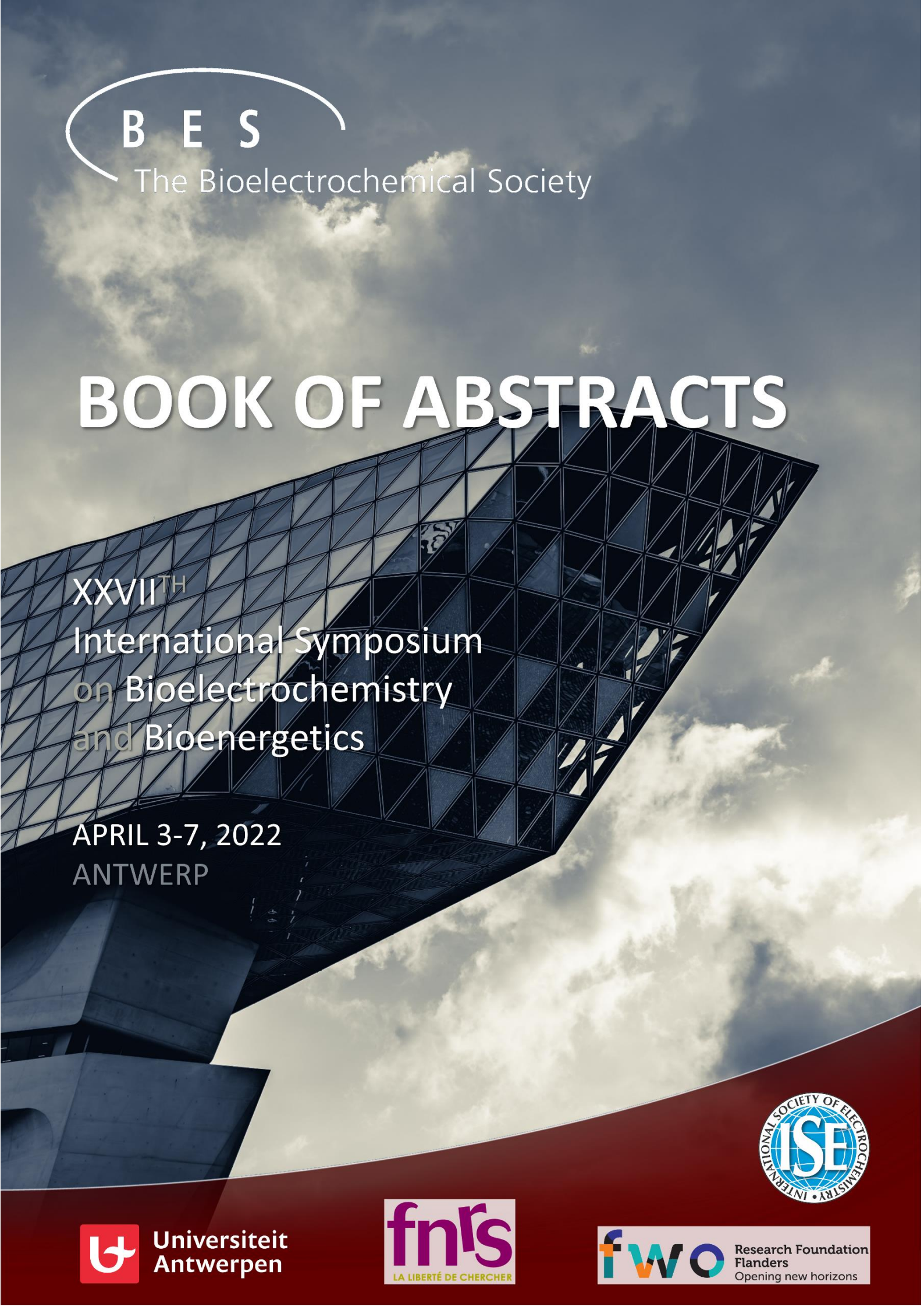
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B E S
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Plenary sessions

P1: Electrochemical Control over Metalloprotein Single Crystals: Linking Structure, Function and Spectroscopy to Elucidate Mechanism for NiFe Hydrogenases

Kylie A. Vincent,[1] Philip A. Ash,[2] Sophie Kendall-Price,[1] Stephen Carr,[1] Wangzhe Li,[1] Rhiannon Evans,[1]

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Electrochemical control has been used widely in studies of redox metalloenzymes, yielding insight into catalysis, inhibition and inactivation, and leading to applications of bio-electrocatalytic oxidation or reduction processes. For the hydrogenases, electrochemical control has been coupled with infrared (IR) spectroscopy to enable spectroscopic study of electrochemically-generated redox states and intermediates during enzyme catalysis.(1, 2) We now show how electrochemical control can also be extended to structural study of hydrogenases in the crystalline state. In structural work on metalloprotein crystals, it is notoriously difficult to achieve well-defined redox states. Protein crystals may be treated with chemical oxidants or reductants, or soaked with substrate/product in attempts to modify redox state, but the resulting crystals are often in mixed redox states that are difficult to characterise. Recently, we have shown the possibility of electrochemically manipulating single crystals of hydrogenases, and using IR microspectroscopy, simultaneously, to report on the active site redox state.(3,4) In this way, it has been possible to achieve single crystals of hydrogenase in substantially pure redox states. The electrochemically manipulated crystals maintain diffraction to high resolution, offering important opportunities for precisely-controlled structural studies. Furthermore, some of the chemical steps relevant to the hydrogenase mechanism are slowed in crystallo, making it possible to resolve transformations that are too fast to observe in solution.(3) Overall, we show how electrochemical control over crystals of a complex metalloprotein such as hydrogenase opens up exciting new opportunities to unify structure-function-activity insight and bridges diverse research areas across biological chemistry.

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2.Lubitz, W. et al. Chem. Rev. 2014, 114, 4081.

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P2: Expanding the Genetic Code of Bioelectrochemical Systems

Lital Alfonta

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Genetic code expansion is a robust technology that enables the site-specific modification of proteins theoretically at will, with more than 300 unnatural amino acids. This ability enables the modification of proteins with biorthogonal chemical handles, biophysical probes, and redox-active amino acids that do not exist in nature as amino acids, among other possibilities. In the past several years we have expanded the genetic code of several microorganisms: *Synechococcus sp. cyanobacteria, Pseudomonas aeruginosa, Vibrio natriegens, and Chlamydomonas reinhardtii*. Thus, we now have a set of molecular tools that allow us to modify proteins in these microorganisms in addition to *E. coli*. In those different microorganisms, proteins and enzymes were modified with unnatural amino acids to tether them site-specifically to electrodes to allow direct electron transfer between the enzyme active site and an electrode. Enzymes were both oxidative enzymes where the electrons flow to the electrode as well as reducing enzymes where electrons were effectively injected to the enzyme active site. In my talk, I will demonstrate how catalytic and thermodynamic parameters of the tethered enzymes have been dramatically changed upon their site-specific attachment to electrodes. I will also demonstrate that these concepts can be extrapolated to more complex systems such as whole microorganisms for future electrochemical control of microorganisms and their utilization for different applications.

P3: From Single Molecules to Living Electronics: Unraveling Mechanisms of Microbial Electron Transport

Moh El-Naggar, Grace Chong, Marko Chavez, Joshua Atkinson, Tingting Yang, Christina Cole, Fengjie Zhao, Sahand Pirbadian, James Boedicker, Yunke Zhao, Ron Naaman, Fabien Pinaud

Departments of Physics, Biological Sciences, and Chemistry, University of Southern California

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Electronic components that bridge the biotic-abiotic interface will have vast implications for both studying and harnessing the activity of living cells. While much ongoing research focuses on applying traditional rigid electronics to biology, an alternative is to discover bioelectronic solutions that life itself evolved to interact with the abiotic world. Towards realizing this vision, recent studies at the interface of microbiology, electrochemistry, and physics have uncovered metalloprotein electron conduits and nanowires that electronically link bacteria to extracellular surfaces ranging from environmental minerals to solid-state electrodes. Since this extracellular electron transport naturally evolved to interact with external surfaces, a fundamental understanding has special implications for new bioelectrochemical technologies and living electronics that harness the advantages of microbes in detecting external signals or hosting synthetic genetic circuits.

We will describe our recent progress in understanding extracellular electron transport at multiple length scales, from the biophysics of individual multiheme cytochromes to the electrophysiology of whole bacteria and multicellular communities ranging from biofilms to cable bacteria. Using single molecule tracking, stochastic simulations of cell surface multiheme cytochromes, and lithographic patterning of electrode attached biofilms, we describe how the interplay of cytochrome dynamics and electron hopping can give rise to long-distance electron conduction along bacterial membrane surfaces. In addition, we describe strategies to characterize and harness the electrochemical activity, spin filtering, and conduction properties of bacterial electron conduits in both synthetic structures and living biofilms.

P4: Treating Cancer Using Electroporation – Understanding Mechanisms and Implementing Clinical Applications

Julie Gehl, Professor and Chief Physician

Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen and Department of Clinical Oncology and Palliative Care, Zealand University Hospital, Vestermarksvej 9, 4000 Roskilde, Denmark

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Brief electric pulses can cause permeabilization of the lipid bilayer of cell membranes (electroporation). Over the past three decades this principle has been refined and exploited for medical use. Electroporation can be either irreversible, where cells do not recover after extensive permeabilization, or reversible where the permeabilized state is temporary. Reversible electroporation can be used for delivery of drugs, nucleic acids and other molecules, to meet medical needs. By combining electroporation with the chemotherapeutic agent bleomycin, which does not easily traverse the cell membrane, a dramatic enhancement of cytotoxicity can be achieved (electrochemotherapy, ECT). ECT is now widely used for the treatment of cutaneous tumors and further indications for deep seated tumors are being investigated. Calcium electroporation (CaEP) is a novel treatment where supraphysiological doses of calcium can be internalized by electroporation, causing acute cell death associated with severe ATP-depletion. Electroporation can also be used to deliver DNA, RNA and other oligonucleotides, and this is used in vaccination as well as cancer treatment. Irreversible electroporation is used for e.g. cancer treatment or cardiac ablation.

This talk will give a range of examples of medical use of electroporation technology, as well as dig into the basic mechanisms involved in electroporation at the cellular level, and illustrate how the technology may further evolve in concert with increased understanding of the underlying mechanisms.

P5: Bipolar Bioelectrochemistry

Alexander Kuhn

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A normal electrochemical or bioelectrochemical conversion takes place in most cases in a classical way, i.e. on the surface of electrodes which are connected to a source of electricity. However, there is an alternative way to trigger electrochemical processes at a distance on objects that are not in physical contact with a voltage or current generator. This "wireless electrochemistry", or more scientifically called "bipolar electrochemistry", has a long history [1]. The concept is currently experiencing a real renaissance [2] in different fields, especially because of a large variety of potential applications that have been highlighted during the last decade [3,4]. The approach allows not only the very controlled modification of surfaces or the generation of motion, but, most importantly, also the development of new concepts in (bio)analytical chemistry [5, 6]. In this presentation we will discuss the main research directions in this field and illustrate the power of the concept by focusing on some recent bioelectrochemical applications [7-11].

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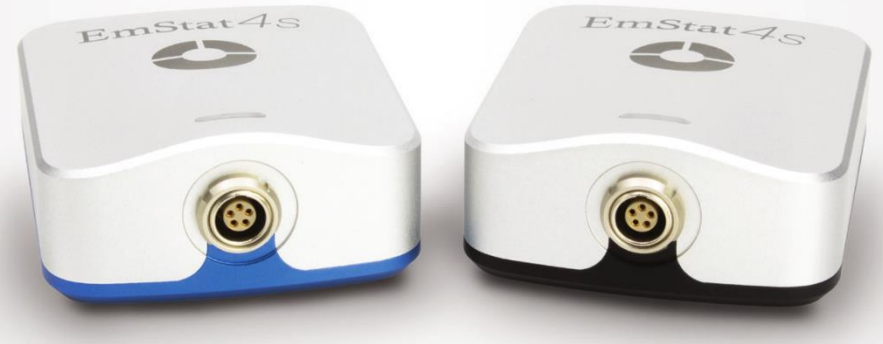

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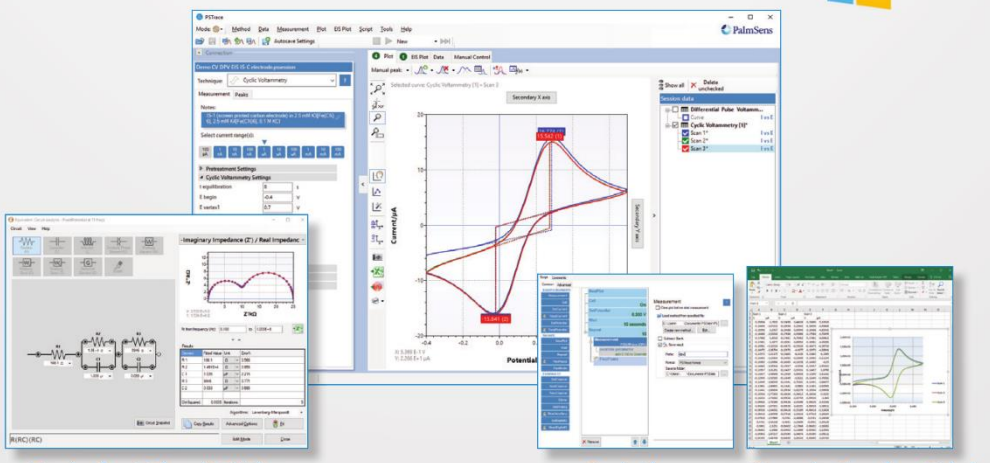

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S1: Bio(inspired) sensors & diagnostics

Keynote speakers

S1K1: Magneto-Hydrodynamic Extraction of Interstitial Fluid for Noninvasive Wearable Biosensing

Johan Bobacka[1,2], Tuuli A. Hakala[2], Alejandro García Pérez[2,3], Melissa Wardale[2], Ida A. Ruuth[2], Risto T. Vänskä[2,3], Teemu A. Nurminen[2], Emily Kemp[2], Zhanna A. Boeva[1,2], Juha-Matti Alakoskela[2], Kim Pettersson-Fernholm[2], Edward Hæggström[2,3]

[1]Åbo Akademi University, Faculty of Science and Engineering, Henriksgatan 2, 20500 Turku/Åbo, Finland 2GlucoModicum Ltd, A.I. Virtasen Aukio 1, 00560 Helsinki, Finland [3]University of Helsinki, Department of Physics, Gustaf Hällströminkatu 2, 00560 Helsinki, Finland

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Noninvasive wearable biosensing requires needle-free access to a representative biological sample, such as interstitial fluid (ISF). In this work we present and discuss a magneto-hydrodynamic (MHD) extraction technique where a small electric current is combined with a magnetic field to accelerate fluid transport through skin via the Lorentz force [1]. The extraction efficiency of MHD is evaluated by utilizing porcine skin as an ex-vivo model and glucose as a model analyte. When applying a 0.3 mA current and a 300 mT magnetic field, the MHD technique was found to increase the rate of glucose transport through porcine skin by an order of magnitude compared with reverse iontophoresis (0.3 mA). Hence, MHD opens exciting possibilities for noninvasive monitoring of glucose and other biomarkers in ISF. The MHD technology was integrated with a glucose biosensor into a wearable device that is currently being evaluated in clinical trials. The performance offered by MHD for noninvasive determination of biomarkers in ISF will be highlighted in this presentation.

