based on aptamers with redox markers are among the most sensitive experimental tools of this type. We developed and optimized the redox-labeled electrochemical aptasensors for the detection of Jurkat leukemia cells. The sgc8c DNA aptamers specific to the protein tyrosine kinase 7 (PTK7), which is important membrane protein cancer marker that is over expressed in Jurkat cells were used. We compared the sensitivity of aptasensors for aptamers modified either by methylene blue (MB) and ferrocene carboxylic acid (Fc), respectively. Both aptasensors were tested in the presence of Jurkat cells at concentration range 50-5000 cells/mL using differential pulse voltammetry. In both cases the comparable sensitivity was obtained with limit of detection: 37±6 cells/mL for Fc labeled aptamers and 38±8 cells/mL for MB labeled aptamers based on 3.3S/N (signal to noise) rule. The interaction of the sensing surface with control U266 cells was less significant [1]. The sensitivity of electrochemical sensors has been compared with label-free thickness shear mode (TSM) acoustic method. In this case binding of the Jurkat cells with the sensing surface composed of the sgc8c aptamers were monitored by changes in resonant frequency and motional resistance. TSM method has been less sensitive in comparison with electrochemical one (limit of detection: 195±20 cells/mL). However, evaluation of the sensor response was easier. The peculiarities of detection of the cells by TSM are discussed also in respect of the viscoelastic contribution [2].

Acknowledgements This research was financially supported by Science Agency VEGA, project No. 1/0419/20.

References

[1] C. Slabý, L. Babelová, T. Hianik, *Proceedings of IECB 2020*, **2020**, *60*, 5. doi:10.3390/IECB2020-07082
[2] A. Poturnayová, M. Buríková, J. Bízik, T. Hianik, *ChemPhysChem* **2019**, *20*, 545-554. doi: 10.1002/cphc.201801126.

S2-O-25

Transition Metal Hexacyanoferrates Based (Bio)sensors for Medical Diagnostics

E.V. Karpova, E.V. Shcherbacheva, D.V. Tikhonov, A.A. Karyakin
M.V. Lomonosov Moscow State University
119991, Lenin Hills, Moscow, Russia
karpowa.ew@gmail.com

Prussian Blue (PB) based (bio)sensors have been known and used for different medical applications since many years. It was shown that ferric hexacyanoferrate (HCF) possesses unique properties in electrocatalysis of H_2O_2 reduction. Non-iron HCFs, especially nickel hexacyanoferrate (NiHCF), are suitable matrixes for PB stabilization [1]. The usage of PB nanoparticles [2] opens new horizons in (bio)sensor production.

In the current work hydrogen peroxide sensors were fabricated by casting a droplet of suspension containing PB or PB-NiHCF nanoparticles onto the working electrode surface of planar sensor structures. The sensitivity of sensors based on PB-NiHCF nanozymes is similar to it based on PB. However PB-NiHCF nanoparticles based sensors are characterized by dramatically improved operational stability: under severe for PB conditions upon continuous monitoring of 1 mM H_2O_2 the PB-NiHCF nanozymes based sensor retains 78% of its initial response after 25 minutes. At the same time the response of the solely PB based sensor decreases down to 7% of its initial value.

Besides a significantly enhanced operational stability of glucose biosensors based on PB-NiHCF nanoparticles, they can be used for long-term glucose monitoring in excretory liquid, such as sweat. PB nanoparticles were furthermore used to obtain lactate biosensors applied in non-invasive diagnostics development. Within this contribution, we report on the suitability of sweat lactate for diagnostics. We demonstrate that a significant increase in lactate concentration in sweat from the working muscle area is assigned to a simultaneously rise of blood lactate content during exhaustive physical exercise. The variation rates of lactate concentration in sweat from working and latent muscle areas correlate positively with blood lactate levels ($r > 0.8$ and $r = 0.7$, respectively), thus offering the prospect of a non-invasive approach for the monitoring of sportsperson training.

1. A.A. Karyakin. Current opinion in electrochemistry, 5(1):92, 2017.
2. M.A. Komkova, E.E. Karyakina, A.A. Karyakin. JACS, 140:11302, 2018.

Financial support through RSF (grant # 19-13-00131) is greatly acknowledged.

S2-O-26

Real-time determination of cellobiose and glucose formation during enzymatic biomass hydrolysis

Hucheng Chang, Lena Wohlschlager, Adrian Ruff, Wolfgang Schuhmann, Florian Csarman, Stefan Scheiblbrandner, Roland Ludwig
Department of Food Science and Technology,
University of Natural Resources and Life Sciences, Vienna
Muthgasse 11, 1190 Vienna, Austria
hucheng.chang@students.boku.ac.at

Enzymatic hydrolysis of lignocellulosic biomass for biofuel production relies on complex multi-enzyme cocktails. Continuous and accurate measurement of the released key products is crucial to optimize the industrial degradation process and also to investigate the activity and interaction of the involved enzymes on the insoluble substrate. Amperometric biosensors have been applied to perform continuous cellobiose measurements during the enzymatic hydrolysis of pure cellulose powders. However, the oxygen-sensitive mediators used in those biosensors restricted their function under physiological or industrial conditions. Also, the combined measurements of the hydrolysis products cellobiose and glucose require a high selectivity of the biorecognition elements. We employed an ([Os(2,2'-bipyridine)2Cl]Cl)-modified polymer and cellobiose dehydrogenase to fabricate a cellobiose biosensor, which can accurately and specifically detect cellobiose even in the presence of oxygen and the other main product glucose. Additionally, a glucose biosensor was fabricated to simultaneously measure glucose produced from cellobiose by β-glucosidases. The cellobiose and glucose biosensors work at applied potentials of +0.25 V and +0.45 V vs. Ag|AgCl, respectively, and can selectively detect their substrate. Both biosensors were used in combination to monitor the hydrolysis of pure cellulose of low crystallinity or industrial corncobs samples. The obtained results correlate with HPLC-PAD analysis and demonstrate that neither oxygen nor the presence of redox-active compounds from the lignin fraction of corncobs interfere with the measurements.

References

1. Ferrari, A. R.; Gaber, Y.; Fraaije, M. W. Biotechnol. Biofuels 7 (2014) 2–9.
2. Tokin, R.; Väljamäe, P.; Cruys-Bagger, N.; Tatsumi, H.; Borch, K.; Badino, S. F.; Sørensen, T. H.; Fathalinejad, S.; Gontsarik, M.; Westh, P.; Jensen, K.; Toscano, M. D. Enzyme Microb. Technol. 58 (2014) 68–74.
3. Marquitan, M.; Bobrowski, T.; Ernst, A.; Wilde, P.; Clausmeyer, J.; Ruff, A.; Schuhmann, W. J. Electrochem. Soc. 165 (2018) G3008–G3014.

S2-O-27

Disposable Stochastic Sensor Based on Deposition of a Nanolayer of Silver on Silk for Molecular Recognition of CA 19-9, CEA, and p53

Sorin Sebastian Gheorghe[1], Ruxandra-Maria Ilie-Mihai[2],
Raluca-Ioana Ștefan-van Staden[1,2], Marius Bădulescu[3]
[1]*Faculty of Applied Chemistry and Material Science, University Politehnica of Bucharest, Bucharest, Romania.*
[2]*Laboratory of Electrochemistry and PATLAB, National Institute of Research for Electrochemistry and Condensed Matter, 202 Splaiul Independentei Str., 060021, Bucharest-6, Romania*
[3]*Low Temperature Plasma Laboratory, National Institute for Lasers, Plasma and Radiation Physics (NILPRP), Str. Atomiștilor 409, Măgurele, Romania*
sebastiangheorghe09@yahoo.com

A cold plasma system was used for Silver nanofilm coating of silk material. The Silver coated material was further modified by soaking in a 10^{-3}mol/L solution of α-cyclodextrin. The modified silk material was served as working electrode for simultaneous molecular recognition of specific biomarkers. CA19-9, CEA and p53 served as model analytes to prove that the disposable stochastic sensor can be used for simultaneous molecular recognition of specific biomarkers. The signatures (t_{off} value) obtained for p53, CEA and CA19-9 were different, proving that the proposed disposable stochastic sensor is selective, and can be used for molecular recognition of these biomarkers in whole blood and tumoral tissue samples.

S2-O-28

Acrylate Derivatives Based Molecularly Imprinted Polymer Nanoparticles for the Fabrication of Cilostazol Electrochemical Sensor

Jyoti,[1] Carlo Gonzato,[2] Karsten Haupt,[2] Teresa Żołek,[3] Dorota Maciejewska,[3] Andrzej Kutner,[4] Piyush Sindhu Sharma,[1] Krzysztof Noworyta,[1] and Włodzimierz Kutner[1,5]

[1] *Institute of Physical Chemistry, Polish Academy of Sciences (IPC PAS), Kasprzaka 44/52, 01-224, Warsaw, Poland*

[2] *Université de Technologie de Compiègne, CNRS laboratory for Enzyme and Cell Engineering, CS 60 319 - 60 203 Compiègne Cedex, France*

[3] *Department of Organic Chemistry, Faculty of Pharmacy, Medical University of Warsaw, Banacha 1, 02-097 Warsaw, Poland*

[4] *Medical University of Warsaw, Faculty of Pharmacy, Department of Bioanalysis and Drug Analysis, 1 Stefana Banacha, 02-097 Warsaw, Poland*

[5] *Faculty of Mathematics and Natural Sciences. School of Sciences, Cardinal Stefan Wyszynski University, Woycickiego 1/3, 01-815 Warsaw, Poland*

jyoti@ichf.edu.pl

Electrochemical chemosensor for cilostazol and its primary metabolite, 3,4-dehydrocilostazol, selective determination, with molecularly imprinted polymer nanoparticles as the recognition unit, was devised, fabricated, and tested. Cilostazol is an oral selective cyclic nucleotide phosphodiesterase 3 (PDE_3) inhibitor.[1] It is used to treat intermittent claudication (IC), a symptom of peripheral arterial disease (PAD).[2]

Interactions of the polymer cavities formed by functional acrylic monomers with cilostazol were simulated with molecular mechanics (MM) and density functional theory (DFT). The chemosensor's linear dynamic concentration range was 0.135 to 2.58 µM, and the limit of detection was 90.4 nM. The chemosensor response to cilostazol and its primary metabolite was tested using voltammetric and electrochemical impedance spectroscopy techniques showing appreciable selectivity to glucose, cholesterol, and 3,4-dehydroaripiprazole interferences of 8.25, 7.9, and 3.5, respectively.

Funding: Polish National Science Center (grant No. 2015/19/B/ST4/03743).

References

1. Y-W. Chi, C. J. Lavie, R. V. Milani, C. J. White, *Vasc. Health Risk Manag.*, 2008, **4**, 1197-1203.
2. S. L. Bramer, W. P. Forbes, and S. Mallikaarjun, *Clin. Pharmacokinet.*, 1999, **37**, 1-11.

S2-O-29

Antimcrobial Activity of Silver Nanoparticle-Based Films Studied by Scanning Probe Microscopy

Giada Caniglia[1], Anna Heinzmann[1], Maria Chiara Sportelli[2], Antonio Valentini[3], Nicola Cioffi[2], Christine Kranz[1]

[1] *Institute of Analytical and Bioanalytical Chemistry, Ulm University, Albert-Einstein-Allee, 11 – 89081 Ulm, Germany*
[2] *Chemistry Department, University of Bari "Aldo Moro", V. Orabona, 4, 70126, Bari, Italy*
[3] *Physics Department, University of Bari "Aldo Moro", V. Orabona 4, 70126, Bari, Italy*
giada.caniglia@uni-ulm.de

Biofilms are well-organized sessile communities which exhibit an increased tolerance against antimicrobial and antibiotic treatments in comparison with their planktonic counterparts. Within the last decades, novel strategies to prevent the formation of biofilms have been developed and, among others, metal-based nanoparticles (NPs) have been intensively studied [1]. Silver (I) nanoparticles (Ag-NPs) are known to be effective antimicrobial agents, as silver(I) can penetrate the bacterial cells and produce oxidative stress via the generation of reactive oxygen species (ROS) [2]. In this contribution, biocompatible AgNPs-fluoropolymers [3] are investigated as antimicrobial films. The mechanism of the silver(I) release is studied in-situ by scanning electrochemical microscopy (SECM) in combination with square-wave stripping voltammetry (Fig. 1). Besides, the relation between silver(I) release and the swelling behavior of the polymeric films will be presented, combining electrochemical techniques and atomic force microscopy (AFM).

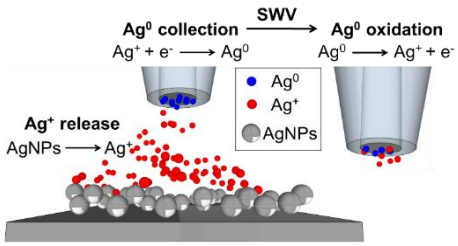


Fig. 1. Scheme of Ag(I) release study with SECM-SWV.

References

[1] M.C. Sportelli, et al. Nano-Antimicrobials Based on Metals. In Novel Antimicrobial Agents and Strategies; John Wiley & Sons, Ltd, 2014; pp 181–218.
[2] N. Durán, et al. *Nanomedicine,* **2016**, 12(*3*), 789-799.
[3] M.C. Sportelli, et al. *Sci. Rep*. **2017**, 7 (*1*), 11870.

S2-O-30

Detection of Catechol and Azinphos Methyl Using Poly(3,4-Ethylenedioxythiophene)- Iridium Oxide Nanocomposite Based Tyrosinase Biosensor

Sevinc Kurbanoglu, Cem Erkmen, Bengi Uslu
Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry, 06560, Ankara/TURKEY
skurbanoglu@gmail.com

The increasing of synthetic pesticides in well waters, tap waters and wastewater is a worldwide problem because of their widespread use against pests in agriculture or crops [1,2]. Due to the use of pesticides in agriculture; air, soil and water are polluted over time. Depending on their physicochemical properties, pesticides can cause environmental problems by being dragged to other places by wind and rain. Some of them drift from the soil with rain, flood and snow waters and pollute river, lake and seawaters. Pesticides are directly and indirectly affect human health. In this study, a novel, sensitive and simple amperometric biosensor for the determination of catechol and pesticide azinphos methyl based on Tyrosinase enzyme inhibition has been developed. The biosensor was prepared from iridium (IV) oxide, Poly (3,4 ethylenedioxythiophene) nanocomposite.

Screen printed electrodes were used as the transducer and Tyrosinase was immobilized in IrOx/PEDOT nanocomposite with the help of crosslinking agent glutaraldehyde. Nanobiosensor was successfully used for the dual determination of catechol and azinphos methyl using fixed potential amperometry. Nanobiosensor performance was optimized in terms of iridium (IV) oxide, Poly (3,4 ethylenedioxythiophene) and Tyrosinase. The surface morphology of the enzyme electrode was characterized by scanning electrochemical microscopy, cyclic voltammetry and electrochemical impedance. Under optimized conditions, linear relationships were achieved in the range 0.05-10.65 µM for catechol and 10-200 µM for azinphos methyl. Limit of detection was found as 0.017 µM for catechol and 2.964 µM for azinphos methyl with acceptable repeatability and reproducibility. Finally, developed biosensor was successfully applied to the detection of azinphos methyl tap water, waste water, well water, human urine, serum and saliva. For future studies, the designed biosensor can easily be used on-site. Moreover, the designed dual phenolic compound/pesticide detection nanobiosensor can be extended to other enzymes and other pesticides pollutants if desired.

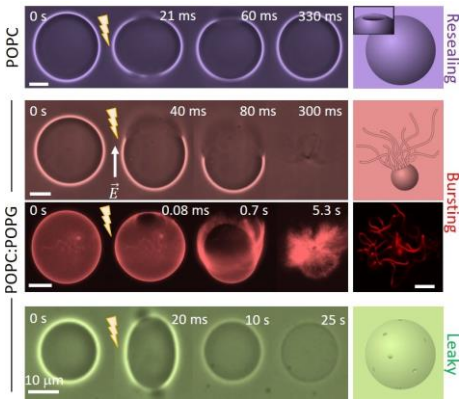
S3 Pulsed electric and magnetic fields in biology, medicine and biotechnology

S3-O-01

The Role of Charges on Membrane Stability upon Electroporation

Fernanda S.C. Leomil[1,2], R. B. Lira[2,3], M. Zoccoler[4], K. A. Riske[1], R. Dimova[2]
[1]*Biophysics Department, Federal University of São Paulo, São Paulo, Brazil;*
[2]*Max Planck Institute of Colloids and Interfaces, 14424 Potsdam, Germany;*
[3]*Moleculaire Biofysica, Zernike, Groningen, The Netherlands*
[4]*Department of Physiology and Biophysics, University of São Paulo, Brazil;*
fernanda.leomil@unifesp.br

Membrane stability is vital for cell survival. When subjected to strong stimuli, such as electric pulses, membrane pores open (electroporation). In model membrane systems, such as giant unilamellar vesicles (GUVs, tens of microns in size) the pores can be directly observed under the microscope. In vesicles made of neutral lipids (phosphatidylcholine, PC), pores reseal and membrane integrity is restored. However, in charged membranes, the vesicles are destabilized after electro-poration: long-living submicroscopic pores are generated or the GUVs collapse completely, restructuring into membrane nanotubes (see figure below). In this work, we used neutral PC GUVs containing increasing fractions of the anionic lipid phosphatidylglycerol (PG) to show that the origin of these phenomena is related to the membrane pore edge tension (γ), which governs pore closure, and is significantly decreased in membranes containing 50 mol% of PG [Adv. Sci. 2021, 2004068]. The pore edge tension was determined from the pore closure dynamics [Biophys. J. 2010, 99:3264] using a software [manuscript in preparation] that automatically performs the image processing steps, discloses pore dynamics and calculates the pore edge tension. We also observed that destabilization propensity is enhanced for membranes made of lipids with higher degree of unsaturation and that it can be reversed by membrane charge screening by calcium ions.



S3-O-02

Evaluation of Calcium Electroporation for the Treatment of Cutaneous Metastases: a Double Blinded Randomized Controlled Phase II trial

Dóra Ágoston, Sándor Rátkai, Eszter Baltás, Judit Oláh, Lajos Kemény, Erika Kis
University of Szeged, Department of Dermatology and Allergology
Korányi fasor 6, 6720 Szeged, Hungary
kis.erika.gabriella@med.u-szeged.hu

Bleomycine based electrochemotherapy (ECT) is a widespread method in the treatment of cutaneous tumours. Calcium electroporation (Ca-EP) is a novel treatment which anticancer efficacy was confirmed by numerous preclinical studies. In this randomized double blinded phase II study, the efficacy of Ca-EP was examined and compared with bleomycin based ECT in the treatment of cutaneous metastases of different tumour histotypes.

Patients were included with up to 10 cutaneous metastases which sizes ranged between 0,5 and 3 cm. Metastases were randomized into one of the two treatment arms. In one arm intratumoral calcium (Ca), in the other intratumoral bleomycin was given and followed by electroporation (EP) in both arms. Response rate was evaluated clinically 6 months after treatment according to RECIST-like guidelines, and histologically by taking biopsies from both Ca and bleomycin treated lesions. Adverse events were evaluated and graded according to Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

Seven patients with a total of 44 cutaneous metastases (34 malignant melanoma, 10 breast cancer metastases) were treated according to the protocol. Thirty-three metastases were randomized into the two treatment arms; 18 lesions were treated with Ca-EP and 15 with ECT. The objective response rate (ORR) for Ca-EP was 33% (6/18), complete response rate (CRR) was 22% (4/18). For ECT ORR was 53% (8/15), CRR was 40% (6/15). Six months after treatment 13 biopsies were taken from Ca- (7) and from bleomycin- EP (6) treated lesions. Forty-six percent (6/13) of the biopsies were taken from clinically completely responded lesions, which were confirmed by histology in 83% (5/6). From the biopsies no tumor cells were identified in 100% (3/3) of the lesions treated with Ca-EP and in 67% (2/3) of the lesions treated with ECT. After 1 year, none of the 10 completely responded metastases had relapsed. Ulceration, itching and exudation were found slightly more frequently in metastases treated with bleomycin, and less hyperpigmentation was seen in metastases treated with Ca-EP.

Ca-EP was non-inferior to ECT, therefore, it should be considered as a feasible, effective and safe treatment option.

S3-O-03

GM1 Leaflet Asymmetry Stabilizes Membrane Pores

Mina Aleksanyan, Rafael B. Lira, Jan Steinkühler, Rumiana Dimova
Max Planck Institute of Colloids and Interfaces, 14424, Potsdam, Germany
mina.aleksanyan@mpikg.mpg.de

For effective cell transfection and electrochemotherapy treatments, the cell plasma membrane needs to be transiently porated by electric fields and reseal afterwards. Model membranes, such as giant unilamellar vesicles (Dimova, Ann. Rev. Biophys. 48:93, 2019) can be used to unravel the role of factors governing electroporation and pore lifetimes. Because cell membranes are highly asymmetric, we investigated the influence of membrane asymmetry on electroporation of model membranes. We employed giant vesicles, in which the GM1 is asymmetrically distributed in the leaflets (Dasgupta et al., Proc. Natl. Acad. Sci. USA. 115:5756, 2018). GM1 is a ganglioside which is enriched and asymmetrically distributed in neuronal plasma membranes. Upon electroporation of asymmetric GM1-doped vesicles (see Fig. 1), we identified series of membrane remodeling events which were distinctly different from those in symmetric membranes. After electroporation, asymmetric vesicles displayed a high density of membrane nanotubes, which slowed down the pore resealing and resulted in lower values of the measured pore edge tension. Furthermore, we observed the formation of a large number of membrane defects on the vesicles after pore closure and subsequent increased membrane permeability to small molecules indicating the generation and stabilization of pores with suboptical resolution. Our findings suggest that neuronal membranes, which are asymmetrically enriched in GM1, may have lower propensity for pore resealing and thus lower stability upon poration.

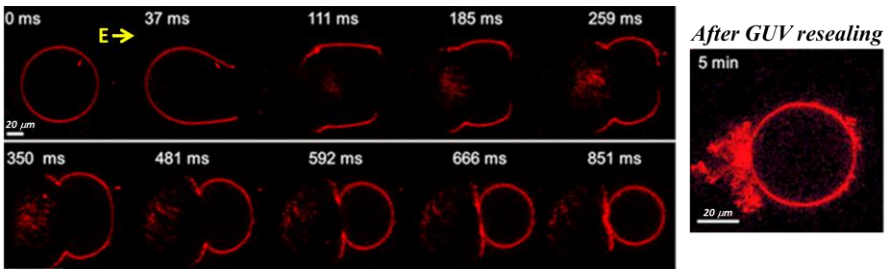


Figure 1: Electroporation of a 4 mol% GM1-doped asymmetric giant vesicle exhibiting a high density of nanotubes which slow down the pore closure. Left: A sequence of confocal cross sections of the vesicle during and after application of a DC pulse (3kV/cm, 50 ms). The indicated time is relative to the beginning of the pulse. Right: Cross section of the vesicle 5 minutes after the pulse. Scale bars are 20 μm.

S3-O-04

Evaluation of Dynamic Cell Response to Electroporation by Means of Digital Holographic Microscopy

Mihaela G. Moisescu[1], Violeta L. Calin[1], Mona Mihailescu[2], Nicolae Tarba[3], Ana Maria Sandu[4], Eugen Scarlat[2], Tudor Savopol[1]
[1]*Biophysics and Cellular Biotechnology Dept., Carol Davila University of Medicine and Pharmacy, 8 Eroii Sanitari Blvd., Bucharest, 050474, Romania*
[2]*Digital Holography Imaging and Processing Laboratory*
[3]*Physics Dept., Doctoral School of Automatic Control and Computers*
[4]*CAMPUS Research Center, Doctoral School of Electrical Engineering, University "Politehnica" of Bucharest, 313 Splaiul Independentei, Bucharest, 060042, Romania*
mihaela.moisescu@umfcd.ro

Changes in the optical and shape-related characteristics of electroporated cells were studied using an off-axis Digital Holographic Microscopy (DHM) setup. DHM is adequate for studying fast processes of living cells in their natural environment because does not imply sample scanning, providing quantitative phase images (QPIs) of nanometric resolution on the z axis, without using any staining.
Holograms of murine B16F10 cells were acquired with a high-speed camera (60fps) integrated to a DHM setup with double stabilized HeNe laser 632.8nm, (900nm and 10nm, lateral and vertical resolutions, respectively). For 1.6s before and 90s after electric pulses delivery (trains of 4 bipolar rectangular pulses, 100μs, 1kHz, 0.6, 0.8 and 1kV/cm) series of QPIs were obtained after hologram reconstruction using Koala®. After cell image segmentation (Matlab), phase related parameters were calculated either on the whole cell (global parameters like projected area, dry mass density and entropy) or in specifically defined areas of 3x3 pixels regions of interest (autocorrelation function coefficients of optical phase fluctuations). The time evolution of these parameters was analyzed.
Projected area, dry mass density and entropy proved to be predictors for permeabilized cells which swell or collapse when the pulses amplitude was increased.
The autocorrelation functions coefficients for cell membrane regions which are less prone to electropermeabilization, are not affected by the pulses, while those corresponding to regions in which the membrane permeabilization is favored, are strongly modified by the electric pulses. The analysis of time autocorrelation functions of the phase fluctuations is a novel approach in DHM as well as in the field of electroporation and allows to reveal details of cellular response to electroporation pulses in different regions.

S3-O-05

All-atom Molecular Dynamics Simulation of Strong Electric Field Effect on Microtubules

Jiří Průša[1], Ahmed Taha Ayoub[2], Djamel Eddine Chafai[1], Daniel Havelka[1], Michal Cifra[1]
[1]*Institute of Photonics and Electronics of the Czech Academy of Sciences, Chaberská 57, 182 51 Praha 8 - Kobylisy, Czechia*
[2]*Biomolecular Simulation Center, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Heliopolis University, Cairo 11777, Egypt*
cifra@ufe.cz

Microtubules are cellular structures essential in cell division, intracellular transport and other cellular functions. Therefore, potential biomedical interventions in these processes require the capability to modulate the function of microtubules.
Tubulin, which constitutes microtubules, has an exceptionally high structural electric charge and dipole moment [1]. Hence, electric field seems to be an appropriate tool to influence microtubules. To bring more insight into this proposal, we carried out an all-atom molecular dynamics simulation with the purpose of modelling [2]. We found that a nanosecond-scale electric field opens the microtubule lattice longitudinally, and that this effect depends on the electric field strength and temperature. Our results indicate that intense nanosecond electric field might be a tool for modification of microtubule structure.

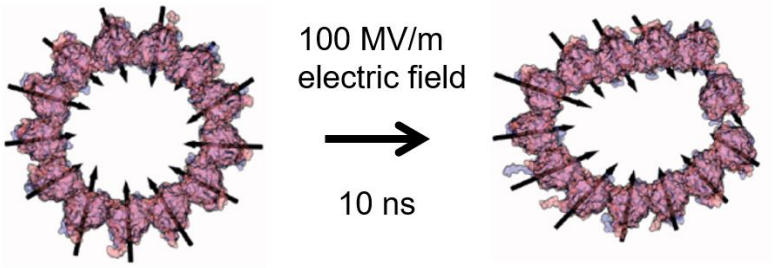


Fig. 1 Electric field deforms and opens microtubule lattice on cathode side.

Acknowledgements to Czech Science Foundation, project GA20-06873X. Authors also participate in an exchange project between Czech and Slovak Academies of Sciences, no. SAV-18-11.

[1] Marracino et al. 2019, *Sci. Rep.*, 9:10477
[2] Průša et al. 2021, *Comp. Struct. Biotech. J.*, in press.

S3-O-06

Pulsed Electric Fields As Physical Tool To Indirectly Remodel Dermal Extracellular Matrix

Sara Gouarderes[1], Camille Ober[2], Layal Doumard[1], Jany Dandurand[2], Alexander Golberg[3], Valérie Samouillan[2], Laure Gibot[1]

[1] *IMRCP CNRS UMR 5623, Université Toulouse III - Paul Sabatier, France*

[2] *CIRIMAT UMR 5085, Université Toulouse III - Paul Sabatier, France*

[3] *Porter School of Environment and Earth Sciences Studies, Tel Aviv University, Tel Aviv, Israel*

gouarderes@chimie.ups-tlse.fr

Impairment in extracellular matrix remodeling is involved in diseases such as cancers or fibrosis. Excessive collagen production and/or decreased degradation by metalloproteinases (MMPs) proteolytic activity result in an abnormal collagen accumulation and tissue loss of function. Due to their local and transient effects, physical stimuli appear as very attractive tools to remodel extracellular matrix[1]. In this study, we assess the potential of pulsed electric fields used in electroporation to locally and transiently stimulate human cells to remodel the ECM themselves. Two distinct calibrated electric protocols already used in human and vet clinics for gene therapy (10 square-wave pulses of 5 ms, 1 Hz, 600 V/cm) and electrochemotherapy (8 square-wave pulses of 100 µs, 1 Hz, 1000 V/cm) were used in our experiments[3]. Thanks to a tissue-engineered human dermal substitute model rich in endogenous extracellular matrix[3], we demonstrated that microsecond and millisecond pulsed electric fields induced 1) mRNA modulation of matrisome-related genes, 2) transient decrease in pro-collagens production and hydroxyproline content in tissue, 3) long-lasting over-activation of MMPs activity and 4) down-regulation at mRNA and protein level of TGF-β, a key player in fibrosis. Thus, pulsed electric fields appear to be a particularly promising therapeutic tool in human health and present a high and easy translational potential in the field of matrix remodeling since they are nowadays used in clinics as physical vector for drug delivery in cancer therapy.

1. Gouarderes, S., Mingotaud, A.-F., Vicendo, P. & Gibot, L. Vascular and extracellular matrix remodeling by physical approaches to improve drug delivery at the tumor site. *Expert Opin. Drug Deliv.* **0**, 1–24 (2020).
2. Gehl, J. *et al.* Updated standard operating procedures for electrochemotherapy of cutaneous tumours and skin metastases. *Acta Oncol.* **57**, 874–882 (2018).
3. Madi M, Rols MP, Gibot L. Efficient In vitro Electropermeabilization of Reconstructed Human Dermal Tissue. J Membr Biol. 2015 Oct;248(5):903-8

S3-O-07

Bleomycin Based Electrochemotherapy for Deep-Seated Soft Tissue Sarcomas - Initial Results in Szeged, Hungary

Aurel Ottlakan[1], Gyorgy Lazar[1], Judit Olah[2], Renata Koszo[2], Katalin Hideghety[2], Erika Gabriella Kis[3]

[1]*University of Szeged, Department of Surgery, 6720, Szeged, Semmelweis str. 8, Hungary*
[2]*University of Szeged, Department of Oncotherapy, 6720, Szeged, Korányi alley 12, Hungary*
[3]*University of Szeged, Department of Dermatology and Allergology, 6720, Korányi alley 6, Hungary*

ottlakan.aurel@med.u-szeged.hu; kis.erika.gabriella@med.u-szeged.hu

Recently bleomycin/cisplatin based electrochemotherapy (ECT) has become an emerging technique not only in the treatment of superficial skin tumors, but also in case of advanced, metastatic and surgically inoperable deep seated lesions.

During a 2-year period (February 2019- February 2021) seven cases of soft tissue sarcomas (STS) [3 fibromyxoid sarcomas, 3 epitheloid sarcomas, 1 liposarcoma] have been treated by bleomycin based electrochemotherapy with intratracheal anesthesia, with the use of standard-, and VEG (variable electrode geometry) electrodes in a prospective study setting at the University of Szeged Department of Surgery. Each procedure was started 8 minutes after an intravenous bleomycin administration and ECT lasted for a maximum of 40 minutes. Timing of postoperative follow-up was at 1 week, 1-2-4-6 months, with follow-up imaging performed at 2-4-6 months.

Among the 4 male- and 2 female patients (of the 7 cases, there was 1 patient treated twice, thus 6 outcome results) mean age was 63.66 years, mean BMI (Body Mass Index) 29.21, mean ASA (American Score of Anaesthesiologists) 2.5, mean CCI (Charlson Co-morbidity Index) 6.66, mean operative time (between intubation and extubation) 40 minutes. Mean hospital stay was 1.83 days, mean VAS (visual analog scale) for posteoperative pain was 2. There were no major postoperative adverse avents. According to the 2 month follow-up CT/MRI, 2 cases of complete response (CR), 3 cases of partial response (PR) and 1 progressing disease (PD) were confirmed.

On the basis of our results it can be confirmed, that bleomycin based ECT for deep-seated advanced-, metastatic and surgically inoperable tumors is a safe and effective non-surgical procedure resulting in low perioperative burden to patients, with potentially prolonged overall survival and improved quality of life.

S3-O-08

Changes in the Packing of Bilayer Lipids triggered by Electroporation

Ioan Tivig, Mihaela G. Moisescu, Eugenia Kovacs, Tudor Savopol
Biophysics and Cellular Biotechnology Dept., Carol Davila University of Medicine and Pharmacy
8 Eroii Sanitari Blvd., 050474, Bucharest, Romania
ioan.tivig@gmail.com

One of the intensely studied effects of oscillating or pulsed electric fields on cells is the increased permeability of the cell membrane, a phenomenon known as electroporation or electropermeabilization. The remanence of cell membrane disorganization induced by the applied electric pulses is not fully understood in terms of lipid bilayer reorganization. In our study we propose a method based on real-time fluorescence quantification of water penetration into the lipid bilayer in case of electroporated cells in suspension.
An integrated experimental setup was used (1). In brief, it includes a pulse generator, electroporation electrodes (fitting a fluorometer cuvette, with a window for the excitation beam), a spectrofluorometer (with 2 emission channels), an optical fiber thermometer and a trigger system.
Laurdan labeled NIH 3T3 cells were suspended in three conductivity buffers (0.14, 0.04, 0.01 S/m) and *Generalized Polarization* (GP) parameter was computed based on fluorescence intensities measured at 440 and 490 nm (2). Trains of bipolar rectangular pulses (1 kV/cm, 100 µs pulse duration, 10 Hz, 1 to 50 pairs of pulses) were applied during fluorescence and temperature data acquisition. Efficacy and reversibility of electroporation was checked by the Propidium Iodide based method. Inhibitors of endocytosis and scavengers of reactive oxygen species were used to check whether the variation of GP due to electroporation was caused by a cellular process or a specific peroxidation.
Depending on the number of the applied pulses, two kinds of evolution were observed in the GP variation: a *negative deflection* at high number of pulses and high conductivity buffers (> 10 pulses, 0.14 S/m) and a *positive deflection* at low number of pulses or low conductivity buffers. The negative deflection (corresponding to a looser packing of the membrane bilayer lipids) was attributed mainly to the thermal effect of the pulses, while the positive deflection was attributed to a lipid peroxidation triggered by electroporation. The permeabilization conditions in which one of these two GP variations is prevailing, are discussed in correlation with the presence of endocytosis and peroxidation inhibitors.

1. Tivig I et al. Eur Biophys J. 2020;49(1):105-11.
2. Tivig I et al. Bioelectrochemistry. 2021;138:107689.

S4 Bioenergetics and biosynthesis

S4-O-01

Electrochemical Monitoring in Real-time of the Redox State of Mitochondria

Emmanuel Suraniti[(a)], Camille Colin[(a)], Yumeng Ma[(a)], Neso Sojic[(a)], Philippe Diolez [(b)], Stéphane Arbault [(a, c)]

(a) University of Bordeaux, ISM, CNRS UMR 5255, NSysA - ENSCBP,
(b) 33600 Pessac, France
(c) *IHU LIRYC, CRCTB Inserm U1045 – Hôpital Xavier Arnozan, 33604 Pessac, France*
(d) *University of Bordeaux, CBMN, CNRS UMR 5248, 33600 Pessac, France*
stephane.arbault@u-bordeaux.fr

The functioning of the respiratory chain, where dioxygen is reduced, in mitochondria is dependent on electron and proton transfers between enzymatic complexes. Reduced substrates (NADH, succinate) provide the respiratory chain with electrons, transferred via quinones and cytochrome c, until complex IV; protons are transferred across the inner membrane to create a potential gradient used as an energetic leap by the ATP Synthase to phosphorylate ADP into ATP. Quinones in their semi-quinone state can reduce oxygen directly and form reactive oxygen species (ROS, e.g. H_2O_2). The redox status of mitochondria thus directly determines their functioning (ATP formation) as well as general cell activities. The balance between oxidative phosphorylation and glycolytic processes is rendered by the settling of the Warburg effect, involved in carcinogenesis. Consequently, there is a true need to decipher on the redox state of the respiratory chain and of redox mediators (*Bioessays*, 2019, 1, 1800180), the embedded quinones (Q_{10} or Q_9).

Based on seminal works by AL Moore et al. (*Febs Letters*, 1988, 235, 76), we developed an electrochemical method in order to monitor indirectly and in real-time the redox state of internal quinones of mammalian cardiac mitochondria. A short-length side chain quinone species, typically Q_2, is added to the mitochondria solution to be oxidized at a carbon electrode in its reduced form Q_2H_2. Q_2 is sufficiently lipophile to permeate across mitochondrial membranes and poise at redox equilibrium with the internal, membrane embedded, pool of quinones. Thus, when respiratory chain substrates are added, quinones are reduced and lead to an increase of oxidation current. Similarly, at phosphorylated state, quinones are less reduced since they allow to transfer electrons between complexes. In full correlation with the oxygen consumption detection by a Pt Clark electrode, the redox state of internal quinones is monitored in real-time. Further works combine this approach with the detection of ROS release by a fluorescence method to gain a full picture of the redox state of working mitochondria.