1. Tuuli A. Hakala, Alejandro García Pérez, Melissa Wardale, Ida A. Ruuth, Risto T. Vänskä, Teemu A. Nurminen, Emily Kemp, Zhanna A. Boeva, Juha-Matti Alakoskela, Kim Pettersson-Fernholm, Edward Hæggström, and Johan Bobacka, “Sampling of fluid through skin with magneto-hydrodynamics for noninvasive glucose monitoring”, Scientific Reports, 11 (2021) article no. 7609.

S1K2: DNA Electrochemistry and Electrochemical DNA Biosensors for Clinical Diagnostics

Elena Ferapontova

Interdisciplinary Nanoscience Center (iNANO), Natural Sciences, Aarhus University Gustav Wieds Vej 1590-14, DK-8000 Aarhus C, Denmark

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Electrical properties of self-assembling DNA nanostructures underlie the paradigm of nanoscale bioelectronics and allow the development of sensitive and accurate, yet simple, inexpensive and robust analytical platforms, which can successfully compete with other approaches [1]. Recent efforts are further concentrated on the development of robust and non-invasive assays for early diagnosis and prognosis of a variety of sever chronic and infectious diseases, including cancer, neurodegeneration, and pathogenic infections [2-4].

Here, I overview basic electrochemistry of DNA and our current achievements in construction of label-free electrochemical DNA-based biosensors for clinical diagnostics, and electrocatalytic strategies for signal amplification providing the optimal signal resolution and sensitivities of the biomarker's detection sufficient for clinical applications. Some selected examples of electrochemical biosensors for cancer biomarker proteins, neurotransmitters, and bacteria developed in my group will be discussed.

Acknowledgement: Support from the Novo Nordisk Foundation, grant reference number NNF20OC0065428 is greatly acknowledged

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4 Ferapontova, E., Electrochemical analysis of dopamine: Perspectives of specific in vivo detection, Electrochim. Acta 2017, 245, 664–671

Invited speakers

S1I1: Paper-based (bio)sensors as smart and sustainable point-of-care devices

Fabiana Arduini [a,b], *Veronica Caratelli* [a,b], *Noemi Colozza* [a,b], *Laura Fabiani* [a], *Luca Fiore* [a,b], *Vincenzo Mazzaracchio* [a], *Danila Moscone* [a]

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[b] *SENSE4MED S.R.L., Via Renato Rascel 30, 00133 Rome, Italy*

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In the last decade, electrochemical paper-based (bio)sensors have garnered much attention in the sensing field thanks to their cost-effectiveness, easiness to use, and miniaturization. Besides these characteristics in common with the other electrochemical (bio)sensors, the features of paper such as foldability and porosity have opened new unprecedented electrochemical (bio)sensor configurations allowing for reagent-free measurements, origami-like set-up, and the absence of sample treatment. Furthermore, paper-based electrochemical devices have overcome the limitation of other electrochemical sensors, being able to detect the target analytes not only in solution but also in aerosol phase and surface without any additional instrument, matching one of the top 10 emerging technologies of 2021, namely diagnosing diseases with a puff of breath. In addition, after the measure, the device can be burned reducing waste management with a relevant decrease of analysis costs in the case of biological fluids. In the invited presentation, I will report how we have exploited the features of paper to design smart biosensors able to treat the sample, to contain any reagent needed for the measurement, to on-site synthesize nanomaterials, and to make the measurement delivering novel paper-based point-of-care devices.

Oral presentations

S1O1: Brain tissue oxygen pressure monitoring using polyphenylenediamine-polyurethane-coated carbon fiber microelectrodes

Stephane Marinesco, Shuting Chen, Anne Meiller

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Brain tissue oxygen pressure (PbtO2) monitoring is an integral part of clinical bedside brain monitoring techniques for severely brain-injured patients in neurological intensive care units. It is also important in pre-clinical research to understand brain metabolism. Electrochemistry provides high spatial and temporal resolution to achieve PbtO2 monitoring, especially platinum (so-called Clark electrodes) and carbon fibre microelectrodes. However, a major problem with long-term PbtO2 recordings is a significant loss of sensitivity due to electrode passivation in the living brain tissue. Here, we tested a polyphenylenediamine-polyurethane (PPD-PU) coating to decrease electrode fouling in vivo and improve the stability PbtO2 recordings. PPD-PU deposition was tested on bare carbon fibre microelectrodes (7 µm diameter, 100 µm long, 4400 µm2) as well as on platinized carbon fibre microelectrodes. Oxygen reduction started at 0 mV vs Ag/AgCl on Pt microelectrodes, whereas it occurred around -500 mV vs Ag/AgCl on carbon, resulting in increased sensitivity for oxygen on Pt. However, when the microelectrodes were coated with PPD-PU, the sensitivity for oxygen was more diminished on carbon (-78 ±3%), than on Pt (+36 ± 25%). In vivo, however, carbon fibre microelectrodes coated with PPD-PU were used for 2.5-5 h PbtO2 monitoring and showed an increase in oxygen sensitivity (+60 ±30 %) whereas platinized carbon fibres displayed a dramatic decrease in sensitivity (-81 ±6 %). These results indicate that PPD-PU coating on carbon fibre microelectrodes can increase the stability of PbtO2 monitoring over time and prevent electrode fouling in vivo. Future experiments will be aimed at evaluating their potential use in long term brain monitoring sessions lasting several days.

S1O2: Peroxidase-based sensors using mediated electrochemistry and in-situ area normalization

Tanushree Mandal, Donal Leech

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Redox enzymes such as horseradish peroxidase (HRP), glucose oxidase (GOx) etc. have long been used to design specific, sensitive, and selective electrochemical sensors. For example, a hydrogen peroxide sensor based on HRP bioelectrocatalysis can be used in many diagnostics, industrial and environmental sectors. Mass produced sensors using screen-printing of electrodes are cheap and can be easily modified, but with one disadvantage that their surface areas can be irregular.

A major challenge in electrochemical sensor design is estimation of electrochemical surface area (ECSA) of an electrode sensor because the current is proportional to ECSA and variability in the electrode surface areas generate variability in the corresponding currents. ECSA can be estimated using charge associated with stripping of underpotentially deposited hydrogen from platinum, stripping of oxide layer from gold, use of a redox probe, or by measurement of double-layer charging (DLC) capacitance1. Here we report on a comparison of these methods. We will show that normalization of electrode responses having different surface areas can be achieved using in-situ experimental methods.

Peroxide sensors are developed using redox-active complexes of osmium coordinated with polypyridyl ligands. A range of Os(II/III) complexes were studied to find the best mediator for detection of hydrogen peroxide using HRP catalysis. The results show that the [Os(bpy)2Cl2]Cl complex can be used as an effective mediator in designing a peroxide sensor.

Using an optimized mediator and a simple method to estimate carbon electrode surface area, peroxidase-based sensors can be designed with improved peroxide detection having potential application across a range of areas, such as immunoassays for detection of cancer biomarkers2.

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S1O3: Cross-linked dextran-methacrylate enzymatic biosensors for transdermal glucose monitoring

Bastien Darmau[(1,2)], *Isabelle Texier*[(2)], *Andrew J. Gross*[(1)]

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Electrochemical biosensors have attracted considerable interest for portable and rapid personal health monitoring. Amperometric biosensors exploiting enzymes such as glucose oxidase (GOx) have already revolutionised the treatment of diabetes[1]. Limitations and barriers of entry for in-vivo biosensors include cost, accuracy, biocompatibility, and comfort, as well as stability limitations[2]. Herein, we describe a new 2nd generation glucose biosensor that combines the emerging FAD glucose dehydrogenase (FAD-GDH)[2] with adsorbed mediator and photocross-linked dextran-methacrylate (Dex-MA) biopolymer at carbon nanotube electrodes. Biopolymers with different degrees of substitution have been synthesized and their important effects on catalytic and biosensing performance explored. The cross-linked Dex-MA are very promising for sensing in simple buffer as well as synthetic interstitial fluid. We will briefly present our first steps towards the development of microneedle devices for transdermal sensing.

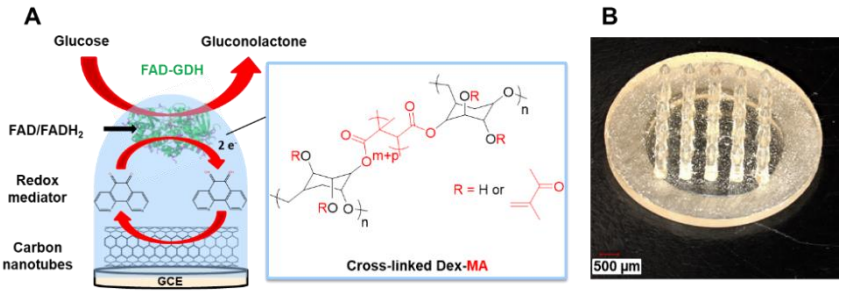

Figure 1. (A) Schematic of the glucose biosensor and (B) Polymer microneedle

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S1O4: Sandwich Sensor Based on Electrocatalytic Reduction of Oxygen by G4-Hemin-Aptamer Complex for the Femtomolar Detection of HER2/neu in Human Serum.

Ana Díaz Fernández, Elena E. Ferapontova

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Breast cancer is the most commonly diagnosed cancer in females and the leading cause of cancer death.1 For its better clinical prognosis and choosing the best-targeted therapy, the levels of the Human Epidermal growth factor Receptor-2 (HER2/neu) are monitored. Her2/neu is a glycoprotein complex belonging to the family of receptor tyrosine kinases, that is overexpressed in tumours with aggressive growth and spreading. Current clinical methods for the analysis of HER2/neu relies on invasive methods and thus, are poorly suited for continuous monitoring. Therefore, there is a clear need for complementary or alternative non-invasive techniques. Electrochemical sensors could be a good alternative to achieve the required sensitivity and selectivity for a precise determination of HER2/neu.2

In this work, we present an electrochemical sandwich apta and immuno sensor on magnetic beads exploiting the covalent hemin-G-quadruplex (G4) complex as an electrocatalytic label.3 The analysis of HER2/neu is followed via the electrocatalytic reduction of oxygen on graphite electrodes electrocatalyzed directly by hemin intercalated into the G4 structure linked to the detection aptamer. The assay exploits the direct oxidase activity of the hemin-G4 covalent complex4,5 and thus doesn´t require any additional substrate or mediator species, since it uses solely oxygen already present in the solution. The sensors detect HER2/neu in the femtomolar range in both buffer and serum, with a negligible interference from other proteins. The analytical characteristics are adequate for clinical applications and may complement the existing methods for HER2/neu monitoring.

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S1O5: Construction of an enzymatic, oxygen-insensitive L-lactate sensor using soluble or grafted redox mediators

Roy Cohen, Nidaa S. Herzallh, Matan M. Meirovich, Oren Bachar, Liora Frech, Yifat Cohen, Omer Yehezkeli

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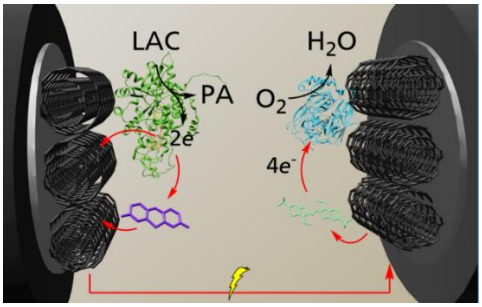

Lactate is involved in many diseases and conditions and is, therefore, an important biomarker. L-lactate is the common isoform found in our bodies and is implicated with lactic acidosis, heart conditions, tissue damage, systemic shock, cancer, and more. Similar to glucose sensing, L-lactate biosensors were studied and fabricated, based mainly on L-lactate oxidase. However, these systems are sensitive to the changing concentration of oxygen in our bodies. In contrast, FMN-dependent lactate dehydrogenase is oxygen insensitive and therefore doesn't have this limitation. I will present an amperometric L-lactate biosensor, capable of lactate detection or as a bioanode in lactate/oxygen biofuel-cell configurations. Furthermore, I will show that the grafting of various redox mediators to multi-walled carbon-nanotubes significantly improves the lifetime of the biosensor while still enabling both applications.

Utilization of FAD-Glucose Dehydrogenase from T. emersonii for Amperometric Biosensing and Biofuel Cell Devices, Roy Cohen, Rachel E. Bitton, Nidaa S. Herzallh, Yifat Cohen, and Omer Yehezkeli, Analytical Chemistry 2021 93 (33), 11585-11591

An Oxygen-Insensitive Biosensor and a Biofuel Cell Device based on FMN L-lactate Dehydrogenase, Roy Cohen, Nidaa S. Herzallh, Matan M. Meirovich, Oren Bachar, Liora Frech, Yifat Cohen, Omer Yehezkeli, Under Review

S1O6: Deep Eutectic Solvents with Bio-Based Chiral HBD or HBA: a New Electroanalytical Approach for the Enantiodiscrimination of Chiral Probes

Sara Grecchi[1], Serena Arnaboldi[1], Elisa Emanuele[1], Lorenzo Guazzelli[2], Mariangela Longhi[1], Fabiana Arduini[3], Laura Micheli[3], Patrizia R. Mussini[1]

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Deep Eutectic Solvents (DESs) offer advantages similar to Ionic Liquids (ILs), but with easier and more sustainable synthesis; moreover, bio-based DESs often include chiral components, surprisingly underexploited. Herein we intent to present the use of enantiopure DESs as chiral media for enantioselective electroanalysis. Three chiral DESs, consisting in a molecular salt with bio-based chiral cation $[NopolMIm]^+$ combined with three natural and low-cost partners (levulinic acid, glycerol and urea), are introduced and investigated as chiral voltammetry media [1]. Significant potential differences were observed for the enantiomers of a model chiral probe, also changing the achiral partner and reaching an impressive ~0.5 V in the levulinic acid case. With the same medium good enantiodiscrimination was also observed for the aminoacid tryptophan, a quite different probe of applicative interest [1].