S4-O-02

High Viologen Loading Polymer for Highly Efficient Wiring of Hydrogenases

Anna Lielpetere[1], Jana Becker[1], Julian Szczesny[1], Felipe Conzuelo[1], Adrian Ruff[1], James Birrell[2], Wolfgang Lubitz[2], Wolfgang Schuhmann[1]

[1]*Analytical Chemistry – Center for Electrochemical Sciences (CES), Faculty of Chemistry and Biochemistry, Ruhr-University Bochum, Universitätsstr. 150, D-44780 Bochum, Germany*
[2]*Max Planck Institute for Chemical Energy Conversion, Stiftstr. 34-36, D-45470 Mülheim an der Ruhr, Germany*
anna.lielpetere@rub.de

Hydrogenases are powerful but sensitive biocatalysts for hydrogen oxidation and evolution that can be wired to electrode surfaces and protected against oxygen by low potential redox polymers [1]. To date, several hydrogenase-based bioanodes have been reported, providing high hydrogen oxidation currents by using multilayer electrode architectures [2, 3].
This work explores the influence of the mediator loading in a redox polymer matrix used for immobilization and electrical wiring of the biomolecules, with the aim of improving even further the performance of hydrogenase-based bioelectrodes, by increasing the mediator density and thus overcoming electron transport limitations in the active enzyme/polymer films. The electron transfer and resulting catalytic currents by wiring [NiFe] and [FeFe] hydrogenases with a high mediator loading redox polymer are evaluated. The viologen-modified polymer **P(N_3MA-GMA)-vio** ((poly(3-azido-propyl methacrylate-co-glycidyl methacrylate)-viologen, with N_3MA:GMA ratio of 85:15) was synthesized in a two-step process. The polymer backbone was obtained using a free radical polymerization followed by addition of the mediator via Cu(I)-mediated 1,3-dipolar cycloaddition ("click reaction") to the N_3MA group, ensuring a maximum mediator loading of up to 85 %. The polymer was used to fabricate hydrogenase-based bioanodes for hydrogen oxidation on gas diffusion electrodes. Due to the high hydrophilicity of the redox polymer, electrografting of ethylenediamine in combination with bifunctional crosslinking was employed for an increased stability of the assembly. This strategy resulted in highly stable bioelectrodes with high hydrogen oxidation current densities.

References: 1. N. Plumeré, O. Rüdiger, A. A. Oughli, R. Williams, J. Vivekananthan, S. Pöller, W. Schuhmann, W. Lubitz, *Nat. Chem.* **2014**, *6*, 822. 2. A. Ruff, J. Szczesny, N. Marković, F. Conzuelo, S. Zacarias, I. A. C. Pereira, W. Lubitz, W. Schuhmann. *Nat. Commun.* **2018,** *9*, 3675. 3. J. Szczesny, N. Marković, F. Conzuelo, S. Zacarias, I. A. C. Pereira, W. Lubitz, N. Plumeré, W. Schuhmann, A. Ruff, *Nat. Commun.* **2018**, *9*, 4715.

Acknowledgements: This work was supported by the European Commission in the framework of the ITN "ImplantSens" (AMD-813006-9).

S4-O-03

CO_2-fixating Bioelectrocatalytic Cascades in Redox-Active Hydrogel for Stereoselective C-C Bond Formation via Reductive Carboxylation

Leonardo Castañeda-Losada[1,2], David Adam[3], Nicole Paczia[3], Darren Buesen[1,5], Fabian Steffler[2,6], Volker Sieber[4], Tobias Erb[3], Michael Richter[2], Nicolas Plumeré[1,5]

[1]Center for Electrochemical Sciences, Ruhr-Universität Bochum; [2]Fraunhofer IGB; [3]Department of Biochemistry and Synthetic Metabolism, Max-Planck Institute for Terrestrial Microbiology; [4]Chemistry of Biogenic Resources, Technical University Munich; [5]Electrobiotechnology, Technical University Munich; [6]Fraunhofer CBP.

Schulgasse 11a, 94315 Straubing, Germany.

leonardo.castaneda@igb.fraunhofer.de

Selectivity of bioelectrocatalysis offers important advantages over classical electrocatalysis for direct CO_2 utilization into high value-added C_{2+} products[1]. However, wiring of CO_2 reducing enzymes to electrodes is demanding because many of these enzymes require NADPH as natural cofactor for electron transfer. Here, we report the regio- and stereoselective incorporation of CO_2 into crotonyl-CoA by an NADPH-dependent enzymatic reductive carboxylation. Specifically, the immobilization of ferredoxin $NADP^+$ reductase within a 2,2´-viologen modified hydrogel[2] allowed the iterative reduction of $NADP^+$ with a faraday efficiency of 98 ± 3 % and a rate of 1.9 ± 0.2 µmol.cm^{-2}.h^{-1}. Co-immobilization of crotonyl-CoA carboxylase/reductase within the hydrogel led to the stereoselective formation of (2*S*)-ethylmalonyl-CoA with 92 ± 6 % faradaic efficiency and a product rate formation of 1.6 ± 0.4 µmol cm^{-2} h^{-1}. Together, the co-immobilized enzymes constitute an electrically driven cofactor regeneration and coupled CO_2 fixation system for stereoselective (2*S*)-ethylmalonyl-CoA formation at high rates. Our system provides the proof-of-principle for the electro-biocatalyzed CO_2-fixation into structurally complex substrates with high regio- and stereocontrol during C-C bond formation. Altogether, the biohybrid system fosters the role of bioelectrochmical CO_2 fixation and represents an important step towards the synthetic applications of NADPH-dependent enzymes.

[1] H. Chen, O. Simoska, K. Lim, M. Grattieri, M. Yuan, F. Dong, Y. S. Lee, K. Beaver, S. Weliwatte, E. M. Gaffney, S. D. Minteer, Chemical reviews 2020, 120, 12903.
[2] S. Hardt, S. Stapf, D. T. Filmon, O. Rüdiger, J. A. Birrell, F. Fourmond, C. Léger, N. Plumeré, Nature Catalysis 2021. DOI: 10.1038/s41929-021-00586-1.

S4-O-04

Protein Film Electrochemistry Studies of CO_2-reducing Enzymes

Vincent Fourmond[a], Marta Meneghello[a], Ana Rita Oliveira[b], Aurore Jacq-Bailly[a], Inês A. C. Pereira[b], Christophe Léger[a]
[a]*CNRS,Aix-Marseille Université, BIP UMR 7281, IMM, IM2B, 31 Chemin J. Aiguier, CS70071, F-13402 Marseille Cedex 20, (France)*
[b]*Instituto de Tecnologia Química e Biológica António Xavier (ITQB NOVA), Universidade Nova de Lisboa, Oeiras (Portugal)Affiliation*
vincent.fourmond@imm.cnrs.fr

There are only two enzymes in Nature that can directly reduce CO_2: the formate dehydrogenase[1](Fdh), which produces formate, and CO dehydrogenases[2,3] (CODH), which produces CO. They are both fast and very energy-efficient, capable of reducing CO_2 with hardly any driving force[4]. There is therefore a strong incentive in studying the catalytic mechanism of these enzymes and understanding the molecular origins of their high performance.
We used Protein Film Electrochemistry, a technique in which the enzyme is wired to an electrode in a configuration in which electron transfer is direct, to study the catalytic mechanism of both CODH and Fdh[5].
We have recently designed a simple method to discriminate between CO_2 and HCO_3^- as a substrate for the reduction of "CO_2"; we were able to show that both enzymes reduce CO_2 and not carbonate[6]. This method is general and can be applied to other redox catalysts.
We also used a site-directed mutagenesis approach to explore the intramolecular diffusion of CO and CO_2 inside the CODH; we will present also some of these results.

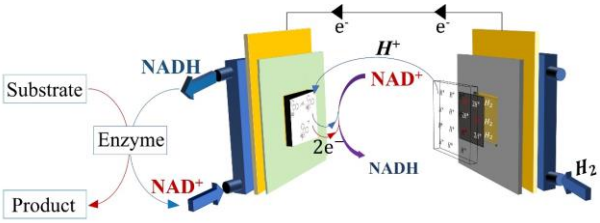
[1] Oliveira, A. R. et al, *ACS Catalysis*, 2020, **10**, 3844-3856
[2] Jeoung, J.-H. & Dobbek, H. *Science*, 2007, **318**, 1461-1464
[3] Benvenuti, M. et al, *Biochim Biophys Acta Bioenerg*, 2020, **1861**, 148188
[4] Fourmond, V. et al. *J. Am. Chem. Soc.*, 2019, **141**, 11269-11285
[5] Fourmond, V. & Léger, C. *Adv. Biochem. Eng. Biotechnol.*, 2016, **158**, 1-41
[6] Meneghello, M. et al. *Angewandte Chemie International Edition*, 2021

S4-O-05

Efficient Electrochemical Regeneration of the NAD(P)H cofactor

Wassim El Housseini[a,b,c], François Lapicque[b], Alain Walcarius[a], Neus Vilà[a], Elisabeth Lojou[c], Mathieu Etienne[a*]
[a] Laboratoire de Chimie Physique et Microbiologie pour les Matériaux et l'Environnement (LCPME), Villers-lès-Nancy, France
[b] Laboratoire Réactions et Génie des Procédés (LRGP), Nancy, France
[c] Bioénergétique et Ingénierie des Protéines (BIP), Marseille, France
wassim.el-housseini@univ-lorraine.fr; *mathieu.etienne@univ-lorraine.fr

The NAD(P)H cofactor plays an important role in biological systems and can be applied as an electron carrier in enzymatic systems catalyzing stereo- and regio-selective reactions having a great biotechnological potential. Due to its high cost, the usage of the NAD(P)H cofactor in biosynthetic applications requires its regeneration. Many regeneration techniques have been developed such as chemical, biological, enzymatic and electrochemical regeneration techniques. We are interested in the electrochemical regeneration which offers a high control of the process and an easy separation of products. Rhodium complex, acting as an electrochemical mediator, has proved its efficient activity towards the electrochemical regeneration of the NADH cofactor. [1,2]



Scheme 1. Functional scheme of the biosynthetic flow bioreactor

In this work, we combined in a single system the electrochemical regeneration of NADH mediated by a rhodium complex in the cathodic compartment with the hydrogen oxidation at the anodic compartment. H_2 oxidation proceeds on a gas diffusion electrode and a Nafion membrane between the anodic and the cathodic compartments allows transfer of H^+ from the anodic to the cathodic compartment for NAD^+ to be reduced. This study was initiated with the rhodium complex in solution. The proposed process was optimized by evaluating the effect of various factors such as hydrogen and solution flow rates, NAD^+ concentration, and rhodium complex amount.[3] In order to test the efficiency of the system in a biosynthetic application, the NADH dependent bioconversion of pyruvate to lactate by lactate dehydrogenase was studied. Later, the rhodium complex was immobilized chemically on a carbon paper covered with multi-walled carbon nanotubes according to a two-step protocol described by us recently.[1,4] The optimal reactor displayed constant activity for more than 7 days and the overall system presented TTNs more than 24000, 2500 and $1.8*10^5$ for the rhodium complex, the NAD^+ cofactor, and the enzymatic reaction respectively, which represent one of the best reported performances to date for bioelectrochemical reactors.[5]

[1] Ruppert, R., Herrmann, S., Steckhan, E. (1987). Efficient indirect electrochemical in-situ regeneration of NADH: electrochemically driven enzymatic reduction of pyruvate catalyzed by d-ldh. *Tetrahedron letters*, *28*(52), 6583-6586.
[2] Zhang, L., Etienne, M., Vilà, N., Le, T. X. H., Kohring, G. W., Walcarius, A. (2018). Electrocatalytic Biosynthesis using a Bucky Paper Functionalized by [Cp* Rh (bpy) Cl]+ and a Renewable Enzymatic Layer. *ChemCatChem*, *10*(18), 4067-4073.
[3] El Housseini, W. , Lapicque, F. , Walcarius, A., & Etienne, M. A hybrid electrochemical flow reactor to couple H_2 oxidation to NADH regeneration for biochemical reactions, *Under review.*
[4] Le, T. X. H., Etienne, M., Lapicque, F., Hehn, A., Vilà, N., Walcarius, A. (2020). Local removal of oxygen for NAD (P)+ detection in aerated solutions. *Electrochim Acta*, *353*, 136546.
[5] Cheng, B., Wan, L., Armstrong, F. A. (2020). Progress in Scaling up and Streamlining a Nanoconfined, Enzyme-Catalyzed Electrochemical Nicotinamide Recycling System for Biocatalytic Synthesis. *ChemElectroChem*,7(22), 4672

S4-O-06

Reversible Catalysis for H_2 Oxidation and Evolution by a [FeFe] Hydrogenase in a Viologen Modified Film[1]

Steffen Hardt, Stefanie Stapf, Dawit Tedros Filmon, James A. Birrell, Olaf Rüdiger, Vincent Fourmond, Christophe Léger, Nicolas Plumeré

Center for Electrochemical Sciences (CES), Faculty of Chemistry and Biochemistry, Ruhr University Bochum, Universitätsstr. 150, D-44780 Bochum, Germany.
steffen.hardt@rub.de

The most energy efficient electrocatalysts function reversibly delivering significant catalytic current densities at a minimal overpotential. Redox-active films are capable of embedding and stabilising redox enzymes. However, mediated electron transfer through the film typically results in loss of the intrinsic catalytic reversibility of the enzyme, as shown by the unidirectional catalytic current observed for such catalytic films. Here, we describe a redox-active, low-potential, 2,2´-viologen modified hydrogel, which exhibits reversible hydrogen conversion, when [FeFe] hydrogenase is embedded. Electrodes functionalised with the catalytic film were applied as anodes in an H_2/O_2 biofuel cell (BFC). Connected to a bilirubin oxidase cathode, the BFCs revealed an open circuit voltage of 1.16 V, a value close to the thermodynamic limit of 1.23 V, which sets a benchmark for a system relying on a catalytic film. The same film acted as a highly efficient H_2 evolving cathode. The catalytic properties were explained by a kinetic model, which shows that reversibility can be achieved under conditions of both fast and slow electron transfer. These films, therefore, combine the high efficiency usually exclusively observed for hydrogenase under conditions of direct electron transfer with the stabilising properties of redox active films; an achievement that may advance the implementation of highly active bio- and molecular catalysts in energy conversion.

(1) Hardt, Stapf, Filmon, Birrell, Rüdiger, Fourmond, Léger, Plumeré, *Nature Catalysis*, **2021**, *accepted* 10.1038/s41929-021-00586-1

S4-O-07

Bio Photo Voltaic (BPV): From Fundamental Principles to Practical Applications

Paolo Bombelli[1,2], Christopher J. Howe[2]

[1]*Dipartimento di Scienze e Politiche Ambientali, Università degli Studi di Milano, Italy*
[2]*Department of Biochemistry, University of Cambridge,*
Tennis Court Road, Cambridge CB2 1QW, UK

Photosynthetic (micro)organisms are capable to generate electrons that can be harvested by a suitable electrochemical setup and be used as a source of electrical current. This concept forms the basis of Bio Photo Voltaic (BPV)[1,2]. The electrical output obtained from these photosynthetic driven bio electrochemical systems has improved considerably over the last few years, with the maximum reported being in the region of ca.4A m-2 for the systems operated with cyanobacteria cells [3].
A number of aspects have been considered for enhancing the electrical output and make the BPV systems suitable for actual applications. These include the availability of electrons from the organisms involved, the transfer of electrons outside the cellular body and interface to the electrode, and the nature of the materials used to build the electrochemical setup.
With the aim to focus on possible areas of application I will present ongoing projects where BPV systems constitute a useful source of electricity in off-grid and remote locations. I will discuss, for example the use of BPV systems to power small electronic devices for sensing and transmitting environmental data. In addition, I will also promote the idea to use of BPVs as educational toolkit for disseminating knowledge related with energy, photosynthesis, bio-electrochemistry and sustainability in schools.

[1] Energy Environ. Sci., 2015, 8: 1092-1109.
[2] Joule, 2020, 4 (10), 2065-2069.
[3] Nature Energy, 2018, 3: 75–81.

S4-O-08

The Cell-electrode Interface in Cyanobacterial Exoelectrogenesis

Laura Wey, Jenny Zhang, Christopher Howe
Department of Biochemistry, University of Cambridge
Hopkins Building, 8 Tennis Court Road, Cambridge CB2 1QW
ltw28@cam.ac.uk

Photosynthetic micro-organisms can export electrons outside their cells, a phenomenon called exoelectrogenesis, which can be harnessed in solar energy conversion. However, the biological mechanisms of exoelectrogenesis, especially the route electrons take from the thylakoid membranes to the cell exterior across the many outer layers of the cell, are not understood (**Fig. 1A**). The complex cell-electrode interface also needs to be better understood, and then taken into consideration to compare the photocurrent outputs different biological samples wired to electrodes. Here, we used photoelectrochemistry (**Fig. 1B**) to measure analytically exoelectrogenesis of mutants of the model cyanobacteria *Synechocystis sp*. PCC6803 with different outer layers and extracellular features. We reveal the exopolysaccharide matrix and type IV pili have no role in electron transfer but contribute significantly to cell-electrode adherence. In addition, we reveal that the periplasmic space contributes significantly to the photocurrent profile complexity of whole cells. This also establishes that analytical photoelectrochemistry and molecular microbiology provide a powerful combination to study exoelectrogenesis.

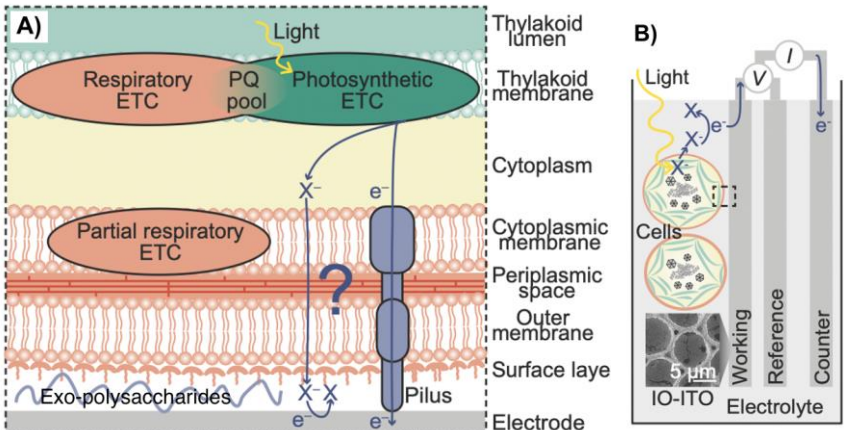


Figure 1 A) Schematic of the cell-electrode interface showing cyanobacterial cell topology and the cell-electrode interface. **B**) Schematic of the photoelectrochemical set-up and scanning electron micrograph of an inverse-opal

S4-O-09

Photosystem I Integrated in 3D Structured Reduced Graphene Oxide for Scalable Biophotovoltaics

Sascha Morlock[a,b], Senthil Kumar Subramanian[b], Athina Zouni[b], Fred Lisdat[a]
[a] *Biosystems Technology, Institute of Life Sciences and Biomedical Technologies, Technical University Wildau, Hochschulring 1, 15745 Wildau, Germany*
[b] *Biophysiks of Photosynthesis, Institute for Biology, Humboldt University of Berlin, Phillippstraße 13, 10115 Berlin, Germany*
sascha.morlock@th-wildau.de

Bioenergy is a field which includes many novel bio-hybrid approaches such as biophotovoltaics. Here, artificial electrode materials are interconnected to photoactive proteins, thylakoid membranes, or whole cells to convert light into electrical current and special chemicals. In the present study a three-dimensional electrode structure made of reduced graphene oxide (rGO) has been combined with photosystem I (PSI) to fabricate a photobioelectrode.[1] Only for PSII a similar system has been described in literture.[2]
A template-based spin-coating process is applied to build the 3D structure. Thereto, a mixture of graphene oxide (GO) and latex beads (LB) is dropped on a rotating glassy carbon electrode (GCE). After removal of the LB, GO is reduced by hydrazine. Cyclic voltammetry, UV/VIS spectroscopy and scanning electron microscopy are used to characterize the basic electrode. Next, a biophotovoltaic is manufactured by immobilizing PSI within the 3D structure. The assembly of the protein results in a direct electron transfer (DET) from the artificial surface to the reaction center of PSI. The photocurrent can be enhanced more than tenfold by the addition of the small redox protein cytochrome *c* (cyt *c*) as mediator. The biophoto-voltaic system is characterized with various (photo)electrochemical methods such as cyclic voltammetry, chopped light voltammetry, and photo action spectroscopy.
By varying the number of spin-coating steps the thickness of the electrode material can be adjusted. As the structure remains semi-transparent, scalability of the biophotovoltaic is feasible and has been studied up to thicknesses of 19 µm. For the electron transfer via cyt *c* towards PSI only a low overpotential is applied as the cathodic photocurrent already starts at positive potentials (vs Ag/AgCl). Rather high efficiencies have been observed at low light intensities.

References

(1) Morlock, S.; Subramanian, S. K.; Zouni, A.; Lisdat, F. *ACS Appl. Mater. Interfaces* **2021**, published. DOI: 10.1021/acsami.1c01142.
(2) Fang, X.; Sokol, K. P.; Heidary, N.; Kandiel, T. A.; Zhang, J. Z.; Reisner, E. *Nano Letters* **2019**, *19* (3), 1844–1850. DOI: 10.1021/acs.nanolett.8b04935.

S4-O-10

Evaluation of Biocathode Materials for CO_2 Bioelectroconversion in Microbial Electrosynthesis Cells

Abraham Gomez Vidales[1], Hongbo Li[1], Sasha Omanovic[2], Boris Tartakovsky[1]
[1]*National Research Council of Canada, Montreal, QC, H4P 2R2, Canada*
[2]*McGill University, Department of Chemical Engineering, Montréal, QC, H3A 0C5, Canada*
e-mail address: abraham.gomezvidales@nrc-cnrc.gc.ca

Bioelectrochemical conversion of carbon dioxide (CO_2) to methane (CH_4) and C2-C4 compounds is significantly limited by cathodic (bio)reactions. Also, cathode materials represent a significant part of the system cost, thus attracting significant research efforts. This study examined and compared three different carbon-based cathode materials: carbon felt (CF), granular activated carbon (GAC), and conductive polymer (CP) in terms of CH_4 and acetate electrosynthesis efficiency, energy efficiency, and energy consumption.

The cathode materials were tested in microbial electrosynthesis cell (MESC) with a 500 mL cathode compartment. In each test, the cathode compartment was filled with one of the carbon-based materials. The anode compartment housed a Ti/IrO_2 mesh (DSA anode). The MESC used a proton exchange membraneless design with the compartments separated by two layers of Nylon cloth with a total interface area of 96 cm^2. In each test, the cathode compartment was inoculated with 20 mL of anaerobic sludge, then maintained at a constant temperature of 30°C and continuously supplied with CO_2 and a solution of nutrients.

At an applied voltage of 2.8 V, the MESC with a CF cathode showed the highest current (310 mA) and CH_4 production rate (750 mL d^{-1}). This MESC also had the highest Coulombic efficiency (CE) of 92%. The GAC cathode and CP cathode MESCs showed 84% and 57% CE, respectively. The higher efficiency of the CF cathode was attributed to its large surface area and porous structure, which allowed the formation of attached microorganisms (biofilm) responsible for catalyzing the bioelectrochemical CH_4 formation. Electrochemical techniques, such as cyclic voltammetry (CV), and electrochemical impedance spectroscopy (EIS) showed that the biofilm on the CF cathode had higher density and catalytic activity towards CO_2 conversion than that one on the GAC and CP cathodes. These results provide a good reference point for developing alternative carbon-based materials for CO_2 electromethanogenesis.

S4-O-11

A Bioinspired Organic Microbattery Using a Self-Gelling Anthraquinone Derivative

Thiago Bertaglia[1], Graziela C. Sedenho[1], Emily F. Kerr[2], Roy G. Gordon[2,3], Michael J. Aziz[3], Frank N. Crespilho[1]
[1]*São Carlos Institute of Chemistry, University of São Paulo, Avenida Trabalhador São-Carlense, 400, CEP 13566-590 - São Carlos – SP - Brasil*
[2]*Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, Massachusetts 02138, USA*
[3]*Harvard School of Engineering and Applied Sciences, 29 Oxford Street, Cambridge, Massachusetts 02138, USA*
thiago.bertaglia@usp.br

Organic and organometallic molecules, like BTMAP-Vi and BTMAP-Fc, were recently applied in the development of a redox flow battery[1] (RFB) and further in a semi-solid microbattery[2], which demonstrate resemblance between both technologies. As anthraquinones are one of the most promising active molecules in organic-based RFB, it seems natural that their use can go towards microbatteries. Thus, this work aims to build up a bioinspired organic microbattery (BOM) using an anthraquinone derivative (AD) with self-gelling property. Cyclic voltammetry measurements in solution showed that the electrochemical reaction half-wave potential of the AD was -0.66 V vs Ag/AgCl/KClsat, which makes it suitable to application as anodes in batteries. Also, linear sweep voltammetry with rotating disk electrode was performed and the diffusion coefficient of the molecule calculated as 3.04×10^{-6} cm^2 s^{-1}, which is similar to other ADs used in energy storage applications[3]. Finally, to build up the BOM, the AD gel was coupled with potassium ferrocyanide incorporated in xanthan gum hydrogel. The results show that the BOM built up was suitable to power devices with small size and low power consumption, like healthcare electronic devices.

References

(1) Beh, E. S.; De Porcellinis, D.; Gracia, R. L.; Xia, K. T.; Gordon, R. G.; Aziz, M. J. *ACS Energy Lett.* **2017**, *2* (3), 639–644.
(2) Crespilho, F. N.; Sedenho, G. C.; De Porcellinis, D.; Kerr, E.; Granados-Focil, S.; Gordon, R. G.; Aziz, M. J. *J. Mater. Chem. A* **2019**, *7* (43), 24784–24787.
(3) Wu, M.; Jing, Y.; Wong, A. A.; Fell, E. M.; Jin, S.; Tang, Z.; Gordon, R. G.; Aziz, M. J. *Chem* **2020**, *6* (6), 1432–1442.

S4-O-12

Multi-Enzyme Anode based Biosupercapacitor Operating in Sucrose Solution

Krzysztof Stolarczyk, Michał Kizling, Maciej Dzwonek, Anna Nowak, Łukasz Tymecki, Agnieszka Więckowska, Renata Bilewicz

Faculty of Chemistry, University of Warsaw, Pasteura 1 Str., 02-093 Warsaw, Poland

Enzymatic fuel cells may become more adapted for applications powering low-power portable devices by broadening the range of usable fuels and storing the gathered charge during periods when no high power is required [1-3]. In this work we demonstrate the operation of an integrated, yet versatile multi-substrate biosupercapacitor utilising glucose, fructose, sucrose or a combination of them as the anode fuel and molecular oxygen as the oxidant at the cathode. To achieve this goal, we designed an enzymatic cascade-functionalised anode consisting of invertase, mutarotase, FAD-dependent glucose dehydrogenase and fructose dehydrogenase. The latter was deposited on a cellulose/polypyrrole composite also containing naphthoquinone-functionalised gold nanoparticles as mediators. The anode was then conjugated to a compatible enzymatic cathode employing laccase, adsorbed on the same type of naphthoquinone derivatised gold nanoparticles and placed in a solution of sucrose. Interestingly, the naphthoquinone derivatised gold nanoparticles act in the device merely as enzyme orienting units (and not as mediator) to catalyze oxygen reduction according to the direct electron transfer mechanism. The improved parameters of the constructed device, achieved by combining good catalytic and capacitive properties include power 0.91 mW cm^{-2} and specific capacitance 1.8 F cm^{-2}at a discharge current density of 1 mA cm^{-2}.

References

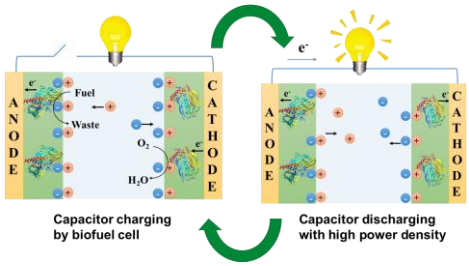
1. M. Kizling, M. Dzwonek, A. Nowak, Ł. Tymecki, K. Stolarczyk, A. Więckowska, R. Bilewicz, „Multi-substrate biofuel cell utilizing glucose, fructose and sucrose as the anode fuels", Nanomaterials. 10(8) (2020) 1534,
2. M. Kizling, K. Stolarczyk, P. Tammela, Z. Wang, L. Nyholm, J. Golimowski, R. Bilewicz, „Bioelectrodes based on pseudocapacitive cellulose/polypyrrole composite improve performance of biofuel cell", Bioelectrochemistry 112 (2016) 184 – 190,
3. M. Kizling, K. Stolarczyk, J. Sim Sin Kiat, P. Tammela, Z. Wang, L. Nyholm, R. Bilewicz, „Pseudocapacitive polypyrrole–nanocellulose composite for sugar-air enzymatic fuel cells", Electrochemistry Communications 50 (2015) 55 – 59.

S4-O-13

Revisit Mediated Bioelectrodes: How Open-circuit Potential is Determined by Enzymatic Kinetics?

Xinxin Xiao
Department of Chemistry, Technical University of Denmark, Kongens Lyngby 2800, Denmark; E-mail address: xixiao@kemi.dtu.dk

Enzymatic biofuel cell (EBFC)/supercapacitor hybrid devices, or so-called "biosupercapacitors", are gaining attention recently (**Scheme 1**) [1-6]. Biosupercapacitor allows to generate much higher power density compared to the power output from the EBFC itself. Now, it's important to have a close look on the mechanism and kinetics underling the spontaneous open-circuit potential (OCP) reset of bioelectrodes. Mediated bioelectrodes undergoing mediated electron transfer (MET) between the electrode surface and enzymes are typically the scenario for biosupercapacitors.
This report attempts to understand how the OCP of a mediated bioelectrode is determined by enzymatic kinetics. Thin film bioelectrode using osmium modified redox polymer is prepared and studied. Mathematic descriptions are derived to describe the relationship between OCP and enzymatic kinetics.



Scheme 1. Working principle of a biosupercapacitor.

References

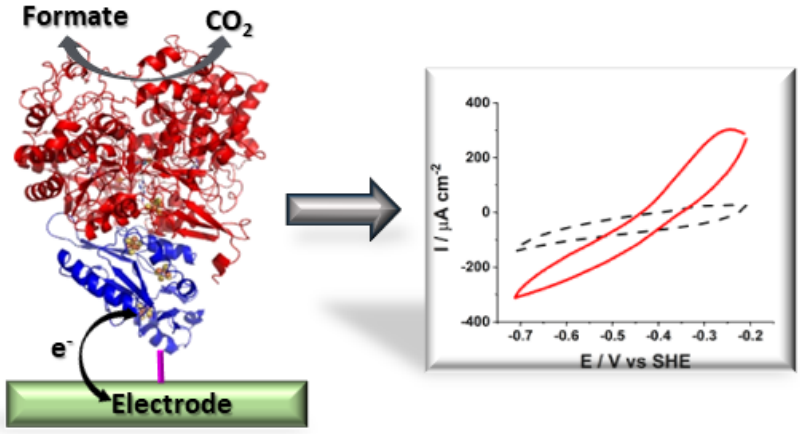
[1] D. Pankratov, Z. Blum, D.B. Suyatin, V.O. Popov, S. Shleev, ChemElectroChem, 1 (2014) 343-346.
[2] C. Agnes, M. Holzinger, A. Le Goff, B. Reuillard, K. Elouarzaki, S. Tingry, S. Cosnier, Energy Environ. Sci., 7 (2014) 1884-1888.
[3] D. Pankratov, F. Conzuelo, P. Pinyou, S. Alsaoub, W. Schuhmann, S. Shleev, Angew. Chem. Int. Ed., 55 (2016) 15434-15438.
[4] M. Kizling, S. Draminska, K. Stolarczyk, P. Tammela, Z. Wang, L. Nyholm, R. Bilewicz, Bioelectrochem., 106, Part A (2015) 34-40.
[5] X. Xiao, P.Ó. Conghaile, D. Leech, R. Ludwig, E. Magner, Biosens. Bioelectron., 90 (2017) 96-102.
[6] K.L. Knoche, D.P. Hickey, R.D. Milton, C.L. Curchoe, S.D. Minteer, ACS Energy Lett., (2016) 380-385.

S4-O-14

Bioelectrocatalytic Activity of W-Formate Dehydrogenase Covalently Immobilized on Functionalized Gold and Graphite Electrodes

Marcos Pita[a], Julia Alvarez-Malmagro[a], Ana R. Oliveira[b], Cristina Gutiérrez-Sánchez[a], Beatriz Villajos[a], Inês A.C. Pereira[b], Marisela Vélez[a], Antonio L. De Lacey[a]
[a]*Instituto de Catálisis y Petroleoquímica, CSIC, c/Marie Curie 2, L10. 28049 Madrid, Spain.*
[b]*Instituto de Tecnologia Química e Biologica, Universidade Nova de Lisboa, Apartado 127, 2781-901 Oeiras, Portugal.*
marcospita@icp.csic.es

Decrease of greenhouse gases like CO_2 has become a key challenge for human kind and new approaches need to be explored, such as electrocatalytic reduction of CO_2 to formate using biocatalysts. In this work we study the covalent bonding of Desulfovibrio vulgaris Hildenborough FdhAB formate dehydrogenase to chemically modified gold and low-density graphite electrodes, using electrostatic interactions for favoring oriented immobilization of the enzyme. Electrochemical measurements show both bioelectrocatalytic oxidation of formate and reduction of CO_2. Atomic force microscopy and quartz crystal microbalance characterization, as well as comparison of direct and mediated electrocatalysis, suggest that a compact layer of formate dehydrogenase was anchored to the electrode surface with some crosslinked aggregates. Furthermore, operational stability for CO_2 electroreduction to formate by direct electron transfer is shown with 100% faradic yield.



S5 Microbial Films and Biocorrosion

S5-O-01

How Comparable are Microbial Electrochemical Systems around the Globe? An Electrochemical and Microbiological Cross-laboratory Study

*Carlo Santoro[1], Kateryna Artyushkova[2], Plamen Atanassov[3], Sofia Babanova[4], Alain Bergel[5], Orianna Bretschger[4], Robert K. Brown[6], Kayla Carpenter[7], Alessandra Colombo[8], Rachel Cortese[7], Pierangela Cristiani[9], Benjamin Erable[5], Falk Harnisch[10], Mounika Kodali[3], Sujal Phadke[7], Sebastian Riedl[6], Luis F. M. Rosa[10], Uwe Schröder[6].

* *carlo.santoro@unimib.it*

[1] *University of Milano-Bicocca, Dept. of Material Science, Via Cozzi, Milan, Italy*

[2] *Physical Electronics, 18725 Lake Dr. East, Chanhassen, Minnesota 55317, USA*

[3] *Dept. of Chemical & Biomolecular Engineering, Univ. of California, Irvine, USA*

[4] *Aquacycl LLC,2180 Chablis Court, Suite 102, Escondido, CA 92029, USA*

[5] *Lab. de Génie Chimique, Univ. Toulouse, CNRS–INPT–UPS, Toulouse, France*

[6] *Institute of Environmental & Sustainable Chemistry, TU Braunschweig, Germany*

[7] *J. Craig Venter Institute, 4120 Capricorn Lane, La Jolla, CA 92037, USA*

[8] *Department of Chemistry, Università degli Studi di Milano, Milan, Italy*

[9] *Ricerca sul Sistema Energetico S.p.A. Milan, Italy*

[10] *Department of Environmental Microbiology, Helmholtz-Centre for Environmental Research – UFZ, Leipzig, Germany*

A cross-laboratory study using Microbial Fuel Cells (MFC) is presented in this work. Five different institutions were involved in this work: 1) Univ. of New Mexico (UNM); (2) Ricerca sul Sistema Energetico (RSE), Milan; (3) CNRS, Univ. de Toulouse (CNR); (4) Technische Universität Braunschweig (TUB); (5) Helmholtz-Centre for Environmental Research (UFZ), Leipzig. Identical MFCs were operated using local domestic wastewater as inoculum source and i) the performance was assessed, and ii) the initial bacterial pools and the biofilm formed on the electrodes were identified. The overall study run for 6 weeks. The inoculum affected importantly the startup phase. Notably, all the MFCs (except one case, CNR) reached similar maximum power outputs and COD removal efficiencies, despite the large diversity found in their bacterial pool. The syntrophic interaction of fermentative and electroactive bacteria at anode lead to the similar performance of the MFCs. Different bacterial groups compose the microbial consortia. However, they share similar functions both on anode and cathode of the different MFCs. Particularly, *Clostridiales, Bacteroidales, Synergistales, Lactobacillales* and *Desulfovibrionales* are the key bacteria orders found in MFCs of each institution. Faster energy recovery was positively associated with the abundance of *Desulfovibrionales* or *Lactobacillales* at the anode, and higher COD removal rate was clustered with bacteria such as Clostridiales, Bacteriodales and Synergistrales.

S5-O-02

Sticky Like a Mussel: Polydopamine for Purple Bacteria Biohybrid Photoanodes

Matteo Grattieri[1,2], Gabriella Buscemi[1,2], Rossella Labarile[1,2], Pinalysa Cosma[1,2], Angela Agostiano[1,2], Massimo Trotta[2], Gianluca M. Farinola[1]
[1] *Dipartimento di Chimica, Università degli Studi di Bari "Aldo Moro" via E. Orabona 4, Bari, 70125, Italy*
[2] *IPCF-CNR Istituto per i Processi Chimico Fisici, Consiglio Nazionale delle Ricerche, via E. Orabona 4, Bari, 70125, Italy*
matteo.grattieri@uniba.it

Interfacing intact photosynthetic organisms and abiotic electrodes enables converting solar energy into electrical (and/or chemical) energy while providing the enzymatic machinery for self-repair and replication of the biocatalysts. Among various photosynthetic organisms, purple bacteria are anoxygenic microorganisms with an extremely versatile metabolism that utilize sunlight to oxidize a broad variety of organic compounds. Thanks to this versatile metabolism, the interest in applying purple bacteria in biohybrid systems performing semi-artificial photosynthesis has recently grown.[1, 2] However, various challenges must be overcome prior to implement the technology in the field, spanning from enhancing photoexcited electron transfer from the biocatalyst to the electrode, to maximizing stability of the biohybrid architecture.