Such proof-of-concept results also suggest many interesting developments, including a deeper investigation of the molecular interactions within the three-actors (probe and the two DES components: HBD and HBA) system, as such and at the interphase with the charged electrode surface, and a further analysis of the role of acid/base interactions. For this reason, many other chiral DESs (with different HBD and HBA, and in different ratios) were studied, considering the effect of the water amount in the enantioselection ability.

These findings can be considered as a remarkable step further in chiral electroanalysis as well as in the development of task-specific enantioselective media.

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S1O7: Novel polymers for bioelectrochemical applications with unusual properties and varied redox potentials

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The use of redox polymers in bioelectrochemical devices provides a range of advantages. Redox polymers provide high electron transfer rates by electrically connecting the redox centers of enzymes to the electrode. Redox polymers increase the possible loading of the enzyme onto electrode and wire the enzyme regardless of its orientation. Polymer hydrogels also provide a hydrated environment for enzymes and increase the stability of the bioelectrode [1].

Depending on the application, the properties of the redox polymer have to be adjusted to ensure the necessary redox potential, hydrophobicity and hydrophilicity, charge interaction and stability over the electrode.

Examples of application of the newly synthesized polymers (Fig. 1) in biodevices will be provided.

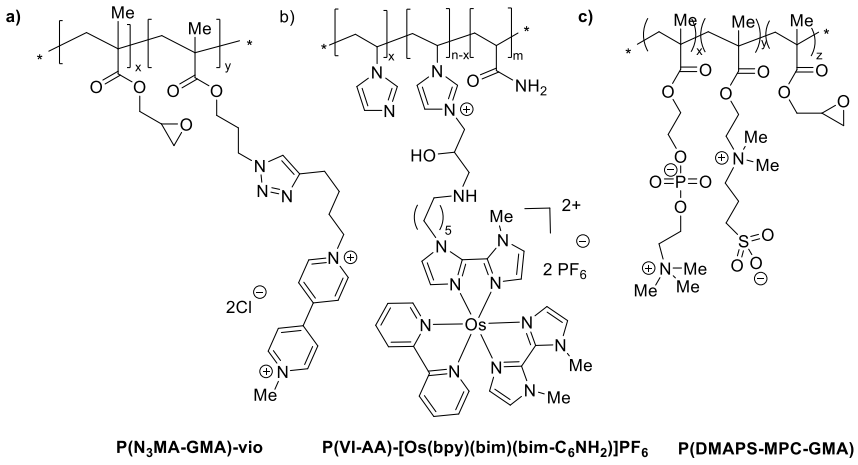

Figure 1. Examples of tailored polymers for bioelectrochemical applications: a) high loading viologen-modified redox polymer for wiring hydrogenases; b) osmium-complex modified redox polymer with optimized redox potential; c) zwitterionic redox-silent polymer for anti-interference shields and improved stability of the bioelectrode.

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S1O8: The Effect Of Polymer Shields On Mediated Enzymatic Glucose Biosensors

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One of the major challenges for development of implanted devices, including biosensors, is non-specific adsorption of cells and biomolecules that generates inflammatory response and leads to reduced performance[1]. When dealing with electrochemical biosensors, electrochemical interferences must also be taken into account. For example, in enzymatic glucose biosensor current density detected in artificial serum is lower compared to the response in PBS, an effect previously observed and attributed to electrochemical interferences and protein adsorption2,3. To achieve a functional implantable sensor, shields must be employed target both these interferences. Most work regarding electrochemical biosensors mainly target the elimination of electrochemical interferences using anionic polymer coatings such as Nafion[4]. In terms of biocompatible coatings, a promising class of materials is zwitterionic polymers that have great biocompatibility and have been proven to reduce fouling on implantation[5]. The objective of our research was to investigate Nafion and a commercial zwitterionic polymer as coating on mediated enzymatic glucose biosensors and how they compared to a library of synthesised zwitterionic polymers with tuneable properties. Protein ELISA and cell adhesion studies were used as a screening to investigate which of the synthesised polymers showed the best compatibility followed by electrochemical testing with and without common electrochemical interferents to determine how the overcoats affected sensor performance. The effect of polymer composition on stability and current response will be discussed.

S1O9: PFAS Sensing: Concept design of electrochemical and optical biostrategies

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PFAS are a class of more than 3000 manmade chemicals representing a global issue due to their bioaccumulation and toxic behaviour. Aiming to transpose toxicological studies in PFAS biosensing design, delipidated human serum albumin was considered for application in perfluorooctanoic acid (PFOA) sensing, here PFOA was selected as representative of PFAS class. Albumin was applied as bioreceptor in impedimetric and optical label-free platforms. In the impedimetric one, the protein bioreceptor was immobilized at screen-printed electrodes previously modified via electropolymerisation developing an immobilization method compatible with EDC/NHS chemistry. The biosensing was tested in presence of increasing concentrations of PFOA and the changes at interfacial electron transfer were correlated to the formation of the albumin-PFOA complex. Extracting the absolute impedance values at 10 Hz from the Bode phase plot was possible to build a calibration plot in the nanomolar range. The interpretation of the impedimetric data was supported by crystallographic study of the conformational changes in the albumin-PFOA complex.

In the optical platform, albumin was immobilized also at D-shaped optical fibers developing a Lossy-Mode Resonance-based label-free platform. This proof-of-concept study is one of the first examples in which this kind of optical sensors is used for the determination of small molecules, such as PFOA. After these preliminary tests these sensing strategies were compared underling their potentials and limitations. By combing and comparing different sensing platforms we aimed answering the need of investigating new biosensing strategies. Overall, this study offers useful guidelines for the development of new sensing strategies for PFAS monitoring in different environmental matrices.

S1O10: Electrochemical monitoring of metabolites for detection and label-free identification of microorganisms

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Persistent bacteremia are most often associated with sepsis, some of the most urgent and life-threatening situations in medical practice. Clinical laboratories currently employ a two-step strategy, with the detection of pathogen in blood culture bottle, followed by its identification once the microorganism is separated from the patient's blood. In this report, we propose a breakthrough approach to reduce time diagnosis of bacteremia. Firstly, our smart blood culture bottles enable a continuous monitoring of blood culture, away from the lab. Secondly, these instrumented bottles can yield identification without separating the pathogen from blood.

We have instrumented blood culture bottles with electrochemical sensors made of different conductive inks that incorporate either conducting polymers or metal oxides. In our experiments, we spiked defibrinated horse blood and human blood with reference bacterial strains in our blood culture bottles previously sterilised by autoclave and containing liquid bacterial growth medium. We conducted 70 experiments with different species and various initial biomasses to measure the variability of our results. Nine of them were negatives (sterile) in order to calculate our detection limits.

Every spiked culture led to a detection, so that we had no false negative. Our time-to-detection is comparable to the one's yieldied by current commercial systems through optical transduction (Bactec™ and BacT/ALERT®).

Electrochemical monitoring provided Gram-typing one hour only after culture positivity. Four hours after positivity it was also possible to determine bacterial species, without any further manipulation.

Blood culture bottles instrumented with electrodes provide a continuous monitoring of the culture at the patient's bedside, just after sample collecting. Furthermore they can yield, for monomicrobial infections, Gram-typing and then identification, without any further handling, provided that incubation is extended a few minutes longer (< 90min). Altogether, these smart blood culture bottles constitute a promising technology for shortening the time of sepsis diagnosis, thereby improving clinical outcomes.

S1O11: A Cholesterol Biosensor Based on Modified Carbon Nanodots and a Dehydrogenase Enzyme

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Monitoring cholesterol physiological concentrations is of great importance in the medical field, as elevated concentrations are associated to high risk of coronary heart disease. In this work, we present a cholesterol biosensor based on the electrocatalytic oxidation of the NADH generated during the enzymatic reaction of cholesterol dehydrogenase (ChDH) as an alternative to the H2O2 biosensing strategies used with cholesterol oxidase-bioelectrodes. Azure A functionalized-carbon nanodots were used as NADH oxidation electrocatalysts and for ChDH covalent immobilization. The biosensor responded linearly to cholesterol concentrations up to 1.7 mM with good sensitivity (4.50 mA cm-2 M-1). Furthermore, the bioelectrode was combined with an O2-reducing bilirubin oxidase cathode to produce electrical energy using cholesterol as fuel and O2 as oxidant. The resulting enzymatic fuel cell was tested in human serum naturally containing free cholesterol; a maximum power density of 1.57 µW cm-2 at 0.25 V and an OCP of 470 mV were reached.

S1O12: Detection of Bacterial Rhamnolipid Toxin by Redox Liposome Single Impact Electrochemistry

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The detection of Rhamnolipid virulence factor produced by Pseudomonas aeruginosa involved in nosocomial infections is reported by using the redox liposome single impact electrochemistry. Redox liposomes based on 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) as a pure phospholipid and potassium ferrocyanide as an encapsulated redox content are designed for using the interaction of the target toxin with the lipid membrane as a sensing strategy. The electrochemical sensing principle is based on the weakening of the liposomes lipid membrane upon interaction with Rhamnolipid (RL) toxin which leads upon impact at an ultramicroelectrode to the breakdown of the liposomes and the release/electrolysis of its encapsulated redox probe.

We present as a proof of concept the sensitive and fast sensing of a submicromolar concentration of Rhamnolipid which is detected after less than 30 minutes of incubation with the liposomes, by the appearing of current spikes in the chronoamperometry measurement.

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Posters

S1P1: Development of a combi-electrosensor for the detection of phenol by combining photoelectrochemistry and square wave voltammetry

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The high toxicity, endocrine-disrupting effects and low (bio)degradability commonly attributed to phenolic compounds have promoted their recognition as priority toxic pollutants by the European Commission and Environmental Protection Agency of the United States. For this reason, the monitoring of these compounds in industrial, domestic and agricultural streams is crucial to prevent and decrease their toxicity in our daily life. To confront this relevant environmental issue, we propose the use of a combi-electrosensor which combines singlet oxygen (1O2)-based photoelectrochemistry (PEC) with square wave voltammetry (SWV). The high sensitivity of the PEC sensor ensures the detection of nmol L−1 levels of phenolic compounds while the SWV measurements allow the differentiation between phenolic compounds. Consequently, this synergy will lead to a powerful tool since each technique improves the general performance by overcoming the inherent drawbacks that they display independently. Moreover, the on-site detection will be reached by integrating the use of screen-printed electrodes (SPEs) with wireless potentiostats. Herein, we report on the development of such a combi-electrosensor for the sensitive and selective detection of phenol (PHOH) in the presence of related phenolic compounds such as hydroquinone (HQ), bisphenol A (BPA), resorcinol (RC) and catechol (CC). The PEC sensor was able to determine the concentration of PHOH in spiked river samples containing only PHOH with a recovery between 96% and 111%. The SWV measurement elucidated the presence of PHOH, HQ and CC in the spiked samples containing multiple phenol compounds. As a result, the combination of the two techniques is a powerful and valuable tool in the analysis of phenolic samples where the PEC sensor determines the total phenolic concentration (being a faster alternative for traditional COD measurements) and the SWV sensor identifies the phenolic compounds present in the sample (being faster than the color test kits). Finally, the practicality of the combi-electrosensor set-up with a dual SPE containing two working electrodes and shared reference and counter electrodes was demonstrated.

S1P2: An electrochemical aptamer-based biosensor for arginin-vasopressin

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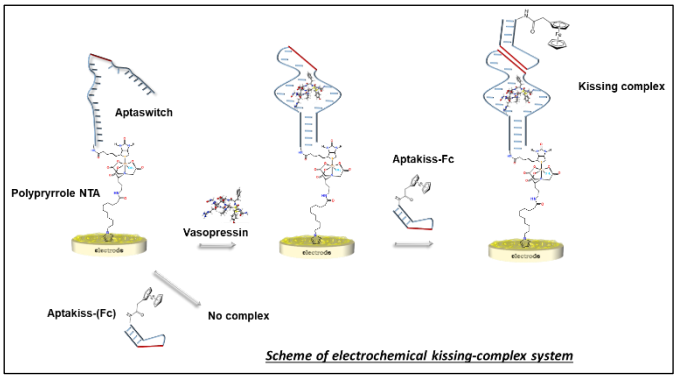
Electrochemical biosensors became essential tools for biomarker detection due to their high sensitivity (detection limits to the femtomolar level[1]) and the possibility to be miniaturized into portable devices. For small molecules detection, aptamers have gained widespread attention as biorecognition elements [2]. Here, we present the elaboration of an electrochemical aptasensor using a pseudo sandwich recognition system, known as kissing complex[3], for arginin vasopressin which is an important diagnostic biomarker for diseases such as diabetes insipidus or SIADH syndrome. This new technique aims to overcome the complexity of the currently used techniques such as radioimmunoassays (RIA) and chromatography (LC) mass spectrometry (MS).



Scheme of electrochemical kissing-complex system

S1P3: Simultaneous detection of Escherichia coli and Pseudomonas aeruginosa from microbiological samples

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Escherichia coli and Pseudomonas aeruginosa are pathogens that contaminate food, water and other environmental sources determining infectious diseases, of varying severity, including nosocomial ones, oftentimes resulting in damaging effects to the health of the population. Their early diagnostic could improve the therapeutic approach and prevent complications in patients and could guide the measures taken to limit the spread of contamination in environmental settings.

Electrochemical sensors offer promising perspectives for the selective and sensitive detection of microorganisms (1). One strategy is the indirect detection of bacteria through specific markers. Enterobactin (Ent), a siderophore produced by E. coli, and pyocyanin (PyoC), a metabolite of P. aeruginosa, were selected as target markers, which were previously studied individually (2).

In this study, Ent and PyoC were simultaneously detected using commercial graphite screen-printed electrodes in culture media. The voltammetric measurements were carried out with a portable potentiostat. The results obtained with the electrochemical method were compared with microbiology tests performed on the same samples. The matrix effect was also assessed.

The proposed setup can be successfully used for the rapid detection of both markers and could indirectly and simultaneously prove the presence of P. aeruginosa and E. coli. This method can be further developed to increase the sensitivity toward the detection of the two pathogens.

Acknowledgements: This study was supported by the European Union's Horizon 2020 research and innovation programme under grant agreement No 883484, PathoCERT.