In this context, we report a bio-inspired approach to achieve a firmly anchored layer of purple bacteria on the electrode surface while providing exogenous redox mediators to enhance photoexcited electrons harvesting. Polydopamine (PDA), containing both catechol and amine groups, is a polymer inspired by the adhesive plaque of mussel byssus, which has been recently utilized to encapsulate isolated photosynthetic apparatus.[3] Here, the biohybrid photoanode was obtained by a one-step immobilization of intact purple bacteria, PDA, and quinone-based redox mediators. Electrochemical and spectroscopic evidence for the obtained biohybrid photoanodes will be discussed together with future research possibilities.

References

1. M. Grattieri, *Photochem. Photobiol. Sci.*, 2020, 19, 424-435.
2. M. Grattieri et al., *Chem. Commun.*, 2020, 56, 8553-8568.
3. M. Lo Presti et al., *Advanced Electronic Materials*, 2020, 6, 2000140.

S5-O-03

Unravelling Performance Limitations of Microbial Anodes: A Real-time Microscale Approach

Lucila Martinez Ostormujof, Sébastien Teychené, Benjamin Erable
Laboratoire de Génie Chimique, Université de Toulouse,
4 allée Emile Monso – CS 44362 – 31030 Toulouse Cedex 4, France
lucila.marinezostormujof@toulouse-inp.fr

Multi-species electroactive biofilms (EAB) are capable of oxidizing a wide spectrum of organic wastes, allowing the processing of complex organic substances in the bioanode of a bioelectrochemical system (BES). However, no BES has yet reached the industrialization stage until today because the stability of operation over the long term is not robust enough. The loss of electrochemical activity of multi-species EAB on bioanodes is seen to act as the main barrier to overcome in the short-term sustainability of BES. Current density of multi-species EAB in anodes classically reaches a maximum value (J_{max}) that drops beyond a few dozen days of operation [1].

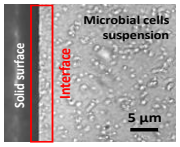


Figure 1: microBES

The new challenge of understanding the origin of the decrease in electrochemical activity of EAB is therefore of prime importance. Identifying what happens at the frontier between the anode and the biofilm, during the colonisation of the electrodes, might provide novel explanations. To this end, we combined the virtues of microfluidics, electroanalysis, real-time microscopy and imaging as to focus the exploration at the µm scale on the electrode-EAB interface. Thus, access to local phenomena allows us to understand and elucidate the cell-cell and cell-electrode interactions, in addition to the coupled bioelectrochemical processes occurring within the EAB structure. To scale down BES experiments, developing and validating the operation of a microfluidic BES was the first stage of our work. This consisted of two consecutive steps: *i) Downsizing of the anode:* We reproduced the classical behaviour of biofilms as observed for large-scaled microbial electrodes in stainless steel (SS) and platinum (Pt) microelectrodes (Ø=50µm). SS excelled Pt in terms of repeatability and reproducibility. J_{max} was of 10.3 A/m^2 and 11.3 A/m^2 for salt marsh and garden compost biofilms respectively when formed under polarization for a minimum time of 30 days. *ii) Downsizing of the complete BES:* A transparent OSTEMER microfluidic BES was developed using ITO conductive glass and SS microwire as electrodes.

This design permits simultaneous SS microwire potential polarization, flow control and real-time observation with a transmission optical microscope. Actual work lies in transferring the experience into the microfluidic BES. Short-term experiments focus in pioneer bacterial adhesion to the electrode. Longer experiments help us to understand biofilm development dynamics, by analyzing its evolution over time and if the mass transfer of substrates or reaction products have changed inside it.

[1] Chong et al. *Int. J. Hydrogen Energy*, 44(2019), 4484-4495.

S5-O-04

Capacitive Bioanodes in Moving Bed Reactors

Annemiek Ter Heijne, Casper Borsje, Tom Sleutels, Cees Buisman
Environmental Technology, Wageningen University & Research,
Bornse Weilanden 9, 6708 WG Wageningen, The Netherlands
annemiek.terheijne@wur.nl

Capacitive bioanodes can be used to improve current production in microbial electrochemical systems for the recovery of energy from wastewater. Here, we studied the use of activated carbon granules as capacitive anodes in moving bed reactors. We investigated the effect of operational parameters on the charging and discharging performance of the granules, and compared bioanodes in moving bed reactors to those in fixed bed reactors. For capacitive bioanodes in a fixed bed reactor, we observed that charge recovery was dependent on the position of the current collector, and most capacitive charge was recovered from the current collector closest to the membrane. Increasing bed thickness from 5 mm to 10 mm resulted in a 1.6 times higher current density per membrane area. These findings were used to improve the design of a moving bed reactor, where activated carbon granules with electro-active biofilm moved through a discharge cell and were recirculated using a gas lift. The moving bed bioanode produced a current of 43 A/m^2.The relatively short discharge time and long charging time of the moving bed as compared to the fixed bed bioanodes led to higher capacitive currents.
Properties of the moving bed reactor were further studied in abiotic experiments. The capacitive discharging was most improved by changes in potential difference between the current collector and charged granules (ΔE 0.3 and 0.5 V). Increasing the bulk electrolyte conductivity also increased the transferred charge, which could originate from the increased capacitance, as measured in a separate setup. Discharging from both sides of the granular bed, as compared to discharging from one side, reduced the maximum distance to the current collector, which increased the transferred charge, irrespective of an increase in bulk electrolyte conductivity. This showed the electrical resistance was more important in determining the transferred charge than the ionic resistance. Further analysis of the discharging process showed that discharging increased the local conductivity through the release of ions from the granules. This offers opportunities for the treatment of low conductivity wastewaters. These results provide new insights to further improve capacitive bioanodes.

S5-O-05

Gram-positive Bacteria Covered Bioanode in a Membrane Electrode Assembly of Bioelectrochemical Systems

Yolina Hubenova[1, 2], Galin Borisov[1], Evelina Slavcheva[1], Mario Mitov[3]
[1]*Institute of Electrochemistry and Energy Systems "Acad. E. Budevski" – Bulgarian Academy of Sciences, Sofia, Bulgaria*
jolinahubenova@yahoo.com
[2]*Plovdiv University "Paisii Hilendarski", Plovdiv, Bulgaria*
[3]*Innovative Center for Eco Energy Technologies, South-West University "Neofit Rilski", Blagoevgrad, Bulgaria*

Electroactive biofilms of Gram-positive bacteria *Paenibacillus dendritiformi*s [1] were prepared on a graphitized paper at applied potential –0.195 V (vs.SHE). After proving the biofilm formation by DPV, its charge transfer capabilities and redox activity were evaluated by EIS and CV. Besides, a suitable cathode and a separator were selected for preparation of a membrane electrode assembly (MEA) after characterization in a neutral buffer solution. The biofilm-coated anode was assembled with Zifron membrane and ETEK as a cathode and built-in within the fuel cell. For the first time, *P. dendritiformis* is examined as a biocatalyst in a real microbial fuel cell. Under limited air access to the cathode, the maximum current density reached ca. 60 mA m^{-2}. This value was doubled by supplying the cathode with pure oxygen and 4 times higher with air. The maximum power of the latter reached 35 mW m^{-2}, which was 3 times higher than those obtained by the non-purged or purged with pure oxygen fuel cells. The maximal current density was 200 mA m^{-2}, which is a high prognostic value that can be achieved with Gram-positive bacteria. The newly developed bioelectrochemical cell was also tested in a microbial electrolysis mode after the catholyte was deaerated by purging with nitrogen. It was found that when using the bioanodes assembled in MEA, the voltage needed to initiate hydrogen evolution on the cathode is in the range of 0.4 ÷ 0.6 V, which is significantly less than the theoretical voltage of 1.23 V required for the decomposition of water by conventional electrolysis.

Acknowledgments This study was supported by the Bulgarian National Science Fund through contract KP-06-H29/8/2018 and implemented in the integrated laboratory of ESHER (contract DO1-160/2018).

Reference

[1]Yolina Hubenova, Eleonora Hubenova, Mario Mitov (2020) Electroactivity of the Gram-positive bacterium Paenibacillus dendritiformis MA-72. Bioelectrochemistry 136, 107632 https://doi.org/10.1016/j.bioelechem.2020.107632

S6 Electron Transport in Biological Systems - Theory and Experiment

S6-O-01

Copper Activation of *Thermus thermophilus* Laccase: Which Structural Features are involved?

Elisabeth Lojou, R. Clément, X. Wang, F. Biaso, M. Ilbert, I. Mazurenko
Bioénergétique et Ingénierie des Protéines, CNRS, Aix-Marseille Université
31 chemin Joseph Aiguier, 13009 Marseille, France
lojou@imm.cnrs.fr

Laccases (LACs) belong to the multicopper oxidase (MCOs) family that couples the four-electron reduction of molecular oxygen to water with the oxidation of a wide variety of phenolic or non-phenolic molecules. *Thermus thermophilus* LAC (Tt LAC) do deserves attention not only for its biotechnological interest because of stability at high temperatures, but also for the presence of an unusual methionine (M) rich loop covering the T1 Cu site, where substrates bind in classical MCOs. Actually, Tt LAC belongs to the MCO group including the copper efflux oxidase CueO of *Escherichia coli* that are activated by addition of exogenous copper. In addition to classical T1 Cu and T2/T3 Cu sites, these enzymes are proposed to be able to fix additional labile coppers in the M rich domain allowing electron transfer from the substrate to the T1 Cu [1].
We recently performed an in-depth electrochemical study of the intra- and inter-electron transfers using the Tt-LAC immobilized on different electrodes. We showed a Cu^{2+}-related bioelectrocatalytic process proposed to be linked to the binding of additional Cu^{+} to the M-rich domain, and which induces a new electron transfer pathway to the T2/T3 trinuclear Cu active site [2]. Although the exact mechanism is not fully understood *in vivo*, we proposed that this new electron transfer pathway would correspond to a cuprous oxidase activity of Tt-LAC, an activity which is crucial for copper detoxification.
To get further insight in the involved electron transfer pathway, we tune in this work Tt-LAC catalytic properties by directed mutagenesis of both the M-rich domain and of two relevant M residues in the coordination spheres of the T1 Cu site. In addition to elucidate the role of the M-rich domain, our aim is to study the potential impact of these mutations that are expected to affect the T1 Cu redox potential on the activation of the enzyme by Cu^{2+}. Using a multidisciplinary approach combining electrochemistry, enzymology, UV-Vis, circular dichroism (CD) and electron paramagnetic resonance (EPR) spectroscopies, we will highlight that each mutation plays a key role influencing the enzymatic activity in distinct unexpected ways.

[1] Serrano-Posada H. et al., *Acta Crystallographica Section, F* **67** (2011) 1595–1598.
[2] Hitaishi V. et al., *J. Am. Chem. Soc.*, **142** (2020) 1394–1405
[3] R. Clément et al., *Biochimie*, 182 (2021) 228-237.

S6-O-02

Ligand Binding Induces a Shift in the Open-Circuit Voltage of Olfactory Receptor 1A1

Anna Lagunas[a,b], Christine Belloir[c], Loïc Briand[c], Pau Gorostiza[b,a,d], Josep Samitier[b,a,e]
[a] *Biomedical Research Networking Center in Bioengineering, Biomaterials, and Nanomedicine (CIBER-BBN), 28029 Madrid, Spain*
[b] *Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute of Science and Technology (BIST), 08028 Barcelona, Spain*
[c] *Centre des Sciences du Goût et de l'Alimentation, AgroSup Dijon, CNRS, INRAE, Université Bourgogne Franche-Comté, Dijon, France*
[d] *Catalan Institution for Research and Advanced Studies (ICREA), Barcelona 08011, Spain*
[e] *Department of Electronics and Biomedical Engineering, University of Barcelona (UB), 08028 Barcelona, Spain*
alagunas@ibecbarcelona.eu

Olfactory receptors (ORs) belong to class A rhodopsin-like family GPCRs (G-protein coupled receptors)[1], and are expressed in the cell membrane of olfactory neurons. Although there are no resolved structures for any ORs, sequence homologies have been found with opsin[2].
A characteristic of ORs is the promiscuity they show in ligand binding. Therefore, the induced fit model generally accepted for GPCRs seems inaccurate to describe odorant recognition by ORs. The possible mechanisms involved have been a matter of intense debate[3,4].
Proteins are quite efficient electronic conductors and their electron transport (ETp) characteristics have been addressed in many studies[5,6]. Here we studied ETp in human olfactory receptor 1A1 (hOR1A1) by electrochemical scanning tunneling spectroscopy (EC STS) in the presence and absence of its cognate ligand dihydrojasmone. We observed that, although no significant changes were found in the conductance of hOR1A1 obtained from current-voltage (IV) and break-junction experiments, the presence of dihydrojasmone induced a shift in the voltage of open circuit (VOC) measured in IV characteristics.

[1] Buck, L.; Axel, R. A novel multigene family may encode odorant receptors: A molecular basis for odor recognition. *Cell* **1991**, *65*, 175.
[2] Park, J. H.; Morizumi, T.; Li, Y.; Hong, J.E.; Pai, E.F.; Hofmann, K.P. *et al.* Opsin, a structural model for olfactory receptors? *Angew. Chem. Int. Ed.* **2013**, *52*, 11021.
[3] Hoehn, R.D.; Nichols, D.E.; Neven, H.; Kais, S. Status of the vibrational theory of olfaction. *Front. Phys.* **2018**, *6*, 25.
[4] Vosshall, L.B. Laying a controversial smell theory to rest. *Proc. Natl. Acad. Sci. U.S.A.* **2015**, *112*, 6525.
[5] Amdursky, N.; Marchak, D.; Sepunaru, L.; Pecht, I.; Sheves, M.; Cahen, D. Electronic transport via proteins. *Adv. Mater.* **2014**, *26*, 7142.
[6] Lidsay, S. Ubiquitous electron transport in non-electron transfer proteins. *Life (Basel)* **2020**, *10*, 72.

S6-O-03

Probing Iron-Sulfur Cluster Redox States by Incorporating an Unnatural Amino Acid

Jiaao Wei, Miguel Ramirez, Stephen Carr, Patricia Rodriguez-Macia, Rhiannon Evans, Philip Ash and Kylie Vincent*
Department of Chemistry, University of Oxford, South Parks Road, Oxford OX1 3QR, United Kingdom
jiaao.wei@chem.ox.ac.uk

Iron-sulfur clusters (FeS) are crucial redox cofactors in nature. They are involved in complex enzyme catalysis, acting as electron-relays. Techniques focusing on studying the electron loading of FeS-cluster relays, especially during enzyme catalysis, are rather scarce. Here, we describe a novel approach to determine the redox state of FeS clusters by infrared (IR) spectroscopy, exploiting the versatility of this technique across a range of sample conditions. In this work, we have incorporated the unnatural amino acid para-cyano-L-phenylalanine (*p*CNF), into the model protein spinach ferredoxin I, which contains a [2Fe-2S] cluster. We have shown that the unnatural amino can be used as infrared probe for the redox state of the [2Fe-2S] cluster. The integrity and correct functioning of the [2Fe-2S] cluster upon *p*CNF incorporation was confirmed by UV-Vis spectroscopy and electrochemistry. A small (≈2 cm^{-1}) but reproducible shift in the absorption frequency of the CN group of *p*CNF upon chemical or electrochemical reduction of the protein was observed by IR spectroscopy, showing that the pCNF can function as a probe of cluster redox state. Furthermore, crystallographic characterisation was performed to solve and compare the structures of the oxidised and reduced *p*CNF-containing ferredoxin, and showed that the IR shift does not arise from a structural change but rather from the reduction of the [2Fe-2S] cluster.
This method could be applied to more complex metalloenzymes containing FeS cluster relays such as hydrogenases, to investigate how the FeS clusters interplay with the active site to allow efficient catalysis.

S6-O-04

Electrochemical Control Over Single Hydrogenase Protein Crystals, Coupled with IR Microspectroscopy

Kylie Vincent[1], Rhiannon Evans[1], Philip Ash[2], Stephen Carr[1,3], Wangzhe Li[1]
[1]*University of Oxford, Department of Chemistry, Inorganic Chemistry Laboratory, South Parks Road, Oxford, OX1 3QR*
[2]*School of Chemistry, University of Leicester, Leicester, LE1 7RH, UK*
[3]*Research Complex at Harwell, Rutherford Appleton Laboratory, Harwell Campus, Didcot, UK*
kylie.vincent@chem.ox.ac.uk

It is often difficult to reconcile structures of redox enzymes with an emerging catalytic mechanism. Obtaining structures of metalloproteins in well-defined redox states (catalytic intermediates, substrate-bound complexes) remains a key challenge. Crystals obtained from chemically reduced or oxidised protein samples, or by exposure to substrate or product, often contain complex mixtures of redox states.

Here we report on recent work, in which we have found that it is possible to manipulate, electrochemically, single protein crystals of hydrogenase into specific redox states and to simultaneously characterise these using infrared microspectroscopy. This is allowing us to make links between spectroscopic signatures observed for hydrogenases in solution and the states which can be populated and enriched *in crystallo* under defined conditions. The crystals continue to yield high-resolution x-ray diffraction data after electrochemical manipulation, enabling us to obtain crystal structures on electrochemically-generated redox states of hydrogenase.

Of further interest, some of the chemical steps (proton transfers) following an electrochemical step on hydrogenase crystals are slowed in the solid state, thereby enabling insight into intermediates which are transient in solution, and thus are normally only captured using fast time-resolved methods.

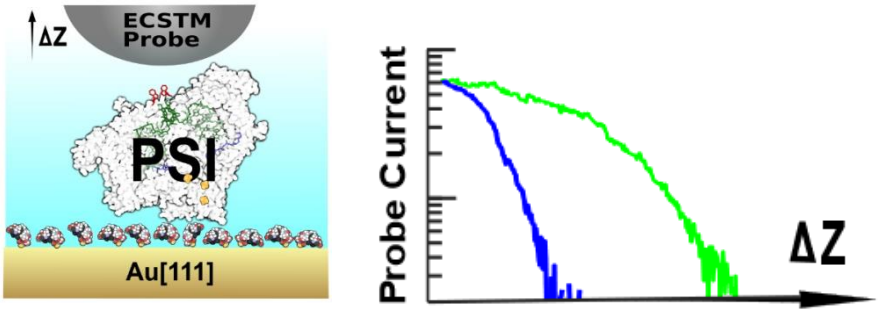
[1] Ash, P.A., et al. 'A method for generating single metalloprotein crystals in well-defined redox states: combined electrochemical control and infrared microspectroscopic imaging of a NiFe hydrogenase crystal', *Chem. Commun.*, 2017, 53, 5858-5861.

S6-O-05

Distance- and Potential-dependent Charge Exchange Through Oriented Single Photosystem I Complexes

Manuel López-Ortiz, Ricardo Zamora-Brito, Marina Inés Giannotti, Nuria Camarero, Chen Hu, Roberta Croce, Pau Gorostiza
Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute of Science and Technology, Baldiri Reixac 10–12, Barcelona 08028
Network Biomedical Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Madrid 28029
mlopezo@ibecbarcelona.eu

"Electron Transfer" (ET) is a charge exchange process driven by the difference in redox potential between donor and acceptor species, while "Electron Transport" term (ETp) has been suggested to describe the electrode biased charge exchange in protein junctions[1]. In particular, the ETp in Photosynthetic Complex I (PSI) in dark has been shown to be favored against the physiological direction of ET[2]. To address the research on this feature, we have chosen to study PSI- photo-switchable redox protein under dark conditions (non-redox active state) by Electrochemical Scanning Tunneling Microscopy (ECSTM). To this end, we have designed a peptide to unidirectionally functionalize plants PSI on atomically flat gold substrates. ECSTM probe was used to investigate the current decay distance (β, nm^{-1})[3] of single PSI complexes under electrochemical control[3]. Our results showed an enhanced charge exchange distance process between PSI and ECSTM probe, and that β values are modulated by probe potential. We suggest that enhanced charge exchange distance process is due to probe potential alignment with the redox potential of the surface exposed electron acceptor cofactor in PSI.



(1) Bostick, C. D.; Mukhopadhyay, S.; Sheves, M.; Cahen, D.; Lederman, D. Protein Bioelectronics : A Review of What We Do and Do Not Know. 1–150.

Castañeda Ocampo, O. E.; Gordiichuk, P.; Catarci, S.; Gautier, D. A.; Herrmann, A.; Chiechi, R. C. Mechanism of Orientation-Dependent Asymmetric Charge Transport in Tunneling Junctions Comprising Photosystem i. *Journal of the American Chemical Society* **2015**, *137* (26), 8419–8427.

Artés, J. M.; Díez-Pérez, I.; Sanz, F.; Gorostiza, P. Direct Measurement of Electron Transfer Distance Decay Constants of Single Redox Proteins by Electrochemical Tunneling Spectroscopy. *ACS Nano* **2011**, *5* (3), 2060–2066.

S6-O-06

Following Electroenzymatic Hydrogen Production with Rotating Ring Disk Electrochemistry and Mass Spectrometry

Jaloliddin Khushvakov,[a] Robin Nussbaum,[a] Cécile Cadoux,[a] Jifu Duan,[b]
Sven Stripp[c] and Ross Milton[a]

[a] *University of Geneva, Department of Inorganic and Analytical Chemistry, Sciences II, Quai Ernest-Ansermet 30, 1211 Geneva 4, Switzerland*
[b] *Ruhr-Universität Bochum, Faculty of Biology and Biotechnology, Photobiotechnology, Universitätsstrasse 150, 44801 Bochum, Germany*
[c] *Freie Universität Berlin, Department of Physics, Experimental Molecular Biophysics, 10623 Berlin, Germany*
ross.milton@inige.ch

Molecular hydrogen (H_2) is a global chemical commodity and future renewable fuel. Metalloenzymes are potential candidates for new hydrogen-producing biotechnologies seeking greener alternatives to the steam-reforming of natural gas (which produces the majority of industrial hydrogen). Electroenzymatic hydrogen production can be achieved by incorporating metalloenzymes into electrochemical systems, where an electrode supplies the reducing equivalents necessary for the reduction of protons (H^+).

Here, the use of rotating ring disk electrochemistry is demonstrated as an electrochemical method to follow the evolution of H_2 by electrode-confined hydrogenases.[1] An [FeFe]-hydrogenase was confined in a redox polymer at a carbon disk electrode and a platinum ring electrode (375 µm gap from the disk electrode) was used to oxidize the electroenzymatically generated H_2. A kinetic isotope effect was observed upon the introduction of deuterons (D^+) to the electrolyte, where the electrocatalytic current was diminished. Further, we demonstrate the use of online mass spectrometry to quantify the products of electroenzymatic H^+ and D^+ reduction (H_2, HD and D_2), where a simple model yields an average kinetic isotope effect of 2.

We anticipate that these techniques will be valuable to mechanistic studies of hydrogenases, in addition to other metalloenzymes that catalyze the reduction of other substrates alongside H^+ (such as nitrogenase).

[1] Khushvakov *et al.*, *Angewandte Chemie – International Edition*, **2021**, DOI: 10.1002/anie.202100863

S6-O-07

First Evidence of Electron Transfers Between Animal Cells and Electrodes

Simon Guette-Marquet[1], Christine Roques[2], Alain Bergel[3]
[1] *INPT*, [2] *UPS*, [3] *CNRS*
Laboratoire de Génie Chimique, Université de Toulouse, CNRS, INPT, UPS
4 allée Emile Monso, 31432 Toulouse, France
alain.bergel@toulouse-inp.fr

The ability of many microorganisms to transfer electrons to conductive surfaces has been extensively described for decades. In contrast, no conclusive proof has been reported regarding the capacity of mammalian cells to achieve similar electron transfer or not.
Vero cells (green monkey kidney epithelial cells) were cultivated on electrodes in a cell culture medium allowing maintenance of cell viability and cell growth (37°C/5 % CO_2). A potential of +690 mV/SHE (450 mV/SCE) was applied to electrodes during the incubation. Control data were obtained under the same conditions but without Vero cells on the electrodes.
Chronoamperometry pattern in the presence of cells are very different from those recorded in the absence of cells (Figure 1). The increase in current in the presence of cells suggests that the cells transfer electrons to the electrode. A correlation between the initial quantity of cells on the electrodes and the shape of chronoamperometries was demonstrated. Furthermore, assays using various electrolytes revealed that glutamine could be one of the sources of the electrons transferred to the electrodes. Finally, measurements in anaerobic conditions indicate that the phenomenon observed is strongly dependent of the presence of oxygen, which suggests that respiratory metabolism may be involved in the occurrence of the current.
These results are the first to highlight the possibility of electron transfers between animal cells and electrodes in chronoamperometric conditions and pave the way for promising uses of electrochemistry in cell biology.

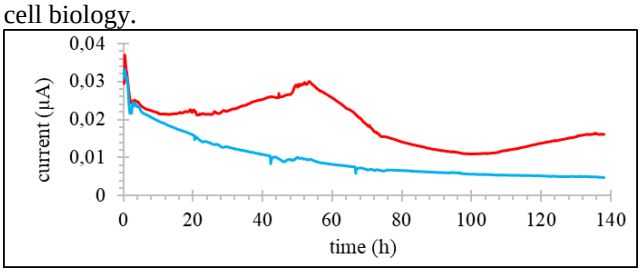


Figure 1 : Chronoamperometry at 690 mV/SHE showing electron transfer in presence of Vero cells (red curve) and control without cells (blue curve)

S6-O-08

Digging at the Catalytic Cycle of [FeFe] Hydrogenase by Single Crystal Electrochemical IR Microspectroscopy

Patricia Rodriguez-Macia[1], Jifu Duan[2], Amy Lian[1], Simone Morra[3], Martin Winkler[2], Thomas Happe[2] and Kylie Vincent[1*]

[1]*University of Oxford, Department of Chemistry, Inorganic Chemistry Laboratory, South Parks Road, Oxford, OX1 3QR, UK*

[2]*Faculty of Biology and Biotechnology, Photobiotechnology, Ruhr University Bochum, 44801 Bochum, Germany.*

[3]*Faculty of Engineering, University of Nottingham, University Park, Nottingham NG7 2RD, UK*

patricia.rodriguezmacia@chem.ox.ac.uk

[FeFe] hydrogenases are known to be the fastest H_2 converting catalysts in nature and use the earth abundant metal Fe in their active site.[1] As such, these enzymes are currently the target of multiple studies aiming to unravel their active site chemistry and translate the concepts learnt into the design of new catalysts. Although [FeFe] hydrogenases have been extensively studied by various techniques including electrochemistry, infrared (IR) spectroscopy and electron paramagnetic resonance; their catalytic mechanism still remains unclear, and there is controversy regarding some suggested catalytic intermediates.[2] It is often difficult to harmonise the information obtained by different techniques as well as to correlate the crystal structures with individual states of the catalytic cycle of these enzymes. Thus, one of the major challenges in the hydrogenase field is the unification of findings.

It has recently been demonstrated that some of the chemical steps are slower *in crystallo*, thereby enabling to trap catalytic intermediates that are too fleeting to be observed in solution.[3] In this work, single crystals of the prototypical [FeFe] hydrogenase I from *Clostridium pasteurianum* (*Cp*HydA1) have been studied by single crystal electrochemical IR microspectroscopy. In this technique, the electrochemical potential can be precisely controlled, while the redox state of the protein crystal is simultaneously probed via IR microspectroscopy, following how the vibrational bands of the intrinsic active site CO and CN^- ligands change with the applied potential. Crystals of wild-type *Cp*HydA1 and genetic variants have been investigated and compared under various defined conditions.[4] The excellent sensitivity and exquisite redox control offered by this technique have allowed the detection of new catalytic intermediates. Furthermore, this enables us to link structural and spectroscopic information to gain a holistic picture of the catalytic mechanism of these important metalloenzymes.

S6-O-09

Mathematical Modeling of Multiple Extracellular Electron Transfer Pathways

Andrew Marcus
Biodesign Swette Center for Environmental Biotechnology,
Arizona State University, Tempe, AZ, 85287
the.andrew@asu.edu

Microorganisms performing extracellular electron transfer (EET) are exposed to a wide continuum of electrochemical potentials. In response to the anode potential, *Geobacter sulfurreducens* regulates multiple EET pathways. Here we develop a model for the regulation of multiple EET pathways for two strains for *Geobacter sulfurreducens* by modifying the previously developed Nernst-Monod equation in three ways: i) an on-off switch to describe potential-dependent inhibition of EET pathway in response to a high anode potential; ii) a pathway regulation function to describe up- and down-regulation of an EET pathway in response to a shift in the anode potential; iii) a lag term in the pathway regulation function to describe the history effect of cell regulation. The model is parameterized and evaluated using electrochemical experiments of two different strains of *Geobacter sulfurreducens*. The model developed here captures essential features of regulatory of multiple EET pathways regulated by the anode potential. Multiple EET pathway model has important implications for how multiple EET pathways are regulated in different potential environments.

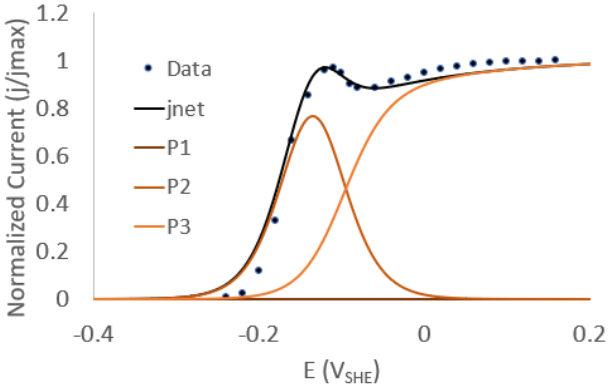


Figure. An example model ouput of a cyclic voltammogram of *Geobacter sulfurreducens*. P1-P3 are the current from each EET pathway.

Abstracts of
Poster presentations

S1 Smart Materials for Bioelectrochemistry

S1-P-01

3D-Printed Electrochemical Microwells for Quantum Dot-based Bioassays

Vassiliki Katseli[1], Michailia Angelopoulou[1], Christos Kokkinos[1]
[1] *Laboratory of Analytical Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, Athens 157 71, Greece*
christok@chem.uoa.gr

Fully integrated electrochemical microwells were manufactured entirely by 3D printing. The microwells were 3D-printed via a single-step process using a dual extruder 3D printer and were composed of a transparent miniature cell (printed from a polylactic acid (PLA) non-conductive filament) with 3 electrodes integrated on the sides of the vessel (printed from a carbon-loaded PLA filament) [1, 2].The bioanalytical usefulness of the 3D-printed microwells was demonstrated for the voltammetric determination of C-reactive protein (CRP), using quantum dots (QDs) labels [3]. A sandwich-type immunoassay was developed into the 3D-printed microwells involving immobilization of CRP capture antibody, sequential immunoreactions with CRP and biotinylated CRP reporter antibody and finally reaction with streptavidin-conjugated CdSe/ZnS QDs. The anodic stripping voltammetric determination of the Cd(II) released after acidic dissolution of the QDs labels was performed at bismuth electroplated film formed in situ on the working electrode surface during the electrolytic preconcentration step and simultaneously with the accumulation of the Cd on the working electrode surface.
Under optimal conditions, the Cd peak current was linearly related to the logarithm of the concentration of CRP with a correlation coefficient 0.996. The accuracy of the immunoassay for the determination of CRP in human serum samples was determined through recovery experiments. The % recovery values ranged from 97 to 105%, demonstrating satisfactory accuracy of the CRP determination in human serum samples.
The proposed 3D-printed electrochemical microwells provide exceptional detection sensitivity as well as fabrication simplicity. The 3D-printing fabrication procedure offers significant advantages over other manufacturing techniques in terms of equipment size and cost, manufacturing speed, simplicity of operation, design transferability and ecofriendliness. These characteristics make the 3D-printed electrochemical microwell an ideal platform for the ultrasensitive detection of important biomolecules.

References

[1] V. Katseli, A. Economou, C. Kokkinos, Talanta, **2020**, 208,120388
[2] V. Katseli, A. Economou, C. Kokkinos, Electrochem. Commun. **2019**,103,100–103
[3] C. Kokkinos, M. Prodromidis, A. Economou, P. Petrou, S. Kakabakos, Anal. Chim. Acta, **2015**, 886, 29-36

Acknowledgements

This research is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning 2014-2020» in the context of the project "Three dimensional printed microdevices for electrochemical bioassays using quantum dots as labels" (MIS 5047951).

S1-P-02

Electron Transfer in Cellobiose Dehydrogenase Multilayers on Electrodes

Kwankao Karnpakdee, Lorenz Schwaiger, Daniel Kracher, Roland Ludwig
Department of Food Science and Technology,
University of Natural Resources and Life Sciences, Vienna
Muthgasse 11, 1190 Vienna, Austria
kwankao.karnpakdee@students.boku.ac.at

Cellobiose dehydrogenase (CDH) is an extracellular oxidoreductase capable of direct electron transfer to the electrode surface (DET). This enzyme consists of two domains. A catalytic dehydrogenase domain (DH) containing a flavin adenine dinucleotide (FAD) cofactor is connected to an electron transferring b-type cytochrome domain (CYT) by a flexible linker peptide. Due to the CYT domain's capability to perform direct electrochemistry, we sought to utilize the isolated CYT domain as an electron mediator in enzyme film-based biosensors. To this end, we produced the full-length CDH of the fungus *Neurospora crassa* and its isolated CYT domain recombinantly in *Pichia pastoris*. To investigate the electron transfer between the isolated CYT and cytochrome domains found in full-length CDH, a solution of 50 µM CDH was mixed with different concentrations of CYT (0, 50, 100, 200, and 500 µM) and was then electrochemically characterized on thioglycerol-modified gold electrodes. The changes in the enzyme's catalytic and non-catalytic currents with the different ratios of CYT:CDH were measured by cyclic voltammetry. Furthermore, polyethyleneimine (PEI) was added to study the influence of a film-stabilizing positively charged polymer on the electron transfer between CYT and CDH. The results show an increase in the catalytic currents when the concentrations of CYT were higher. These results strongly support our hypothesis that electrons are able to 'hop' from the full-length CDH to the isolated CYT domain. We also discovered that different concentrations of PEI affect the midpoint potential of CYT and CDH. Our findings suggest a novel route for applying CYT domains as electron mediators in enzyme films to improve the output currents of biosensors. Furthermore, this approach could be applied to fabricate multilayer enzyme-mediator films on different electrode materials.

References

1. R. Ludwig, R. Ortiz, C. Schulz, W. Harreither, C. Sygmund, L. Gorton, Anal. Bioanal. Chem. 405 (2013) 3637.
2. S. Ma, R. Ludwig, ChemElectroChem. 6 (2019) 958.
3. S. Scheiblbrandner, R. Ludwig, Bioelectrochemistry. 131 (2020) 10735.

S1-P-03

Cell Adhesive, Bacteriostatic and Bactericidal Conducting Polymer Coatings for Neural Regeneration

Katarzyna Krukiewicz, Dominika Czerwińska-Główka, Małgorzata Skorupa
Department of Physical Chemistry and Technology of Polymers,
Silesian University of Technology
M. Strzody 9, 44-100 Gliwice, Poland
katarzyna.krukiewicz@polsl.pl

Due to their superior electrochemical properties and high biocompatibility, conducting polymers are commonly applied in neural tissue engineering as biomimetic interfaces[1]. By tailoring their surface morphology and chemical composition, it is possible to induce significant neuroprotective effects and address neurological challenges associated with peri-implant gliosis and fibrous encapsulation[2]. Although conducting polymers enhance the process of neural cell adhesion and biointegration[3], their surface characteristics could be also modified to exhibit bacteriostatic and bactericidal effects, allowing to prevent infections on biomaterial surface[4].
In this communication, the recent attempts in the design of multifunctional conducting polymer coatings with diversified biological characteristics will be presented. The presentation will focus on the comparison of the behavior of polythiophenes and polypyrroles modified with biologically active dopants towards neural cells and model bacterial strains, and its correlation with physicochemical properties of coatings. The research attention will be particularly paid to poly(3,4-ethylenedioxythiophene) and poly(3,4-ethylenedioxypyrrole) doped with tetracycline, a wide spectrum antibiotic with a neuroprotective effect. Possessing advanced electrochemical properties and high cytocompatibility coupled with bacteriostatic and bactericidal effects, these conducting polymers are shown as appropriate candidates for neural interface applications.

This research was supported by the National Science Centre, Poland (SONATA 2016/23/D/ST5/01306 and OPUS 2019/35/B/ST5/00995).

References

1 C. Vallejo-Giraldo, et al *Small*, 2018, **14**, 1800863.
2 K. Krukiewicz, et al *Bioelectrochemistry*, 2020, **134**, 107528.
3 R. Green, et al *Adv. Mater.*, 2015, **27**, 7620–7637.
4 S. Gomez-Carretero, et al *Adv. Healthc. Mater.*, 2017, **6**, 1700435.

S1-P-04

Electrochemical Fingerprinting of Cocaine in Street and Water Samples Using a Multi-Walled Carbon Nanotubes-Based Sensor

Florina Maria Truță[1], Ana-Maria Drăgan[1], Mihaela Tertiș[1], Anca Florea[1], Jonas Schram[2], Andreea Cernat[1], Bogdan Feier[1], Karolien de Wael[2,3], Cecilia Cristea[1], Radu Oprean[1]
[1]Analytical Chemistry Department,"Iuliu Hațieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania
[2]Axes research group, University of Antwerp, Antwerp, Belgium
[3]NANOlab Center of Excellence, University of Antwerp, Antwerp, Belgium
Florina.Truta@umfcluj.ro

In the last years, illicit drug consumption has increased tremendously and it seriously affects the public health worldwide. Cocaine is an alkaloid, and is the second most used illicit drug in Europe. This drug is highly addictive because it stimulates the central nervous system and causes euphoria and dependence, and at the same time is very harmful for people's health (1).
For the detection of cocaine numerous techniques were applied. Among them, the most popular are GC–MS, HPLC, LC-MS, IMS, CE, and immunoassays. These techniques have some disadvantages like high cost, complicated operations, and lengthy analysis time. However, electrochemical methods offer a fast, portable, low-cost, and accurate alternative for the analysis of illicit drugs and their metabolites. Nanomaterials have gained much attention over the last decade in the development of sensors for a myriad of applications. The applicability of these nanomaterials, functionalized or not, significantly increases and is therefore highly suitable for use in the detection of drugs of abuse.
In this study the influence of several platforms was investigated such as graphite and graphite modified with nanomaterials (gold, silver, graphene and multi-walled carbon nanotubes), for the detection of cocaine, and the adulterants mentioned in the literature as the most commonly used compounds that are found in the mixture with this drug of abuse. Two electrolytic media with different pH values were used, and real samples were successfully analyzed in order to have enough insights for the detection of cocaine in real scenarios. A calibration curve was also performed and the quantitative analysis of cocaine from different type of real samples such as: waste water, tap water, and seized samples were successfully performed.