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S1P4: Novel electrochemiluminescent assay for the aptamer-based detection of testosterone

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This work presents a proof-of-concept assay for the detection and quantification of small molecules based on aptamer recognition and electrochemiluminescence (ECL) readout. The testosterone-binding (TESS.1) aptamer was used to demonstrate the novel methodology. Upon binding of the target, the TESS.1 aptamer is released from its complementary capture probe – previously immobilized at the surface of the electrode – producing a decrease in the ECL signal after a washing step removing the released (labeled) TESS.1 aptamer. The analytical capability of the ECL assay towards testosterone detection was investigated displaying a linear range from 0.39 to 1.56 µM with a limit of detection of 0.29 µM. The selectivity of the proposed assay was assessed by performing two different negative control experiments; i) detection of testosterone with a randomized ssDNA sequence and ii) detection of two other steroids, i.e. deoxycholic acid and hydrocortisone with the TESS.1 aptamer. In parallel, complementary analytical techniques were employed to confirm the suggested mechanism: i) native nano-electrospray ionization mass spectrometry (native nESI-MS) was used to determine the stoichiometry of the binding, and to characterize aptamer-target interactions; and, ii) isothermal titration calorimetry (ITC) was carried out to elucidate the dissociation constant (Kd) of the complex of testosterone and the TESS.1 aptamer. The combination of these techniques provided a complete understanding of the aptamer performance, the binding mechanism, affinity and selectivity. Furthermore, this important characterization carried out in parallel validates the real functionality of the aptamer (TESS.1) ensuring its use towards selective testosterone binding in further biosensors. This research will pave the way for the development of new aptamer-based assays coupled with ECL sensing for the detection of relevant small molecules.

S1P5: Electrochemical aptasensor for the detection of quorum sensing molecules in Pseudomonas aeruginosa

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Bacterial infections caused by Pseudomonas aeruginosa are associated with high rates of mortality due to its capacity to develop multidrug resistance and form an adhesive biofilm which leads to failure of therapy and systemic dissemination. Early diagnostic is an important tool for increasing the chances of survival. There are new detection methods targeting representative structures such as quorum sensing (QS) molecules. QS is a form of cell-to-cell communication between bacteria, that plays a key role in virulence and biofilm formation. The detection of the molecules involved in QS in P. aeruginosa (derivatives of N-acyl homoserin lactone) would facilitate the rapid identification of nosocomial infections, allowing the establishment of an appropriate treatment (1).

Aptamers are short single stranded oligonucleotides, artificially synthesized to bind targets with high affinity. Due to their advantages (high specificity and stability, low cost, easy functionalization), aptamers have gained great interest as recognition elements in the development of electrochemical sensors (2).

In this study, we developed a sensitive and specific electrochemical aptasensor based on screen printed electrodes for the detection of QS molecules in P. aeruginosa. The specific aptamers for the target molecules were selected based on the information obtained from the literature (2). The aptamers were functionalized with thiol groups and the electrode surface was modified with gold nanoparticles to facilitate the immobilization of the aptamers. A deposition step of 2-mercaptoethanol was applied to eliminate nonspecific interactions at the Au surface. All the modification steps were optimized to determine the optimum conditions for the detection of QS molecules. The modified electrodes were characterized using different electrochemical techniques. The developed sensor showed good results in detecting QS molecules in P. aeruginosa real samples.

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.

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S1P6: Electrochemical Detection of Amphetamine in Street Samples Using an innovative nanoMIPs-based Sensor

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The prevalence of recreational substance abuse amongst young adults has markedly increased over the past two decades and it remains one of the major problems facing our society today worldwide. Amphetamine (AMP) is one of the most potent sympathomimetic amines with respect to stimulatory effects on the central nervous system (1). The direct electrochemical detection of AMP is a challenge because the molecule is non-electroactive at the potential window of conventional graphite SPEs. In this regard, a molecularly imprinted polymer (MIP) for AMP detection was synthesized The MIPs nanoparticles (nanoMIPs) were synthesized in the presence of a template molecule. After polymerization and removal of the template, MIPs are embossed with complementary cavities and functionalities (2). The technology presented herein could potentially help to rapidly determine AMP from confiscated street samples. The voltammetric sensor for AMP detection uses electroactive nanoMIPs, produced by introducing ferrocene monomer into the polymeric structures, which serves as an efficient transducer of electrochemical response. For the immobilization of nanoMIPs onto the surface of graphite SPEs different approaches were tried, and the best results in terms of stability, sensitivity, and specificity were obtained after the direct deposition of a suspension that contains chitosan, nanoMIPs, and graphene oxide.

Acknowledgements: This project has received funding from Horizon 2020 research and innovation programme (grant agreement No 833787, BorderSens)

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S1P7: Electrochemical journey towards glyphosate detection

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Glyphosate is a non-selective and broad-spectrum herbicide, classically used in combination with genetically modified crops. Despite glyphosate being the most used herbicide in the world, its detection remains a real challenge and there is a need for a new tool allowing its detection[1].

In the present project, we propose a new approach for the design of an electrochemical enzymatic biosensor using an engineered glyphosate oxidase. Unlike other biosensors for glyphosate detection, based on enzyme inhibition (HRP, urease, tyrosinase), glyphosate oxidase catalyses the glyphosate oxidation, thus producing H2O2, which can then be detected by electrochemistry.

Expressing oxidases as fusion proteins with Carbohydrate Binding Modules[2] or Carbon Nanotube Binding Peptide[3] allows their direct binding to paper- or carbon nanotube-based electrodes from a clarified cell lysate, avoiding tedious purification steps and simplifying the biosensor assembly. Progress and challenges in this enzymatic glyphosate biosensor conception will be discussed.

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S1P8: Electrochemical Detection of Methamphetamine in Confiscated Samples Using a Portable Device

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Methamphetamine (MA) is a synthetic psychoactive drug with medical applicability, being prescribed for Attention-Deficit and Hyperactivity Disorder and short-term treatment of obesity. Most frequently though, it is abused for various effects such as euphoria and hallucinations. This behavior is obvious in the increased spread and abuse of MA, which had the largest increase in quantities seized in the last decade1. Hence, the present study took advantage of the highly sensitive and accurate detection provided by the electrochemical techniques2,3, aiming for the detection of MA in confiscated samples using a portable device. In this regard, the electrochemical behavior of MA was investigated by means of square wave voltammetry. Firstly, the analytical characterization of the method was performed, exhibiting a LOD of 66.4 µM. Thereafter, the selectivity of the method towards MA in various mixtures was evaluated. Finally, the method was employed for the screening of confiscated samples with 100% true positive results, displaying its potential as a fast and easy to use method for on-site analysis.

Acknowledgements: This project has received funding from the European Union's Horizon 2020 Research and Innovation program under grant agreement No 833787, BorderSens.

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S1P9: Development of a Microbial Fuel Cell – Based Biosensor for BOD and COD

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Biological oxygen demand (BOD) and chemical oxygen demand (COD) are fundamental indicators for the wastewater quality. Due to the practical problems in determining the BOD related to the duration of the analysis, new alternative methods are intensively looking for. The aim of this study is to develop a bioelectrochemical system (BES), performing as a biosensor for simultaneous measurement of both indicators, which will significantly shorten the analysis time.

Carbon felt (CF) was selected as the most appropriate electrode material for formation of electroactive biofilm (EAB), required for the stable performance of the developed biosensors. New 3D-printed electrochemical cells were designed and long-term experiments for EAB formation on the selected CF electrodes were carried out at applied potential (+0.2 V vs. Ag/AgCl) by using activated sludge and wastewater from Municipality Wastewater Treatment Plant. A linear correlation between the generated current and the BOD/COD values was established by addition of BOD standard solutions with different concentrations to real wastewater. The obtained results by this approach will be used for development of a microbial fuel cell-based BOD/COD biosensor.

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S1P10: Amperometric biosensor for galactose detection using engineered Galactose Oxidase

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Galactosemia is a rare, hereditary disorder of carbohydrate metabolism that affects the body's ability to convert galactose to glucose. Accordingly, a deficiency of the enzymes participating in the metabolism of galactose can lead to health issues such as hepatosplenomegaly, bleeding disorders, Escherichia coli sepsis, cataracts, and sometimes death.1 Therefore, an early determination of galactose levels is significant in the diagnosis, allowing for appropriate and timely treatment for preventing these life-threatening disorders. Biosensors using electrochemical detection methods are the most widely investigated, exhibiting high sensitivity, selectivity, and reproducibility.2

It is precisely in this context that the following work is inserted, we propose to achieve an amperometric galactose biosensor using direct electron transfer between galactose oxidase (GOase) engineered by directed evolution and a nanostructured electrode surface. For this purpose, we use low density graphite and engineered GOase for unique point covalent immobilization through cysteines inserted on the surface of this enzyme. Several controls were performed and direct electron transfer and mediated electron transfer responses using osmium polymer were tested. Furthermore, surface modification using electrochemical reduction of aromatic diazonium-(4-nitrophenyl) azanide, directly on the electrode and posterior reaction with 3-Maleimidopropionic acid N-hydroxysuccinimide ester to favor the bound formation directly with the cysteine was extensively studied.

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S1P11: Electrochemical screening of a chemical library of transketolases inhibitors

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High throughput screening (HTS) methods and systems miniaturization are new assets in fundamental research. Besides the limitation of costs of the experimentation, they offer the possibility to assay a set broad of conditions at the same time while remaining fast and easy to use. Among the HTS methods, electrochemical ones are based on the detection of current variations on multiplexed electrodes. Previously we proposed a 96-well electrochemical system based on intermittent pulsed amperometry and screen-printed electrodes operating in parallel and independently. [1, 2] A proof-of-concept for the screening of inhibitors of the transketolase (TK) from E. coli was shown [2].

TK catalyze the transient grafting of two carbon units of a ketose onto thiamin pyrophosphate (TPP) leading to α, β-dihydroxyethylthiamin pyrophosphate (DHETPP). In our screening method, DHETPP is oxidized back to TPP thanks to two ferricyanide molecules. This reaction produces one glycolic acid and two ferrocyanide molecules detected electrochemically [3].

In the present work we apply this methodology on several inhibitors of TK from human pathogens in order to identify lead compounds.

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S1P12: Electrocatalytic Detection of Escherichia coli at DNA Beacon Modified Gold Screen-Printed Electrodes

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Prevention of food spoilage, pathogenic infections, and antimicrobial resistance requires rapid, sensitive, and on-point detection systems allowing fast and inexpensive detection of bacterial species c. Among the microbial communities, the bacterial strain of Escherichia coli (E. coli) is the most commonly found pathogen responsible for bacterial contamination [2]. Here, we have developed an ultrasensitive electrocatalytic assay utilizing gold screen-printed electrodes for the detection of E.coli DNA and ribosomal RNA. The binding of E. coli specific DNA to the capture DNA beacon-immobilized on the gold screen-printed electrode induces hybridization. The formed duplex acts as an electrical wire, mediating electron transfer from the gold surface to the DNA-intercalated redox indicator methylene blue [3] and further to solution-present redox indicator ferricyanide. This coupling results in the electrocatalytic amplification of the ferricyanide signal which was otherwise impeded on the DNA modified electrode surface [4]. The gold screen-printed electrodes used here are widely opted for developing portable detection platforms. However, their immediate use is often hampered due to their insufficient surface quality, resulting in sensing artifacts. We show that different surface pre-treatments of these screen-printed electrodes result in very different patterns of immobilization of thiolated DNA probes, which interfere with DNA hybridization. The developed assay allows down to femtomolar detection of both synthetic DNA and isolated E. coli ribosomal RNA.

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S1P13: Electrochemical Detection of Drug Use in Oral Fluid for Roadside Drug Testing

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Illicit drug consumption remains a problem to public safety and health, with abuse of illicit drugs having increased significantly over the last years. A concern related to this abuse is driving under the influence of drugs (DUID). Currently, police and law enforcement agencies rely on the use of lateral flow immunoassays (LFAs), which suffer from a lack of specificity. In this report, we present a rapid, sensitive, and affordable electrochemical method for the detection of illicit drugs and their metabolites in oral fluid at screen printed electrodes (SPE). For low level detection, the use of modification of the SPE with nanostructured materials is investigated. Importantly, for the first time, the effects of the oral fluid matrix on the electrochemical sensing of illicit drugs and their metabolites is explored. Interestingly, the electrochemical signals for the drugs are shown to be partially suppressed by the biofouling properties of albumin and most probably other proteins in the OF matrix. Strategies to mitigate these biofouling properties are explored. Additionally, the interference of common cutting agents and adulterants on the electrochemical profile of the selected drugs and metabolites is evaluated. Finally, several commercial oral fluid collection devices are evaluated in combination with the electrochemical method. The developed method shows promising potential in on-site testing for recent illicit drug use.

S1P14: Intermittent Pulsed Amperometry and new paper-based electrodes as a tool for characterization of ligninases

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Lignocellulosic biomass is essentially composed of glycoside polymers (cellulose, hemicelluloses) and lignin, which is the most important natural source of aromatic compounds. The valorisation of lignins into molecules of interest requires a fractionation step that can be catalysed by ligninolytic enzymes, notably oxidoreductases from fungi and bacteria (1). Ligninolytic potential of microorganisms is based on their ability to produce enzymatic cocktails composed of laccases, peroxidases, oxidases, and dioxygenases etc. able to act in synergy. The use of such enzymes in biorefinery is limited by their low efficiency and stability. Moreover, the identification of robust biocatalyst exhibiting ligninolytic activities represents a technological barrier. Indeed, spectrophotometric or chromatographic methods classically used for the detection of ligninolytic activities are not fully adapted for a high throughput assay in opaque and heterogeneous media. This technological lock hinders the discovery of biocatalysts efficient on solid substrate.

In this work, we first describe the application of a 96-electrodes system based on intermittent pulse amperometry for exploring the ligninolytic potential of peroxidases by screening several substrates. In a second part, the development of a new tool using paper-based electrodes for screening ligninolytic activities is described. This strategy consists of depositing lignin at the opposite side of paper where electrodes are screen-printed. The results present the application of these two strategies to highlight the ligninolytic potential of oxidoreductases isolated from the bacterial strain Thermobacillus xylanilyticus.