Acknowledgements: This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 833787, BorderSens.

1. https://www.unodc.org/unodc/en/index.html

S1-P-05

Electrochemical Fingerprinting of MDMA for Fast Analysis in Street and Water Samples Using a Graphene-Based Sensor

Ana-Maria Drăgan[1], Florina Maria Truță[1], Mihaela Tertiș[1], Anca Florea[1], Jonas Schram[2], Andreea Cernat[1], Bogdan Feier[1], Karolien de Wael[2,3], Cecilia Cristea[1], Radu Oprean[1]
[1] *"Iuliu Haţieganu" University of Medicine and Pharmacy, 6 Pasteur street, Cluj-Napoca, Romania*
[2] *AXES Research Group, University of Antwerp, Groenenborgerlaan 171, 2010 Antwerp, Belgium*
[3] *NANOlab Center of Excellence, University of Antwerp, Groenenborgerlaan 171, 2010 Antwerp, Belgium*
ana.dragan@umfcluj.ro

Illicit drugs are a constant concern worldwide, due to their increased spread and abuse which impacts society on many levels, from health and environmental implications to increased violence and criminal activities. MDMA (3,4-methylenedioxymethamphetamine) is a synthetic amphetamine-type substance (ATS) which accounted for 3% of the seizures reported in the latest EU report (1). Therefore, an analytical tool for the fast and accurate detection of MDMA is of great importance. In this regard, the present study explored the potential of electrochemical fingerprinting of MDMA for its selective and sensitive detection.

Considering the improvements brought by nanomaterials to the electrochemical sensors, several platforms were investigated: graphite and graphite modified with nanomaterials (gold and silver nanoparticles, graphene and multi-walled carbon nanotubes). Giving the heterogeneous purity of the illicit drugs on the market, the influence of several known adulterants/cutting agents on the electrochemical signal of MDMA was studied by testing a series of binary mixtures on the most suitable platform (graphene). Also, the method was characterized by analytical parameters such as calibration curve, limit of detection and limit of quantification in order to evaluate its performance. Finally, the electrochemical detection of MDMA was performed in street samples and spiked waters, showing the applicability of the presented method in the field of illicit drug detection.

Acknowledgements: This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 833787, BorderSens.

1. EMCDDA. European Drug Report 2020: Trends and Developments. Publications Office of the European Union, Luxembourg

S1-P-06

Enzymatic Polymerisation of 1,10 Phenanthroline-5,6 Dione in Conducting Biocompatible Ink as a Redox Mediator for Glutamate and Glucose Biosensing

Eithne Dempsey, Grace Halpin[1], Glen George[1], Andrea Bacciu [2], Gaia Giovanna Maria Roccitta[2], Pier Andrea Serra [2]

[1]*Department of Chemistry, Kathleen Lonsdale Institute for Human Health, Maynooth University, Maynooth Co. Kildare, Ireland*
[2]*Department Medical, Clinical and Experimental Sciences, Medical School, University of Sassari, Viale San Pietro 43/B, 07100 Sardinia, Italy*

eithne.dempsey@mu.ie

Here we report mediated glucose and glutamate biosensing methodologies based on enzymatically synthesised poly(1,10-phenanthroline-5,6-dione) (pPD) at graphite ink modified carbon transducers. Biocompatibility of the graphite ink was ensured via inclusion of additives/binders (polyethylene glycol, xylitol and chitosan) as part of the formulation. The sensing layer comprised the underlying layer of ink with either glucose or glutamate oxidase (0.25 U and 2 U respectively) sandwiched between two chitosan layers with an outer layer of either 1.5 % poly(ethylene glycol) diglycidyl ether (PEGDGE) or 0.1 % triethylene in the case of glucose and glutamate respectively. The enzymatically driven pPD deposition approach enabled encapsulation of the ortho-quinoidal polymeric species which formed a robust "honeycombed" surface confined redox active layer. The reversibility of the 1,10-phenanthroline-5,6-dione redox active film was made possible at neutral pH through adsorption at the underlying graphite ink layer, avoiding formation of the insoluble 1,10-phenanthroline-5,6-diol species (via intermolecular self-complexation) through interactions with the graphite surface. Film studies included stability (5% decrease in electroactivity over 20 cycles), surface coverage (average 5.33×10^{-7} mol cm^{-2}) and response to glucose and glutamate over the ranges 1- 10 mM and 10 - 100 μM respectively with sensitivity of 3.33×10^{-6} C cm^{-2} mM^{-1} and 8.71×10^{-4} C cm^{-2} mM^{-1} using chronocoulometry (0.007 and -0.05 V vs. Ag/AgCl for 10 s pulse in the case of glucose and glutamate).

S1-P-07

Nitrogen, Sulfur co-doped Graphene: Smart Materials for Electrochemical Applications

M. Coroş, F. Pogăcean, C. Varodi, A. Turza, E. Gal, A. Ciorițǎ, and S. Pruneanu

National Institute for Research and Development of Isotopic and Molecular Technologies, 67-103 Donath street, 400293 Cluj-Napoca, Romania
maria.coros@itim-cj.ro

The nitrogen/sulfur-doped graphene samples were synthesized by the hydrothermal method using thiourea as doping/reducing agent for graphene oxide (GO), previously dispersed in water. The thiourea/GO mixture was poured into an autoclave and placed in the oven at various temperatures (140; 160; 180 and 200°C) for different period of time (3; 8; and 12 hours). The effect of the reaction time, temperature and the GO: thiourea ratio on the morphology and structure of the resulting materials was thoroughly investigated by advanced techniques: SEM, X-Ray powder diffraction, FTIR, XPS, elemental analysis. The percentage of N and S decreased with the increasing of the reaction time, as shown below:

Sample	**Reaction temperature (°C)**	**wt.%**			
		C	**N**	**S**	**H**
NS-Gr-1	140	67.5	**3.9**	**17.8**	0.67
NS-Gr-2	160	71.6	**3.62**	**16.5**	0.88
NS-Gr-3	180	78.6	**2.55**	**9.92**	1.1
NS-Gr-4	200	82.1	**1.42**	**7.1**	0.75

Due to their remarkable properties (high surface area, analyte specific interaction, robustness) graphene were recently included in the smart materials class being highly suitable as electrode materials. Typically, co-doping of nitrogen and sulfur into graphene has been confirmed to be an effective approach to substantially promote the electrocatalytic activity due to the unique electronic structures of S and N atoms that can induce the redistribution of spin and charge densities. The co-doped graphene modified electrochemical sensor offers many advantages, such as high sensitivity, low cost, easy operation, and miniaturization.

Acknowledgement

This work was supported by grant of Romanian Ministry of Research and Innovation, CNCS-UEFISCDI, project number PN-III-P4-ID-PCCF-2016-0006, within PNCDI III.

S1-P-08

A Bio-hybrid Tandem: Coupling Two Photobioelectrodes for High Voltage Energetics

Marc Riedel, Soraya Höfs, Sascha Morlock, Fred Lisdat
Biosystems Technology, Institute of Life Sciences and Biomedical Technologies, Technical University Wildau, Hochschulring 1, 15745 Wildau, Germany
sascha.morlock@th-wildau.de

Over the last decade the coupling of photoactive materials and enzymes has intensified interest in order to modulate biocatalytic and electrochemical reactions with light. These advances can be used in different applications divided into sensorial, synthetic, and bioenergetic setups. For the latter, attractive characteristics regarding the potential behavior have emerged. For instance, the combination of two semiconductors (TiO_2 and PbS) with an enzyme (FAD glucose dehydrogenase) has been established [1]. This photobioanode collects the electrons from sugar oxidation at very low potentials when illuminated. This concept has been extended to photosystem II to improve the efficiency of photoelectrochemical water splitting [2].
The present study investigates $BiFeO_3$ as photocathode material in a photobioelectrode [3]. Thereto, a thin layer of $BiFeO_3$ has been prepared by a spin coating on FTO slides, giving rise to cathodic photocurrents. The performance is boosted in the presence of H_2O_2 (5 µM to 20 mM) and results in an onset potential of about 0.63 V vs Ag/AgCl. In contrast, no current can be detected in the dark underlining the light-directed origin of hydrogen peroxide conversion.
This photocatalytic activity of the $BiFeO_3$ has been combined with glucose oxidase (GOx) to construct a photobiocathode. Here, GOx produces hydrogen peroxide while consuming glucose. Thus, the photocathode is supplied with substrate. Finally, this photobiocathode has been coupled to the glucose consuming photobioanode which was already established. Thereby a photobioelectrochemical biofuel cell has been set up that allows the light-driven generation of electricity under glucose consumption at both, photobioanode and biocathode. This cell results in an open circuit voltage of about 1 V under illumination and demonstrates the potential of coupling suitable semiconductor structures with biocatalytic conversions on electrode surfaces for application in bioenergetics as well as biosensing.

References

[1] *ACS Catal.* **2018,** 8, 5212–5220.
[2] *Angew. Chem. Int. Ed.,* **2019**, 58, 801–805.
[3] *Angew. Chem. Int. Ed*. **2021**, 60, 2078–2083.

S1-P-09

Novel Conductive Peptide Molecular Materials for Electrochemical Sensing of Biomarkers

Lucian-Gabriel Zamfir, Mihaela Puiu, George Mădălin Dănilă, Camelia Bala
LaborQ, University of Bucharest
4-12 Regina Elisabeta Blvd., 030018 Bucharest, Romania
lucian-gabriel.zamfir@cdi.unibuc.ro

Short self-assembling peptides are used in the development of electrochemical biosensors, either as support for the immobilization of ligands, or as conductive layers [1,2]. We report on a novel electrochemical affinity biosensor for protein detection using a methylene Blue (MB)-labelled peptide wire as conductive support for ligand immobilization. First, the peptide wire was anchored on the electrochemically activated gold surface using the gold-thiol chemistry. The ligands for antibody and receptor targets were covalently attached to the peptide support using a bifunctional linker. The electron-transfer (ET) rate constants and density of the surface-bound peptide molecules were estimated through square wave (SWV), alternating current and cyclic voltammetry measurements. The surface densities of labelled peptide were estimated for both flattened and rough surfaces, following various pre-treatment protocols. The highest and most stable signals were recorded for rough surfaces following the optimization of the electrochemical surface-activation protocol. It was observed that moderate rough surfaces favored the formation of a stable peptidic layer, probably through the coordination of histidine residues to neighboring gold atoms. This approach does not involve the addition of thiolic compound to ensure stability.
The developed biosensor was used for the detection of two different high-molecular weight biomarkers: the anti-tumor-associated carbohydrate antigen antibody and the growth hormone secretagogue receptor. The detection followed a signal-off interrogation where the decrease of the SWV signal at the binding of the target was caused by the hindering of ET between MB and the electrode surface. Thus, peptide-wires offer a promising alternative to alkyl thiols for the modification of electrodes due to their conductive properties and stability.

This work was supported by a grant of the Romanian Ministry of Education and Research, CNCS - UEFISCDI, project number PN-III-P4-ID-PCE-2020-0998, within PNCDI III. L.-G. Zamfir was supported by ICUB fellowship 20965/2020 and by RYA, funded by Alexander von Humboldt-Stiftung Foundation.

[1] A. Karimzadeh, M. Hasanzadeh, N. Shadjou, M.d.l. Guardia, TrAC Trends in Analytical Chemistry 107 (2018) 1-20.
[2] M. Puiu, C. Bala, Bioelectrochemistry 120 (2018) 66-75.

S1-P-10

Tracing Potassium Ion Transport through Model Lipid Membranes with Reconstituted Membrane Proteins

Aleksandra Buta[1,2], Ewa Nazaruk[1], Piotr Koprowski[2], Bogusz Kulawiak[2], Adam Szewczyk[2], Renata Bilewicz[1]
[1]*Faculty of Chemistry, University of Warsaw, Pasteura 1, 02-093 Warsaw, Poland*
[2]*Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology Polish Academy of Science, Pasteura 3, 02-093 Warsaw, Poland*
abuta@chem.uw.edu.pl

Lipidic cubic phases (LCP) and black lipid membranes (BLM) are examples of model biological membranes that are used to membrane proteins activity study.
LCP is a transparent and three-dimensional lipidic matrix, which is permeated by an intercommunicating water channels. This membrane model is an intermediate state between a liquid and a solid, i.e. it demonstrates common features to both a liquid (fluidity) and a solid (ordered structure). Due to the large internal surface area of cubic phases (400 m^2/g), it is possible to efficiently incorporate proteins into LCP structure, and thus to conduct research on a larger scale.
The second model of biological membranes is BLM. BLM is a technique that allows the analysis of ion flux through single ion channels embedded in an artificial lipid membrane. The lipid bilayer is formed in the small aperture of the Teflon cup, which is located between two compartments filled with electrolyte. After adding the protein solution to one side of the system, a voltage is applied and the phenomenon of ion transport is studied by registering changes in the conductivity of the membrane.
The aim of the study is to verify whether the ion channels such as ROMK (renal outer medullary potassium channel) and gramicidin, after incorporation into the model biological membrane, enable the transport of potassium ions. The obtained results indicate that the reconstituted molecules are functional in the model biological membranes.

This work implemented as a part of Operational Project Knowledge Education Development 2014–2020 co-financed by European Social Fund, Project No POWR.03.02.00-00-I007/16-00 (POWER 2014-2020). This work was supported by the "Excellence Initiative - Research University" program - an internal grant of the University of Warsaw to increase the research potential of employees (Mikrogrant IDUB UW).

S1-P-11

Cells Under Irradiation – Electrochemical Evaluation

Adrian Enache[1], Melania Onea[1] and Mihaela Bacalum[2]
[1]*National Institute of Materials Physics*
[2"]*Horia Hulubei" National Institute for R&D in Physics and Nuclear Engineering*
Măgurele, Romania
adrian.enache@infim.ro

The interaction of radiation with matter takes place through energy transfer and is accomplished especially by ionized atoms or molecules. Although the biological effects of ionizing radiation on living organism it is known for many years there is a high demand on the development of new devices able to real-time quantify the effects of radiation on biological systems. The effect of radiation on biological systems involves multiple physical, chemical and biological steps. Direct effects result in a large number of reactive oxygen and nitrogen species within and outside cells, which are responsible for oxidative stress. Indirect effects are defined as alteration of normal biological processes and cellular components (DNA, protein, lipids, etc.). The development of sensor platforms to observe in real time the interaction between different kinds of radiation with biological materials has a great relevance in fields such as aeronautics for reducing radiation effects on flying crews and radiobiology for management of various diseases.
In this work, a classical design of an electrochemical (EC) three-electrode system was employed for analysing the effects of proton beam radiation on cell cultures. The EC three electrode system was produced by photolithography on a single silicon strip at National Institute of Materials Physics using silver as reference and gold as counter/auxiliary and working/sensing electrodes. In order to investigate the effect of proton radiation on the biological cell cultures, the cells were grown on the EC surface and irradiated. The experiments for proton irradiation were conducted at the "Horia Hulubei" National Institute for R&D in Physics and Nuclear Engineering using a Cyclotron TR19. After optimization of the experimental set-up and dosimetry, the radiobiological experiments were performed at doses ranging between 0 and 2 Gy and the effect of proton beam irradiation on the cell cultures was evaluated by measuring the open circuit potential of electrochemical sensor component electrodes.

Acknowledgements
Financial support from the Romanian Ministry of Research and Innovation through projects: PNCDI III-P5-5.1-23ELI and PNCDI III-P4-150PCE

S1-P-12

Antimicrobial and Neuroprotective Tetracycline-Loaded poly(3,4-Ethylenedioxypyrrole) Matrix for Biomedical Applications

Dominika Czerwińska-Główka[1], Magdalena Skonieczna[2,3], Sebastian Student[2,3], Wioletta Przystaś[3,4], Ewa Zabłocka-Godlewska[3,4], Beata Cwalina[3,4], Katarzyna Krukiewicz[1]

[1] *Department of Physical Chemistry and Technology of Polymers, Silesian University of Technology, M.Strzody 9, 44-100 Gliwice, Poland*

[2] *Department of Systems Biology and Engineering, Faculty of Automatic Control, Electronics and Computer Science, Silesian University of Technology, Akademicka 16, 44-100 Gliwice, Poland*

[3] *Biotechnology Centre, Silesian University of Technology, Krzywoustego 8, 44-100 Gliwice, Poland*

[4] *Department of Environmental Biotechnology, Faculty of Energy and Environmental Engineering, Silesian University of Technology, Konarskiego 18, 44-100 Gliwice, Poland*

dominika.czerwinska-glowka@polsl.pl

Conducting polymers are regarded as interesting materials for bioengineering, because of combining electrical activity with demonstrated biocompatibility. However, due to the numerous reports of implant-related infections, materials with antibacterial properties are becoming especially desirable.

In this study, we fabricated electrically-responsive polymers through electrochemical oxidative polymerization, obtaining matrices based on poly(3,4-ethylenedioxypyrrole) (PEDOP) as well as PEDOP/Tc containing common antibiotic (tetracycline) within the polymer backbone. Both matrices simultaneously with a sputtered Pt-layer as control were carefully examined by a wide variety of techniques (electrochemical, spectroscopic and microscopic). The microbiological evaluation of matrices was based on culturing Gram-negative *Escherichia coli* on investigated surfaces, and assessing their cell dimensions and surface coverage (SEM), together with demonstrated viability (LIVE/DEAD assay). To evaluate neuroprotective function, in vitro studies with rat neuroblastoma B35 cell line were carried out (MTT assay, cell cycle, apoptosis and SEM). Obtained results confirmed that PEDOP/Tc exhibited both electrical and biocompatible properties together with evidence antibacterial character, which makes it extremely potential neural interface materials.

This research was supported by the National Science Centre, Poland (SONATA 2016/23/D/ST5/01306 and OPUS 2019/35/B/ST5/00995).

S1-P-13

Electrochemical Sensing of Organohalide Pollutants Using Heme Functionalized SnO_2 Films on Flexible Plastic Substrates

Eleni Anna Economou[1], Apostolos Panagiotopoulos[2], Emmanuel Topoglidis[1]
[1]*Department of Materials Science, University of Patras, Rion 26504, Greece*
[2]*Department of Materials, Imperial College London, London SW7 2AZ, UK*
msci1612@upnet.gr

Organohalides (RX) are widespread environmental pollutants which typically contain a carbon-halogen bond and are resistant to biodegradation. Accidental or deliberate release of these materials into soil or groundwater can exert long-term toxic effects, present serious health risks and therefore their prevalence in ground water is of considerable environmental concern. [1] This work presents a simple and efficient preparation method for hemin modified mesoporous SnO_2 films on plastic, flexible ITO-PET substrates [2] for the electrochemical reduction of RX, such as CBr_4, chloroform and trichloroacetic acid. Scanning Electron Microscopy, X-ray Diffraction (XRD), Fourier Transform-Infrared Spectroscopy (FT-IR) and UV-vis absorption spectroscopy were used to characterize the resultant functionalized electrodes. For the immobilization of hemin on the films surface, Nafion and the drop-casting technique were used. The properties of the SnO_2 films enable a high hemin loading to be achieved in a stable and functional way allowing its direct reduction and oxidation. [2,3] The hemin modified electrodes exhibited significant catalytic activity for the electrochemical reduction of RX in water by using cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The results showed a sensitive response; the cathodic catalytic current was linearly proportional to the concentration of RX in the range 0–150 μM when using DPV with a limit of detection of 1 μM. This type of sensor has demonstrated good repeatability, reproducibility and stability.

Acknowledgement
This research was supported by Grant 80669 from the Research Committee of the University of Patras via "C. CARATHEODORI" program.

References
[1] T. Ito, G. J. Meyer, Environ. Eng. Sci. **2007**, *24*, 31 – 44.
[2] A. Panagiotopoulos, A. Gkouma, A. Vassi, C. J. Johnson, A. E. G. Cass, E. Topoglidis, *Electroanalysis* **2018**, *30*, 1956 – 1964.
[3] G. Samourgkanidis, P. Nikolaou, A. Gkovosdis-Louvaris, E. Sakellis, I. M. Blana, E. Topoglidis, *Coatings* **2018**, *8*, 284.

S1-P-14

Bacteriostatic Properties of Mg-Containing Oxide Coatings Formed on Ti Surface via Plasma Electrolytic Oxidation

Katarzyna Leśniak-Ziółkowska, Kasjana Brodacz, Alicja Kazek-Kęsik, Wojciech Simka
Faculty of Chemistry, Silesian University of Technology
B. Krzywoustego 6 Street, 44-100 Gliwice, Poland
katarzyna.lesniak-ziolkowska@polsl.pl

Due to the raising bacteria resistance to antibiotics action and problematic antibiotic treatment, the alternative antibacterial agents are strongly appealed. One of the cases inducing the risk of dangerous bacterial infection appearance is an implantation process. Among different biomaterials dedicated to hard tissue implants, titanium seems to be an interesting choice. In order to improve its bioactive and antibacterial properties, the plasma electrolytic oxidation (PEO) process can be proceeded. During the PEO treatment, the elements from the anodising bath can be incorporated into the forming porous oxide layer.
Titanium surface was oxidised in baths containing different Mg-containing compounds. The surface morphology and roughness, as well as the oxide layers thickness, were investigated using scanning electron microscopy (SEM). Additionally, the energy-dispersive X-ray spectroscopy (EDX) analysis was performed and the wettability of surfaces was determined using the water dynamic contact angle measurements. The bacteriostatic properties of obtained Mg-containing surfaces were confirmed using the bacteria adhesion tests with Staphylococcus aureus (ATCC 25923) strain.

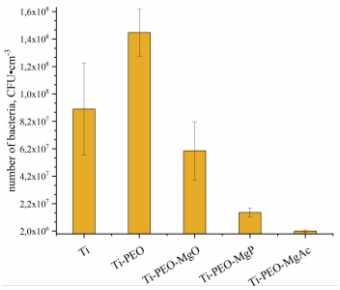


Fig. 1 The number of adhered live Staphylococcus aureus (ATCC 25923) bacteria after 4 h.

S1-P-15

Examination of carbon fibre microcylinder as an electrode for electrochemical sensing

Alexandra Elsakova, Mark Merzlikin, Ali Jafarov, Ausra Baradoke
Institute of Technology, University of Tartu, Nooruse 1, Tartu, 50411, Estonia
alexandra.elsakova@ut.ee

Methods that allow the identification of neurotransmitters as hormone-type biomarkers without a label can potentially make a significant contribution to biomedical applications and individual health. In support of this goal, numerous methods are being developed that take into account the main problems of working with real biological objects, namely the need to measure low concentrations in the presence of other solutes, the concentration of which is several times higher than the concentration of the molecule of interest. These limitations can be solved using electrochemistry methods. Several analytical methodologies using carbon based electrodes were reported [1 - 4]. However, the rapid fabrication of pure carbon electrodes with a large surface area is still challenging.

The carbon fibre sensing interface design allows rapid adjustment of the electrochemical surface area, which in turn modulates the sensor's sensitivity. This methodology is much easier to extend to various analytes in pH neutral buffers. The characteristics of the electrodes were determined using scanning electron microscopy, energy dispersive X-ray spectroscopy. Alternative methods for the characterisation using cyclic voltammetry, rectangular voltammetry and electrochemical impedance will be presented.

Acknowledgments We acknowledge the support from the Intelligent materials and systems lab, Institute of Technology, University of Tartu (Grant Number MOBJD5).

References

[1] Baradoke, A., Hein, R., Li, X., Davis, J.J., 2020. Reagentless Redox Capacitive Assaying of C-Reactive Protein at a Polyaniline Interface. Anal. Chem. 92, 3508–351
[2] Baradoke, A., Pastoriza-Santos, I., González-Romero, E., 2019. Screen-printed GPH electrode modified with Ru nanoplates and PoPD polymer film for NADH sensing: Design and characterization. Electrochim. Acta 300
[3] Baradoke, A., Jose, B., Pauliukaite, R., Forster, R.J., 2019. Properties of Anti-CA125 antibody layers on screen-printed carbon electrodes modified by gold and platinum nanostructures. Electrochim. Acta 306, 299–306
[4] Baradoke, A., Santos, A., Bueno, P.R., Davis, J.J., 2020. Introducing polymer conductance in diagnostically relevant transduction. Biosens. Bioelectron. 112705

S1-P-16

3D printed Electrochemical Cells for Biosensing on Flexible Carbon Electrodes

Ali Jafarov, Alexandra Elsakova, Mark Merzlikin, Ausra Baradoke
Institute of Technology, University of Tartu, Nooruse 1, Tartu, 50411, Estonia
ali.jafarov@ut.ee

Methods that allow the identification of biomarkers without a label can potentially make a significant contribution to biomedical applications and individual health. Several analytical methodologies using carbon-based electrodes were reported [1 - 4]. 3D printing has become an area of widespread use in various branches of science and industry. However, the rapid fabrication of carbon electrodes and fabrication of electrochemical cells for multiplexing is still challenging.
In this work, electrochemical cell was designed, which made it possible to increase the multiplexing of carbon electrodes. For printing, a 3D printer Prusa i3 MK3s was used in support with software PrusaSlicer. Biocompatible, flexible filament was used as a material, as it is elastic and resistant to deformation. The 3D printed electrochemical cell design suits multiplexing of carbon fibre flexible electrodes as sensing interface for dopamine, thereby increasing the measurement accuracy. Alternative methods for the characterisation using cyclic voltammetry, and electrochemical impedance will be presented.

Acknowledgments We acknowledge the support from the Intelligent materials and systems lab, Institute of Technology, University of Tartu (Grant Number MOBJD5).

References

[1] Baradoke, A., Hein, R., Li, X., Davis, J.J., 2020. Reagentless Redox Capacitive Assaying of C-Reactive Protein at a Polyaniline Interface. Anal. Chem. 92, 3508–351
[2] Baradoke, A., Pastoriza-Santos, I., González-Romero, E., 2019. Screen-printed GPH electrode modified with Ru nanoplates and PoPD polymer film for NADH sensing: Design and characterization. Electrochim. Acta 300
[3] Baradoke, A., Jose, B., Pauliukaite, R., Forster, R.J., 2019. Properties of Anti-CA125 antibody layers on screen-printed carbon electrodes modified by gold and platinum nanostructures. Electrochim. Acta 306, 299–306
[4] Baradoke, A., Santos, A., Bueno, P.R., Davis, J.J., 2020. Introducing polymer conductance in diagnostically relevant transduction. Biosens. Bioelectron. 112705

S1-P-17

Carrier Impact on the Properties of the Organo-Mineral Heterostructures Based on Begetable Extracts

K.D. Pershina[1], M.O. Khodykina[1]
[1] *Vernadsky Institute of General and Inorganic Chemistry NAS of Ukraine*
Paladin Avenue, 32/34, Kiyv 03142, Ukraine
pershina@ionc.kiev.ua

Porous clay heterostructures (PCH) are obtained by the insertion of an organic bulky cation with a metal atom in the interlayer spacing of a mineral, causing a swelling of the layers of the mineral. The heterostructures are based on native enzymes from vegetable extracts (cabbage, horseradish, and black radish) and inorganic carriers with different surface nature (bentonite and its acid form, kaolin, aerosil) were synthesized for the study of oxidative activity and electrochemical properties of the new materials. The atomic absorption and IR spectroscopy have been found the differences of the organic components binding with the inorganic carrier, which is based on specific and non-specific binding of the metal ions from organic cation with various carrier surfaces. The increasing of the surface acidity could provide a friendly biocompatible interface for immobilizing biomolecules mixture and increase the oxidative activity of mature structure. Changing the vegetable component didn't change the common dependence of binding of the metal atoms with the carrier, but changing acidity radically changed such binding. In the case of high acidity – the binding has non-selective nature, with decreasing acidity the binding becomes the selective one. In the case of selective binding, it can be realized active elements for energy storage (supercapacitors and capacities). Non-selective binding gives the ability to realize the elements of the sensors (Fig. 1).

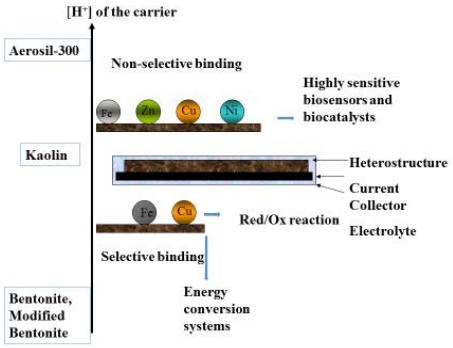


Figure 1: The principal scheme of the carrier impact on the ending properties of the organic-mineral heterostructures based on vegetable extracts

S1-P-18

Nanozymes Based on Stabilized Prussian Blue Nanoparticles as Substitute for Natural Peroxidase

Elizaveta Shcherbacheva, Elena Karpova, Arkady Karyakin
Faculty of Chemistry, Lomonosov Moscow State University
GSP-1, Leninskie Gory, Moscow, Russia
scherbacheva.e@gmail.com

One of the rapidly growing areas of medicine is the development of methods for the early diagnosis of diseases. Immuno-, DNA- and RNA-analyses can determine tumor markers in human blood and effectively monitor the progression of autoimmune diseases. However, available tools still have two major problems - high cost and instability of enzymes. To overcome this, the enzymatic labels–peroxidases can be replaced with nanoparticles that mimic the activity of the enzyme. The unique structural and physicochemical properties of Prussian Blue nanoparticles (PB NP) make them the most promising substitute for peroxidase.

Recently, a method for the synthesis of PB NP was developed [1], which allows preparation of nanozymes that are specific in the reduction reaction of hydrogen peroxide and at the same time exceeds the catalytic activity of the natural enzyme by several thousand times. However, synthesized particles become unstable at pH 5 or higher. To expand the working range of the nanoparticles, composite PB NP - nickel hexacyanoferrate (PB-NiHCF) were synthesized.

Conditioning of PB nanoparticles in a growing solution that contains both nickel and hexacyanoferrate ions results in a significant increase of hydrodynamic diameter. Particle sizes were determined using DLS and confirmed with TEM. A study of the stability of PB-NiHCF nanoparticles was carried out in phosphate-citrate buffer at pH 7.4. The inactivation constant for composite NP is an order of magnitude lower than for PB NP ($3.37 \cdot 10^{-4}$ instead of $3.27 \cdot 10^{-3}$). When studying the catalytic properties of the obtained particles by the oxidation reaction of 3,3',5,5'-tetramethylbenzidine with H_2O_2, the value of k/K_m was $0.34 \cdot 10^6$ $M^{-1} \cdot s^{-1}$, which coincides with the corresponding value for PB NP ($0.35 \cdot 10^6$ $M^{-1} \cdot s^{-1}$ [1]).

Overall, by depositing PB-NiHCF NP on the surface of a three-electrode planar structure we obtained hydrogen peroxide sensors that retain 90% of the initial response value at least 5 times longer than sensors modified with PB NP.

1. M.A. Komkova, E.E. Karyakina, A.A. Karyakin, JACS 2018, 140, 11302.

This work was financially supported by the RSF, grant 19-13-00131.

S1-P-19

Synthesis and Catalytic Properties of Ultrasmall Prussian Blue Nanoparticles with Peroxidase Activity

Zavolskova M.D., Nikitina V.N., Karyakin A.A.
Chemistry Department, M.V. Lomonosov Moscow State University
119991, Moscow 1, GSP-1, 1-3 Leninskiye Gory, Russia
marina-zav@bk.ru

Prussian Blue (PB) is known as "artificial peroxidase," since it is a catalyst for the reduction of hydrogen peroxide. Catalytic synthesis of PB nanoparticles (NPs) defeating natural peroxidase [1] allowed creating sensors that has shown higher sensitivity to hydrogen peroxide than PB films. Nanoparticles of Prussian Blue possessing the peroxidase-like activity are also usable for lactate biosensors production [2]. At the same time, the decrease in size of PB particles can improve the sensors characteristics due to higher surface area of electrocatalyst. Ultrasmall PB NPs corresponding to the enzymes in their size, less than 10 nm in diameter, hasn't been synthesized yet and their catalytic properties remained unknown.

We report on the synthesis of ultrasmall PB nanoparticles. We used reversed micelles, also called "nanoreactors" for controlled synthesis of NPs.

Instead of direct mixing of Fe(II) and Fe(III) salts the above mentioned results were achieved in case of Fe(III) salts and reducing agent. It was shown that reduction of the Fe(III) salts mixture captured in reverse micelles with aniline leads to the smallest particles. Aniline is able to polymerize upon oxidation and stabilize the resulting particles.

Size of the synthesized particles was investigated by dynamic light scattering and confirmed with transmission electron microscopy images. Thus, the particles synthesized in reverse micelles are 4.7 ± 0.8 nm.

Synthesized BL NPs were extracted from inverted micelles with glycol and transferred to a new micellar system (w = 10; citrate-phosphate buffer pH 5.0/isooctane/AOT). The particle size in the new system appeared to be 6.6 ± 0.5 nm. The peroxidase activity of the synthesized particles in reversed micelles has been investigated spectrophotometrically. The appearance of guaiacol oxidation product has been monitored at 470 nm. The resulting PB nanoparticles were used to create electrochemical sensors for hydrogen peroxide detection.

Authors thank the Grant № 19-13-00131(Russian Science Foundation).

References

[1] M.A. Komkova, E.E. Karyakina, A.A. Karyakin, JACS, 2018, 140, 11302
[2] D.V.Vokhmyanina, K.D. Andreeva, M.A. Komkova, E.E. Karyakina, A.A. Karyakin, Talanta, 2020, 208,120393

S1-P-20

An Electroanalytical Approach to Measure the Pore Size Distribution of Inverse Opal Electrodes

Yoshua Moore[1], Darren Buesen[1], Huaiguang Li[1], Xialong Chen[2], Jenny Zhang[2] and Nicolas Plumeré[1]
TUM Campus Straubing for Biotechnology and Sustainability[1]
Schulgasse 22, 94315 Straubing, Germany
Department of Chemistry, University of Cambridge[2]
Lensfield Road, Cambridge CB2 1EW, U.K
yoshua.moore@tum.de

Bioelectrochemical devices have in recent times become a focal research topic in renewable energy and power production, with recent developments utilising highly porous materials to immobilise enzymes/organisms directly at the electrode surface. Key requisites for increasing the power outputs of such devices include well-defined geometries, large interfacial surface areas and openness of the scaffold, all of which have led many to develop hierarchical porous structures. One geometry with great applicable relevance is that of the mesoporous inverse opal (meso-IO) structure; the ability to fine-tune the dimensions of the IO lattice means that one can optimally integrate biomolecules of a broad range of sizes. However, high quality IO structures are challenging to fabricate and can oftentimes result in defects and inhomogeneities of the scaffold. We are currently developing an electroanalytical technique based on linear sweep voltammetry that aims to complement existing surface characterisation techniques by providing additional information pertaining to the ensemble structure of the electrode. This includes the electrolyte-accessible surface area, the average pore size, and the pore size distribution of the whole electrode. Our method intends to overcome the shortcomings that are prevalent when attempting to determine the metrics of spherical macropores with traditional analyses that rely on sorption isotherms or mercury intrusion porosimetry. Fundamentally, our electrochemical method exploits the different mass transport regimes that occur for a freely diffusing redox probe with respect to the size of the pore within which it is situated; experimentally observed current responses will differ accordingly and, by extension, a whole electrode containing a distribution of pore sizes will generate a fingerprinted current response conforming to its size distribution. Through the combination of experimental data acquisition and an open-source App, these pore metrics can be estimated relatively easily when compared to other available methods.

S1-P-21

Gold Nanoparticles Decorated Reduced Graphene Oxide for Highly Sensitive Electrochemical Monitoring of Fortified Infant Food and Formulae

Patrick Severin Sfragano[a], Francesca Bettazzi[a], Chiara Ingrosso[b], Valentina Pifferi[c], Luigi Falciola[c], M. Lucia Curri[b,d], Ilaria Palchetti[a]
[a]*Dipartimento di Chimica "Ugo Schiff", Università degli Studi di Firenze, Via della Lastruccia 3, 50019 Sesto Fiorentino (FI), Italy*
[b]*CNR-IPCF, Sez. Bari, c/o Dip. Chimica Via Orabona 4, 70126 Bari, Italy*
[c]*Dipartimento di Chimica, Università degli Studi di Milano, Via Golgi 19, 20133 Milano, Italy*
[d]*Dipartimento di Chimica, Università degli Studi di Bari, Via Orabona 4, 70126 Bari, Italy*
patrickseverin.sfragano@unifi.it

Fortified formulae and fortified foods are means to increase the intake of vitamins, minerals and other nutrients in infants and young children with inadequate or at risk of inadequate status of these nutrients. Indeed, a reliable analytical tool is needed in quality control (QC) laboratories for the assessment of commercial formulae and foods for infants and young children, where a rapid and cost-effective monitoring of vitamin C content is essential. Since results are needed quickly, so that corrective actions can be implemented promptly, QC procedures demand fast and simple analytical assays to monitor possible vitamin losses during food process and storage. For this purpose, in this work, an easy and reliable method based on a novel electroanalytical nanostructured sensor has been developed. In particular, we have modified the sensor surface with Au nanoparticles (NPs) decorated Reduced Graphene Oxide (RGO) flakes, obtaining an *ad hoc* designed sensor surface. The novel nanostructured sensor platform exhibits a LOD of 0.088 mg L^{-1} and RSD of ca. 8%. This LOD is lower than enzymatic-based spectrophotometric methods routinely used in QC laboratories. Data on different commercially available infant food and formulae confirm the reliability of the proposed method.