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S1P15: Characterisation of a Ru(II) Complex for PEC sensing of Phenolic Compounds and Alkaline Phosphatase activity

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The ability of a Ru(II) complex to produce singlet oxygen (1O2) upon photoexcitation under visible light was exploited for bioanalytical applications. Particularly, such complex was used as photosensitizer of a nanostructured TiO2 surface on indium-tin oxide (ITO) electrodes. A (photo)electrochemical characterisation of the Ru(II) complex was conducted. The production of 1O2 was assessed by spectrophotometric measurements. Furthermore, this highly reactive state of oxygen was used to trigger the oxidation of reversible and quasi-reversible phenolic compounds when irradiated. For instance, hydroquinone reacted with 1O2, producing benzoquinone, which was reduced back at the electrode surface in a redox cycle, hence generating a detectable photocurrent correlated to its concentration. Various parameters (pH, potential, etc.) were evaluated towards the overall performances of the platform. Moreover, harnessing the activity of alkaline phosphatase (AlkP), the Ru(II) complex-modified TiO2/ITO electrode was tested for the detection of p-aminophenol (PAP). Notably, PAP was in-situ produced from the enzymatic reaction of p-aminophenyl phosphate (PAPP) with AlkP on the electrode surface. As a result, a detectable photocurrent was observed and PAP production over time allowed assessing the enzyme activity.

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S1P16: Wearable wristband-based electrochemical sensor for the detection of phenylalanine in biofluids

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Wearable electrochemical sensors are driven by the user-friendly capability of on-site detection of key biomarkers for health management.[1] Despite the advances in biomolecule monitoring such as glucose, still, several unmet clinical challenges need to be addressed. For example, patients suffering from phenylketonuria (PKU) should be able to monitor their phenylalanine (PHE) level in a rapid, decentralized, and affordable manner to avoid high levels of PHE in the body which can lead to a profound and irreversible mental disability. Herein, we report a wearable wristband electrochemical sensor for the monitoring of PHE tackling the necessity of controlling PHE levels in PHE hydroxylase deficiency patients.[2] The proposed electrochemical sensor is based on a screen-printed electrode (SPE) modified with a membrane consisting of Nafion, to avoid interferences in bio- fluids. The membrane also consists of sodium 1,2-naphthoquinone-4-sulphonate for the in situ derivatization of PHE into an electroactive product, allowing its electrochemical oxidation at the surface of the SPE in alkaline conditions. Importantly, the electrochemical sensor is integrated into a wristband configuration to enhance user interaction and engage the patient with PHE self-monitoring. Besides, a paper-based sampling strategy is designed to alkalinize the real sample without the need for sample pretreatment, and thus simplify the analytical process. Finally, the wearable device is tested for the determination of PHE in saliva and blood serum. The proposed wristband-based sensor is expected to impact the PKU self-monitoring, facilitating the daily lives of PKU patients toward optimal therapy.

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S1P17: Biosensors operating in physiological fluids under homeostatic conditions

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Implantable biosensors for real time and continuous monitoring of biomarkers require extensive testing to investigate biocompatibility, stability, and performance. During the development of these biosensors, experimental flow set-ups can be used which simulate in vivo environment more realistically than conventional, stagnant experimental set-ups. Previously, we have characterized a flow system for an enzymatic fuel cell which can generate power from human blood 1. Herein, we have adapted this system to investigate biosensors.

In the present work, we modified a tubular working electrode with an engineered enzyme based on Cellobiose Dehydrogenase (CDH) which is capable of direct electron transfer and oxygen insensitive to create a glucose sensor. Additionally, we used an osmium redox polymer [Os(2,2'-bipyridine)2(polyvinylimidazole)10Cl]+ (Os(bpy)PVI) with a redox potential of +220mV (vs. Ag/AgCl (3M KCl).

Our results indicate a glucose concentration dependency in PBS (1 – 50 mM) for the electrodes with CDH, as well as modified with the redox polymer. Furthermore, a pulse test was performed with the aim of studying the equilibrium characteristics of the system. The observed results imply that an enzyme deactivation is very unlikely since the initial current was reached and stabilized fast. To complete this set of experiments we simulated a glucose event after food intake: here we could show a glucose dependency in a pulsed and a non-pulsed sensor mode by performing a continuous measurement with varying glucose concentrations. First flow stability tests showed a stable current for 2 hours. Moreover, when the medium was changed from physiological buffer to real physiological fluids, similar results were obtained.

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S1P18: Cytochromes fused to enzymes create electron transferring biorecognition elements – a study of direct electron transfer on MWCNT/GC electrodes

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Electrochemical biosensors utilize biorecognition elements, e.g. enzymes. Glucose oxidase, to name one example, is employed in commercial sensors. The redox centers of glucose oxidase and most related enzymes are buried deep within the enzyme. In this case, there is no direct electron transfer (DET) to the electrode and sensors based on such biorecognition elements inevitably require small molecule mediators to shuttle electrons to the electrode. Third generation biosensor development focuses on the development and application of enzymes capable of DET to the electrode without the need of a mediator. Cytochromes fused to oxidoreductases have been reported to facilitate DET. This concept enables the application of additional enzymes in mediator-free biosensors for many more interesting analytes than those already available. Prototype versions of such fusion enzymes often have low, difficult to detect currents and a quantitative immobilization of the fusion enzyme on the electrode is challenging. This inevitably leads to underestimated kinetic constants. Here, we present progress on generating a protocol for an electrode modification that allows the detection of low catalytic currents. Multi-walled carbon nanotubes (MWCNT) with distinct functional groups deposited on glassy carbon (GC) were the basis of the study. Conditions were further optimized towards our final aim – the detailed electrochemical characterization of an engineered fusion enzyme – intended for the application in blood glucose monitors.

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S2: Microbial electrochemical technologies and electron transport system

Keynote speakers

S2K1: Microbial Electrochemical Technologies (MET) already grew up to play a promising role in the water sector: case studies

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Two decades after Geobacter was found to be the key actor for generating electric current in a sediment we can find real applications for removing pollutants from water (electrobioremediaton) o monitoring water quality (electrobiosensors). Wastewater was certainly the most tested matrix for hosting MET due to the potential conversion of the chemical energy from pollutants into electrical power. Still we cannot harvest energy per m2 to compete with renewables energies in the market, but researchers have been able to design, construct and operate MET-based devices capable of treating wastewater from single house to real communities (1000 habitants). The largest application so far corresponds to a technology so-called METland, where electrochemical concepts are integrated in already existing wastewater treatment solution: the constructed wetlands. The hybrid solution is indeed an electroconductive biofilter made of sustainable materials to enhance microbial oxidation of pollutants and reduce the footprint of this nature-based solution. The system has evolved to modular construction so it can be used as plug and play solutions for treating also industrial water from oil&gas sector. In conventional microbial electrochemistry, solid electrodes (eg. rods, plates, granules, and felts) are typically used as electroconductive materials9 to support biofilm growth. Under such conditions diffusion and migration processes become a limiting factor for achieving optimal biodegradation rates. In contrast with such static configurations, an innovative design, the microbial electrochemical fluidized bed reactor (ME-FBR) uses a fluid-like electrode to minimize mass transfer and energy limitations while simultaneously enhancing the activity of both electroactive planktonic and electroactive biofilms in the bioreactor. Indeed, a fluid-like anode has been shown to be efficient for removing organic pollutants and nitrogen from industrial brewery wastewater at a pilot scale (http://life-answer.eu/en/), demonstrating the scalability of the process.

Invited speakers

S2I1: Biomolecular Wires for Electromicrobiology and Liposome Microreactors

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Electron exchange between enzymes inside bacteria and redox partners outside those bacteria, often termed extracellular electron transfer (EET), is a fascinating process. EET evolved to support anaerobic respiration by allowing extracellular mineral oxides to serve as terminal respiratory electron acceptors in place of oxygen. EET also allows bacteria to exchange electrons with electrodes supporting green electricity production in microbial fuel cells and chemical electrosynthesis by microbial enzymology. At the heart of EET is conductivity across the bacterial cell wall. For Shewanella oneidensis, a model organism for studies of EET, the cell wall is a lipid bilayer with a hydrophobic core approximately 35 Å wide and too large for direct electron transfer at a rate sufficient to support respiration. To facilitate EET the bacterium assembles a complex of three proteins, MtrCAB, and positions that complex to span the cell wall lipid bilayer [1]. A chain of close-packed redox-active heme cofactors runs through MtrCAB to facilitate rapid electron exchange [2] between redox partners inside and outside the bacterium. Thus, the MtrCAB complex is a biomolecular wire capable of electron transfer between spatially separated aqueous compartments [3,4]. This contribution reviews recent studies in which purified MtrCAB is incorporated into the lipid bilayer of a liposome vesicle containing entrapped redox enzyme. The resulting assembly is shown to perform as a microreactor for light-driven reduction, and so removal, of greenhouse gas by virtue of the ability of MtrCAB to perform trans-membrane electron transfer.

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Oral presentations

S2O1: Increased nitrate reduction with microbial electrochemical snorkel

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Microbial Electrochemical Snorkel (MES) is a short-circuited Microbial Fuel Cell (MFC), which can be applied for water treatment. In MES, anodic and cathodic sides are immersed in electron donor-rich and electron-acceptor rich media, respectively. Electron donors are oxidized by anodic bacterial biofilm on anodic side and electrons are transported through MES to be used in reduction of electron acceptors by cathodic bacterial biofilm.

In this study, a stainless steel MES was studied to use nitrate as electron acceptors and therefore treat nitrate-polluted wastewaters. In literature, to obtain a nitrate reducing biocathode of MFC, usually a potential in range -0.3 to -0.1 V vs SHE is applied. Here, by creating a proper MES setup, potential of -0.2 V vs SHE was reached [1]. Increased cathodic signals were observed on cyclic voltammetry of biocathodes (Fig. A) after nitrate addition to the reactor. Installing MES improved nitrate reduction by 40-60% (Fig. B). Microbial analysis showed increased population of bacteria from order Beggiatoales on biocathodes, which were not found in sediment and in water.

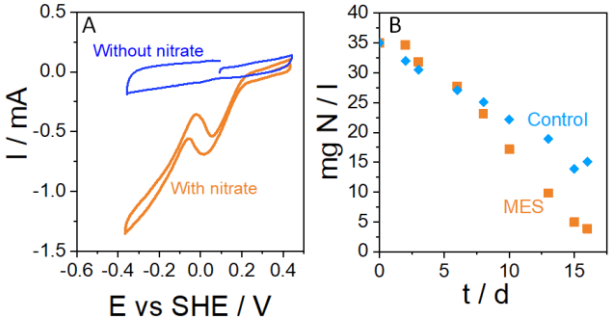

Fig. A - Cyclic voltammetry of biocathodic part of MES in presence (orange) and absence (blue) of nitrate.

Fig. B. Results of nitrate reduction with MES (organe) and for control (blue).

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S2O2: Investigating the role surface charge of polydopamine as antimicrobial coating via scanning probe microscopy

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Surface charge density and distribution play an important role in almost all interfacial processes, e.g., for the adsorption of biomolecules and for the adhesion of cells. Bacterial adhesion and the associated biofilm formation may be impeded by appropriate antimicrobial coatings, e.g., nanoparticle containing films [1]. In recent years, polydopamine (PDA) has gained substantial interest among the antimicrobial polymers (AMP) as a bio-derived AMP offering a plethora of functional groups, versatility for chemical modifications, and last but not least - its antimicrobial potential [2]. PDA films can be formed on literally any surface via dip coating processes or – especially on conductive substrates - via electropolymerization. Given the wealth of functional groups provided by PDA, the surface charge and correspondingly the adhesion properties of PDA [3] are strongly influenced by pH and the oxidation state of the polymer [4, 5].

In this contribution, we present the utility of scanning probe microscopy techniques and vibrational spectroscopy to determine the surface charge of PDA in dependence of the applied potential and pH value, and its consequence for biofilm formation. Moreover, we investigate the influence of these parameters on bacterial adhesion, e.g., E. coli via single cell force spectroscopy using colloidal conductive atomic force-scanning electrochemical microscopy (AFM-SECM) probes. In addition, infrared attenuated total reflection spectroscopy (IR-ATR) was applied to study the relation of the PDA surface charge density with the early stages of bacterial adhesion.

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S2O3: Genome Analysis of Cable Bacteria Reveals Unique Proteins Involved in the Metabolism of Long-Distance Electron Transport

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Filamentous cable bacteria have a unique metabolism that depends on centimetre-scale electron transfer through a periplasmic conductive structure. Detailed genome information can improve our understanding of the “electrical metabolism” and evolutionary history of cable bacteria. Yet, genomic studies of cable bacteria are particularly challenging as pure cultures are lacking. In our initial approach, individual cable bacterium filaments were hand-picked from sediment for whole genome amplification and second-generation Illumina sequencing. These single filament genomes are however incomplete and fragmented in many contigs. To resolve this, we developed a novel metagenome approach, which combines second and third generation sequencing to DNA extracted from bulk sediment of enrichment cultures. Assembly of Nanopore long reads followed by polishing with high-accuracy Illumina short reads resulted in the first closed genomes of cable bacteria. Genome analysis allowed to identify proteins involved the metabolism of long-distance electron transport. This way, we identified a novel putative terminal oxidase with characteristics that are specifically suited to cable bacteria metabolism.

S2O4: Temperature dependence of conductivity in cable bacteria

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The mechanism behind the extremely high electrical conductivity of the fiber network inside cable bacteria is currently unknown. The temperature dependence of the conductance of materials can provide critical insight into the underlying conduction mechanism. We investigated the current-voltage characteristics of the conductive fiber network inside cable bacteria over a wide temperature range (4 K – 300 K). To this end, single filaments of cable bacteria were chemically extracted and placed on electrode-patterned chips. For high temperatures (100 K – 300 K) we see an Arrhenius type dependence with a low activation energy 40 ± 8 meV ($N = 44$). Both two- and four-probe measurements show this dependence, indicating that contact resistances are not modulating the signal. For low temperatures (T < 100 K), a conspicuous deviation from the Arrhenius dependence is observed. At these low temperatures, the conductance responds more weakly to temperature than the Arrhenius dependence, and becomes even temperature independent at the lowest temperatures (T < 10 K). This peculiar temperature dependence of the conductance has not been previously observed in biological structures. By fitting models to the obtained data, we can delineate possible mechanisms to explain this intriguing response.

S2O5: Real-time bioelectronic sensing of environmental contaminants

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Real-time chemical sensing is needed to counter the global threats posed by pollution. Synthetic biology holds great promise for engineering cells as bioelectronic sensors that respond to diverse chemical and physical stimuli by producing extracellular electrical signals. It can be challenging to interface engineered cells with electronics to maintain a high signal-to-noise ratio in diverse environmental settings. Here, we combine synthetic biology and materials engineering approaches to develop a living bioelectronic sensor platform with minute detection times in environmental samples. Escherichia coli was programmed to reduce an electrode in a chemical-dependent manner using a modular, eight-component, synthetic electron transport chain. Encapsulation of the engineered cells with electrodes and conductive nanomaterials yielded a living bioelectronic sensor that produced significantly more current upon exposure to thiosulfate, an anion that causes microbial blooms. To diversify the ligands that can be sensed to include chemicals that affect vertebrate reproduction and health, we incorporated an engineered protein switch to gate electron transfer within the synthetic pathway that enabled the detection of an endocrine disruptor within two minutes in riverine water, implicating the signal as mass transport limited. These findings provide a new platform for miniature, low-power sensors that can be used to safeguard ecological and human health.

S2O6: Small molecule mediated exogenous electron flow into live cyanobacteria that lack the ability to split water

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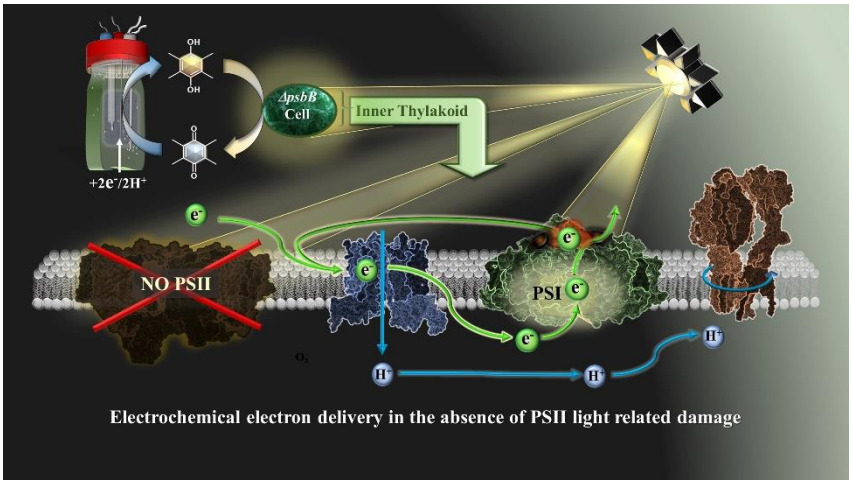
Light-activated Photosystem II (PSII) carries out the critical step of splitting water in photosynthesis. However, PSII is susceptible to light-induced damage. Here, results are presented from a novel bioreactor system using microbial electro-photosynthesis (MEPS) where redox mediators, in conjunction with an electrode, drive electron flow into live Synechocystis (ΔpsbB) cells that lack PSII. In the absence of PSII downregulation, our preliminary data shows that MEPS can generate light-dependent current which increases with light intensity up to 2050 µmol photons m–2 s–1, delivering 113 µmol electrons h–1 mg-chl–1, and an average current density of 150 A m–2 s–1 mg-chl–1. In this work, we look more closely at our MEPS system and characterize the use of different small molecules mediators. This work provides a platform for photosynthetic foundational studies and has the potential to improve photosynthetic performance at high light intensities beyond the point where natural photosynthesis can endure.



S2O7: Electrochemical and Microbiological Response of Exoelectrogenic Biofilm to Polyethylene Microplastics

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Exoelectrogenic biofilm and the associated microbial electrochemical processes have recently been intensively studied for water treatment, but their response to and interaction with polyethylene (PE) microplastics which are widespread in various aquatic environments has never been reported. Here, we investigated how and to what extent PE microplastics would affect the electrochemistry and microbiology of exoelectrogenic biofilm in both microbial fuel cells (MFCs) and microbial electrolysis cells (MECs). When the PE microplastics concentration was increased from 0 to 75 mg/L in the MECs, an apparent decline in the maximum current density (from 1.99 to 0.74 A/m2) and abundance of electroactive bacteria (EAB) in the exoelectrogenic biofilm was noticed. While in the MFCs, the current output was not significantly influenced and the abundance of EAB lightly increased at 25 mg/L microplastics. Moreover, the microbial community richness and the microplastics-related operational taxonomic units decreased with PE microplastics. Furthermore, the electron transfer-related genes (e.g., pilA and mtrC) and cytochrome c concentration decreased after adding microplastics. This study provides the first glimpse into the influence of PE microplastics on the exoelectrogenic biofilm with the potential mechanisms revealed at the gene level, laying a methodological foundation for the future development of efficient water treatment technologies.

S2O8: Electrochemical Characterization of Mammalian Respiratory Complex I and III in Intact Mitochondrial Membranes.

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Energy production by the mitochondrial respiratory chain is essential for life. Malfunction of any of the participating enzymes can result in life threatening diseases. Measuring mitochondrial oxygen consumption or ATP production can detect alterations in the oxidative phosphorylation chain (OXPHOS), but these general assays cannot identify the individual redox proteins responsible for the malfunction. Spectroscopically based biochemical assays carried out in mitochondrial fragments are frequently used to identify the redox activity of the individual respiratory chain proteins[1]. Electrochemical and spectroelectrochemical methods have also provided a wealth of information regarding reaction mechanisms of these enzymes[2]. However, these experiments have been mainly carried out with purified and reconstituted mitochondrial proteins adsorbed on electrodes using sample preparation protocols difficult to implement for the direct study of isolated mitochondria. We will present results of the electrochemical detection of Complex I and III on mitochondrial membranes adsorbed on a gold electrode. Catalytic activity was revealed through the oxidation and reduction on the electrode of a membrane associated quinone. The response to specific substrates and inhibitors support our interpretation that we monitor independently the activity of these two membrane proteins included in their native environment.

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Posters

S2P1: Chlorella biocathodes in microbial fuel cells

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Bioelectrochemical systems (BES) utilise microorganisms to catalyse electrochemical reactions. The most common type of BES is a microbial fuel cell (MFC), commonly used to produce current from organic matter during wastewater treatment. The oxygen reduction reaction, which takes place in the cathode chamber of an MFC, is an important reaction in the system. It has traditionally been catalysed by platinum and carbon-based electrodes. However, in recent times microbial catalysts are gaining relevance in this application because of their environmental friendliness. Furthermore, microalgae are being pitched as cathodic catalyst because they produce oxygen required for the oxygen reduction reaction in the cathode. This research explored the use of Chlorella vulgaris as a cathodic biocatalyst.

MFCs were set up using activated sludge at the anode with or without Chlorella vulgaris at the cathode. Initially, blank MFCs produced a peak current of 204 ± 15 µA within the first 19 days of experimental run while the algal MFCs achieved 189 ± 5 µA.

Subsequently, with a change of light regime from 12-hour dark / 12-hour light cycle to continuous illumination, the algae achieved a current of 131 µA in light and 94.6 µA in the dark.

S2P2: Unraveling the Conductive Structures of Cable Bacteria

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Filamentous cable bacteria display highly efficient electron transport over centimeter distance [1]. This long-distance electron transport is mediated by conductive structures that are embedded in the cell envelope and contain fibers. These periplasmic fibers are ~ 50 nm in diameter and run in parallel along the cell and across the cell-cell division plane over the entire length of the bacterial filament [2,3]. The periplasmic fiber network can be extracted from the cable bacteria. Studies on these extracted fiber sheaths reveal that the fibers are composed of protein and incorporate nickel, which was shown to impact electron transport [4]. However, the exact chemical structure and composition of the conductive structures remain elusive. Here, we further take apart the periplasmic fiber network and combine advanced microscopy (SEM, TEM, AFM) techniques to characterize the conductive fibers and hence achieve a deeper understanding of this new form of biological electron transport.

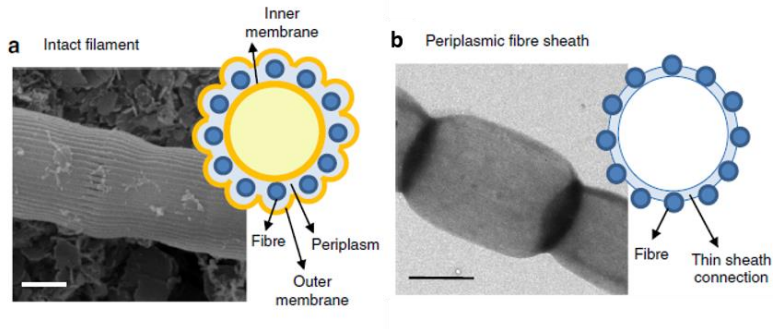

Fig. 1. a) SEM image (×7500) of an intact cable bacterium filament with schematic of the structure in cross-section revealing the periplasmic embedding of the fibers (blue circles). Scale bar = 1 µm. b) TEM image of an extracted cable bacterium filament retaining the fiber sheath with schematic of cross-section. Scale bar = 2 µm [3].

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S2P3: Controlling the conductivity of light-patterned biofilms

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Electroactive biofilms have been shown to transfer electrons over multiple cell lengths through formation of a gradient of reduced and oxidized cytochromes. This long-distance biological electron conduction process is thought to arise from the interplay of membrane-bound cytochrome diffusion and electron hopping between neighboring cytochromes. However, the impact of cytochrome quantity on the rate of electron diffusion through biofilms has not been assessed. Here, we utilize biofilm lithography to pattern Shewanella oneidensis biofilms with defined geometries and independently control biofilm conductivity by tuning expression of cytochromes in a strain devoid of cytochromes essential for extracellular electron transfer. Direct control over cytochrome content in a biofilm is enabling the evaluation of how the diffusion-assisted hopping mechanism translates to the observed rates of electron conduction in biofilms. These studies provide new insight into the quantity of cell-surface cytochromes required to facilitate electron percolation through a biofilm and the conductivity of biofilms required to electrically connect the metabolism of spatially separated cells within a biofilm.

S2P4: Understanding the Effects of Kinetic Limitations on Degradation Rates for Different Substrates in MFCs

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Increasing global demand for clean energy and water has sparked recent interest in Microbial Fuel Cells (MFCs). Despite significant progress in improving this technology over the last 25 years, there remains a lack of understanding of the processes occurring within MFCs, such as degradation rates of complex organic compounds. Limited literature on what these rates are under realistic conditions hinders this technology's optimisation. This research presented aims to help fill this research gap by investigating the degradation rates of different substrates (acetate, glucose and starch) in kinetic and non-kinetic limited systems and at varying temperatures.

Previous research has shown that power outputs when using simpler compounds (acetate) are significantly higher when compared to complex compounds (starch) due to acetate not needing to be broken down by fermentation, indicating hydrolysis is the rate-limiting step in these systems[1]. However, this was under fully stirred conditions. In reality, many MFCs operate in batch mode, and those which are continuous, have such low flow rates that turbulence does not create fully stirred conditions. If MFCs are to be used for real wastewater treatment, the degradation rates and limitations need to be fully understood. Such rates are needed to model and engineer MFC reactors effectively.

To determine whether the systems are kinetically limited, two bench-scale reactor configurations are used; one that is fully stirred, allowing the basic limitations of the main degradation pathways to be determined; and one that is not stirred, giving an indication as to whether the processes within the system are taking place in the bulk liquid or on the biofilm. Stirring and non-stirring is used to determine the mass transfer limitations within the MFCs, and ultimately help design reactors and operational conditions which maximise their efficacy for treating complex wastes.

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S2P5: A self-sustainable sensor platform to monitor the water quality with the use of a sediment microbial fuel cell

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Microbial fuel cells (MFC) are versatile bioelectrochemical systems which from an environmental sensing perspective can also be used as a biochemical oxygen demand (BOD) sensor[1]. In this contribution, we report on a prototype of an Arduino-based sensor platform raft including an MFC as BOD sensor. The goal was to build a self-sustainable and compact platform to monitor the water quality of remote water bodies. The raft was based on a sediment MFC where the anode functioned as an anchor, buried in the anaerobic sediment while the cathode was connected to the raft, i.e. a more oxygen-rich environment, both electrodes consisted of graphite felt. Besides measuring BOD with the MFC, pH, conductivity, dissolved oxygen (DO), total dissolved solids, and temperature and pressure (above water) were determined using additional sensors. The sensors were powered by a solar cell and data was transmitted via 4G. Introductory, short term tests have been performed in fresh water environments in Belgium and Scandinavia. A small current was produced by the MFC which could be correlated to the DO levels. As the latter are measured at the surface near the cathode, a high concentration would be beneficial for the MFC. With a prototype in place for a prolonged period, research could continue by adding/varying the sensors on the platform (e.g. temperature sensor near the anode) and should include longer measurements. A second platform is in development for comparative measurements and to link environmental parameters to the data.

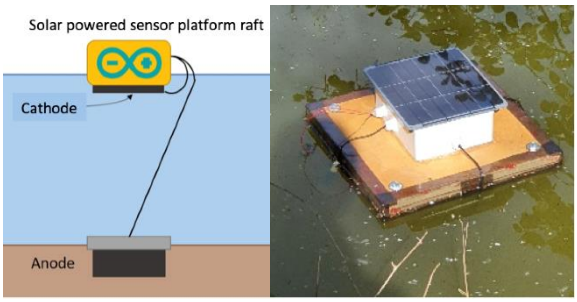

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Figure 1: Scheme and photograph of the prototype

S2P6: A Novel Bioelectrochemical Approach for Gold and Silver Recovery from Their Dithiosulfate Complexes

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In this study, we demonstrate a novel bioelectrochemical approach for gold and silver recovery from Au(I)- and Ag(I)-dithiosulfate complexes, simulating industrial effluents. For this purpose, dual-chamber bioelectrochemical reactors, comprising of a bio-anode and an abiotic cathode, separated by an ion-exchange membrane, were operated in a short-circuited (Microbial Electrochemical Snorkel (MES)) mode. The performance of the developed MES was monitored by measuring the current and potentials over time and compared with that of a microbial fuel cell (MFC), loaded by 510 Ω external resistor. Higher removal and recovery efficiencies, comparable with those of previously reported with AgNO3 and HAuCl4 solutions, respectively, were achieved at operation in an MES mode, supporting the theoretical predictions. Experiments with real industrial effluents, containing [Au(S2O3)2]3− and [Ag(S2O3)2]3− complexes, are in a progress.

Acknowledgments: The authors are kindly acknowledged for financial support to the Bulgarian National Science Fund through the Contract KP06-H29/2018 and to project № BG05M2OP001-1.002-0014 „Center of competence HITMOBIL - Technologies and systems for generation, storage and consumption of clean energy", funded by Operational Programme "Science and Education For Smart Growth" 2014-2020, co-funded by the EU from European Regional Development Fund.