References

[1] F. Bettazzi, C. Ingrosso, P.S. Sfragano, V. Pifferi, L. Falciola, M.L. Curri, I. Palchetti, *Food Chem.* 344 (2021) 128692.

[2] C. Ingrosso, M. Corricelli, F. Bettazzi, E. Konstantinidou, G. V. Bianco, N. Depalo, M. Striccoli, A. Agostiano, M.L. Curri, I. Palchetti, *J. Mater. Chem. B.* 7 (5), (2019), 768.

S1-P-22

A Novel Bicyclic Peptide as Bioreceptor in Electrochemical Biosensing of Clinical Markers

Patrick Severin Sfragano[1], Giulia Moro[2], Alessandro Angelini[2], Federico Polo[2] and Ilaria Palchetti[1]
[1]*Department of Chemistry "Ugo Schiff", University of Florence, Via della Lastruccia 3, 50019 Sesto Fiorentino (FI), Italy*
[2]*Department of Molecular Sciences and Nanosystems, Ca' Foscari University of Venice, Via Torino 155, 30172 Venice, Italy*
patrickseverin.sfragano@unifi.it

In a world that constantly moves towards the selection of non-invasive clinical approaches, such as the liquid biopsy of biological fluids, and personalised medicine, the scientific community is always on the lookout for reliable biomarkers and new methods for their detection. To this end, our group synthesized and used a chemically constrained bicyclic peptide that shows a great affinity for a specific protease of the plasminogen activator system, namely the human urokinase-type plasminogen activator (uPA). uPA is a literature-reported protein often associated with – among other diseases – breast cancer growth and invasion; high levels of uPA are found in biological fluids in conjunction with metastatic evolutions of the neoplasia. Currently, ELISA is the gold standard technique for its determination. In our work, the role of the primary antibody in ELISAs is played instead by the bicyclic peptide mentioned, which is immobilized onto the surface of superparamagnetic microbeads. Due to the small size of such bicyclic peptide and since both of its loops interact with uPA, a large interaction surface for uPA is achieved, thus heightening the sensitivity of the method. Differential pulse voltammetry on disposable screen-printed electrodes was used to detect the oxidation current of the electroactive affinity reaction marker, which is proportional to the concentration of uPA in the sample. The solid performances of the proposed method may allow clinical analyses of this biomarker, even in point-of-care applications.

S1-P-23

Poly(Caffeic acid)@Carbon Nanotube Decorated with CuO for Glucose Detection

Maria Kuznowicz, Artur Jędrzak, Teofil Jesionowski
Chemical Technology and Engineering Institute, Faculty of Chemical Technology, Poznan University of Technology, Berdychowo 4, PL-60965 Poznan, Poland
maria.m.kuznowicz@doctorate.put.poznan.pl

In recent years, nanomaterials have been more widely used in sensors as a result of their unique physical and chemical properties. Nanoscience and nanotechnology advances have allowed the development of novel nanomaterials that can be used in enzyme-free glucose detection. Common enzyme-based sensors with electrochemical transducer can accurately calculate glucose levels in human blood, but they are limited by the enzyme's low stability [1].
Nanoscale metals, metal oxides, doped metal oxide composites, organic and inorganic composite materials have also been used with the electrode material. Metal oxide nanoparticles have received enormous attention for their promising sensing applications among different types of engineered nanomaterials. Specific features, such as controllable size, functional biocompatibility, bio-safe, chemical stability, and catalytic properties, exhibit the metal oxide nanoparticles [2].
A novel nanohybrid PCA@MWCNT-CuO material was developed and synthesized. Transmission electron microscopy (TEM), atomic force microscopy (AFM), X-ray Photoelectron Spectroscopy (XPS), and Raman spectroscopy were used to examine the nanocomposite's morphology. The presented nanoplatform has been tested in terms of its potential application in the construction of amperometric sensors. The glucose concentration in various solutions was then assessed using a hybrid-support sensor system. The excellent glucose detection properties were most likely due to a synergistic effect of the individual components (poly(caffeic acid), multi-walled carbon nanotube, and CuO). Furthermore, for quantifying glucose concentrations in human serum samples, good accuracy and precision have been demonstrated.

This work is financed and prepared as part of a research project supported by the National Science Center Poland, no. 2017/27/B/ST8/01506

References

[1] T. Jesionowski, M. Kuznowicz, A. Jędrzak, T. Rębiś, Encyclopedia of Sensors and Biosensors, Elsevier, 2021
[2] M. Adeel, M.M. Rahman, I. Caligiuri, V. Canzonieri, F. Rizzolio, S. Daniele, Biosens. Bioelectron. 2020, 165, 112331

S2 Electrochemical Sensors for Diagnostics and Therapy Monitoring

S2-P-01

Fabrication of an Electrochemical Enzymatic Biosensor for Glycose using a Dual Pen-on-Paper Approach

V. Pagkali, D. Soulis, E. Stavra, A. Economou*
Department of Chemistry, National and Kapodistrian University of Athens
Athens 157 71, Greece
**aeconomo@chem.uoa.gr*

Paper is a material that offers several advantages for the fabrication of analytical devices. Paper-based analytical devices (PADs) exploit manipulation of liquids on hydrophilic paper channels featuring hydrophobic barriers; therefore, many types of PADs have been developed and applied to various fields such as clinical diagnostics and POC testing, environmental monitoring and food quality control [1,2].
The patterning of the hydrophobic barriers and electrodes is a critical step in the fabrication of electrochemical PADs and several approaches have been proposed in the literature [3]. Pen-on-paper (PoP) strategies involve the use of suitable writing tools (pens or pencils) to deposit functional materials on paper substrates with the view to create hydrophobic patterns and conductive areas (electrodes) [4].
The aim of this work was the development of a simple and fast programmable dual PoP approach for the fabrication of an electrochemical enzymatic PAD for glucose monitoring. For this purpose, a fluidic device with a sample introduction zone and a detection zone was designed and patterned by x-y plotting with commercial marker pens. Electrodes were further deposited in the detection zone by x-y plotting with a commercial writing pencil and, finally, glucose oxidase was immobilized in the detection zone. Glucose monitoring was based on electrochemical detection of the hydrogen peroxide generated enzymatically in the detection zone.

References

[1] L.M. Fu, Y.N. Wang, TrAC **2018**, *107*,196.
[2] T. Ozer, C. McMahon, C.S. Henry, *Annu. Rev. Anal. Chem.* **2020,** *13*, 85.
[3] H. Lim 1, A.T. Jafry, J. Lee, *Molecules* **2019**, *24*, 2869.f
[4] Z. Li, H. Liu, X. He, F. Xu, F. Li, *TrAC* **2018**, *108*, 50.

Acknowledgements

This research is co-financed by Greece and the European Union (European Social Fund-ESF) through the Operational Programme "Human Resources Development, Education and Lifelong Learning 2014-2020" in the context of the project "Development of paper-based integrated fluidic electrochemical biosensors using nanomaterial labels" (MIS 5047930).

S2-P-02

Molecular Recognition of Guanine with Mixed Monolayers of a Nucleolipid and a Phospholipid Supported on Gold (111) Electrodes

Francisco Prieto Dapena[a], ZhangFei Su[b], Estrella Drago[a], Julia Alvarez Malmagro[a,b], Manuela Rueda, Jacek Lipkowski[b]

[a] *Department of Physical Chemistry, University of Seville, C/Professor García González n◦ 2, 41012 Seville, Spain*

[b] *Department of Chemistry, University of Guelph, Guelph, Ontario, Canada N1G 2W1*

dapena@us.es

Recently we have described potential controlled guanine-cytosine recognition reaction in gold (111) electrodes modified with monolayers of 1,2-dipalmitoyl-sn-glycero-3-(cytidine diphosphate) nucleolipid (DG-CDP) by measuring IR spectra of the complex with the help of photon polarization modulation infrared reflection absorption spectroscopy (PM-IRRAS). [1] The lateral hydrophobic interactions between adjacent acyl chains play the main role to the stability of the monolayer. However, the Langmuir isotherms for DG-CDP and for 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), with the same acyl chains length, show that the minimum area per molecule in the DG-CDP monolayer is higher than in the DPPC monolayer. This difference indicates that repulsive interactions between between the polar heads of nucleolipid molecules are significant.[2]

In this work, the thermodynamic analysis of mixed monolayers of DG-CDP/DPPC at the air/water and air/electrolyte interfaces permitted us to select the optimum composition of the mixture minimizing the polar heads repulsive interactions between nucleolipid molecules. The molecular recognition capabilities of gold electrodes modified by this mixed monolayer are investigated using electrochemical and PM-IRRAS measurements.

(1) Alvarez-Malmagro, J.; Su, Z.; Leitch, J. J.; Prieto, F.; Rueda, M.; Lipkowski, J. Electric-Field-Driven Molecular Recognition Reactions of Guanine with 1,2-Dipalmitoyl-Sn-Glycero -3-Cytidine Monolayers Deposited on Gold Electrodes. *Langmuir* **2019**, *35* (28), 9297–9307

(2) Alvarez-Malmagro, J.; Su, Z.; Jay Leitch, J.; Prieto, F.; Rueda, M.; Lipkowski, J. Spectroelectrochemical Characterization of 1,2-Dipalmitoyl- Sn-Glycero-3-Cytidine Diphosphate Nucleolipid Monolayer Supported on Gold (111) Electrode. *Langmuir* **2019**, *35* (4), 901–910.

S2-P-03

Cytochrome–enzyme Chimeric Proteins for Direct Electron Transfer-based Glucose Biosensors

Franziska Schachinger[1], Stefan Scheiblbrandner[1], Su Ma[1], Roland Ludwig[1]

[1]*Biocatalysis and Biosensing Laboratory, Department of Food Science and Technology, BOKU – University of Natural Resources and Life Sciences, Muthgasse 18, 1190 Vienna, Austria*
franziska.schachinger@boku.ac.at

In biosensors, enzymatic recognition elements are either coupled via mediated electron transfer (MET) using redox mediators, or by direct electron transfer (DET) to the electrode. The advantage obtained with DET-based biosensors is the development of simple analytical devices (reagentless sensors) with high sensitivity [1]. We visually screened 2550 cytochromes of which a structure was published for suitability as "built-in" redox mediators for FAD-dependent glucose dehydrogenases in glucose biosensors. Four distinct *b*-type and three *c*-type cytochromes were recombinantly produced using different expression systems. The purified cytochromes were then electrochemically analysed by cyclic voltammetry on thioglycerol-modified gold electrodes in a pH range from 4.00 to 9.00. Midpoint potentials, the reversibility of electrochemical processes and peak currents were analyzed in detail. The resulting data are the basis for further design of a chimeric protein in which the cytochrome domain is linked with via a ~25 amino acids long flexible linker to a glucose dehydrogenase from *Glomerella cingulata* for application in a glucose biosensor [2]. We are currently generating variants of the chimeric protein with the purpose to optimize the interdomain electron transfer. Those are then tested in detail by electrochemical methods. The challenge is to optimize the protocol for detection of catalytic currents for the low electron transfer rates observed of the wild type chimeric protein. Different electrode materials are tested with focus on increasing the electrode surface by using carbon (nano)materials.

[1] Zayats M, Willner B, Willner I, "Design of amperometric biosensors and biofuel cells by the reconstitution of electrically contacted enzyme electrodes." Electroanal. 2008;20:583-601.
[2] Ma, S., and Ludwig, R., "Direct electron transfer of enzymes facilitated by cytochromes", Wiley-VCH Review, 2019

S2-P-04

Measuring lytic Polysaccharide Monooxygenase Activity on Solid Substrates

Lorenz Schwaiger, Hucheng Chang, Stefan Scheiblbrandner, Roland Ludwig
Department of Food Science and Technology, University of Natural Resources and Life Sciences, Muthgasse 11, 1190 Vienna, Austria
Lorenz.Schwaiger@boku.ac.at

Lytic polysaccharide monooxygenases (LPMOs) are secreted by lignolytic organisms to assist hydrolytic enzymes in the depolymerization of biogenic polymers [1]. Their unique ability to cleave crystalline substrates proceeds via an oxidative mechanism and depends on an electron donor and hydrogen peroxide utilized as co-substrate. LPMOs are activated via an initial reduction step of the exposed type 2 copper active site [2]. Once reduced, LPMOs bind to polymeric substrates and enter their catalytic cycle. Methods to analyze the substrate specificity of many LPMOs and the resulting oligosaccharide products by end-point HPLC-MS are well established [3]. However, there is no on-line method available which is able to directly quantify LPMO activity on solid substrates. We are developing an amperometric real-time method which enables us to assess LPMO activity during catalysis. In principle, our biosensor relies on the electrocatalytic properties of noble metal electrodes such as gold or platinum towards H_2O_2, already realized in first generation biosensors, and the concomitant provision of electrons for LPMO activation via MET. In the reductive milieu, we assess LPMO activity by comparing cathodic currents of H_2O_2 reduction in the presence and absence of LPMO. Activated LPMOs will consume a fraction of the not yet reduced H_2O_2 which is then no longer available for turnover by the electrode, resulting in a smaller current dependent on the type of substrate present. Although initial studies were performed with soluble xyloglucan, cellulose as a substrate was introduced by placing a common dialysis membrane made of regenerated cellulose in close proximity to the employed metal electrode. A challenging part of the biosensor development was the selection of a suitable electron donor, i.e. a redox mediator, which does not interfere with the detection of hydrogen peroxide and is stable on electrodes. By studying diffusion-controlled hydrogen peroxide consumption as a function of LPMO activity enables us to determine the kinetics of a process largely inaccessible by any other approach. Here, two LPMOs from *Neurospora crassa* (9C and 9F) with different substrate preferences [4] were tested but the by all LPMOs commonly shared active site architecture and similar mechanism suggests that this approach can be applied to other LPMOs and other crystalline substrates. Thereby, our system is opening up a route to characterize these versatile enzymes under almost natural turn-over conditions.

[1] Cragg, S.M. et al.; 2015; vol 29 108–119; [2] Forsberg, Z. et al.; 2019; vol 59 54–64 [3] Eijsink, V. G. H et al.; 2019; vol 12 58; [4] Agger, J. W. et al.; 2014;111, 6287–6292;

S2-P-05

Electrochemical Detection of L-Tryptophan with Graphene-Modified Electrode

Florina Pogăcean, Codruța Varodi, Maria Coroș, Alexandra Cioriță,
Valentin Mirel and Stela Pruneanu

National Institute for Research and Development of Isotopic and Molecular Technologies,
67-103 Donath street, 400293 Cluj-Napoca, Romania;
florina.pogacean@itim-cj.ro

In this work, we report an easy electrochemical method for the synthesis of good-quality triple-doped graphene (N, S, B) in aqueous electrolytes containing ammonium sulfate, boric acid, and sodium chloride. Our method has the advantage of short processing time and mild reaction conditions. Incorporating N, S, B into the electrochemically exfoliated graphene sheets showed favorable electro-catalytic activity towards tryptophan detection.
The graphene sample was prepared by electrochemical exfoliation of graphite rods in solution containing 0.05 M $(NH_4)_2SO_4$ + 0.1 M H_3BO_3 + 0.05 M NaCl. The exfoliation was performed by applying a constant voltage (12 V) between the graphite rods, while the temperature was kept constant (18° C) with a temperature-controlled cryostat. The structural investigation of the graphene sample, performed by X-ray powder diffraction (XRD), revealed that the sample consists of a mixture of few-layer (69%), multi-layer graphene (14%) and graphene oxide (17%). In addition, XPS analysis proved that the sample was triple-doped with hetero atoms such as nitrogen (1.7 at%), sulfur (2.5 at%), and boron (3 at%). The sample was deposited onto the surface of a clean, glassy carbon electrode (GC) and investigated for the non-enzymatic electrochemical detection of L-tryptophan. The electro-catalytic properties of the EGr/GC electrode led to a considerable decrease in the oxidation potential from +0.9 V (bare GC) to +0.72 V. In addition, the EGr/GC electrode has higher sensitivity (two times) and a lower detection limit (ten times) in comparison with the bare GC electrode.

Acknowledgement

This work was supported by grant of Romanian Ministry of Research and Innovation, CNCS-UEFISCDI, project number PN-III-P4-ID-PCCF-2016-0006, within PNCDI III.

S2-P-06

Amperometric Detection of L-Cysteine with N and S co-doped Graphene-Modified Electrodes Physics and Technology of Isotopes

Codruța Varodi, Maria Coroș, Florina Pogăcean, Alexandra Ciorîță and Stela Pruneanu

National Institute for Research and Development of Isotopic and Molecular Technologies, 67-103 Donath street, 400293 Cluj-Napoca, Romania; codruta.varodi@itim-cj.ro

Doping graphene with heteroatom (nitrogen, sulfur) has become an important strategy for changing the electrochemical properties of graphene based materials.
The nitrogen and sulfur co-doped graphene samples were chemically synthesized in our group by the hydrothermal method, using graphene oxide (GO) as starting material and thiourea as doping/reducing agent. The reaction temperatures were 120^{o} C and 200^{o} C, so the samples were correspondingly denoted as NSGr-120 and NSGr-200. After synthesis, the samples were characterized both morphological and structural by different techniques: SEM, X-Ray powder diffraction, FTIR, XPS, and elemental analysis. According to elemental analysis, the percentage (wt%) of N and S decreased in the graphene samples with the increase of the reaction time. The new co-doped graphene samples were deposited on two glassy carbon (GC) electrodes and next investigated for the non-enzymatic electrochemical detection of L-cysteine in pH 6 PBS, using cyclic voltammetric and chronoamperometric techniques. There were marked differences between the bare GC and the graphene-modified electrodes, both in terms of peak potential and peak current. In the case of bare GC electrode, no oxidation peak was recorded, indicating that the L-cysteine molecules cannot be easily oxidized on its surface. For GC/NSGr-120 and GC/NSGr-200 electrodes the corresponding CV exhibits a broad oxidation peak at around +0.62 V and +0.72 V respectively, proving the electro-catalytic effect of NS-doped graphene. As expected, the effect is higher in the case of NSGr-120 sample, which may be related to the higher concentration of sulfur heteroatom within the graphene structure.

Acknowledgement

This work was supported by grant of Romanian Ministry of Research and Innovation, CNCS-UEFISCDI, project number PN-III-P4-ID-PCCF-2016-0006, within PNCDI III.

S2-P-07

Electrochemical Sensor based on Molecularly Imprinted Polymer for the Detection of bacterial Quorum Sensing Molecules

Denisa Căpățînă, Cecilia Cristea, Bogdan Feier
Analytical Chemistry Department, Faculty of Pharmacy,
"Iuliu Haţieganu" University of Medicine and Pharmacy,
Cluj-Napoca, Romania
denisa.elen.capatina@elearn.umfcluj.ro

Quorum sensing (QS) is a form of cell to cell communication between bacteria with the aid of substances called autoinducers, such as N-acyl derivatives of homoserine lactone (AHL). QS plays a key role in determining virulence and in biofilm formation, which poses a great threat to human health due to the films' resistance to antimicrobial agents. With antibiotic resistence on the rise, it is crucial to develop new methods to determine quorum sensing autoinducers (1).
Due to their advantages (high selectivity, wide versatility, good resistance and low costs), molecularly imprinted polymers (MIPs) have played an increasingly important role in the development of (bio)sensors and have been very useful in the dosage of a wide variety of molecules. In situ electropolymerization of MIP, using electropolymerizable monomers, represents a simple one-step method to obtain thin layers directly at the electrode surface that allow a selective detection of the analyte of interest from the sample (2).
In this study, we developed a sensitive and specific electrochemical sensor based on MIP for the detection of molecules involved in bacterial quorum sensing.
In order to select the monomer that exhibits the highest binding interactions with our molecules of interest, we tested several electropolymerizable monomers. Based on the results, we selected methylene green as the monomer for our study.
The composition of the polymerization mixture, the electropolymerization method, the template extraction and the rebinding process were optimized in order to determine the optimum condition for the detection of QS molecules. The modified electrodes were characterized using different electrochemical techniques. Finally, the sensor was tested for the analysis of QS molecules from real samples.

Acknowledgements This work was supported by a grant of the Romanian Ministry of Education and Research, CNCS - UEFISCDI, Project no. PN-III-P1-1.1-TE-2019-1360, within PNCDI III.

1. Bettenworth V, *et al*. J Mol Biol. 2019; 431 (23): 4530–46
2. Beluomini MA, *et al*. J Electroanal Chem. 2019; 840(April): 343–66

S2-P-08

The Development of an Electrochemical Method for the Detection of Gentamicin in Biological Samples

Alexandra Pusta, Denisa Căpățînă, Adrian Blidar, Bogdan Feier, Cecilia Cristea
"Iuliu Haţieganu" University of Medicine and Pharmacy,
Department of Analytical Chemistry and Instrumental Analysis,
4 Pasteur Street, 400349, Cluj-Napoca, Romania
alexandra.pusta@elearn.umfcluj.ro

Gentamicin (GEN) is an aminoglycoside antibiotic used in the treatment and prophylaxis of a wide variety of infections caused by Gram-negative bacteria. Despite its benefits, gentamicin has a low therapeutic index and its use can lead to serious adverse reactions such as nephrotoxicity and ototoxicity (1). For this reason, it is essential to develop new detection methods that can be used for GEN therapeutic drug monitoring.
In this study, a new direct electrochemical method for the detection of GEN from biological samples was developed.
Different types of screen-printed electrodes (SPE), such as carbon based, graphene based, gold based and platinum based SPEs, as well as glassy carbon electrodes, (GCE) were tested in order to study the electrochemical behavior of GEN. The influence of the supporting electrolyte and pH were studied using cyclic voltammetry (CV) on a pH range between 2 and 13. The influence of the scan rate was also tested using CV and a differential pulse voltammetry method was also optimized for the detection of GEN.
In order to apply the method to biological sample analysis, different pretreatment strategies were investigated, both for serum and urine samples. Finally, the method was successfully applied to spiked and non-spiked commercial human serum samples and urine samples, after the application of the optimized pretreatment protocols.

Acknowledgements This work was supported by a grant of the Romanian Ministry of Education and Research, CNCS - UEFISCDI, project number PN-III-P1-1.1-TE-2019-1360, within PNCDI III. Alexandra Pusta acknowledges UMF grant no. 1032/57/13.01.2021.

1. Krause KM, Serio AW, Kane TR, Connolly LE. Aminoglycosides: An Overview. Cold Spring Harb Perspect Med. 2016 Jun 1;6(6):a027029.

S2-P-09

Applications of Conductive Electrospun Polymeric Fibers in DNA Biosensing

Anca Aldea, Elena Matei, Ionuț Enculescu, Victor C. Diculescu
National Institute of Materials Physics,
Atomiștilor 405A, 077125, Măgurele, Romania
anca.aldea@infim.ro

The sensitive and specific detection of analytes has a great significance in clinical diagnosis. Nanostructuring electrodes surface improves the sensing performance of the devices and facilitates the detection of analytes in specific environments [1,2]. An easy and low cost method to obtain nanostructured surfaces is by electrospinning. This technique is suitable for obtaining polymeric fibers, which can be easily coated with metal layers in order to obtain a conductive path.

Nucleic acid-based biosensors involve short, synthetic oligonucleotide (ODN) sequences which are ideal elements for determination of complementary sequences and genetic mutations.

This work describes the fabrication and characterization of poly-methyl methacrylate (PMMA) electrospun fibers coated with a gold layer and their applications in DNA biosensing, for the discrimination of specific mutations with high selectivity and sensitivity.

The adsorption of DNA oligonucleotides with sulfur substituents onto the Au-coated PMMA electrospun fibers was investigated by scanning electron microscopy (SEM), Raman spectroscopy, surface plasmon resonance (SPR), X-ray photoelectron spectroscopy (XPS) as well as electrochemical techniques. It has been shown that the immobilization procedure involves a strong interaction between the sulfur-containing groups and the gold surface, while the use of conductive electrospun structures drastically reduces the effects of non-specific adsorption and leads to high sensitivity of the biosensor, when compared to planar gold electrodes. In order to evaluated the selectivity of the biosensor, control experiments with non- complementary ODNs were performed.

References

[1] A. Aldea, E. Matei, R. J. B. Leote, I Rau, I. Enculescu, V.C. Diculescu Electrochim. Acta, 363, (2020), 137239

[2] A. Aldea, R.J.B. Leote, E. Matei, A. Evanghelidis, I. Enculescu, V.C. Diculescu, Microchemical Journal, 2021, 106108

Acknowledgements

Financial support from Romanian Minister of Scientific Research and Innovation through Operational Programme Competitiveness 2014-2020, Project: NANOBIOSURF-SMIS 103528

S2-P-10

Disposable SOD Biosensors based on Metallized Electrospun Polymeric Fibers for the Detection of Superoxide in Cell Culture Media

Caroline G. Sanz, Anca Aldea, Ricardo J.B. Leote, Mădălina M. Bârsan
National Institute of Materials Physics (NIMP)
Str. Atomiștilor 405A, Măgurele 077125, Romania
caroline.sanz@infim.ro

Superoxide ($O_2^{\bullet-}$) is one of the main reactive oxygen species present in biological systems, and critical $O_2^{\bullet-}$ levels, that increase due to a deficiency in antioxidant species, lead to oxidative damage and cell death. Superoxide dismutase (SOD) poses as an important part of the antioxidant defense towards the control of oxidative stress, given that it catalyzes the dismutation of $O_2^{\bullet-}$ to O_2 and H_2O_2 via a cyclic electron-transfer mechanism. In this work, disposable SOD biosensors were developed to monitor the concentration of $O_2^{\bullet-}$ in cell culture media. The biosensor was constructed using metallized electrospun polymeric fibers as support, viable alternatives in the construction of disposable biosensors given their low cost, whilst also presenting increased surface area, flexibility and biocompatibility to be employed in cell culture analysis. Cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and scanning electron microscopy (SEM) were primarily performed in order to characterize the surface of the metallized fiber support prior to the enzyme immobilization. Different immobilization techniques were conducted towards the construction of the biosensor, which included cross-linking, physical adsorption and covalent bonding on thiol self-assembled monolayers, previously deposited on the electrodes. Moreover, the incorporation of thin polymeric films was evaluated to increase the stability of the immobilized enzyme and mediate the electron transfer process between the enzyme and the electrode. The biosensors were characterized by CV, EIS and fixed potential amperometry in the presence of increasing concentration of $O_2^{\bullet-}$, whilst the analytical parameters were optimized in terms of applied potential and pH of the media. An interference study was performed to analyze the selectivity of the biosensor in complex matrices. Finally, the optimized SOD biosensor was employed in the determination of $O_2^{\bullet-}$ in cell culture media and the results will be discussed.

References

[1] Braik, B. Barsan, M.M. Dridi, C. Ben Ali, M. Brett, C.M.A. *Sensors Actuators, B Chem.* 236 (2016) 574–582.
[2] Balamurugan *et al*, *Biosens. Bioelectron.* 116 (2018) 89–99.

Acknowledgements

Financial support from UEFISCDI, PN-III-P1-1.1-TE-2019-0387.

S2-P-11

Aptamer Selection for Vancomycin Using Magnetic Beads-Based SELEX Technology

Geanina Ștefan[1,2], Oana Hosu[1], María Jesús Lobo-Castañón[2], Noemí de-los-Santos-Álvarez[2], Cecilia Cristea[1]
[1]*Department of Analytical Chemistry, Faculty of Pharmacy, "Iuliu Haţieganu" University of Medicine and Pharmacy, 4 Pasteur street, 400349 Cluj-Napoca, Romania*
[2]*Departamento de Química Física y Analítica, Universidad de Oviedo, Av. Julián Clavería 8, 33006 Oviedo, Spain*
geanina.stefan@umfcluj.ro

The emergence of resistance to most available antibiotics makes treatment a major clinical challenge. Vancomycin (VAN) is a glycopeptide antibiotic being one of the most common antimicrobials used worldwide; it can cause diverse mild side-effects as hypersensitivity reactions or severe reactions as systemic symptoms syndrome [1]. Hence, it is critical to accurately measure the concentration of VAN from biological and environmental samples, having the aim to improve the patient compliance to treatment and to overcome the multi-antibiotic resistance issue.
Innovation has brought new cutting-edge technologies that have been designed and used in diagnosis in the last recent years. Aptamers can be harnessed as diagnosis agents because of their recognition capacity and high affinity for target molecules. Aptamers are obtained via an *in vitro* chemical process named as systematic evolution of ligands by exponential enrichment (SELEX). The SELEX technology achieved high improvements the use of magnetic-beads for the aptamer-target molecule selection has speeded up the process and improved the key separation step. Owing to high affinity and specificity of aptamers, a variety of aptamer-based biosensors have been designed and applied for clinical and environmental biomarker detection [2].
Our current progress in the selection of a new aptamer for vancomycin selected through magnetic beads-based SELEX technology is presented in this communication. Further applications in the detection of vancomycin from biological samples and waste waters are envisioned.

References
[1] W.R. Miller et al., *Infect. Dis. Clin. North Am.*, **2020**, 34, 751-771
[2] A. D. Fernández et al., *Analytica Chimica Acta*, **2020**, 1124, 1-19

Acknowledgments
This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CCCDI-UEFISCDI, project number ERANET-RUS-PLUS-PLASMON-ELECTROLIGHT/46/2018, within PNCDI III. GS thanks UMF for the internal grant number 1033/63/13.01.2021. OH thanks to the Romanian Ministry of Education and Research, CNCS - UEFISCDI, project number PN-III-P1-1.1-PD-2019-0631, within PNCDI III.

S2-P-12

Electrochemical Detection of Enterobactin as a Biomarker for *Escherichia coli* with a Hydrogel and Nanoparticle Layer-based Sensor

Alexandra Canciu, Andreea Cernat, Mihaela Tertiș, Cecilia Cristea
Analytical Chemistry Department, Faculty of Pharmacy,
"Iuliu Haţieganu" University of Medicine and Pharmacy
4 Louis Pasteur St, Cluj-Napoca, Cluj, Romania
alexandra.canciu@elearn.umfcluj.ro

Pathogenic strains of *Escherichia coli (E. coli)* are a major threat to human health causing foodborne and waterborne diseases from gastrointestinal symptoms and diarrhea to even fatal hemolytic uremic syndrome. Standard detection techniques such as plate culture, polymerase chain reaction, enzyme-linked immunosorbent assay, are often laborious and require extensive assay time or experienced personnel, making them inconvenient for testing in various on-field settings or in cases of outbreaks when a quick response is needed; hence, the necessity for rapid, reliable and portable detection tools of this contaminant microorganism.
Electrochemical sensors have gained considerable attention lately due to their sensitivity, versatility, low cost and miniaturization capability (1). One such approach is the detection of bacteria through their specific virulence markers (2). Enterobactin (also known as enterochelin) is the principal siderophore secreted by *E. coli*, important in bacterial growth, replication and infection process.
In this study, the electrochemical fingerprint of enterobactin on commercially available graphite screen-printed electrodes was achieved. The modified electrodes with a hybrid layer consisting of agar hydrogel and Au/Ag nanoparticles were successfully employed for the quantification of enterobactin from standard solutions, as well as from lysogeny broth (Miller). Given the short assay time, this method can be further developed for fast and accurate detection of *E. coli* in industrial and environmental applications.

Acknowledgements This study was supported by the European Union's Horizon 2020 research and innovation programme under grant agreement No 883484, PathoCERT.

1. Razmi N, Hasanzadeh M, Willander M, Nur O. Biosensors. 2020;10(5):54.
2. Cernat A, Tertis M, Gandouzi I, Bakhrouf A, Suciu M, Cristea C. Electrochem commun. 2018;88:5–9.

S2-P-13

Controlled Immobilization of Enzymes in Biocatalytic Reactors

Simin Arshi, Edmond Magner
Department of Chemical Sciences and Bernal Institute, University of Limerick, V94 T9PX Limerick, Ireland.
simin.arshi@ul.ie

The controllable positioning of enzymes in a flow reactor plays an essential role in the performance of enzyme cascade reactions (1). The use of electrodes as enzyme supports enables the stable immobilization of enzymes at defined positions and over defined areas (2). Such an approach can be used to pattern enzymes in a sequential manner and opens the way to developing biocatalytic cascade systems. The immobilization of redox enzymes such as glucose oxidase (GOx) can be used to deliver the potent oxidant, H_2O_2, in a controlled manner and can be utilized as a step in an enzyme cascade. A range of immobilization methods have been screened to ascertain the optimal system for the immobilization of glucose oxidase. These methods include the direct coupling of the enzyme to the electrode using a diazonium linker, immobilization in silica layers, and immobilization in polymer films. Immobilized GOx produced hydrogen peroxide in a stable manner, under several hours of continuous operation. The rate of production of hydrogen peroxide differed and depended on the method of immobilization. The production of H_2O_2 at steady-state concentrations was achieved and then was incorporated into a cascade reaction in a flow reactor.

1. Wu Z-Q, Li Z-Q, Li J-Y, Gu J, Xia X-H. Contribution of convection and diffusion to the cascade reaction kinetics of β-galactosidase/glucose oxidase confined in a microchannel. Physical Chemistry Chemical Physics. 2016;18(21):14460-5.
2. Xiao X, Siepenkoetter T, Whelan R, Salaj-Kosla U, Magner E. A continuous fluidic bioreactor utilising electrodeposited silica for lipase immobilisation onto nanoporous gold. Journal of Electroanalytical Chemistry. 2018;812:180-5.

S2-P-14

Gemcitabine Electrochemical Direct Detection from Serum and Pharmaceutical Formulations using Boron Doped Diamond Electrode

Iulia Rus, Alexandra Pusta, Mihaela Tertiș, Robert Săndulescu, Cecilia Cristea
Department of Analytical Chemistry and Instrumental Analysis, Faculty of Pharmacy, "Iuliu Haţieganu" University of Medicine and Pharmacy, 4 Louis Pasteur, 400349, Romania
rus.iulia@umfcluj.ro

Gemcitabine is an antitumor drug which acts as a nucleoside analogue, being incorporated in DNA instead of cytidine by DNA-polymerases and stopping thus DNA synthesis. It is a widely used antitumor drug, very effective in pancreatic, ovarian, breast and non-small cell lung cancer (1). The purpose of this study was to develop a simple and fast electrochemical strategy for the detection of gemcitabine from pharmaceutical formulations as well as human serum.
An electrochemical characterization of gemcitabine was performed, regarding the influence of the electrode material, electrolyte solution, pH and scan rate on the detection of the target molecule. Based on the results obtained, a DPV electrochemical procedure was optimized, calibration curves were built and tests on real samples were performed.
Different electrode materials were tested, like graphite, gold and platinum based screen printed electrode, glassy carbon electrode and boron doped diamond electrode (BDDE). An analytical signal was observed for the oxidation of gemcitabine only on BDDE, at a potential around 2 V. The results showed that the intensity of the analytical signal of gemcitabine is the highest at pH 5 and at higher values of scan rate. However, further tests were done at pH 7.4, simulating the media of the real samples. The tests performed on real samples of gemcitabine solution for infusion and on spiked serum showed good recovery.
To conclude, a new successful electrochemical method for the detection of gemcitabine from various samples was developed using BDDE.

References

1. Lucas de Sousa Cavalcante, Gisele Monteiro. Gemcitabine: Metabolism and molecular mechanisms of action, sensitivity and chemoresistance in pancreatic cancer. *European Journal of Pharmacology* 741 (2014), 8-16. https://doi.org/10.1016/j.ejphar.2014.07.041

Acknowledgements This work was supported by a grant of the Romanian Minister of Research and Innovation, CCCDI – UEFISCDI, project number PNIII-P1-1.2-PCCDI-2017-0221/59PCCDI/2018 (IMPROVE), within PNCDI III. Iulia Rus acknowledges UMF Grant no. 1034/5/13.01.2021.

S2-P-15

An Os polymer and Galactose Oxidase Modified Mesoporous Gold Biosensor for the Determination of Galactose

Denise Demurtas, Edmond Magner
Department of Chemical Sciences and Bernal Institute, University of Limerick, V94 T9PX Limerick, Ireland; denise.demurtas@ul.ie

Among the materials employed for implantable electrochemical biosensors, gold has always been very attractive due to its high biocompatibility, chemical stability and good electrical conductivity. In comparison to polycrystalline gold (PG), mesoporous gold (MPG), possesses a much higher surface-to-volume ratio that can accommodate higher enzyme loadings. The presence of pores protects the enzyme from the bulk solution, limiting interference effects and protecting the enzyme from denaturation [1]. Galactose oxidase is a Cu metalloenzyme that catalyzes primary alcohols oxidation such as D-galactose. Its catalytic activity can be detected using electrochemical methods through mediators. A Os polymer was chosen to improve the electron transfer (ET) from the enzyme towards the electrodes.
We prepared mesoporous (MPG) electrodes and compared them to planar gold (PG) electrodes as supports for *Dactylium dendroides* galactose oxidase (E.C. 1.1.3.9). A solution containing the enzyme, $Os[(bpy)_2PVI]Cl^+$ and the cross-linking agent poly(ethylene glycol)diglycidyl ether (PEDGE) was drop cast onto the surface of the electrode and allowed to dry. The concentrations of Os polymer and enzyme were varied using the ratios: 1:1, 3:1 and 1:2. The response on MPG with varying pore sizes (15, 40 and 78 nm) was obtained [2]. The optimal response was obtained with a ratio (by mass) of Os polymer:enzyme of 1:1 and an average pore size of 78 nm. A detailed comparison of the response of the sensor for the determination of galactose was performed.

1. Bollella, P., Porous Gold: A New Frontier for Enzyme-Based Electrodes. *Nanomaterials (Basel)* **2020,** *10* (4)
2. Siepenkoetter, T.; Salaj-Kosla, U.; Xiao, X. X.; Belochapkine, S.; Magner, E., Nanoporous Gold Electrodes with Tuneable Pore Sizes for Bioelectrochemical Applications. *Electroanalysis* **2016,** *28* (10), 2415-2423

S2-P-16

Flexible Electrodes as Platforms for Bio(sensors) for Biomarker Monitoring in Sweat

Ricardo J. B. Leote[1,2], Anca Aldea[1], Elena Matei[1], Victor C. Diculescu[1]
[1]*National Institute of Materials Physics Atomiştilor 405A, Măgurele, Romania*
[2]*Faculty of Physics, University of Bucharest, Atomiştilor 405, Măgurele Romania*
ricardo.leote@infim.ro

A new approach is proposed for developing flexible electrodes based on metalized electrospun polymeric fibers for development of sensors and biosensors for detection of biomarkers in biological fluids especially sweat. The procedure involves the electrospinning of poly(methacrylate) (PMMA) submicronic fibers and subsequent metallization with gold by magnetron sputtering. Then, the gold coated fibers were transferred on poly(ethyleneterephtalate) (PET) foils in order to obtain the Au/PMMA/PET electrodes.
Au/PMMA/PET and Pd/PdO/Au/PMMA/PET electrodes were produced and characterized morphologically by scanning electronic microscopy (SEM) with energy dispersive X-ray spectroscopy (EDX), their crystalline structure was determined by X-ray diffraction (XRD) and the chemical composition and oxidation states of metals determined by X-ray photoelectron spectroscopy (XPS) [1]. Predominant electrochemical processes were characterized by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) [2-3], showing a low resistance to charge transfer reaction and, at the same time, high capacitance values.
The practical applicability of the Au/PMMA/PET and Pd/PdO/Au/PMMA/PET electrodes was tested for: i) potentiometric detection of H^+, Cl^-, Ca^{2+}, NH_4^+ through immobilized ionophores; ii) amperometric detection of H_2O_2 and iii) amperometric detection of glucose and uric acid through immobilized glucose oxidase (Gox) and uricase (Urox). All analytical parameters were calculated and the determination of these biomarkers was carried out in artificial sweat and blood serum samples.

[1] V. C. Diculescu, M. Beregoi, A. Evanghelidis, R. F. Negrea, Nicoleta G. Apostol, I. Enculescu, Sci Rep, 9, (2019), 8902
[2] A. Aldea, E. Matei, R. J. B. Leote, I Rau, I. Enculescu, V. C. Diculescu Electrochim. Acta, 363, (2020), 137239
[3] A. Aldea, R. J. B. Leote, E. Matei, A. Evanghelidis, I. Enculescu, V. C. Diculescu, Microchem. J. (2021), 106108

Acknowledgements
Financial support from the Romanian Ministry of Research and Innovation through Operational Program Competitiveness 2014-2020, Project: NANOBIOSURF SMIS 103528

S2-P-17

Theoretical Studies Using Quantum Mechanical Calculations for 1,3,4-Thiadiazole Derivatives with Electrochemical Applications

Alina-Alexandra Vasile (Corbei)[1], Veronica Anastasoaie[1], Mihaela Cristea[2], Eleonora-Mihaela Ungureanu[1], Amalia Ștefaniu[3*]

[1]*"Politehnica" University of Bucharest, Gheorghe Polizu 1-7, 011061, Sector 1, Bucharest, Romania*

[2]*Institut of Organic Chemistry "C. D. Nenitzescu "of the Romanian Academy, Spl. Independentei 202B, PO Box 15-258, 71141-Bucharest, Romania*

[3]*National Institute for Chemical-Pharmaceutical Research and Development, 112 Vitan av. Bucharest, Romania,*

astefaniu@gmail.com

Structural theoretical insights on 2-(azulen-1-yldiazenyl)-5-(methylsulfonyl)-1,3,4-thiadiazole and 2-(azulen-1-yldiazenyl)-5-(methylsulfinyl)-1,3,4-thiadiazole were obtained form quantum mechanical calculations using density functional theory (DFT) method at B3LYP level 6-31+ G(d,p) basis sets [1] in ground state with Spartan Software [2].

Detailed electronic properties and key molecular descriptors are resulted and used to evaluate the potential use of compounds under study as ligands for heavy metal recognition. Frontier molecular orbitals and molecular electrostatic potential map (EPM) representation derived from electrical charge distribution of the investigated structures, are used to predict global reactivity [3, 4] and the probable nucleophilic and electrophilic areas, respectively. The higher negative region (red) was observed on the sulphonyl and sulfinyl groups and on the N-N atoms of the thiadiazole ring, which is related with probable position for an electrophilic attack. This analysis along with global quantum reactivity parameters results, provide valuable information to forward investigations on the potential application as sensor for heavy metal ions recognition.

References

[1] C. Lee, W. Yang, R.G. Parr, Physical Review B 37 (1988) 785–789

[2] W.J. Hehre, A Guide to Molecular Mechanics and Quantum Chemical Calculations; Wavefunction, Inc.: Irvine, CA, USA, 2003

[3] R. Yankova, S. Genieva, N. Halachev, G. Dimitrova, Journal of Molecular Structure 1106 (2016) 82-88

[4] V.S. Sastri, J. R. Perumareddi, Corrosion Science 53 (1997) 617-622

S2-P-18

Generation of a Chimeric Oxidoreductase Capable of Direct Electron Transfer

Marie C. Viehauser, Roland Ludwig,
Biocatalysis and Biosensing Laboratory, Department of Food Science and Technology, BOKU – University of Natural Resources and Life Sciences, Vienna, Austria
marie.viehauser@boku.ac.at

Since Leland C. Clark invented the biosensor concept in 1962, many attempts have been made to improve their performance. The most critical factor in generating efficient bioelectrocatalysts is the electrical coupling of the enzymatic activity to the transducing electrode. Enzymes capable of direct electron transfer (DET) are independent of redox mediators and represent therefore a promising tool for bioelectrocatalytic oxidation and reduction reactions. However, most enzymes that are currently used in biosensors are not able to perform direct electron transfer. Cellobiose dehydrogenase is an external fungal flavocytochrome and one of the few enzymes capable of DET in its native form. It consists of a catalytically active dehydrogenase (DH) domain connected to a b-type cytochrome domain (CYT) by a flexible linker. The DH domain belongs to the glucose-methanol-choline (GMC-) oxidoreductases, which share a conserved structure around the FAD-cofactor.

One method of generating novel biocatalysts with DET functionality is fusing the enzyme to a cytochrome as it occurs naturally in cellobiose dehydrogenase (CDH). CDH consists of a catalytically active dehydrogenase (DH) domain connected to a b-type cytochrome domain (CYT) by a flexible linker. In this study we used glucose dehydrogenase from *Glomerella cingulata* (*Gc*GDH) to fuse it with the CYT domain (CYT) and the linker from *Neurospora crassa* CDH IIA, generating a chimeric enzyme (*Nc*IIA_*Gc*GDH) with a built-in redox mediator for direct bioelectrocatalysis. The chimeric enzyme was recombinantly produced in Pichia pastoris, purified and characterized with biochemical and electrochemical methods. The data confirmed that the chimeric enzyme is able to perform direct electron transfer via the fused cytochrome domain. The presented results also provide a basis for optimizing direct electron transfer rates and for maximizing the performance of *Nc*IIA_*Gc*GDH in bioelectrocatalytic processes.

S2-P-19

Estimation of Chemical Reactivity Parameters through DFT Investigations on 2-thioxo-thiazolidin-4-one Derivatives

Ovidiu-Teodor Matica[1], Eleonora-Mihaela Ungureanu[1], Mihaela Cristea[2], Lucia Pintilie[3], Amalia Ștefaniu[3*]

[1]*"Politehnica" University of Bucharest, Gheorghe Polizu 1-7, 011061, Sector 1, Bucharest, Romania*

[2]*Institut of Organic Chemistry "C. D. Nenitzescu "of the Romanian Academy, Splaiul Independenței 202B, PO Box 15-258, 71141-Bucharest, Romania*

[3]*National Institute for Chemical-Pharmaceutical Research and Development, 112 Vitan av. Bucharest, Romania,*

astefaniu@gmail.com

Density functional theory based function B3LYP with 6-31 +G(d,P) basis set [1, 2] was employed to perform predictive computations on three 2-thioxo-thiazolidin-4-one derivatives (R1-R3) for estimation of their electrochemical properties. The calculations are done on the optimized structures of compounds, as shown in Figure 1.

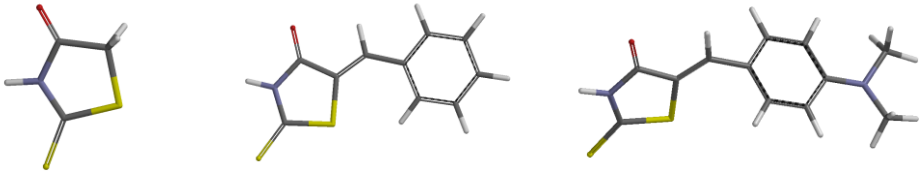


Fig. 1. R1 R2 R3

Geometrical parameters (bond lengths, angles and torsion angles) and molecular electronic properties are reported. Frontier molecular orbitals energies are used to obtain global reactivity parameters related with kinetic stability of the investigated structures. The variation of resulted quantum parameters (molecular frontier energy gap, ionization potential, electron affinity, electronegativity, chemical potential, electrophilicity index) is correlated with the polarity of the compounds and their electrochemical behavior.

References

[1] C. Lee, W. Yang, R.G. Parr, Physical Review B, 37, 785–789, 1988

[2] W.J. Hehre, A Guide to Molecular Mechanics and Quantum Chemical Calculations; Wavefunction, Inc.: Irvine, CA, USA, 2003

S2-P-20

New perspective of Biologically Active Compounds and Thiocompounds Conjugated with Gold Nanoparticles for Nanotechnology Applications

Krzysztof Stolarczyk[a], Elżbieta U. Stolarczyk[b], Marta Łaszcz[b], Andrzej Leś[a], Marek Kubiszewski[b], Katarzyna Sidoryk[b]
[a] *University of Warsaw, Faculty of Chemistry, 1 Pasteura, 02-093 Warsaw, Poland*
[b] *Łukasiewicz Research Network - Pharmaceutical Research Institute, 8 Rydygiera, 01-793 Warsaw, Poland*

Sulfur-containing compounds have been used as excellent ligands for binding to gold-nanopraticles (AuNPs), because of a very strong interaction of sulfur nucleophiles with gold [1,2]. The use of thiol groups as a linker of biologically active compounds to gold nanoparticles is well known and established. Our new approach involves exploring a possible interaction of well-known biologically active compounds with gold nanoparticles to improve therapeutic efficiency of drugs, maintaining their bioavailability and biological activity. In our research, we have developed an innovative method of conjugation of genistein (GE) active substance with gold nanoparticles to obtain a unique nanoconjugate (AuNPs-GE) with the expected anti-cancer properties for biomedical applications [3]. A new form of genistein conjugated with gold nanoparticles (AuNPs-GE) was characterised by a higher level of cytotoxicity compared with a free genistein. This encourages us to intensify studies on AuNPs-GE as a candidate for enhancing the anticancer effect of genistein. To increase the strength of the interaction, and improve the stability of AuNPs-GE conjugates, we have focused on the investigation of an optimal thiol linker as new derivatives - thiolated genistein (HS-GE). We have created an HS-GE monolayer on the gold surface. We have also prepared gold electrodes modified with 1,9-nonanodithiol and gold nanoparticles modified with HS-GE, which was confirmed by the Scanning Electron Microscope (SEM). Voltammetric experiments have confirmed the adsorption of thiogenistein on the gold electrode as well as the immobilization of thiogenistein on gold nanoparticles adsorbed on the gold electrode modified with 1,9-nonanodithiol.

References

1. E.U. Stolarczyk, K. Sidoryk, M. Cybulski, M. Kubiszewski, K. Stolarczyk, Nanomaterials 8 (2018) 1018
2. K. Sidoryk, O. Michalak, M. Kubiszewski, A. Leś, M. Cybulski, E. U. Stolarczyk, J. Doubsky, Molecules 25 (2020) 3470
3. E.U. Stolarczyk, K. Stolarczyk, M. Łaszcz, M. Kubiszewski, W. Maruszak, W. Olejarz, D. Bryk, Eur. J. Pharm. Scien. 96 (2017) 176

S2-P-21

Sensitive Determination of Antidepressant Citalopram with MOF-based Voltammetric Sensor

Maria Madej*, Dariusz Matoga*, Klaudia Skaźnik*, Radosław Porada**, Bogusław Baś**, Jolanta Kochana*
**Jagiellonian University, Faculty of Chemistry, Gronostajowa 2, 30-387, Kraków, Poland*
***AGH University of Science and Technology, Faculty of Materials and Ceramics, A. Mickiewicza 30, 30-059, Kraków, Poland*
marysia.madej@doctoral.uj.edu.pl

Depression is a serious, recurrent mood disorder that affects more than 320 million people worldwide and significantly impairs an individual's ability to function and cope with daily life. According to World Health Organization, depression is ranked as the largest contributor to global disability and major factor in suicide deaths, with almost 800,000 cases per year [1]. Citalopram (CIT), a selective serotonin-reuptake inhibitors representative, exhibits a broad spectrum of therapeutic activity against depressive disorders [2]. As a tertiary amine derivative, citalopram undergoes the irreversible electrooxidation, thus the application of voltammetric sensors for CIT determination could be very promising due to their high sensitivity, simplicity, rapidity and low cost of the apparatus [3].

The main aim of this work was to develop a voltammetric sensor intended for citalopram determination, based on isonicotinate manganese(II) framework (JUK-2) – a two-dimensional metal-organic framework with high proton conductivity. For this purpose, the composition of nanocomposite, as well as experimental conditions were optimized. The JUK-2-MWCNTs-AuNPs/GCE showed a linear response in three CIT concentration ranges, 0.05 – 1.0, 1.0 – 10.0 and 15.0 – 115.0 μmol L^{-1}, with a detection limit of 0.011 μmol L^{-1}. The fabricated sensor was successfully employed for the determination of CIT in synthetic urine and blood samples, as well as pharmaceuticals, with satisfactory recoveries (98.6 – 104.8%).

Acknowledgements
The study was supported by the EU Project POWR.03.02.00-00-I004/16 and by the National Science Centre (Poland) grant no. 2019/35/B/ST5/01067.

References
[1] Depression and Other Common Mental Disorders, Global Health Estimates, World Health Organization, Geneva, 2017
[2] A.F. Joubert et al. Hum Psychopharmacol Clin Exp 15 (2000) 439–451
[3] M. Madej et al. J Electrochem Soc 166 (2019) H359–H369

S2-P-22

Using Robot Mechanics for Virutal Screening of Apelin Receptor as an Alzheimer Target

Laurentiu Spiridon[1], Laura Manoliu[1], Eliza Martin[1], Adina Milac[1†]
[1]*Institute of Biochemistry of the Romanian Academy*
296 Splaiul Independentei, sector 6, Bucharest, Romania, 060031
ls@biochim.ro

Alzheimer's is a neuro-degenerative condition affecting 50 million worldwide and is the leading cause of dementia in elderly. Its treatment is still elusive suggesting the need for novel therapeutic tactics. One of the potential new drug targets is apelin receptor (APJ), a G-protein coupled receptor which binds endogenous apelin peptide, observed to have increased expression in patients with Alzheimer, Huntington's and Parkinson's diseases. Although high-throughput screening procedures are well-established they are time-consuming and cost ineffective rendering virtual screening as an important step in drug design pipelines.

To better understand the APJ ligand binding mechanism, this study used a gradual robot mechanics Brownian to detailed molecular dynamics transition engaging recently developed software Robosample [Spiridon et al, 2020]. Starting robot topology comprised quternion-based Ball joints in protein coil regions. Representative conformers from resultant atomistic simulations were retained for an ensemble docking experiment.

The ligand library was assembled from 8 known AR agonists from published data with the addition of 3000 compounds added by chemical similarity. The library was further augmented with 2237 molecules identified through ligand-based pharmacophore modelling and 2978 FDA approved compounds.

Rigid body constrained dynamics showed at least two anchor points for endogenus ligand binding which were further used for both binding site assessment and ligand property analysis. Enhanced ensemble based virtual screening revealed additional candidates without altering the efficiency and pose prediction resulted in larger ensembles of poses.

We have shown that molecular detail recursivity in docking simulations may improve ensemble docking techniques against APJ because it leads to mechanistic details that are not available in the two aforementioned regimes separately. Anchor points found with such hybrid molecular simulations present themselves into an excellent strategy for pharmacophore modeling in our subsequent studies.

References

Spiridon L, Șulea TA, Minh DDL, Petrescu AJ. Robosample: "*Robosample: A rigid-body molecular simulation program based on robot mechanics.*" Biochim Biophys Acta Gen Subj**.** 2020 Aug;1864(8):129616.

S2-P-23

Electrochemical Detection of miRNA

Marta Jarczewska, Wiktor Bojarski, Magdalena Mieczkowska, Aleksandra Majewska, Elżbieta Malinowska
The Chair of Medical Biotechnology, Faculty of Chemistry, Warsaw University of Technology, Noakowskiego 3. 00-664 Warsaw, Poland
mjarczewska@ch.pw.edu.pl

miRNA are molecules that are known as single-stranded RNA sequences that usually consist of approximately 18-24 nucleotides that are present in different organisms such as plants, animals and viruses [1]. miRNA are responsible for posttranscriptional regulation of gene expression and it is shown that over 60% of genes that encode proteins are regulated by miRNA. miRNAs are engaged in different processes such as differentiation, proliferation and apoptosis. miRNA are considered as potential biomarkers as their abnormal concentration can be linked to the development of cancer, cardiovascular or neurodegenerative diseases. Various techniques are applied for miRNA detection including real-time PCR, northern blotting and microarray assays. An attractive alternative can be the application of electrochemical methods due to their low detection limits, simplicity of operation and possibility of miniaturization.

Herein, we present the results on the studies on the application of single-stranded unlabeled and labeled oligonucleotide probes as well as PNA strands as receptor layers for detection of miRNA sequences. The research was focused on the optimization of immobilization protocol as well as the choice of proper redox indicator that would allow for determination of miRNA strands. In the studies various detection techniques including cyclic and square-wave voltammetry as well as impedance spectroscopy.

[1] K. Chaudhuri and R. Chatterjee, DNA Cell Biol., 2007, 26, 321–337
[2] C. Chen, D.A. Ridzon, A.J. Broomer, Z. Zhou, D.H. Lee, J.T. Nguyen, et al., Nucleic Acids Res., 2005, 33, 1-9

S2-P-24

Enhancement of the Tetracycline Detection Process by using an Aptasensor Modified with Gold Clusters

Marcin Jaskółowski and Agnieszka Więckowska

University of Warsaw, Faculty of Chemistry
1 Pasteur Str., 02-093 Warsaw, Poland
m.jaskolowski2@uw.edu.pl

In recent years, biosensors that consist of biological recognition system - receptor and a physical transducer have gained great popularity. They have been widely used in monitoring biological and synthetic processes in the field of food analysis and in the area of human monitoring and diagnostics [1].
DNA biosensors, whose recognition element consists of oligonucleotide strands, are especially popular due to its excellent stability and the possibility of multiple uses [2].
In the project we present a study on detection of tetracycline, using a gold-based DNA biosensor with gold clusters attached to oligonucleotides. The used oligonucleotides are modified with a short thiol which allows for the formation of self-assembling monolayers on gold transducers [3].
ssDNA used to construct the sensor is also modified with amino group which can bind gold clusters - small nanoparticles that are capable of exchanging electrons with the electrode as a molecular redox probe. We used pulse voltametric methods to detect the characteristic signals corresponding to the redox processes of gold clusters and confirm their presence near the electrode. DNA strand, when interacting with the tetracycline molecule, can change its conformation, which may lead to the cluster approaching closer to the gold surface. Electrochemical impedance spectroscopy was employed to characterize biological monolayer adsorbed on the gold electrode as well as detect the antibiotic. In the presence of tetracycline in solution, resistance of the electron transfer between the solution and the electron surface changes, allowing for determination tetracycline concentration.

References

1. V. Kavita, *J Bioengineer & Biomedical Sci.*, 2017, 7
2. N. Prabhakar, K. Arora, S.P. Singh, H. Singh, B.D. Malhotra, *Anal. Biochem.*, 2007, 366, 71
3. H.C.M. Yau, H.L. Chan, M. Yang, *Biosens. Bioelectron.*, 2003, 18, 873

S2-P-25

Novel Nanobodies for the Differentation of Viral from Bacterial Infection

Justyna Borkowska[1], Sandra Oloketuyi[2], Roberto Bernedo[2], Andreas Christmann[3], Giulia Cazzaniga[2], Horst Wilhelm Schuchmann[3], Harald Kolmar[3], Ario de Marco[2], Joanna Niedziółka-Jönsson[1], Katarzyna Szot-Karpińska[1]
[1]*Institute of Physical Chemistry - Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland,*
[2]*Laboratory for Environmental and Life Sciences – University of Nova Gorica, Vipavska cesta 13, SI-5000 Nova Gorica, Slovenia*
[3]*Applied Biochemistry - Technical University of Darmstadt, Karolinenplatz 5, 64289 Darmstadt, Germany*
jborkowska357@gmail.com

Nanobodies are a novel class of antigen-binding fragments and so recognized as new biosensing elements. They were designed based on heavy-chain antibodies (HCAbs) that naturally occurred in camelid's serum. HCAbs lack light chains and consist of a single variable chain (V_HH) and two constant domains. The isolated V_HH domain is a stable polypeptide with MW of about 12-15kDa. Nanobodies give a lot of opportunities for bio drugs development because of their advantages, e.g. small size, simple structure, high stability, water solubility and strong antigen-binding [1]. Novel nanobodies that bind CRP protein may be an alternative to antibodies and provide the core for constructing new, low-cost, stable and sensitive sensing platforms.
In our research, we have tested the nanobodies selected by phage display technology against CRP using bio-layer interferometry (BLI) and electrochemistry [2]. BLI method was used to characterize CRP-nanobody interactions. We obtained dissociation constants, considered them with statistical parameters and compared them with the result obtained for anti-CRP monoclonal antibodies. In the electrochemical studies, we immobilized nanobodies on ITO electrode and tested immunological recognition of CRP. A combination of results may help understand nanobodies nature and develop a new sensing platform, which will be alternative for existing biosensors.

[1] E. Pardon, T. Laeremans, S. Triest et al. *Nat Protoc*, 9, (2014) 674–693.
[2] S. Oloketuyi, R. Bernedo, A. Christmann, J. Borkowska, G. Cazzaniga, H. W. Schuchmann, J. Niedziółka-Jönsson, K. Szot-Karpińska, H. Kolmar, A. de Marco *Anal. Bioanal. Chem.* (under review 2021)

Acknowledgements
This work was funded by the Polish National Science Centre under the SONATA 13 grant UMO 2017/26/D/ST5/00980 to Dr Katarzyna Szot-Karpińska

S2-P-26

Flow Injection Amperometry as an Alternative to Potentiometry for Solid Contact Ion-Selective Membrane-Based Electrodes

Maksimova E.D., Nikitina V.N., Karyakin A.A.
Department of Chemistry, M. V. Lomonosov Moscow State University,
119991, Moscow, GSP-1, 1-3, Leninskiye Gory, Russia
ekaterina.maksimova@chemistry.msu.ru

Determination of electrolytes in physiological fluids is an important analysis in medical diagnostics. The classical potentiometry with the ion-selective electrodes is used in laboratory analyzers to determine ions. However, due to the complexity of their design, such sensors are not suitable, for example, in compact devices for personal use. Thus, the elaboration of ion-selective electrodes (ISEs) with solid contact instead of internal standard solution is promising. To solve the problem of low background signal stability and long response time of solid contact ISEs, an alternative readout principle: flow injection amperometry at constant potential, is proposed.
We've investigated the origin of amperometric signal generated by conducting polymers in flow injection mode depending on the doping ion. In this work the solid contact K^+ and Na^+SEs were based on poly(ethylene-3,4-dioxythiophene) covered with ion-selective membrane via spin-coating technique. We've shown that flow injection amperometry provides the similar to potentiometry selectivity and improved sensitivity. Moreover, flow injection amperometry in comparison to potentiometry for ion-selective electrodes provides a shorter response times, within 15–30 s, by two orders of magnitude decreased detection limit and 5-6 fold increased signal-to-noise ratio [1]. The analysis of mixed K^+ and Na^+ solutions revealed the possibility to distinguish the abnormal levels of potassium in the presence of physiological sodium fluctuations. Thus, the proposed approach opens new horizons for the improvement of performance characteristics of solid contact ISEs and applicable for express diagnostics of cardiovascular diseases.

The grant № 19-13-00131 of Russian Science Foundation is greatly acknowledged.

1. V.N. Nikitina, E.D. Maksimova, M.D. Zavolskova, A.A. Karyakin, Electrochim. Acta, 2021, 377, 138074.

S2-P-27

Oligonucleotides Monolayers – Comparison Of The Method of Attachment to the Substrate

Marcin Jaskółowski and Agnieszka Więckowska
University of Warsaw, Faculty of Chemistry
1 Pasteur Street, 02-093 Warsaw, Poland
am.wieckowska@uw.edu.pl

Self-assembly process of thiol derivatives on gold substrate is very convenient method for immobilization of compounds of biological importance [1]. Thiol modified oligonucleotides as a recognition element in biosensors are used to create well-organized receptor layers. Among different types of DNA layer preparation this one, employing S-Au bond ensure reproducibility and high values of surface density of stands on the sensor surface [2].
In the project we present a study comparing the properties of aptamer layers on gold electrode or SPR sensor depending on the method of modifying the DNA strand with appropriate binding groups. In order to form a self-assembly monolayer on gold, oligonucleotides are modified with short thiols at one end to form a covalent bond with gold surface. We used the same aptamer modified in three different ways: the aptamer modified with C_3-thiol on 3' end, or with C_6-thiol on 3' or modified with C_6-thiol on the 5' end of oligonucleotides strand. Depending on the type of modification of single-stranded DNA molecules, the monolayers of these molecules showed different degree of organization. We used electrochemical and optical techniques to characterize aptamer layers on gold surface.

References

1. J.C. Love, L.A. Estroff, J.K. Kriebel, R.G. Nuzzo, G.M. Whitesides; *Chem.Rev.*, 2005, 105, 1103
2. L.I. Hernandez, I. Machado, T. Schäfer, F.J. Hernandez; *Curr. Top. Med. Chem.*, 2015, 15, 1066

S2-P-28

Accurate and Rapid Sensor based on Polydopamine Nanomaterial

Artur Jędrzak, Maria Kuznowicz, Teofil Jesionowski
Chemical Technology and Engineering Institute, Faculty of Chemical Technology, Poznan University of Technology, Berdychowo 4, PL-60965 Poznan, Poland
artur.jedrzak@put.poznan.pl

The aim of sensing technology is to change the way we calculate essential parameters in diagnostics, environmental monitoring, safety, and security. Nanostructures have special physicochemical properties that cannot be found in bulk materials. Over the last few decades, nanomaterials have been actively explored and applied as the base of advantageous sensing applications [1, 2].
The deposition of PDA films from aqueous solutions is a novel and versatile method of surface functionalization, and the films are generally considered to be robust, non-toxic, inert, and biocompatible. Polydopamine's properties, which include exceptional adhesion, ease of functionalization and coating, good photothermal properties, and the fact that it is biocompatible, biodegradable, and has suitable mechanical properties, have led to a wide range of applications [3].
The manner in wich polydopamine can be combined with nanomaterials, specifically magnetite (Fe_3O_4), to create a sensitive and selective platform for bio-sensing applications is shown. The fabrication of Fe_3O_4@PDA was achieved using a simple one-step process and glucose oxidase from *Aspergillus niger* was immobilized by adsorption. A biosensor based on a glassy carbon electrode was achived and optimized using cyclic voltammetry and chronoamperometry. Finally, a series of physicochemical analyzes were performed to accurately characterize the obtained systems (TEM, FT-IR, Bradford, PdI, Zeta, NIBS). Aalytical parameters of the sensor (sensitivity, LOD, selectivity, stability over time), characteristics and properties of the created nanoplatform were determined.

This work was financed and prepared as part of research project supported by the National Science Centre Poland, no. 2017/27/B/ST8/01506.

References

[1] A. Hrioua, A. Loudiki, A. Farahi, M. Bakasse, S. Lahrich, S. Saqrane, M.A. El Mhammedi, Bioelectrochemistry, 137 (2021) 107687
[2] H. Liu, Z. Zhang, Y. Xu, X. Tan, Z. Yue, K. Ma, Y. Wang, Bioelectrochemistry 137 (2020) 107675
[3] A. Jędrzak , T. Rębiś, M. Nowicki, K. Synoradzki, R. Mrówczyński, T. Jesionowski, Appl. Surf. Sci. 455 (2018) 455–464

S3 Pulsed electric and magnetic fields in biology, medicine and biotechnology

S3-P-01

Calcium Electroporation Stimulates ROS Release and Alternates ASPH Expression in Human Colon cancer

Anna Szewczyk[1,2], Nina Rembiałkowska[1], Anna Choromańska[1], Katarzyna Bieżuńska-Kusiak[1], Jolanta Saczko[1], Julita Kulbacka[1]
[1]*Department of Molecular and Cellular Biology, Faculty of Pharmacy, Wroclaw Medical University, 50-556 Wroclaw, Poland*
[2]*Department of General Zoology, University of Wroclaw, Sienkiewicza 21 St., 50-335 Wroclaw*
a.szewczyk@umed.wroc.pl

Rapid influx of calcium ions into the cell cytosol caused by electroporation application disturbs calcium homeostasis, which leads to mitochondrion overloading, ATP depletion and cell death. Our studies focused on effects of calcium electroporation – CaEP (micro- and nanosecond) on reactive oxygen species (ROS) production and modulation of expression of aspartate-β-hydroxylase (ASPH) transmembrane protein involved in calcium flux, cell motility and invasiveness.
Three cell lines LoVo (sensitive human colorectal adenocarcinoma cell line), LoVoDX (doxorubicin-resistant human colorectal adenocarcinoma cell line), Hs738st/int (normal human intestine fibroblast) were tested with CaEP (2 mM of $CaCl_2$). The following EP protocols were used: (1) 1.2 kV/cm, 1 Hz, 100 µs, 8 pulses; (2) 37.5 kV/cm and (3) 50 kV/cm, with repetition frequency of 100 Hz, 200 impulses of 10 ns each, and time rise of 2 ns. The viability was measured by MTS assay after 24 hours and ROS level analyzed by ROS-Glo H_2O_2 test (Promega) after 6 hours. The expression of aspartate-β-hydroxylase (ASPH) protein was visualized by immunofluorescence staining using confocal laser scanning microscope (CLSM) 24 hours after CaEP application.
MTS assay shown a viability decrease of malignant cell caused by µs- and nsCaEP. Normal cells were sensitive only to µsCaEP parameters. We obtained the most promising survival ratio between normal and malignant cells for nsCaEP (2 mM of Ca^{2+} with 50 kV/cm). Moreover, in malignant cells the significant release of ROS after nsCaEP was detected. ASPH signal was clearly observed in untreated LoVoDX and LoVo cells what is correlated with the aggressive tumorigenesis. The ASPH signal decreased after each of CaEP parameters. The opposite effect occurred in Hs378st.int cells which presented a low level of ASPH in untreated cells and it was higher after the nsCaEP application (2 mM of Ca^{2+} with 50 kV/cm). The obtained results confirmed that nsCaEP originates significant reduction of colorectal cells viability, increases ROS and reduces ASPH signal that possibly is associated with cell overloaded by calcium influx and cell death.

Financing: This work was supported by the Polish National Science Centre project SONATA BIS 6 (2016/22/E/NZ5/00671, PI: J. Kulbacka).

S3-P-02

Evaluation of Electrochemotherapy Efficacy on a 3D Spheroid Neuroblastoma/Monocyte Co-Culture Model

Artsiom Klimko[1], Mircea-Bogdan Matei[2], Christien Oktaviani Matei[2], Tudor Savopol[2], Mihaela G. Moisescu[2]
[1]*Faculty of Medicine, Carol Davila University of Medicine and Pharmacy*
[2]*Biophysics and Cellular Biotechnology Dept., Carol Davila University of Medicine and Pharmacy, 8 Eroii Sanitari Blvd., 050474, Bucharest, Romania*
bogdan.matei@umfcd.ro

Combining electroporation with cytotoxic drug administration, known as electrochemotherapy (ECT), is a clinical approved procedure to tackle tumor evolution (1). There are reports relating the efficacy of ECT treatment of various solid tumors to the competence of the immune system of the patient, the immunosuppression decreasing the response to ECT (2). Currently, there is a lack of sufficient data of basic science examining the interaction of immune cells with ECT-exposed cells to explain the in vivo observations.

The purpose of this study was twofold: i/to develop and validate a 3D tumoral spheroid culture model to evaluate the effects of ECT on a human neuroblastoma cell line (SHSY-5Y) and ii/to use this model in examining how the presence of tumoral-associated monocytes will influence the response to ECT.

SHSY-5Y spheroids were created on agarose-coated low adherent 96 well plates each. After electric pulses delivery they were co-seeded with undifferentiated monocytes isolated from peripheral venous blood of a healthy donor. The spheroids were electroporated with standard ECT pulses: 8 bipolar rectangular pulses, 100 µs pulse duration 1Hz, up to 1kV/cm (1) in the presence of Bleomycin, Cisplatin or Temozolomide acting as cytotoxic agents. Morphological analysis was performed by means of visible and fluorescent microscopy. Spheroid growth rate was documented before and after ECT and analyzed by Image J and MATLAB software.

The number of seeded cells and days left for the spheroids to grow was optimized. The spheroids showed to be a reliable model for an in vitro study of tumor cells interactions in a 3D matrix. Co-seeding with monocytes resulted in spheroids growth for the non-pulsed categories and in spheroid reduction when ECT was applied. The data are discussed with respect to the categories of cytotoxic agents.

1. Gehl J et al. Acta Oncol. 2018;57(7):874-82.
2. Calvet CY and Mir LM. Cancer Metastasis Rev. 2016;35(2):165-77.

S3-P-03

Intracellular Distribution of Dihydroethidium Oxidation Products after Nanosecond Electrical Stimulation

P. Thomas Vernier, Esin B. Sözer, and Iurii Semenov
Frank Reidy Research Center for Bioelectrics, Old Dominion University
4211 Monarch Way, Norfolk, VA 23508 USA
pvernier@adu.edu

Intracellular formation and distribution of reactive oxygen species (ROS) was assessed quantitatively with imaging microphotometry in Chinese hamster ovary (CHO) cells exposed to 300 ns pulsed electric fields (nsPEF) using the fluorescent, ROS-sensitive dye, dihydroethidium (DHE). Two experimental approaches — "no wash" and "wash" — were evaluated. In the "no wash" procedure, DHE was present in the extracellular medium throughout the experiments. In the "wash" procedure, observations of intracellular ROS production were made after DHE was removed from the extracellular medium. The first approach hinders accurate measurements of intracellular ROS, because the intracellular fluorescence of oxidized DHE continuously increases in time in both pulse-exposed and unexposed cells, which makes it difficult to quantify intracellular ROS generation in individual cells and to compare sets of experimental samples. In the second approach, the intracellular fluorescence of oxidized DHE in untreated cells remains relatively constant for at least 1 hour after loading cells with DHE, enabling reproducible measurements of intracellular ROS induced by nanosecond electric pulses. With this method we characterized production and intracellular distribution of ROS in CHO cells exposed to single or multiple 300 ns electric pulses. One pulse above threshold intensity (1 MV/m) produces an increase in oxidized DHE fluorescence, indicating the generation of intracellular ROS, which can be separated into an immediate fast phase (0–1.5 min) and a subsequent slow phase (1.5–5.0 min), with a plateau after about 4 min. DHE fluorescence increases proportionally with the number of applied pulses, is independent of pulse repetition rate in the range 1–1000 H–z, and is insensitive to the antioxidants Trolox and rosmarinic acid. Imaging analysis reveals cytosolic and nucleolar localization of oxidized DHE fluorescence in untreated cells. nsPEF exposure causes a moderate decrease (after 1–10 pulses) or slight increase (after 20 pulses) in cytosolic DHE fluorescence and a significant increase in nucleolar ROS. Nucleolar oxidation has been identified as a general response to cellular stress [1]. Nanosecond-pulse-induced intracellular ROS production or increased accessibility of nucleolar helical polynucleotides may be a stress response.

[1] Yang, K., M. et al A redox mechanism underlying nucleolar stress sensing by nucleophosmin. *Nat Commun.* (2016) Nov 25;7:13599

S3-P-04

Can the Green Tea Catechin Play a Role in Enhancing the Efficacy of Electroporation in Pancreatic Cancer cells?

Olga Michel[1], Wojciech Szlasa[2], Jolanta Saczko[1], Mounir Tarek[2], Julita Kulbacka[1]

[1]*Department of Molecular and Cellular Biology, Wrocław Medical University, Borowska 211A, 50-556 Wrocław, Poland*
julita.kulbacka@umed.wroc.pl

[2]*Faculty of Medicine, Wroclaw Medical University, Wroclaw, Poland*
wojciech.szlasa@student.umed.wroc.pl

[2]*CNRS, Université de Lorraine, Campus Sciences BP 70239 54506, Vandœuvre-lès-Nancy, France*
mounir.tarek@univ-lorraine.fr

Bioflavonoids derived from green tea are known for their anticancer properties. In preliminary studies we examined the influence of catechin incubation on the effectiveness of electroporation with cisplatin on pancreatic ductal adenocarcinoma (PDA) cell lines [1]. Only short incubation with high (highest non-toxic) catechin concentrations increased cisplatin's toxicity. After excluding the role of enzymes involved in oxidative stress generation and prevention, we hypothesized that catechin can influence electroporation efficiency through non-transcriptional mechanisms such as interaction with plasma membrane or with proteins responsible for multidrug resistance (MDR) phenomenon.