S2P7: Electrochemical impedance spectroscopy analysis of highly conductive fiber networks in cable bacteria

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Cable bacteria are filamentous microorganisms, whose metabolism is powered by electron transport across a centimeter-long chain of >10.000 cells. Electrical currents run through internal protein fibers with a conductivity of up to 500 S cm-1, which is by far the highest conductivity yet observed for any natural biological material.1 This opens promising perspectives for technological applications. Yet in spite of recent advances,2-3 the mechanism of how cable bacteria conduct electrons remains fundamentally unresolved.

Here, we used electrochemical impedance spectroscopy (EIS) to investigate the conductive properties of the fiber network of cable bacteria in both dry and wet states. Importantly, the overall conductance of the fiber network was not affected by humidity and no diffusive response was detected at low frequencies, confirming the electronic nature of conduction. The influence on the EIS response of pH and various ions in solution was additionally studied.

Our results provide a more detailed insight into the electron transfer occurring in the system and contribute to the further development of a model of the conductive pathways in cable bacteria.

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S2P8: Electrochemical characterization of precious-metal-free catalysts in neutral medium

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One of the promising methods for producing hydrogen is microbial electrolysis cell (MEC). A major challenge for the practical application of MEC is finding of cost-effective cathode materials with high electocatalytic activity for the hydrogen evolution reaction (HER) in neutral medium. In this study, precious-metal-free cathodes were produced by electrodeposition of NiW and NiMo on a graphitized paper and the electrochemical performance of the produced materials in neutral phosphate buffer solution was investigated. The modification of graphitized paper with NiW and NiMo electrodeposits results in an increase of the electrocatalytic activity towards HER. NiW modified materials exhibit twice higher electrocatalytic activity than NiMo. Further studies of the produced materials as cathodes in MEC are going to be performed.

Keywords: Precious-metal-free catalysts, NiW and NiMo electrodeposits, electrocatalytic activity, Hydrogen Evolution Reaction, neutral electrolyte.

Acknowledgments: The authors are kindly acknowledged for financial support to project № BG05M2OP001-1.002-0014 „Center of competence HITMOBIL - Technologies and systems for generation, storage and consumption of clean energy", funded by Operational Programme "Science and Education For Smart Growth" 2014-2020, co-funded by the EU from European Regional Development Fund and to the Bulgarian National Science Fund through the Contract KP06-H29/2018.

S2P9: Plant microbial fuel cells from a photovoltaic perspective

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The plant microbial fuel cell (PMFC) is a technology that emerged in 2008, combining plants and bacteria in a fuel cell configuration, able to convert sunlight into electricity and therefor often mentioned in the same breath as photovoltaics(1). To investigate to what extent these two technologies compare, PMFCs were approached from a photovoltaic perspective, and a rough upper limit for the overall energy conversion efficiency of PMFCs is estimated so they can be compared with various classes of solar cells. To this end, the efficiencies of all intermediate steps in the working principle of PMFCs were investigated, going from light absorption in the leaves to electrical losses at the electrodes. Through this approach, the maximum power conversion efficiency was estimated to be around 0.3%, situated two orders of magnitude lower than traditional and emerging photovoltaics(2). The corresponding maximum power density is compared to the historical evolution of reported power output values for PMFCs since their emergence, showing no clear increase through time. Although still various routes towards increased power output are being proposed, it is clear that PMFCs are intrinsically limited and dwarfed compared to PV from the standpoint of solely power output. However, they can be of further interest for dedicated niche applications, ranging from sensing to remediation(3). This critical discussion aids in better placing PMFCs, their expectations, and their applications in the world of green, and particularly of solar energy-related research.

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S2P10: Inhibition of E. coli Growth by Zn^{2+} through the in situ and in vivo formation of ZnO/OH Nanocomposites

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Metal oxide nanoparticles (MOx NP), particularly zinc oxide (ZnO), returned into the spotlight as one of the most potent non-conventional antibacterials due to their wide spectrum of antimicrobial activity, unique physicochemical properties and low-cost.1 Despite that, the antibacterial mechanism of ZnO remains controversial.2, 3 Surprisingly, the use of ZnO NP in certain products have recently raised concerns related to the risk of environmental pollution and increase in antibiotic resistance.4

Here, we scrutinized the antibacterial mechanistic pathways of ZnO NP (<100 nm) and zinc chloride (ZnCl2) as potential less environmentally harmful alternative, in dark conditions. The antibacterial activity tests of ZnO and ZnCl2 showed great inhibitory effects on E. coli DH5a proliferation, with minimal inhibitory concentrations of 5 and 68 µg ml−1, respectively. Electrochemical and microscopic investigations suggest that the antibacterial activity of ZnO NPs can be ascribed to ZnO interactions with bacterial cell walls and generation of reactive oxygen species (ROS). Whereas, the antibacterial activity of ZnCl2 is most likely associated with the disruption of Zn2+ homeostasis in the bacterial cytoplasm and the formation of insoluble ZnO/OH nanocomposite followed by generation of ROS. Overall, the results suggest that ZnCl2 delivered in proper environment can act as a suitable replacement for ZnO NP.

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S3: Bio-electrosynthesis

Keynote speakers

S3K1: Can we produce tunable products from microbial electrochemical systems?

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The microbial electrochemical technology platform has been unique and versatile in producing many value-added products for waste valorization. The fundamental mechanism of these processes involves the interaction between electrochemistry and microbiology, and in many cases the electrical potential affects microbial metabolic pathways. In this talk, I will present our explorations on understanding and testing the feasibility of using electrical potential and efficient catalytic membrane electrodes for tunable organic chemical generation, and I will discuss the opportunities and challenges on regulating such processes using environmentally relevant mixed culture microbial communities (Figure 1).

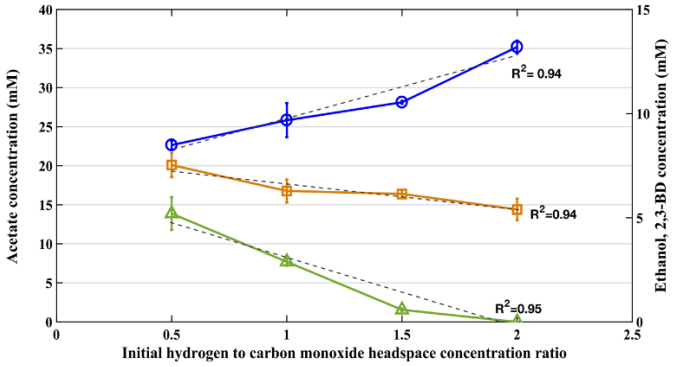

Figure 1: The ratio of syngas generated from microbial electrochemical reactors regulates organic product distribution.

S3K2: Tuning microbial extracellular electron transfer for biotechnology

Miriam A. Rosenbaum, Anthony Chukwubuikem, Santiago Boto, Angel Franco, Desy Askitosari, Carola Berger, Simone Schmitz

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Microbial extracellular electron transfer via diffusible redox-shuttles has some significant advantages for biotechnology over direct electron transfer pathways. Most importantly, it is not dependent on a 2-dimensional electrode biofilm and it can enable distant cells or other microbial partners to engage in extracellular electron transfer. However, today, electron transfer via redox-mediators is much less efficient for the microorganisms than direct electron transfer. In this talk, I will illustrate our recent approaches to tune anodic and cathodic electron mediation to make biocatalysts fit for biotechnological productions.

Our anodic work focuses on phenazine-based electron shuttling with mainly Pseudomonas species and the clarification of the phenazine connection into cellular energy metabolism. We are applying our molecular understanding of phenazine synthesis, regulation, and molecular physiology towards a heterologous utilization of phenazines for mediator-based microbial electrocatalysis. Our recent work, confirms a significant phenazine reduction already in the periplasm but also shows that some reduction happens in the cytosol. A reductive interaction of the phenazines with the elements of the aerobic electron transfer chain could not be confirmed.

In contrast, our cathodic work proves long predicted molecular hydrogen to be the electron shuttle for microbial electrosynthesis with Clostridium ljungdahlii. Suspended cells depending on dissolved hydrogen show a superior performance compared to cells adhering to the electrode. We developed strategies to control the growth phenotype of C. ljungdahlii to deliver desired electrosynthesis performance.

With these approaches, we are tuning future anodic and cathodic microbial electrocatalysts as modular platforms for bioproductions.

Invited speakers

S3I1: What could the efficiency of electromicrobial production be?

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Electromicrobial production could enable synthesis of complex, energy dense organic molecules from CO2 and renewable electricity. In this talk, I'll present a new theory that predicts the efficiency and kinetic constraints on electromicrobial production driven by H2 oxidation, formate oxidation and extracellular electron transfer. This theory predicts that electrical-to-biofuel conversion efficiency could rise to ≥ 52 with engineered in vivo CO2-fixation. Meanwhile, schemes that use electrochemical CO2-reduction could achieve efficiencies of almost 50% with no complications of O2-sensitivity.

I'll also present the results of comprehensive screening of 3,667 genes in the Shewanella oneidensis genome and the discovery of 5 new genes that encode a pathway for electron uptake. This result highlights both distinct electron uptake components and an electronic connection between aerobic and anaerobic electron transport chains that allow electrons from the reversible EET machinery to be coupled to different respiratory processes in S. oneidensis. Homologs to these genes across many different genera suggesting that electron uptake by EET coupled to respiration could be widespread. These gene discoveries provide a foundation for: studying this phenotype in exotic metal-oxidizing microbes, genetic optimization of electron uptake in S. oneidensis; and genetically engineering electron uptake into a highly tractable host like E. coli to complement recent advances in synthetic CO2 fixation.

S3I2: Insights Into The Interactions Of Acetogenic Bacteria With Cathodes During Microbial Electrosynthesis

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Acetogenesis is the reduction of CO2 into acetate or other organic compounds by acetogenic bacteria. Some acetogens are capable of using cathodic electrons for this CO2 reduction and are thus of interest for microbial electrosynthesis. Current microbial electrosynthesis rates remain too low to allow upscaling of this biotechnological process beyond lab scale. My research group investigates the interactions of acetogenic bacteria with cathodes, in order to ultimately improve microbial electrosynthesis rates.

As a first interaction, we are studying the extracellular electron uptake mechanism of acetogenic bacteria. Our working hypothesis is that the cathodic electron uptake is mediated by H2 and that some acetogens maintain low H2 partial pressures on the cathode surface, thereby thermodynamically stimulating the H2 evolution reaction on the cathode. We have recently determined H2 consumption characteristics and found that these differ strongly between different acetogens. During my presentation, I will explain the importance of the H2 consumption characteristics and elaborate on how those parameters can be used to select the acetogenic strain best suited for microbial electrosynthesis.

The second interaction we are investigating is biofilm formation on the cathode surface by acetogens. Very little is known about biofilm formation by acetogens, while these microbes usually form only sparse biofilms on cathodes. We have recently adapted the acetogen Sporomusa ovata towards stronger biofilm formation and have tested its performance on cathodes. During my presentation, I will explain our adaptive evolution strategy and present the properties of our adapted strain.

Oral presentations

S3O1: Electrochemical Immobilisation of Enzymes for Biocatalysis

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Electrochemical based enzyme immobilisation techniques can enable surface control that allows for surface modification at the micron scale. Enzyme immobilisation can be performed using a wide range of approaches that include alkanethiol, diazonium and a large range of polymer modified surfaces. Self-assembly of thiols has been widely used for the surface modification of electrodes and is an attractive method for the immobilisation of enzymes for use in biosensors and biocatalysis In a previous communication we described the electrochemical deposition of SAMs for the spatial and sequential immobilisation of the redox protein cytochrome c (cyt c) [1]. In this report we extend this process, employing the same method of blocking and de-blocking of electrodes surfaces, to successfully immobilise a sequence of three separate enzymes in a sequential manner in aqueous solution. The enzymes selected were alcohol dehydrogenase (ADH) (EC 1.1.1.1), formaldehyde dehydrogenase (FLDH) (EC 1.2.1.46) and formate dehydrogenase (FoDH) (EC 1.2.1.2). Recent work on the use of electrochemical deposition to immobilize Candida antartica lipase B for use in a flow system [2] has been extended to the immobilization of a cascade system based on glucose oxidase, horseradish peroxidase and catalase for use in a model bioreactor and the use of a diaphorase based system for the regeneration of NADH (3, 4).

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S3O2: Electro-enzymatic ATP regeneration system for phosphorylation reactions based on co-immobilization of membrane enzymes on a floating phospholipid bilayer

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The combined immobilization of the membrane-bound NiFeSe hydrogenase from Desulfovibrio vulgaris with E. coli ATPsynthase F1-FO (ATPase) allows using the oxidation of H2 as a fuel for producing the biochemical energy vector adenosine triphosphate (ATP). The ATP regeneration system consists in a gold electrode modified with a floating phospholipid bilayer that allows coupling the catalytic activity of the two membrane-bound enzymes. The biomimetic membrane modified serves as enzyme support and simultaneously provides a confined aqueous phase close to the electrode. The H2 oxidation activity of the hydrogenase covalently bound to the electrode surface generates a change of the local pH at the aqueous interface, leading to a proton gradient across the biomimetic membrane acidification that triggers ATP production by the embedded ATP-synthase [1]. This electro-enzymatic assembly is studied as ATP regeneration system of phosphorylation reactions catalysed by kinases, such as hexokinase and NAD+-kinase.

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S3O3: CO_2 conversion in 3-D printed microbial electrosynthesis cells under moderate saline conditions

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Microbial electrosynthesis (MES) is a promising technology where bacteria catalyse the electrochemical conversion of CO2 to multi-carbon chemicals. However, its adoption in industry is hindered by the high overpotentials deriving from sub-optimised cell design and low electrolyte conductivity. In this study, anaerobic sludge acclimated to saline conditions was evaluated for CO2 valorisation in H-type, galvanostatic MES cells at 5, 10, 15 or 20 g/L NaCl concentration. The acetogenic communities were enriched at both 5 and 10 g/L NaCl, with acetate production rates of 7.3-7.5 g/m2/d at 0.25 mA/cm2 applied current. Cells with 10 g/L NaCl had lower resistance, resulting in higher production per electric power invested (56.4 vs. 46.8 kg/MWh). Higher NaCl concentrations caused >90% lower production, and a Na+ inhibitory threshold of 6 g/L was suggested by ICP analysis. The microbial consortia enriched at 5 and 10 g/L NaCl were then used to inoculate 3-D printed three-chamber cells equipped with a gas diffusion biocathode. The highest acetate production rate of 55.4 g/m2/d (82.4% CE) was obtained at 1 mA/cm2 in the cells with 5 g/L NaCl. With 10 g/L NaCl, acetate production at 1 mA/cm2 was hindered by the electromigration of Na+ from the anode towards the cathode, quickly reaching inhibitory concentrations. As a proof-of-concept, three cells were hydraulically connected in series to simulate an MES stack. At 0.25 mA/cm2, a three-times higher acetic acid production was achieved compared to a single cell, suggesting an efficient CO2 conversion in the whole stack, whereas the productivity quickly deteriorated after increasing the current to 1 mA/cm2.