We implemented molecular dynamics (MD) simulations to examine the localization of catechin on the membrane leaflet, its influence on membrane's thickness and finally, impact of the catechin-membrane interaction on the electroporation threshold. These data were further compared with experimental observation of electroporation threshold of PDA cells using the fluorescent dye. Next, we have compared the viability of PDA cells after application of electroporation with calcium chloride. We concluded that catechin molecules can influence properties of a plasma membrane but not to the extent that could account for a significant enhancement of membrane permeability. Enhanced drug efficacy in multidrug resistant PDA after catechin incubation suggests that this flavonoid may interact with proteins responsible for drug resistance. Therefore, further studies will focus on the impact of green tea catechin on the expression and function of MDR proteins typically present in pancreatic cancer cells.

S3-P-05

Electrochemotherapy Treatment of Multiple Non-melanoma Skin Tumors in a Renal Transplant Patient

Petra Rózsa[1], Dóra Ágoston[1], Krisztina Bottyán[1], Edit Szederkényi[2], Anita Varga[1], Henriette Ócsai[1], Eszter Baltás[1], Lajos Kemény[1], Judit Oláh[3], Erika Kis[1]
[1]*Department of Dermatology and Allergology, University of Szeged, Korányi fasor 6, Szeged, 6724, Hungary*
[2]*Department of Surgery, University of Szeged, Semmelweis u. 8, Szeged, 6725,*
[3]*Department of Oncotherapy, University of Szeged, Korányi fasor 12, Szeged, 6724, Hungary*
rozsa.petra@med.u-szeged.hu

The risk of cutaneous malignancies is significantly higher in long-term immunosuppressed patients than in the general population. These patients develop multiple, high-risk skin tumors, which tend to be aggressive, rapidly growing, and local recurrence is common after surgery. The treatment of these multiple tumors can be challenging, especially in the head and neck region. The objective of this case report is to highlight the possible role of electrochemotherapy (ECT) in the treatment of multiple non-melanoma skin tumors in renal transplant patients.

A 70-year-old male patient was referred to our clinic with multiple (n=15) non-melanoma skin tumors localized in the head and neck region, chest and hands (0,5-2 cm in diameter). The patient had renal transplantation 7 years earlier, and was treated with immunosuppressive medication (tacrolimus, then everolimus). During this time, he developed 27 non-melanoma cutaneous tumors, which were surgically excised. Our multidisciplinary tumor board decided to perform ECT on the new tumors. The treatment was performed according to the Standard Operating Procedures, under general anesthesia, with the use of intravenous Bleomycin (15000IU/m2). After treatment, mild ulceration, and transient postoperative pain (VAS: 2) was observed. Five months after one treatment, all target lesions were in complete remission. 16 months after the first ECT, novel tumors were noticed outside the treated area, therefore a second ECT was performed on the new lesions.

Immunosuppressed patients are a special high-risk sub-group regarding multiple non-melanoma skin tumors. ECT can be a suitable choice of treatment for these patients because of its high objective response rate, good cosmetic result, and transient, low-grade side-effects. During one session of ECT, multiple tumors can be treated, and the treatment is repeatable if necessary. Transplant physicians should be aware of this treatment option.

S3-P-06

Calcium Electroporation (CaEP) Fused with 17β-estradiol in Ovarian Cancer Treatment in vitro

Zofia Łapińska*, Urszula Szwedowicz, Agnieszka Gajewska-Naryniecka Anna Szewczyk, Jolanta Saczko

[1] *Department of Molecular and Cellular Biology, Wroclaw Medical University*
Borowska 211A, 50-556 Wroclaw
**zofia.lapinska@student.umed.wroc.pl*

Ovarian cancer (OC) is an estrogen-dependent carcinoma associated with the highest number of death from among all gynecological malignancies [1]. Estrogens (Es), depending on the concentration could have a stimulating or cytotoxic effect on cancer cells [2]. This phenomenon has been described as an 'estrogen paradox'.

Calcium electroporation (CaEP) is up-and-coming treatment method based on electrochemotherapy (ECT), where the cytostatics have been replaced by calcium ions (Ca^{2+}) [3]. Unfortunately, to date, there are no literature reports about the efficiency of CaEP in ovarian cancer.

Our work aimed to investigate *in vitro* the influence of OC cells (OvBH-1) preincubation with 17β-estradiol ($E_{2;}$ 10μM) on the effectiveness of chemotherapy (CT) and electrochemotherapy (ECT) with cisplatin or calcium chloride ($CaCl_2$). Additionally, we studied the influence of all analyzed treatment methods on the Chinese hamster ovary cell line (CHO-K1).

We used 100μs × 100Hz × 8 pulses electroporation protocol with different voltage [kV/cm]. Cells viability after applied treatment was examined by MTT assay. The efficiency of cell membrane permeabilization was analyzed by a flow cytometer using the Yo-Pro™-1 dye. Moreover, we used fluorescent staining to observe cells' morphological changes.

We discovered that preincubation OvBH-1 cells enhance the effectiveness of CT and ECT with cisplatin and $CaCl_2$. Simultaneously, estradiol reduced the range of cells' membrane permeabilization, which may suggest that the mechanism responsible for the improved efficiency of ECT after OC cells preincubation is not related to changes in cell membrane permeability.

Funding: *Statutory Subsidy Funds of the Department of Molecular and Cellular Biology no. SUB.D260.20.009*

References

[1] Z. Huang *et al.*, *Oncol. Lett.*, vol. 15, no. 1, pp. 588–591, 2018
[2] R. J. Santen, *Breast Cancer Res.*, vol. 9, no. 3, pp. 1–5, 2007
[3] S. K. Frandsen and J. Gehl, *Tech Cancer Res Treat*, vol. 17. pp. 1–6, 2018

S3-P-07

Growth Response of Tomato under Different Direction of External Electric Field

Dahae Kim[1,2], Sora Lee[1,2], and Myung-Min Oh[1,2*]
[1]*Division of Animal, Horticultural and Food Sciences, Chungbuk National University*
[2]*Brain Korea 21 Center for Bio-Health Industry, Chungbuk National University*
Cheongju 28644, Republic of Korea
dahaekim@chungbuk.ac.kr

Numerous studies have revealed that electric field positively affects many physiological responses in plants including seed germination and plant growth. There is still insufficient knowledge in understanding how plants respond to the external electric field. We investigated the effect of an external electric field with various directions on the growth of tomato seedlings. Tomato seedlings were grown in several growth chambers with differently applied electric fields in vertical or horizontal directions. The electric field was generated in the chambers using stainless steel mesh electrodes and a high voltage generator, and control plants were grown without electrodes in the chamber. The 18-day-old tomato seedlings were exposed to 5 kV/m of the electrostatic fields for 3 weeks. Plant height was significantly increased in all treatments by 10-30% compared with the control. Also, the number of nodes showed a similar pattern as plant height. The number of leaves was also increased in all treatment compared with the control. However, the horizontal electric field decreased leaf area by about 30% compared to the control. Shoot fresh weight was increased in the vertical electric fields and root fresh weight was increased in the horizontal field. In conclusion, our results show that the directions of the electric field applied to plants can change the morphogenesis and promote the growth of them. We suggest that the electric field could be used to improve the quality of seedling by controlling the direction.

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (grant number 2020R1I1A3074865).

S4 Bioenergetics and biosynthesis

S4-P-01

CO_2 Conversion to CH_4 and Acetate in a Microbial Electrosynthesis Cell with Conductive Polymer Cathode Enhanced by Electrodeposition of Ni-based Alloys

Emmanuel Nwanebu[1], Abraham Gomez Vidales[1], Sasha Omanovic[2] and Boris Tartakovsky[*1]
[1]*National Research Council of Canada, 6100 Royalmount Av., Montreal, QC, Canada H4P 2R2*
[2]*Department of Chemical Engineering, McGill University, Montreal, QC, H3A 0C5, Canada*
[*]*boris.tartakovsky@cnrc-nrc.gc.ca*

In this study, CO_2 conversion to CH_4 and acetate was achieved in flow-through laboratory-scale microbial electrosynthesis (MES) cells. Each PVC-made MES cell was divided into two compartments separated by an electrically-insulating nylon cloth of an interfacial area of 37cm^2. The anode compartment housed a Ti/IrO_2 mesh, while the cathode compartment contained a 3D printed, electrically-conductive polymer (polylactade, PLA) lattice. The cathode compartment was inoculated with 50 mL of homogenized anaerobic. The MES cells were maintained at a constant temperature of 27°C and continuously fed with 10 g L^{-1} of $NaCO_3$ at an inflow rate of 150 mL/day.

A progressive increase in current from 23 to 33 mA was observed in the MES cell with a conductive PLA lattice. The cathode off-gas contained up to 40-55% (v/v) CH_4 and 20-30% (v/v) H_2 with CO_2 concentration below the detection limit, i.e. carbonate supply limited CH_4 production. Also, the cathode liquid contained 100 – 400 mg L^{-1} of acetate. To improve the MES performance, the PLA lattice surface was coated by electrodeposition of Ni-based materials. Three cathode lattices containing either electrodeposited Ni, Ni-Fe, or Ni-Fe-Mn materials were prepared. Initial electrochemical characterization showed that the electrocatalytic activity of these lattices in the hydrogen evolution reaction were in the following order: Ni > NiFeMn > NiFe. Following the initial electrochemical characterization, these lattices were tested by continuously operating MES cells for at least 20 days with each lattice as a cathode. It was found that electrocoating the conductive PLA lattice with Ni-based materials resulted in increased current (55-70 mA) leading to higher CH_4, H_2 and acetate production at the cathode. The best performing NiFeMn lattice showed stable production of CH_4 (50mL d^{-1}), H_2 (330mL d^{-1}) as well as acetate (500 mg L^{-1}) at 2.8V applied cell voltage and a current of 55 mA. This is by 67% higher than that of the non-coated PLA lattice. Also, this performance corresponded to a Coulombic Efficiency of over 80%.

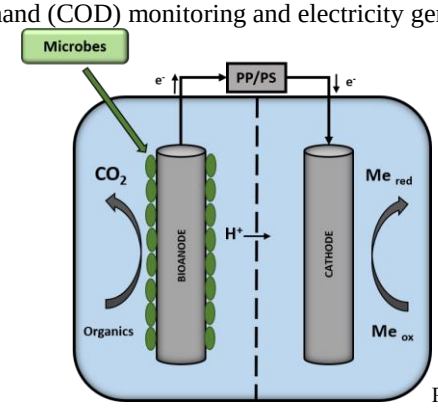
S4-P-02

Bioelectrochemical Recovery of Metals from Ashes and Slags

Marianne Haberbauer[1], Sabine Spiess[1], Sophie Thallner[1], Klemens Kremser[2], Georg M. Gübitz[2], Jiri Kucera[3], Martin Mandl[3]

[1]*K1-MET GmbH, Stahlstraße 14, 4020 Linz, Austria*
[2]*University of Natural Resources and Life Sciences Vienna, Dept. of Agrobiotechnology, IFA-Tulln, Inst. of Environmental Biotechnology, Konrad-Lorenz-Strasse 20, 3430 Tulln an der Donau, Austria*
[3]*Department of Biochemistry, Faculty of Science, Masaryk University, Kotlarska 2, 61137 Brno, Czech Republic*
marianne.haberbauer@k1-met.com

Waste incineration plants generate substantial amounts of ashes and slags which pose environmental risks due to their toxicity. In this project, the combination of bioleaching and bioelectrochemistry as an environmentally friendly strategy for the recovery of valuable metals from incineration residues was investigated. Zinc, cobalt, copper and other metals from different incineration residues were leached biologically. These leachates were further used as electrolyte in the cathode chamber of a bioelectrochemical system (BES, Fig. 1). By applying a constant potential of 0.20 V vs Ag/AgCl (3 M), performance of the BES has been studied by chemical oxygen demand (COD) monitoring and electricity generation.



PP/PS…power production/power supply

Fig.1: Scheme of a BES for metal recovery

The project was supported by the European fund for regional development, the program Interreg V-A Austria – Czech Republic under the project ATCZ183, IRAS (Innovative Recycling technology for Ashes and Slags).

S4-P-03

Enhanced Direct Electron Transfer of Cellobiose Dehydrogenase on Three-Dimensional Graphene Modified Carbon Electrodes

Xiaomei Yan[a], Jing Tang[a], Su Ma[b], David Tanner[a], Roland Ludwig[b], Jens Ulstrup[a], Xinxin Xiao[a,*]
[a]*Department of Chemistry, Technical University of Denmark, Kgs Lyngby 2800, Denmark*
[b]*Biocatalysis and Biosensing Laboratory, Departmentt of Food Science and Technology BOKU – University of Natural Resources and Life Sciences, 1190 Vienna, Austria*
xiyan@kemi.dtu.dk; xixiao@kemi.dtu.dk

Cellobiose dehydrogenase (CDH) is a versatile oxidoreductase with two separate domains: a catalytic dehydrogenase domain (DH) with one flavin adenine dinucleotide (FAD) cofactor and an electron transfer (ET) cytochrome domain (CYT). In addition, the enzyme holds a flexible linker for ET between the two domains.[1,2] We have prepared a three-dimensional (3D) graphene modified carbon electrode to enhance direct electron transfer (DET) between *Myriococcum thermophilum* CDH and the electrode. The 3D electrode is fabricated by electrochemical reduction of graphene oxide and *in-situ* growth of a positively charged polyethylenimine (PET) adlayer on carbon papers (CPs). This surface improves electrostatic adsorption of overall negatively charged CDH and facilitates the DET process. The strategy presented leads to controlled PET modification and CDH immobilization, and the efficient DET of CDH bioelectrodes offers novel opportunities in fabrication of glucose/oxygen biofuel cells and glucose sensors.

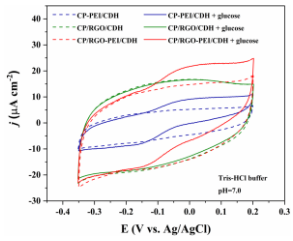


Figure 1. Cyclic voltammetry of CDH bioelectrodes in 0.1 M oxygen-free Tris-HCl buffer containing 30 mM $CaCl_2$ in the absence or presence of 25 mM glucose. Scan rate: 5 mV/s

Acknowledgments
The PhD project is supported by the China Scholarship Council (CSC).

References
[1] Yan, X.; Tang J., B.; Tanner, D.; Ulstrup J., Xiao, X. *Catalysts.* **2020**, *10*, 1458
[2] Ma, S. Laurent, C. V. F. P., Meneghello, M., Tuoriniemi, J., Oostenbrink, C., Gorton, L., Bartlett, P. N., Ludwig, R. *ACS Catal.* **2019**, *9*, 7607-7615

S4-P-04

Carbon-based Electrodes for Application in Bio-inspired Organic Batteries

Luana C. I. Faria, Graziela C. Sedenho, Frank N. Crespilho
São Carlos Institute of Chemistry, University of São Paulo, Avenida Trabalhador São Carlense, 400, CEP 13566-590 - São Carlos - SP – Brasil
luitalianofaria@usp.br

Great efforts have been made in the development of bioinspired energy storage system, which use analogues of substances present in bioenergetic cycles. In this context, Redox Flow Batteries (RFBs) emerged as a promising candidate for energy storage, once can use quinones like in the respiratory chain. Carbon-based electrodes have been widely used in aqueous organic and organometallic RFBs, since they have high electronic conductivity, high chemical and electrochemical stability, high surface area, and low cost.[1] Thus, the objective is to study the effectiveness of different carbon-based electrode surfaces, as well as to compare them in order to evaluate the best performance for implementation in RFBs. For this purpose, here we use chemically treated flexible carbon fibers (FCF)[2] and thermally treated Carbon Paper Sigracet 39 AA (SGL)[3]. To understand the modifications promoted by the treatment processes on both electrodes surfaces, 4,5-dihydroxy-1,3-benzenedisulfonic acid (BQDS) was employed as an electrochemical probe in cyclic voltammetry measurements. The obtained results show that the electrochemical reaction of BQDS on FCF is almost reversible, in SGL it is less reversible than the first. An $E_{1/2}$ of approximately 0.70 V for FCF and 0.75 V for SGL is observed. The current dependence on the scan rate was studied, proving that the BQDS in the FCF is freely diffusing, since the peak current increases linearly with the square root of the scan rate. The results obtained with BQDS in SGL, on the other hand, suggest a joint control of diffusion and adsorption. Finally, the stability of both electrodes at extreme potential was also tested, which simulates the charge condition of an RFB. After applying a potential of 1.30 V, the peak currents have no significant influence on electrochemical kinetics. Thus, the results indicate that FCF has a better electrochemical response compared to SGL, being more promising for application in batteries.

References

(1) Winsberg, J.; Hagemann, T.; Janoschka, T.; Hager, M. D.; Schubert, U. S. *Angew. Chemie Int. Ed.* **2017**, *56* (3), 686–711
(2) Pereira, A. R.; de Souza, J. C. P.; Iost, R. M.; Sales, F. C. P. F.; Crespilho, F. N. *J. Electroanal. Chem.* **2016**, *780*, 396–406
(3) Chen, Q.; Gerhardt, M. R.; Hartle, L.; Aziz, M. J. *J. Electrochem. Soc.* **2016**, *163* (1), A5010–A5013

S4-P-05

In-situ FTIR and UV-Vis for the Mapping of Redox Reactions of Symmetrical Quinones

Jose E. S. Clarindo, Graziela C. Sedenho, Lucyano J. A. Macedo, Frank N. Crespilho
São Carlos Institute of Chemistry, University of São Paulo
Av. Trabalhador São Carlense, 400
CEP 13566-590, São Carlos - SP – Brazil
clarindo.eduardo@usp.br

Quinones are one of the most important and well-studied types of organic redox couples and are well-represented in biological systems containing electron transfer processes. Alizarin Red S (ARS) contains quinones (–C(=O)–) and phenolics functional groups that can be used in redox reactions of symmetrical batteries: two redox couples, one at positive side and other at negative side. Here, we investigate ARS as a promising candidate able of developing an organic batterie enables cell potentials above 1.0 V. In-situ UV-Vis and FTIR spectromicroscopy[1] and electrochemistry measurements were used to investigate the electron-transfer reactions at carbon surfaces and how each surface reacts to charge-transfer kinetics with ARS. In-situ techniques suggest there are changes in electronic transitions and stretches and formed intermediate with applied potential for different surfaces. In-situ UV-Vis shows at higher potentials during ARS reduction process a change in electronic transition at 430 nm (n—π*) of partially ionized hydroxyl groups indicating different mechanisms of the expected reversible mechanism $2e^-/2H^+$. In-situ FTIR measurements from 1.0 to -0.3 V suggest that it is possible to correlate the C=O groups with its respective conjugated cyclic dione, and the structures can be differentiated consequently, for instance, using 2-C-O stretching (1288 cm^{-1}), 1-C-O stretching (1262 cm^{-1}) and C=O stretching/C-C-C in-plane bending (1201 cm^{-1}). Electrochemistry measurements corroborate in-situ FTIR/UV-Vis showing different voltametric profiles for different carbon surfaces implying different overall redox mechanism for spontaneous proton-coupled electron transfer processes[2].

References

(1) Macedo, L. J. A.; Crespilho, F. N. *Anal. Chem.* **2018**, *90*, 1487–1491
(2) Sedenho, G. C.; De Porcellinis, D.; Jing, Y.; Kerr, E.; Mejia-Mendoza, L. M.; Vazquez-Mayagoitia, Á.; Aspuru-Guzik, A.; Gordon, R. G.; Crespilho, F. N.; Aziz, M. J. *ACS Appl. Energy Mater.* **2020**, *3*, 1933–1943

S4-P-06

Microbial Electrogenicity Evaluation in Domestic Wastewater

Bruno R. Rossi[1], Graziela C. Sedenho[1], Wilson T. L. da Silva[2], Frank N. Crespilho[1]
[1]*São Carlos Institute of Chemistry, University of São Paulo,*
Avenida Trabalhador São-Carlense, 400
CEP 13566-590 - São Carlos - SP – Brasil
[2]*Brazilian Agricultural Research Corporation (EMBRAPA), Brazil*
R. 15 de Novembro, 1452,
CEP 13560-970 São Carlos - SP – Brazil
brunorossi@usp.br

Domestic wastewater treatment has a large cost associated with it due to large amount of electrical energy consumtion[1]. As an alternative, microbial fuel cells have been proposed to accomplish wastewater treatment and energy production due to their ability to generate electricity from the degradation of organic matter in the wastewater[2]. In a microbial fuel cell system, an exoelectrogenic bacteria on the anode transfer the electrons from the degradation of the organic matter in wastewater to the cathode where the oxygen reduction reaction completes the circuit[3]. In this way, it is important to understand and evaluate the electrogenic behavior of the microorganisms in wastewater treatment systems. In this work, we evaluate the electrogenicity of microorganisms at the anode surface of microbial fuel cell in a domestic wastewater system by the addition of cattle manure. For this the electrode was incubated in a domestic wastewater sample for different days, followed by the characterization of the modified electrode through cyclic voltammetry and the scanning electron microscopy (SEM) measurements. The results show that the microorganisms colonize the electrode surface and produces electricity from the organic matter degradation.

References

(1) McCarty, P. L.; Bae, J.; Kim, J. *Environ. Sci. Technol.* **2011**, *45*, 7100–7106
(2) Rabaey, K.; Verstraete, W. *Trends Biotechnol.* **2005**, *23* (6), 291–298
(3) Logan, B. E.; Rossi, R.; Ragab, A.; Saikaly, P. E. *Nat. Rev. Microbiol.* **2019**, *17* (5), 307–319

S4-P-07

Polystyrene-β-cyclodextrin Nanoparticles for Oriented Electro Connection of Enzyme

Paulo Henrique M. Buzzetti[1], Marie Carrière[1,2], Karine Gorgy[1], Fabien Giroud[1], Mumtaz Muhammad[2], Redouane Borsali[2] and Serge Cosnier [1]

[1]*Université Grenoble Alpes, DCM UMR 5250, F-38000 Grenoble, France*
CNRS, DCM UMR 5250, F-38000 Grenoble, France
[2] *Univ Grenoble Alpes, CNRS, CERMAV, F-38000 Grenoble, France*
paulo-henrique.maciel-buzzetti@univ-grenoble-alpes.fr

This work deals about the self-assembly of diblock copolymer consisting of a hydrophilic block β-cyclodextrin (β-CD) and hydrophobic polystyrene block (PS) to form PS-βCD glyconanoparticles (NPs). A post-functionalization by host-guest interaction between sodium anthraquinone-2-sulfonate (AQS) and β-CD was performed. The resulting NPs-AQS were used to obtain a specific orientation of bilirubin oxidase (BOD) during its adsorption on an electrode modified by a deposit of carbon nanotubes. SEM images showed the formation of spherical NPs-AQS exhibiting diameter of 20 nm in agreement with DLS (Dynamic Light scattering), where the first of three size distributions showed particles with 25 nm in diameter. In addition, the NPs-AQS present a reversible redox system at $E_{1/2}$ = - 0.454 V *vs.* SCE (pH 6.0), with a positive shift of 186 mV compared to the conventional value of the redox couple of free AQS. Thanks to NPs-AQS, the oriented BOD were tested in O_2 saturated electrolyte. The O_2 catalytic reduction currents were recorded for the biocathodes in the presence and the absence of the NPs-AQS on the CNT films. It appears that NPs-AQS facilitate the enzyme wiring with the electrode and increase the efficiency of the direct electron transfer (Fig. 1).

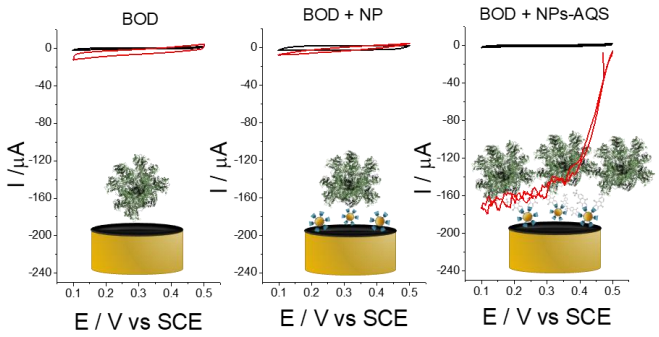


Figure 1. CVs recorded to the biocathodes at 10 mV s^{-1} (pH 7.0) with argon saturation (black) and O_2 saturation (red).

S4-P-08

Implementation of Oxygen-Sensitive Catalysts in Fuel-cells

Huaiguang Li, Ute Münchberg, Alaa A. Oughli, Darren Buesen, Wolfgang Lubitz, Erik Freier & Nicolas Plumeré

Campus Straubing for Biotechnology and Sustainability, Technical University Munich
Schulgasse 22, 94315 Straubing, Germany
Huaiguang.li@tum.de

Biological and bioinspired catalysts for energy conversion schemes hold great potential for meeting sustainable energy needs, but widespread implementation necessitates solving their O_2 instability. Redox-active films were proposed as protective matrices for preventing oxidative deactivation of oxygen-sensitive catalysts such as hydrogenases for their use in fuel cells[1-4]. While theoretical models predict quasi-infinite protection from oxygen, experimental data for hydrogenase-catalyzed hydrogen oxidation within redox films show half-lives of only about a day. Here, we employ *operando* confocal microscopy to elucidate the deactivation processes. H_2O_2 generated from the incomplete reduction of O_2 induces the decomposition of the polymer matrix rather than the deactivation of the biocatalyst. We show that efficient dismutation of H_2O_2 by iodide extends the aerobic half-life of the catalytic film containing an oxygen-sensitive [NiFe] hydrogenase to over one week, approaching the experimental anaerobic half-life. Altogether, our data support the theory that redox films make the hydrogenases immune against the direct deactivation by O_2 and highlight the importance of suppressing H_2O_2 production to reach complete protection from oxidative stress. These findings are of particular interest for the implementation of biological and bioinspired catalysts for sustainable energy conversions.

1. Li, H. et al. Complete protection of O_2-sensitive catalysts in thin films. *J. Am. Chem. Soc.* **141,** 16734–16742 (2019).
2. Plumeré, N. et al. A redox hydrogel protects hydrogenase from high-potential deactivation and oxygen damage. *Nat. Chem.* **6,** 822–827 (2014).
3. Fourmond, V. et al. Mechanism of protection of catalysts supported in redox hydrogel films. *J. Am. Chem. Soc. 137,* 5494–5505 (2015).
4. Li, H. et al. Suppressing hydrogen peroxide generation to achieve oxygen-insensitivity of a [NiFe] hydrogenase in redox active films. *Nat. Commun.* **11,** 920 (2020).

S5 Microbial Films and Biocorrosion

S5-P-01

Bioelectrochemical Methanation of CO_2 from Untreated Steel Mill Gas

Marianne Haberbauer[1], Sabine Spiess[1], Amaia Sasiain Conde[1], Sophie Thallner[1], Niels Waldmann[2], Eva Neuhauser[2], Andreas Paul Loibner[3], Nina Kieberger[4]
[1] *K1-MET GmbH, Stahlstrasse 14, 4020 Linz, Austria*
[2] *acib GmbH, Konrad-Lorenz-Strasse 20, 3430 Tulln an der Donau, Austria*
[3] *University of Natural Resources and Life Sciences Vienna, Dept. of Agrobiotechnology, IFA-Tulln, Inst. of Environmental Biotechnology, Konrad-Lorenz-Strasse 20, 3430 Tulln an der Donau, Austria*
[4] *voestalpine Stahl GmbH, voestalpine-Strasse 3, 4020 Linz, Austria*
marianne.haberbauer@k1-met.com

Steel production is one of the most energy-intensive processes in which 7% of global CO_2 is emitted. Extensive efforts have been made in the past few decades, especially in Austria, to increase the energy efficiency of production and to reduce CO_2 emissions. The optimization potential in terms of resource and efficiency, and thus the reduction of greenhouse gas emissions of modern steel plants is almost completely exploited regarding conventional process optimizations. Therefore, the national project LOCON (Low energy CO_2 conversion and utilization at the example of steel industry) focuses on the biological methanation of CO_2 from untreated steel mill gas. Two innovative processes are being considered for biological methanation with naturally occurring microorganisms: geo-methanation and bioelectrochemical methanation.
Here we want to present our work regarding bioelectrochemical methanation with a bioanode and a biocathode. In a bioelectrochemical system (BES) at least one electrode is covered by an electroactive biofilm, which catalyzes oxidation or reduction processes. At the bioanode microbial oxidation of organics (e.g. wastewater) occurs, whereby electrons are produced which are supplied to a CO_2 reducing biocathode. To test the biological methanation of CO_2 from untreated steel mill gas, a lab-scale BES was set up and an innovative adaptation strategy of BES polarization was adopted, consisting in the shift of the potentiostatic control of the process from the anode (at + 0.4 V vs Ag/AgCl (3M)) to the cathode (at -1.00 vs Ag/AgCl (3M)). During long-term operation different process parameters like chemical oxygen demand removal, current generation and CH4 production were monitored. In the first stage the biocathode was flushed with pure CO2. After successful methanation (max. 3.6 L/m²/d) and optimization of process parameters, the CO2 conversion of untreated steel mill gas will be tested and further improved.

This project has received funding from the Austrian Federal Ministry for Digital and Economic Affairs (BMDW) program "Beyond Europe".

S5-P-02

Precious Metals Recovery by Microbial Electrochemical Snorkel

Mario Mitov[1,2], Elitsa Chorbadziyska[1,2], Ivo Bardarov[3], Krassimir L. Kostov[4], Yolina Hubenova[3,5]
[1]*Innovative Center for Eco Energy Technologies (ICEET), South-West University "Neofit Rilski", 66 Ivan Mihajlov str., 2700 Blagoevgrad, Bulgaria*
[2]*Department of Chemistry, South-West University, Blagoevgrad, Bulgaria*
[3]*Institute of Electrochemistry and Energy Systems "Acad. Evgeny Budevski" – Bulgarian Academy of Sciences, Sofia, Bulgaria*
[4]*Institute of General and Inorganic Chemistry – BAS, Sofia, Bulgaria*
[5]*Department of Biochemistry and Microbiology, Plovdiv University "Paisii Hilendarski", Plovdiv, Bulgaria*
mitovmario@swu.bg

Recently, we reported for the first time the proof-of-concept for metal recovery by microbial electrochemical snorkel (MES) [1]. It has been demonstrated that the operation at short-circuit conditions results in higher efficiency of copper recovery compared to that obtained with a microbial fuel cell (MFC) with the same construction. In this report, we summarize the new experimental results for silver and gold recovery by using a similar MES set-up. Different types of cathodes, immersed in 1 g/L Ag(I) or Au(III) solutions, were short-circuited with the bioanodes of sediment MFCs. In parallel, the experiments were also carried out in an MFC mode, where the cathode and the anode were connected through an external resistor. The concentration of the precious metal in the catholyte was measured spectrophotometrically over time. The results confirmed that the current, respectively the reaction rate was higher in the MES mode than in MFC mode. The precious metal removal reached ca. 97±2% in the short-circuited mode. The carried out XPS analysis shows that the biggest part of the dissolved metallic ions was recovered on the cathodes as Ag^0 or Au^0 nanoparticles.

Acknowledgments This study was supported by the Bulgarian National Science Fund through contract KP-06-H29/8/2018 and the National Scientific Program "EPLUS" (contract D01-214/2018).

Reference

[1] M. Mitov, I. Bardarov, E. Chorbadzhiyska, K. Kostov, Y. Hubenova, First Evidence for Applicability of the Microbial Electrochemical Snorkel for Metal Recovery, Electrochem. Commun. 122 (2021) 106889; https://doi.org/10.1016/j.elecom.2020.106889.

S6 Electron Transport in Biological Systems - Theory and Experiment

S6-P-01

Voltammetric Study of the Binding of Phosphoric Acid-Swollen Cellulose with Immobilized Lytic Polysaccharide Monooxygenases

D. Zouraris[1], A. Karnaouri[2], P. K. Pandis[3], C. Argirusis[3], E. Topakas[2], A. Karantonis[1]
[1]*Laboratory of Physical Chemistry and Applied Electrochemistry, School of Chemical Engineering, National Technical University of Athens, 15780 Zografou, Athens, Greece*
[2]*IndBioCat Group, Biotechnology Laboratory, School of Chemical Engineering, National Technical University of Athens, 15780 Zografou, Athens, Greece*
[3] *Laboratory of Inorganic Materials Technology, School of Chemical Engineering, National Technical University of Athens, 15780 Zografou, Athens, Greece*
dimzouraris@mail.ntua.gr

Lytic polysaccharide monooxygenases (LPMOs) are copper dependent redox enzymes that act on recalcitrant polysaccharides, generating oxidized and non-oxidized chain ends. Although they have gathered a lot of interest, their mechanism of action has not been fully clarified yet and the kinetic data regarding these enzymes are scarce. In order to contribute to some degree to the study of these enzymes, this work focuses on an LPMO belonging to the AA9 family from the filamentous fungus *Thermothelomyces thermophilus*, *Mt*LPMO9H [1]. This enzyme, which is heterologously expressed and produced in *P. pastoris*, is immobilized on an immobilization matrix consisting of cobalt modified mutli-walled carbon nanotubes, taking advantage of its C-terminal hisidine tag. The binding step of phosphoric acid swollen cellulose (PASC) to the immobilized LPMO is studied using large amplitude fast Fourier Transform alternating current Voltammetry (FTacV) [2] at different temperatures. The interpretation of the experimental data is based on a proposed kinetic model, similar to an electrochemical (EC) mechanism, but for immobilized species. The model is studied analytically and numerically and it is shown that the experimental results can be exploited for the verification of the occurrence of binding and the determination of the kinetic constant of the binding step. Therefore, the experimental findings support the case of binding of PASC to LPMO and are analyzed in order to obtain the kinetic constant at different temperatures as well as an estimation of the activation energy for the binding step.

[1] A. Karnaouri, *et al.* Biotechnol. Biofuels 10 (1) (2017) 126.
[2] D. Zouraris, *et al.* Bioelectrochem. 124 (2018) 149-155

S6-P-02

Extracellular Electron Transfer in *Saccharomyces cerevisiae*: The Origin of the Bioelectricity

Graziela C. Sedenho, Iago Modenez, Giovana R. Mendes, Frank N. Crespilho
São Carlos Institute of Chemistry, University of São Paulo,
Avenida Trabalhador São-Carlense, 400
CEP 13566-590 - São Carlos - SP – Brasil
grazielacs@usp.br

Electron transfer (ET) processes have fundamental importance in all living organisms. In a diversity of microorganisms, including bacteria and fungi, ET between the cell and the environment, named extracellular electron transfer (EET), is essential for survival in cases where the terminal electron acceptor is not easily accessible.[1] Understanding how electrons flow in biological cells have direct implication in the fields of microbial physiology, microbial ecology, biotechnology, and green energy. *Saccharomyces cerevisiae* is one of the first microorganism proved to be able to convert chemical energy into electricity, however its EET mechanism is still not clear. In this context, the aim of this work is to contribute to the elucidation of *Saccharomyces cerevisiae* EET mechanism under fermentative conditions, through electrochemical, spectroscopic, and microscopic evidences. The studies show that the yeast cells adsorb and interact on hydrophobic surfaces through the extracellular polymeric substances (EPS) film secreted by the cell, and the electrochemical response comes from a redox specie mostly confined into this film on the yeast cells surface. Spectroscopic and electrophoretic results strongly point out that the compound responsible for the *Saccharomyces cerevisiae* EET is a flavoprotein. Therefore, for the first time, it was possible to understand the EET mechanism of *Saccharomyces cerevisiae*, and the elucidation of this mechanism combined to electrode engineering can contribute to the advancement of biosystems in green energy generation.

Reference

(1) Y. Xiao, *et al.*, Extracellular polymeric substances are transient media for microbial extracellular electron transfer. *Sci. Adv.* **3**, e1700623 (2017)

S6-P-03

Unlocking Efficiency: Electro-Molecular Investigations of Photosynthetic Energy Flow with Microbial Electro Photosynthetic System

Christine Lewis[a], Justin Flory[a], Sidney Hecht[a], Thomas Moore[a], Ana Moore[a], Bruce Rittmann[a], Willem Vermaas [a], César Torres[a], Petra Fromme[a]

Biodesign Institute, Arizona State University, Tempe Arizona 85287, USA

christine.lewis@asu.edu

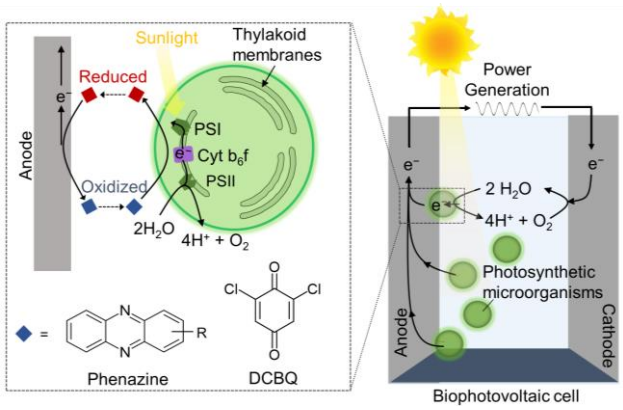
Photosynthesis converts solar energy into chemical energy driving all higher life on Earth. Photosystem II (PSII) carries out a critical step by using light energy to split water into protons, electrons and O_2. However, PSII is damaged under even modest light intensities limiting electron flow. Like PSII, photosystem I (PSI) is extremely efficient at energy transfer, but it is not susceptible to the same level of damage. Therefore, if provided an alternative electron source, it can drive the second half of photosynthesis culminating in fixing CO_2 into carbohydrates. Here, we present results from a novel microbial electro photosynthetic (reactor) system in which small molecule redox shuttles deliver protons and electrons from a cathode to the light driven electron transport cascade of whole *Synechocystis* cyanobacterial cells lacking PSII, to reductively activate PSI and downstream energy storage processes. Joliot-type spectroscopy confirms that select redox shuttles can reduce PSI at kinetic rates that match or exceed reduction by PSII in both low and extremely high light exposure. Chronoamperometry experiments demonstrate that light-dependent current increased with light intensity up to 2050 µmol photons m^{-2} s^{-1} to yield 113 µmol electrons m^{-2} s^{-1} (cathodic surface area where PSII related photodamage is known to occur. Overall, this work provides methodology to probe the complex structures of the photosynthetic electron transport chain to identify and utilize the most efficient parts of photosynthesis while bypassing the processes that slow it down. We anticipate that optimizing these methodologies will increase energy conversion and storage efficiencies to produce biofuel and bio products in lieu of petroleum.

S6-P-04

Phenazines as low-midpoint potential electron shuttles for photosynthetic bioelectrochemical systems

Eleanor R. Clifford[a], Robert W. Bradley[b], Laura T. Wey[c], Joshua M. Lawrence[c], Xiaolong Chen[a], Christopher J. Howe[c]*, Jenny Z. Zhang[a]*
[a]*Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, UK*
[b]*Department of Life Sciences, Sir Alexander Fleming Building, Imperial College, SW7 2AZ, UK*
[c]*Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QW, UK*
jml203@cam.ac.uk

Bioelectrochemical energy conversion depends on efficient wiring of electron transport chains to electrodes, which is often achieved with exogenous electron mediators. However, many widely used mediators are cytotoxic after prolonged exposure, as well as giving rise to sizeable energy losses. In order to assess a new criterion for selecting exogenous electron mediators, we assessed the ability of phenazines to wire the photosynthetic electron transport chain of biofilms of the cyanobacterium Synechocystis sp. PCC 6803 to electrodes. The low-midpoint potential phenazine pyocyanin (PYO) was shown to effectively permeate cells and harvest electrons from multiple points of the photosynthetic electron transport chain, providing a significant enhancement to biofilm photocurrents. PYO was compared with the commonly used high-midpoint potential mediator dichloro-1,4-benzoquinone (DCBQ) in its electron mediation activity and cytotoxicity. Furthermore, cyanobacteria were genetically modified to biosynthesise PYO. The presented results and methodology will aid the design of future electron mediators for use in bioelectrochemical systems.



S6-P-05

Recombinant Expression and Artificial Maturation of *Clostridium acetobutylicum* [FeFe] Hydrogenase: Electrochemical and Spectroscopic Characterization

Tin Lai[1], Patricia Rodriguez-Macia[1], Simone Morra[2],
Miguel Ramirez-Hernandez[1], and Kylie Vincent[1*]
[1]*University of Oxford, Department of Chemistry, Inorganic Chemistry Laboratory, South Parks Road, Oxford, OX1 3QR, UK*
[2]*Faculty of Biology and Biotechnology, Photobiotechnology, Ruhr University Bochum, 44801 Bochum, Germany*
tin.lai@stx.ox.ac.uk

Hydrogenases are nature's H_2 converting catalysts, with [FeFe] hydrogenases being the most active type. It has been demonstrated that [FeFe] hydrogenases can be prepared by overexpressing the apo-hydrogenase (the enzyme lacking the metal active site) in *E. coli* and later on incorporating the synthetic [2Fe] cofactor mimic to form the holo-enzyme, which is completely active and essentially identical to the native enzyme. [1] This method has been used extensively to generate various [FeFe] hydrogenases, however, the [FeFe] hydrogenase from *Clostridium acetobutylicum* (*Ca*) has never been prepared by artificial maturation. Thus, this enzyme remains less characterized than other [FeFe] hydrogenases. Here we establish a heterologous overexpression system for the apo-*Ca*HydA [FeFe] hydrogenase. This system generates higher yields of very pure apo-enzyme, which is afterwards artificially maturated with various synthetic [2Fe] cofactor mimics. This allows detailed electrochemical, spectroscopic, and spectroelectrochemical characterisation of the wild-type enzyme and the [2Fe] cofactor variants, providing a better understanding of this highly active H_2-producer enzyme, and setting the ground for further advanced studies.

[1] G. Berggren, A. Adamska, C. Lambertz, T. Simmons, J. Esselborn, M. Atta, S. Gambarelli, J. Mouesca, E. Reijerse, W. Lubitz, T. Happe, V. Artero and M. Fontecave, Nature, 2013, 499, 66-69.

S6-P-06

Correlating Cyclic Voltammetry and mRNA transcriptomics in *Geobacter sulfurreducens*

Ethan Howley, Rosa Krajmalnik-Brown, César Torres
Arizona State University
Tempe, Arizona USA
ehowley@asu.edu

The metal-reducing bacterium *Geobacter sulfurreducens* is capable of respiring solid electron acceptors at a wide range of redox potentials, but the mechanism by which it performs extracellular electron transfer is not fully understood. We used both mRNA differential expression transcriptomics and cyclic voltammetry to identify genes that may be related to extracellular electron transfer in *G. sulfurreducens* under different conditions. It is likely that *G. sulfurreducens* changes its gene expression to adapt to different electron acceptor potentials, and our experiment was designed to observe that change.

We grew *G. sulfurreducens PCA* under seven different conditions and extracted mRNA from each condition for analysis. The conditions included cells grown on an anode in a microbial electrochemical cell at three different potentials using either acetate or formate as the electron donor. We also studied planktonic cells using fumarate as the electron acceptor.
For each electrode potential we used for mRNA extraction, we performed cyclic voltammetry (CV). By differentiating the CV results and fitting with multiple Nernst-Monod functions, we saw signals of multiple pathways with different midpoint potentials.

In this presentation, I will present our work correlating differential gene expression data in *G. sulfurreducens* with the different electrochemical responses we collected through cyclic voltammetry. With cyclic voltammetry, we were able to identify three pathways with different midpoint potentials in the conditions we studied. The transcriptomic differences between samples at different anode potentials with acetate as the electron donor were subtle, but there were differences in expression of some previously identified electron transfer proteins. We did not detect differential expression of the genes encoding CbcL and ImcH, two proteins implicated in respiration of electrodes at the anode potentials that we used. We also observed significant changes in expression between samples grown using formate vs. acetate samples at the same anode potential. This study will add to our understanding of the complex metabolism of *G. sulfurreducens*.

Authors Index

A

Abardia-Serrano, Clara S2-O-16
Actis, Paolo S1-KL-01
Adam, David S4-O-03
Agostiano, Angela S5-O-02
Ágoston, Dóra S3-O-02 S3-P-05
Aldea, Anca S2-P-09 S2-P-10 S2-P-16
Aleksanyan, Mina S3-O-03
Aleksejeva, Olga S1-O-04
Alexandrov, Kirill S1-O-07
Alfonta, Lital S2-O-21
Algov, Itay S2-O-21
Alvarez Malmagro, Julia S2-P-02 S4-O-14
Anastasoaie, Veronica S2-P-17
Anastasova, S. S2-O-13
Andoralov, Victor S1-O-04
Angelini, Alessandro S1-P-22
Angelopoulou, Michailia S1-P-01
Apetrei, Constantin S2-O-15
Apetrei, Irina Mirela S2-O-15
Arbault, Stéphane S4-O-01
Argirusis, C. S6-P-01
Arshi, Simin S2-P-13
Artyushkova, Kateryna S5-O-01
Ash, Philip S6-O-03 S6-O-04
Atanassov, Plamen S5-O-01
Axente, E. S3-IL-01
Aziz, Micahel J. S4-O-11

B

Babanova, Sofia S5-O-01
Bacalum, Mihaela S1-P-11
Bacciu, Andrea S1-P-06
Bădulescu, Marius S2-O-27
Bala, Camelia S1-P-09 S2-O-03
Baltás, Eszter S3-O-02 S3-P-05
Baradoke, Ausra S1-P-15 S1-P-16
Bard, Allen J. S2-O-07
Bardarov, Ivo S5-P-02
Barrière, Frédéric S2-O-07
Bârsan, Mădălina M. S1-IL-01 S2-P-10

Bartkowiak, Aleksandra	S2-O-17		
Bartlett, Phil N.	S6-KL-01		
Baś, Bogusław	S2-P-21		
Bazan, Guillermo C.	PL-01		
Becker, Jana	S4-O-02		
Belloir, Christine	S6-O-02		
Bergel, Alain	S5-O-01	S6-O-07	
Bernedo, Roberto	S2-P-25		
Bertaglia, Thiago	S4-O-11		
Bettazzi, Francesca	S1-P-21		
Biaso, F.	S6-O-01		
Bieżuńska-Kusiak, Katrzyna	S3-P-01		
Bilewicz, Renata	S1-P-10	S2-O-17	S4-O-12
Birrell, Janes A.	S1-O-05	S4-O-02	S4-O-06
Blidar, Adrian	S2-O-02	S2-P-08	
Bloom, Brian	S6-KL-02		
Bodoki, Ede	S1-O-01		
Bogdan, Diana	S2-O-02		
Bojarski, Wiktor	S2-P-23		
Bollella, Paolo	S1-O-07		
Bombelli, Paolo	S4-O-07		
Borisov, Galin	S5-O-05		
Borkowska, Justyna	S1-O-02	S2-P-25	
Borsali, Redouane	S4-P-07		
Borsje, Casper	S5-O-04		
Bottyán, Krisztina	S3-P-05		
Bradley, Robert W.	S6-P-04		
Brand, Izabella	S1-O-13		
Bretschger, Orianna	S5-O-01		
Brett, Christopher M. A.	S2-O-01		
Briand, Loïc	S6-O-02		
Brodacz, Kasjana	S1-P-14		
Brown, Robert K.	S5-O-01		
Buesen, Darren	S1-P-20	S4-O-03	S4-P-08
Buisman, Cees	S5-O-04		
Buscemi, Gabriella	S5-O-02		
Buta, Aleksandra	S1-P-10		
Butnaru, C.	S3-IL-01		
Buzzetti, Paulo Henrique M.	S4-P-07		

C

Cadoux, Cécile	S6-O-06		
Cahen, David	PL-02		
Călin, Violeta L.	S3-O-04		

Camarero, Nuria	S6-O-05			
Canciu, Alexandra	S2-P-12			
Caniglia, Giada	S2-O-29			
Căpățînă, Denisa	S2-P-07	S2-P-08		
Carpenter, Kayla	S5-O-01			
Carr, Stephen	S6-O-03	S6-O-04		
Carriere, Marie	S4-P-07			
Castañeda-Losada, Leonardo	S4-O-03			
Cazzaniga, Giulia	S2-P-25			
Cernat, Andreea	S1-O-12	S1-P-04	S1-P-05	S2-P-12
Chang, Hucheng	S2-O-26	S2-P-04		
Chen, Xialong	S1-P-20	S6-P-04		
Chiorcea-Paquim, Ana-Maria	S2-O-08			
Chorbadziyska, Elitsa	S5-P-02			
Choromańska, Anna	S3-KL-02	S3-P-01		
Christmann, Andreas	S2-P-25			
Chudinov, Alexander V.	S2-O-19			
Chwiłkowska, Agnieszka	S3-KL-02			
Cieplak, Maciej	S2-O-14			
Cifra, Michal	S3-O-05			
Cioffi, Nicola	S2-O-29			
Ciorită, Alexandra	S1-P-07	S2-O-09	S2-P-05	S2-P-06
Ciornii, D.	S1-O-10			
Clarindo, Jose E. S.	S4-P-05			
Clément, R.	S6-O-01			
Clever, Caleb	S6-KL-02			
Clifford, Eleanor R.	S6-P-04			
Colin, Camille	S4-O-01			
Colombo, Alessandra	S5-O-01			
Conzuelo, Felipe	S1-O-03	S2-O-11	S4-O-02	
Coroș, Maria	S1-P-07	S2-O-09	S2-P-05	S2-P-06
Cortese, Rachel	S5-O-01			
Cosma, Pinalysa	S5-O-02			
Cosnier, Serge	PL-03	S1-O-14	S4-P-07	
Crespilho, Frank N.	S2-O-23	S4-O-11	S4-P-04	S4-P-06
	S6-P-02	S4-P-05		
Cristea, Mihaela	S2-P-17	S2-P-19		
Cristea , Cecilia	S1-O-12	S1-P-04	S1-P-05	S2-O-02
	S2-P-08	S2-P-11	S2-P-12	S2-P-14
	S2-P-07			
Cristiani, Pierangela	S5-O-01			
Croce, Roberta	S6-O-05			
Csarman, Florian	S2-O-26			
Curri, M. Lucia	S1-P-21			

Cwalina, Beata	S1-P-12			
Czerwińska-Główka, Dominika	S1-P-03	S1-P-12		

D

da Silva Oliveira, W.	PL-04			
da Silva, Wanderson	S2-O-01			
da Silva, Wilson T. L.	S4-P-06			
Danhel, Ales	S1-O-08	S2-O-10		
Dănilă, George Mădălin	S1-P-09	S2-O-03		
Darvishi, S.	PL-04			
de Lacey, Antonio L.	S1-O-04	S4-O-14		
de Marco, Ario	S2-P-25			
de Wael, Karolien	S1-P-04	S1-P-05	S2-O-02	
de Zio, Simona	S2-O-06			
de-los-Santos Álvarez, Noemi	S2-KL-04	S2-O-16	S2-P-11	
Dempsey, Eithne	S1-P-06			
Demurtas, Denise	S2-P-15			
di Bari, Chiara	S1-O-04			
Díaz-Fernández, Ana	S2-KL-04			
Diculescu, Victor C.	S1-IL-01	S2-O-22	S2-P-09	S2-P-16
Dimova, Rumiana	S3-O-01	S3-O-03		
Diolez, Philippe	S4-O-01			
Dobrovodsky, Daniel	S2-O-10			
Doumard, Layal	S3-O-06			
Drăgan, Ana-Maria	S1-P-04	S1-P-05		
Drago, Estrella	S2-P-02			
Duan, Jufu	S6-O-06	S6-O-08		
Dziubak, Damian	S2-O-17			
Dzwonek, Maciej	S4-O-12			

E

Economou, Eleni Anna	S1-P-13	S2-P-01		
Eddine Chafai, Djamel	S3-O-05			
Edwardraja, Selvakumar	S1-O-07			
El Housseini, Wassim	S4-O-05			
Elsakova, Alexandra	S1-P-15	S1-P-16		
Enache, Adrian	S1-P-11			
Enculescu, Ionuț	S1-IL-01	S2-P-09		
Engelbrekt, Christian	S1-O-15			
Erable, Benjamin	S5-O-01	S5-O-03		
Erb, Tobias	S4-O-03			
Erkmen, Cem	S2-O-30			
Etienne, Mathieu	S4-O-05			
Evanghelidis, Alexandru	S1-IL-01			
Evans, Rhiannon	S6-O-03	S6-O-04		

F

Falciola, Luigi	S1-P-21			
Faria, Luana C. I.	S4-P-04			
Farinola, Gianluca M.	S5-O-02			
Feier, Bogdan	S1-P-04	S1-P-05	S2-P-07	S2-P-08
Feiertag, Aviv	S2-O-21			
Filipek, Sławomir	S1-O-02			
Filmon, Dawit Tedros	S4-O-06			
Florea, Anca	S1-P-04	S1-P-05		
Flory, Justin	S6-P-03			
Fojt, Lukas	S1-O-08			
Fojta, Miroslav	S1-O-08	S2-O-10		
Fourmond, Vincent	S4-O-04	S4-O-06		
Frank, Anna	S1-O-03			
Freier, Erik	S4-P-08			
Fritea, Luminița	S1-O-14			
Fromme, Petra	S6-P-03			

G

Gajewska-Naryniecka, Agnieszka	S3-P-06			
Gal, E.	S1-P-07			
Garido Fernandes, Isabel	S2-O-08			
Gáspár, Szilveszter	S2-O-20			
George, Glen	S1-P-06			
Gheorghe, Sorin Sebastian	S2-O-27			
Gheorghiu, Mihaela	S2-O-20			
Giannotti, Marina Inés	S6-O-05			
Gibot, Laure	S3-O-06			
Girault, Hubert H.	PL-04			
Giroud, Fabien	S1-O-14	S4-P-07		
Golberg, Alexander	S3-O-06			
Gomez Vidales, Abraham	S4-O-10	S4-P-01		
Gonzalez-Arribas, Elena	S1-O-04			
Gonzato, Carlo	S2-O-28			
Gordon, Roy G.	S4-O-11			
Gorgy, Karine	S4-P-07			
Gorostiza, Pau	S6-O-02	S6-O-05		
Gouarderes, Sara	S3-O-06			
Grattieri, Matteo	S5-O-02			
Gross, Andrew J.	S1-O-14			
Gübitz, Georg M.	S4-P-02			
Guette-Marquet, Simon	S6-O-07			
Guo, Zhong	S1-O-07			
Gutiérrez-Sánchez, Cristina	S4-O-14			

H

Haberbauer, Marianne	S5-P-01	S4-P-02	
Halpin, Grace	S1-P-06		
Happe, Thomas	S6-O-08		
Hardt, Steffen	S1-O-05	S4-O-06	
Harnisch, Falk	S5-O-01		
Hartmut, Michel	S1-KL-02		
Hason, Stanislav	S2-O-10		
Hassan, Ayaz	S2-O-23		
Haupt, Karsten	S2-O-28		
Havelka, Daniel	S3-O-05		
Hecht, Sidney	S6-P-03		
Heinzmann, Anna	S2-O-29		
Hellwig, Petra	S1-KL-02		
Hianik, Tibor	S2-O-24		
Hideghety, Katalin	S3-O-07		
Höfs, Soraya	S1-P-08		
Holzinger, Michael	S1-O-14		
Hosu, Oana	S1-O-12	S2-O-02	S2-P-11
Howe, Christopher J.	S4-O-07	S4-O-08	S6-P-04
Howley, Ethan	S6-P-06		
Hu, Chen	S6-O-05		
Huang, Wei	S1-O-15		
Hubenova, Yolina	S5-O-05	S5-P-02	

I

Iacob, Bogdan-Cezar	S1-O-01		
Ilbert, M.	S6-O-01		
Ilie-Mihai, Ruxandra-Maria	S2-O-27		
Ingrosso, Chiara	S1-P-21		
Ivorra, Antoni	S3-KL-01		

J

Jachimska, Barbara	S1-O-09	S1-O-13	
Jacq-Bailly, Aurore	S4-O-04		
Jafarov, Alicja	S1-P-15	S1-P-16	
Jarczewska, Marta	S2-P-23		
Jaskółowski, Marcin	S2-P-24	S2-P-27	
Jędrzak, Artur	S1-P-23	S2-P-28	
Jesionowski, Teofil	S1-P-23	S2-P-28	
Jipa, F.	S3-IL-01		
Jose, Catherine	S2-O-18		
Jyoti,	S2-O-28		

K

Kampakdee, Kwankao	S1-P-02		

Karantonis, A.	S6-P-01			
Karnaouri, A.	S6-P-01			
Karpova, Elena	S1-P-18	S2-O-25		
Karyakin, Arkady A.	S2-KL-03	S1-P-18	S1-P-19	S2-O-25
	S2-P-26			
Katseli, Vassiliki	S1-P-01			
Katz, Evgheny	S1-O-07			
Kawano, H.	S3-IL-01			
Kazek-Kęsik, Alicja	S1-P-14			
Kemény, Lajos	S3-O-02	S3-P-05		
Kerr, Emily F.	S4-O-11			
Khmeleva, Svetlana A.	S2-O-19			
Khodykina, M. O.	S1-P-17			
Khushvakov, Jaloliddin	S6-O-06			
Kieberger, Nina	S5-P-01			
Kim, Dahae	S3-P-07			
Kis, Erika Gabriella	S3-O-02	S3-O-07	S3-P-05	
Kizling, Michał	S4-O-12			
Klimko, Artsiom	S3-P-02			
Kochana, Jolanta	S2-P-21			
Kodali, Mounika	S5-O-01			
Kokkinos, Christos	S1-P-01			
Kolmar, Harald	S2-P-25			
Komorek, Paulina	S1-O-13			
Koprowski, Piotr	S1-P-10			
Kostov, Krassimir L.	S5-P-02			
Koszo, Renata	S3-O-07			
Kovacs, Eugenia	S3-O-08			
Kracher, Daniel	S1-P-02			
Krajmalnik-Brown, Rosa	S6-P-06			
Kranz, Christine	S2-O-29			
Kremser, Klemens	S4-P-02			
Krishna Kadambar, Vashanta	S1-O-07			
Krukiewicz, Katarzyna	S1-P-03	S1-P-12		
Kubiszewski, Marek	S2-P-20			
Kucera, Jiri	S4-P-02			
Kudła, Patryk	S1-O-02			
Kulawiak, Bogusz	S1-P-10			
Kulbacka, Julita	S3-KL-02	S3-P-01	S3-P-04	
Kurbanoglu, Sevinc	S2-O-30			
Kusumoto, Tomoichirou	S1-KL-02			
Kutdusova, Gulnaz R.	S2-O-19			
Kutner, Wlodzimierz	S2-O-14	S2-O-28		
Kutner, Andrzej	S2-O-28			

Kuznetsova, Viktoriya E.	S2-O-19			
Kuznowicz, Maria	S1-P-23	S2-P-28		

L

Labarile, Rossella	S5-O-02			
Lach, Patrycja	S2-O-14			
Lagarde, Florence	S2-O-18			
Lagunas, Anna	S6-O-02			
Lai, Tin	S6-P-05			
Lapa, Sergey A.	S2-O-19			
Lapicque, François	S4-O-05			
Łapińska, Zofia	S3-P-06			
Łaszcz, Marta	S2-P-20			
Lawrence, Joshua M.	S6-P-04			
Lazar, Gyorgy	S3-O-07			
Lebègue, Estelle	S2-O-07			
Lee, Andrew J.	S1-KL-01			
Lee, Sora	S3-P-07			
Léger, Christophe	S4-O-04	S4-O-06		
Leomil, Fernanda S.C.	S3-O-01			
Leote, Ricardo J. B.	S2-O-22	S2-P-10	S2-P-16	
Leś, Andrzej	S2-P-20			
Leśniak-Ziółkowska, Katarzyna	S1-P-14			
Lewis, Christine	S6-P-03			
Li, Huaiguang	S1-P-20	S4-P-08		
Li, Hongbo	S4-O-10			
Li, Wangzhe	S6-O-04			
Lian, Amy	S6-O-08			
Lielpetere, Anna	S4-O-02			
Lipkowski, Jacek	S2-P-02			
Lira, Rafael B.	S3-O-01	S3-O-03		
Lisdat, Fred	S1-O-10	S1-P-08	S2-O-11	S4-O-09
Lobo-Castañón, María Jesús	S2-KL-04	S2-O-16	S2-P-11	
Loibner, Andreas Paul	S5-P-01			
Lojou, Elisabeth	S4-O-05	S6-O-01		
Long, Yi-Tao	S2-O-12			
López-Ortiz, Manuel	S6-O-05			
Lorenzo-Gómez, Ramón	S2-KL-04			
Lubitz, Wolfgang	S4-O-02	S4-P-08		
Ludwig, Roland	S1-O-04	S1-P-02	S2-O-26	S2-P-03
	S2-P-18	S4-P-03	S2-P-04	

M

Ma, Su	S2-P-03	S4-P-03		
Ma, Yumeng	S4-O-01			

Macedo, Lucyano J. A.	S4-P-05		
Machini, W. B. S.	S2-O-04		
Maciejewska, Dorota	S2-O-28		
Madej, Maria	S2-P-21		
Magner, Edmond	S2-O-05	S2-P-13	S2-P-15
Majewska, Marta	S2-O-14		
Majewska, Aleksandra	S2-P-23		
Makarchuk, Iryna	S1-KL-02		
Maksimova, Ekaterina D.	S2-P-26		
Malferrari, Marcos	S2-O-06		
Malinowska, Elżbieta	S2-P-23		
Mandl, Martin	S4-P-02		
Manoliu, Laura	S2-P-22		
Marcaccio, Massimo	S2-KL-01		
Marcus, Andrew	S6-O-09		
Marian, Iuliu O.	S1-O-01		
Marrazza, Giovanna	S1-O-06	S1-O-12	
Martin, Eliza	S2-P-22		
Martinez Ostormujof, Lucila	S5-O-03		
Matei, Mircea-Bogdan	S3-P-02		
Matei, Elena	S2-P-09	S2-P-16	
Matica, Ovidiu-Teodor	S2-P-19		
Matoga, Dariusz	S2-P-21		
Mattioli, Isabela A.	S2-O-23		
Matyszewska, Dorota	S2-O-17		
Mazurenko, I.	S6-O-01		
Melin, Frederic	S1-KL-02		
Melinte, Gheorghe	S1-O-12		
Melman, Artem	S1-O-07		
Mendes, Giovana R.	S6-P-02		
Meneghello, Marta	S4-O-04		
Merzlikin, Mark M.	S1-P-15	S1-P-16	
Michel, Olga	S3-KL-02	S3-P-04	
Mieczkowska, Magdalena	S2-P-23		
Mihăilescu, Mona	S3-O-04		
Miklavčič, Damijan	PL-05		
Milac, Adina	S2-P-22		
Milton, Ross	S6-O-06		
Mir, Lluis	Milazzo Prize		
Miranda-Castro, Rebeca	S2-KL-04	S2-O-16	
Mirel, Valentin	S2-O-09	S2-P-05	
Mitov, Mario	S5-O-05	S5-P-02	
Miyawaki, A.	S3-IL-01		
Modenez, Iago	S6-P-02		

Moisescu, Mihaela G.	S3-O-04	S3-O-08	S3-P-02
Moore, Yoshua	S1-P-20		
Moore, Thomas	S6-P-03		
Moore, Ana	S6-P-03		
Morlock, Sascha	S1-P-08	S4-O-09	
Mornay, Manon	S2-O-18		
Moro, Giulia	S1-P-22		
Morra, Simone	S6-O-08	S6-P-05	
Muhammad, Mumtaz	S4-P-07		
Münchberg, Ute	S4-P-08		
Munteanu, Raluca-Elena	S2-O-20		

N

Nagar, B.	PL-04		
Narajczyk, Magdalena	S1-O-02		
Nazaruk, Ewa	S1-P-10		
Neuhauser, Eva	S5-P-01		
Niedzółka-Jönsson, Joanna	S1-O-02	S2-P-25	
Nikitina, V. N.	S1-P-19	S2-P-26	
Nikolaev, Anton	S1-KL-02		
Novickij, Vitalij	S3-KL-02		
Nowaczyk, Mark M.	S1-O-03		
Nowak, Anna	S4-O-12		
Noworyta, Krzysztof R.	S2-O-14	S2-O-28	
Nussbaum, Robin	S6-O-06		
Nwanebu, Emmanuel	S4-P-01		

O

Obata, K.	S3-IL-01		
Ober, Camille	S3-O-06		
Ócsai, Henriette	S3-P-05		
Oh, Myung-Min	S3-P-07		
Oktaviani Matei, Christien	S3-P-02		
Oláh, Judit	S3-O-02	S3-O-07	
Oliveira, Maria C.	S1-O-11		
Oliveira, Ana Rita	S4-O-04		
Oliveira-Brett, Ana Maria	S2-O-04	S2-O-08	
Øllgaard Duus, Jens	S1-O-15		
Oloketuyi, Sandra	S2-P-25		
Omanovic, Sasha	S4-O-10	S4-P-01	
Onea, Melania	S1-P-11		
Oprean, Radu	S1-O-01	S1-P-04	S1-P-05
Orobeti, S.	S3-IL-01		
Ostatna, Veronika	S2-O-10		
Otero, Fernando	S2-O-05		

Ottlakan, Aurel	S3-O-07			
Oughli, Alaa A.	S1-O-05	S4-P-08		
Outla, Martin	S2-O-10			

P

Paczia, Nicole	S4-O-03			
Pagkali, V.	S2-P-01			
Palchetti, Ilaria	S1-P-21	S1-P-22		
Panagiotopoulos, Apostolos	S1-P-13			
Pandis, P. K.	S6-P-01			
Paolucci, Francesco	S2-KL-01			
Pereira, Iñes A.C.	S4-O-04	S4-O-14		
Pershina, Katherine D.	S1-P-17			
Phadke, Sujal	S5-O-01			
Pifferi, Valentina	S1-P-21			
Pintilie, Lucia	S2-P-19			
Pita, Marcos	S1-O-04	S4-O-14		
Pivonkova, Hana	S2-O-10			
Plumeré, Nicolas	S1-O-05	S1-P-20	S4-KL-02	S4-O-03
	S4-P-08	S4-O-06		
Pogăcean, Florina	S1-P-07	S2-O-09	S2-P-05	S2-P-06
Polo, Federico	S1-P-22			
Porada, Radosław	S2-P-21			
Prates Ramalho, João P.	S1-O-01			
Prieto Dapena, Francisco	S2-P-02			
Pruneanu, Stela	S1-P-07	S2-O-09	S2-P-05	S2-P-06
Průša, Jiři	S3-O-05			
Przytaś, Wioletta	S1-P-12			
Ptitsyn, Konstantin G.	S2-O-19			
Puiu, Mihaela	S1-P-09	S2-O-03		
Pusta , Alexandra	S2-P-08	S2-P-14		

R

Radko, Sergey P.	S2-O-19			
Rakowski, Kamil	S1-O-09			
Ramirez, Miguel	S6-O-03			
Ramirez-Hernandez, Miguel	S6-P-05			
Rapino, Stefania	S2-O-06			
Rátkai, Sándor	S3-O-02			
Raveendran, Mukhil	S1-KL-01			
Rebeccani, Sara	S2-KL-01			
Rembiałlkowska, Nina	S3-KL-02	S3-P-01		
Ricci, Francesco	Galvani Prize			
Richter, Michael	S4-O-03			
Riedel, Marc	S1-O-10	S1-P-08	S2-O-11	

Riedl, Sebastian	S5-O-01			
Riske, K. A.	S3-O-01			
Rittmann, Bruce	S6-P-03			
Rivas, Jose	S6-KL-02			
Roccitta, Gaia Giovanna Maria	S1-P-06			
Rodriguez-Macia, Patricia	S6-O-03	S6-O-08	S6-P-05	
Rolandi, Marco	S6-IL-01			
Roques, Christine	S6-O-07			
Rosa, Luis F. M.	S5-O-01			
Rossi, Bruno R.	S4-P-06			
Rossowska, Joanna	S3-KL-02			
Rózsa, Petra	S3-P-05			
Rüdiger, Olaf	S1-O-05	S4-O-06		
Rudno-Rudzińska, Julia	S3-KL-02			
Rueda, Manuela	S2-P-02			
Ruff, Adrian	S1-O-03	S2-O-11	S2-O-26	S4-O-02
Rus, Iulia	S2-P-14			
Ruzgas, Tautgirdas	S2-KL-02			

S

Saczko, Jolanta	S3-KL-02	S3-P-01	S3-P-04	S3-P-06
Safarian, Schara	S1-KL-02			
Sakamoto, Junshi	S1-KL-02			
Salaj-Kosla, Urszula	S2-O-05			
Samitier, Josep	S6-O-02			
Samouillan, Valérie	S3-O-06			
Sanches, Natalia M.	S2-O-23			
Sandu, Ana Maria	S3-O-04			
Săndulescu, Robert	S2-P-14			
Santoro, Carlo	S5-O-01			
Sanz, Caroline G.	S1-IL-01	S2-O-22	S2-P-10	
Sasiain Conde, Amaia	S5-P-01			
Saulnier, Joelle	S2-O-18			
Savopol, Tudor	S3-O-04	S3-O-08	S3-P-02	
Scarlat, Eugen	S3-O-04			
Schachinger, Franziska	S2-P-03			
Scheiblbrandner, Stefan	S2-O-26	S2-P-03	S2-P-04	
Schram, Jonas	S1-P-04	S1-P-05		
Schröder, Uwe	S5-KL-01	S5-O-01		
Schuhmann, Wolfgang	S1-O-03	S2-O-11	S2-O-26	S4-O-02
Schuhmann, Horst Wilhelm	S2-P-25			
Schwaiger, Lorenz	S1-P-02	S2-P-04		
Sedenho, Graziela C.	S4-O-11	S4-P-04	S4-P-05	S4-P-06
	S6-P-02			

Selvolini, Giulia	S1-O-06		
Semenov, Iurii	S3-P-03		
Șerban, Ariana	S1-IL-01		
Serien, D.	S3-IL-01		
Serra, Pier Andrea	S1-P-06		
Sfragano, Patrick Severin	S1-P-21	S1-P-22	
Shcherbacheva, Elizaveta	S1-P-18	S2-O-25	
Shleev, Sergey	S1-O-04		
Sidoryk, Katarzyna	S2-P-20		
Sima , Felix	S3-IL-01		
Simka, Wojciech	S1-P-14		
Sindhu Sharma, Piyush	S2-O-14	S2-O-28	
Skaźnik, Klaudia	S2-P-21		
Skonieczna, Magdalena	S1-P-12		
Skorupa, Małgorzata	S1-P-03		
Slavcheva, Evelina	S5-O-05		
Sleutels, Tom	S5-O-04		
Sojic, Neso	S4-O-01		
Soldanova, Zuzana	S2-O-10		
Soulis, D.	S2-P-01		
Sözer, Esin B.	S3-P-03		
Spehar-Délèze, A.	S2-O-13		
Spiess, Sabine	S5-P-01	S4-P-02	
Spiridon, Laurențiu	S2-P-22		
Sportelli, Maria Chiara	S2-O-29		
Stănică, Luciana	S2-O-20		
Stapf, Stefanie	S4-O-06		
Stavra, E.	S2-P-01		
Ștefan, Geanina	S1-O-12	S2-O-02	S2-P-11
Ștefaniu, Amalia	S2-P-17	S2-P-19	
Ștefan-van Staden, Raluca-Ioana	S2-O-27		
Steffer, Fabian	S4-O-03		
Steinkühler, Jan	S3-O-03		
Stoian, Ioan-Adrian	S1-O-01		
Stolarczyk, Elżbieta U.	S2-P-20		
Stolarczyk, Krzysztof	S2-P-20	S4-O-12	
Stripp, Sven	S6-O-06		
Student, Sebastian	S1-P-12		
Su, ZhangfFei	S2-P-02		
Subramanian, Senthil Kumar	S4-O-09		
Sugioka, K.	S3-IL-01		
Suprun, Elena V.	S2-O-19		
Suraniti, Emmanuel	S4-O-01		
Syed, Tofail	S2-O-05		

Szczesny, Julian	S4-O-02			
Szederkényi, Edit	S3-P-05			
Szewczyk, Adam	S1-P-10			
Szewczyk, Anna	S3-KL-02	S3-P-01	S3-P-06	
Szlasa, Wojciech	S3-P-04			
Szota, Maddalena	S1-O-09			
Szot-Karpińska, Katarzyna	S1-O-02	S2-P-25		
Szwedowicz, Urszula	S3-P-06			

T

Taha Ayoub, Ahmed	S3-O-05			
Tanaka, Shunya	S1-O-14			
Tang, Jing	S1-O-15	S4-P-03		
Tanner, David	S4-P-03			
Tarba, Nicolae	S3-O-04			
Tarek, Mounir	S3-P-04			
Tartakovsky, Boris	S4-O-10	S4-P-01		
Ter Heijne, Annemiek	S5-O-04			
Tertiș, Mihaela	S1-P-04	S1-P-05	S2-P-12	S2-P-14
Teychené, Sébastien	S5-O-03			
Thallner, Sophie	S5-P-01	S4-P-02		
Thesseling, Alexander	S1-KL-02			
Thomas Vernier, P.	S3-P-03			
Thorsten, Friedrich	S1-KL-02			
Tikhonov, D. V.	S2-O-25			
Tivig, Ioan	S3-O-08			
Topakas, E.	S6-P-01			
Topoglidis, Emmanuel	S1-P-13			
Torres, César	S6-P-03	S6-P-06		
Trotta, Massimo	S5-O-02			
Truță, Florina Maria	S1-P-04	S1-P-05		
Tsujimura, Seiya	S1-O-14			
Turza, A.	S1-P-07			
Tymecki, Łukasz	S4-O-12			

U

Ulstrup, Jens	S1-O-15	S4-P-03		
Ungureanu, Eleonora-Mihaela	S2-P-17	S2-P-19		
Uslu, Bengi	S2-O-30			

V

Vadgama, Pankaj	S2-O-13			
Valenti, Giovanni	S2-KL-01			
Valentini, Antonio	S2-O-29			
Varga, Anita	S3-P-05			
Varodi, Codruța	S1-P-07	S2-O-09	S2-P-05	S2-P-06

Vasile (Corbei), Alina-Alexandra	S2-P-17			
Vélez, Marisela	S4-O-14			
Veloso, Andreia D.	S1-O-11			
Vermaas, Willem	S6-P-03			
Videira, Romeu A.	S1-O-11			
Viehauser, Marie-Christin	S2-P-18			
Vieira, Nirton C. S.	S2-O-23			
Vilà, Neus	S4-O-05			
Villajos, Beatriz	S4-O-14			
Vincent, Kylie	S6-O-03	S6-O-04	S6-O-08	S6-P-05
Volker, Sieber	S4-O-03			

W

Walcarius, Alain	S4-O-05			
Waldeck, David H.	S6-KL-02			
Waldmann, Niels	S5-P-01			
Wälti, Christoph	S1-KL-01			
Wang, Panpan	S1-O-03			
Wang, X.	S6-O-01			
Wei, Jimeng	S6-KL-02			
Wei, Jiaao	S6-O-03			
Wey, Laura T.	S4-O-08	S6-P-04		
Więckowska, Agnieszka	S2-P-24	S2-P-27	S4-O-12	
Winkler, Martin	S6-O-08			
Wohlschlager, Lena	S2-O-26			

X

Xiao, Xinxin	S1-O-15	S4-O-13	S4-P-03	

Y

Yan, Xiaomei	S1-O-15	S4-P-03		

Z

Zabłoczka-Godlewska, Ewa	S1-P-12			
Zaborowska, Michalina	S2-O-17			
Zamfir, Lucian-Gabriel	S1-P-09	S2-O-03		
Zamora-Brito, Ricardo	S6-O-05			
Zavolskova, Marina D.	S1-P-19			
Zhang, Jingdong	S1-O-15			
Zhang, Jenny Z.	S1-P-20	S4-KL-01	S4-O-08	S6-P-04
Zhao, Fangyuan	S1-O-03			
Zhu, Y.	PL-04			
Zoccoler, M.	S3-O-01			
Żołek, Teresa	S2-O-28			
Zouni, Athina	S1-O-10	S4-O-09		
Zouraris, D.	S6-P-01			

B E S
The Bioelectrochemical Society

bes2021.org