S3O4: The development of a redox-hydrogel bioanode for the bioelectrosynthesis of gluconate

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Gluconate is a bulk chemical widely used in the food, pharmaceutical, and textile industry, with a worldwide annual production of 80 000 tons by 2021 [1]. Industrially, gluconate is produced by fermentation of D-glucose with the enzymes glucose oxidase (GOx) or glucose dehydrogenase (GDH). The use of GOx requires the addition of catalase to decompose H2O2, a side product from GOx regeneration with O2[2]. However, the addition of a second enzyme increases the overall process cost and the complexity of product purification. Therefore, H2O2-free pathways are attractive alternatives for gluconate production with GOx. Herein we utilized a bioelectrochemical approach to oxidize glucose, in which GOx was immobilized on a ferrocene-based redox hydrogel, namely branched polyethylenimine (BPEI). In this kind of polymer matrix, the substrate and product are the only diffusing species. This approach avoids the formation of H2O2 by replacing the natural mediator (O2) with ferrocene (Fc) as an artificial. The Fc-BPEI redox hydrogel showed a redox potential of 550 mV vs. Ag/AgCl, which is considerably more positive than the redox potential of the GOx (-400 mV vs. Ag/AgCl)[3], providing a force to transport the electrons from the enzyme to the electrode. Electrosynthesis with the GOx/Fc-BPEI bioanode in an H-cell setup led to a gluconate formation rate of 6.08 ± 0.74 µg mg-1 GOx s-1 cm-2 with a faradaic efficiency of 93.66 ± 10.06 %. Although redox hydrogels containing immobilized Fc and GOx have been extensively utilized for glucose sensors [4], this is the first report of its use for the quantitative anaerobic electrosynthesis of gluconate.

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S3O5: Fluidized and fixed granular activated carbon bed cathodes for microbial electrosynthesis of carboxylates from CO2

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Microbial electrosynthesis (MES) can use renewable electricity to power microbial conversion of the greenhouse gas carbon dioxide (CO2) into value-added multi-carbon products.1 Ensuring high productivities in MES the microorganisms need to be sufficiently supplied with reducing equivalents by the cathode. Therefore, the cathode material and its configuration play a key role in the optimization of MES systems.

Granular activated carbon (GAC) is an attractive cathode material due to its high biocompatibility, conductivity, and high specific surface area.2 In addition, the fluidization of the GAC bed may improve mixing and mass transfer and result in the enhanced conversion of CO2 into multi-carbon products.

Therefore, the authors compared fluidized with fixed GAC bed cathodes for MES of acetate from CO2. The acetate production rate and current density were almost double as high in fixed GAC bed reactor as in the fluidized bed reactor (204 ± 2 mg L-1 d-1 vs. 119 ± 20 mg L-1 d-1 and 34 ± 13 mA cm-2 vs. 18 ± 2 mA cm-2). However, higher 16S rRNA gene copies in biofilm and planktonic cells were obtained with the fluidized GAC bed reactor. The results indicate that fluidization of the GAC bed charges the bed cathode less efficiently compared to the fixed bed likely due to the decreased contact between the granules and reduced conductivity in the granular bed.

To enhance the conductivity of the granules, GAC was impregnated with copper or nickel. Abiotic characterization of the metal impregnated GAC resulted in considerably higher current densities and hydrogen evolution compared to GAC without metal impregnation. Preliminary results using the impregnated GAC as bed cathodes in MES show improved acetate productivity compared to non-impregnated GAC bed cathodes. The results, however, need to be verified further.

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Posters

S3P1: Electrochemical immobilization of glucose oxidase for the controlled production of H_2O_2 in a biocatalytic flow reactor

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The controlled immobilization of enzymes plays a crucial role in the performance of enzymatic cascades. Electrochemistry offers the ability of controlling the immobilisation of enzymes in a sequential manner for the development of enzymatic cascade reactions (1, 2). The immobilization of glucose oxidase (GOx) at a defined position in a flow reactor can be used to provide the potent oxidant H2O2 for subsequent enzymatic oxidation reactions. GOx has been immobilized on a glassy carbon surface using a range of approaches that include polypyrrole, silica films and diazonium linkers. The rate of production of H2O2 was dependent on the immobilization method. Encapsulated GOx in polypyrrole resulted in the production of H2O2 at a stable rate for 4 hours of continuous operation. Further investigations were performed using carbon electrodes that can be easily incorporated in flow reactors. GOx was immobilized on graphite rods with a surface area of 5.76 cm2 producing 602 ± 57 µM h-1 H2O2 retaining 100% stability after 7 h of continuous operation. A flow reactor was constructed using a GOx modified graphite rod to produce H2O2 in a continuous manner and then coupled to a range of oxidation reactions.

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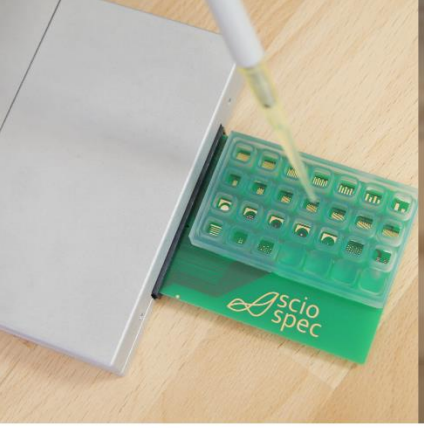


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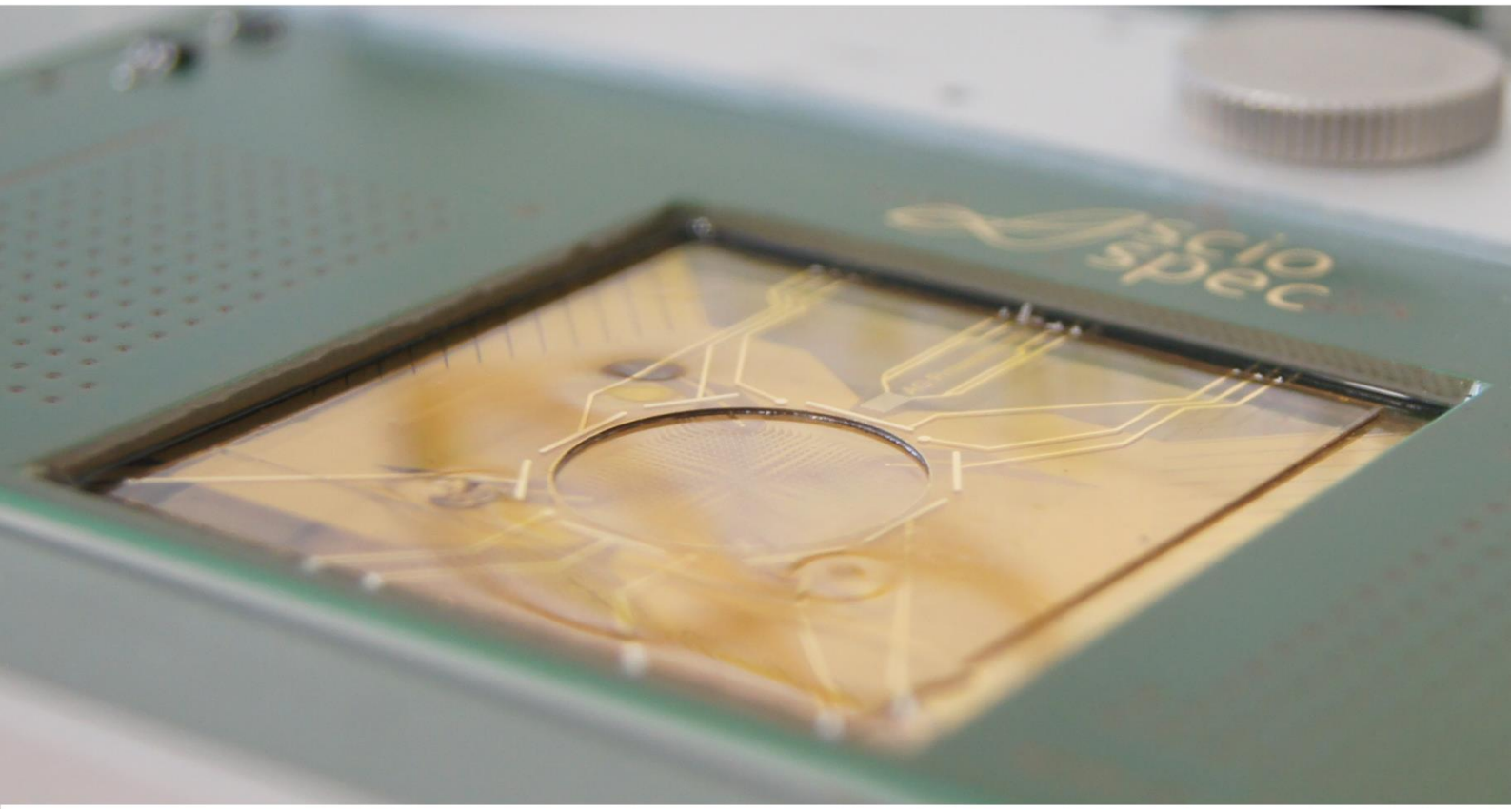

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S4: Electroporation & biomembrane electrochemistry

Keynote speakers

S4K1: Electroporation for the delivery of DNA vaccine

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Electroporation allows efficient delivery of DNA into cells and tissues, thereby improving the expression of therapeutic or immunogenic proteins that are encoded by plasmid DNA. Therapeutic DNA cancer vaccines are a very promising strategy to activate the immune system against cancer. Several clinical trials using plasmid DNA vaccines demonstrated a good safety profile and the activation of a broad and specific immune response. However, these vaccines often demonstrated only modest therapeutic effects in clinical trials. DNA vaccines could be improved i) by increasing their immunogenicity by selecting and optimizing the antigen(s) to be inserted into the plasmid DNA and ii) by combining DNA vaccines with other complementary therapies to attenuate immunosuppression in the tumor microenvironment or to increase the immune responses. We investigated the influence of the site of administration of DNA vaccine delivered by electroporation on the induced immune response. Compared to skin electroporation, muscle electroporation induced the strongest protein expression and the strongest humoral immune response and also elicited cellular immunity. We designed a new generation of DNA vaccines, encoding an engineered vesicular stomatitis virus glycoprotein as a carrier of foreign T cell tumor epitopes (pTOP). The combination of pTOP with immune checkpoint blockade improved the survival of mice bearing a subcutaneous melanoma. We also combined a new poly-epitope DNA vaccine encoding melanoma tumor associated antigens and B16F1-specific neoantigens with an oncolytic virus administered intratumorally. This combination significantly increased the immune activity into the tumor and generated antigen-specific T cells in the spleen. In a GL261 orthotopic glioblastoma, pTOP immunization prior to tumor debulking resulted in durable remission and long-term survival and induced a decrease of the number of immunosuppressive cells and an increase of immunologically active cytotoxic T cells in the brain. These results highlight i) the potential of electroporation for DNA vaccine delivery and ii) the potential of DNA-based immunotherapies coding for viral proteins to induce potent and specific antitumor responses iii) the need to address different arms of the immune response.

S4K2: Electrochemical Impedance Spectroscopy: A Tool for Structure and Function Studies of Bilayers Populated with Ion-Conducting Pores

Gintaras Valinčius, Filipas Ambrulevičius, Tomas Raila, Tadas Penkauskas and Tadas Meškauskas, Marija Jankunec, Rima Budvytyte

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Biological membranes exhibit several essential functions in living organisms among which the electric insulation is one of the utmost importance. This function ensures charge separation and regulation of electric potential in cells/organelles via an appropriate protein machinery. No surprise, various pathogens, both endogenous and exogenous, such as pore-forming toxins exert their detrimental function by damaging the integrity of phospholipid bilayers, which leads to the impairment of ionic homeostasis and cell death.

Tethered bilayer membranes (tBLMs) are convenient, solid supported models of biological membranes, which have a significant potential for a variety of bioelectrochemical applications. In this presentation we will discuss the utility of the tBLMs and the electrochemical impedance spectroscopy (EIS) as a versatile tool to access functional and structural information about the nano-size defects (pores) in model membranes.

We will present and discuss the EIS data analysis methods developed in our laboratory to access information about the density of defects in tBLMs. Such information is important in the activity assays of bacterial pore-forming toxins, and in discriminating between bacterial strains of different pathogenicity. It can be used to evaluate neutralizing effects of antibodies against such pathogens. We will show that the specific molecular architecture of surface supported membranes results in the EIS response that contains information about the lateral arrangement of conducting, water-filled pores in tBLMs. We argue that in some cases the size of nanopores can be evaluated from the EIS data, though with limited precision. Finally, we will demonstrate the utility of EIS to assess the type of lateral distribution of defects: homogeneous/heterogeneous/clustered, and by solving an inverse problem to determine the density distribution function of defects in model membranes, tBLMs.

Invited speakers

S4I1: Effects of Intense Electric Field on Structure and Function of Proteins

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In this talk, we present an overview of electric field effects on proteins and protein structures, focusing on intense electric field from static (pulsed) to THz range. Furthermore, on selected computational and experimental examples (primarily, but not limited to cytoskeletal proteins [1,2]), we demonstrate the primary mechanisms of the effects of electric field on structure of the proteins. Furthermore, if the effect persists, the protein function (enzymatic activity, multi/polymerization capability, etc.), can be affected as we show in experimental examples [3]. The deeper understanding of molecular level effects of electric field on proteins opens-up new possibilities in biomedical and bionanotechnological application.

Acknowledgements to Czech Science Foundation, project GA20-06873X.

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S4I2: Water structure in the sub-membrane region of a floating bilayer

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The structure of water in the sub-membrane region of the bilayer of DPhPC floating (fBLM) on a monolayer of 1-thio-β-D-glucose (β-Tg) modified gold nanoparticle film was studied by the surface enhanced infrared absorption spectroscopy (SEIRAS). SEIRAS employs surface enhancement of the mean square electric field of the photon which is acting on a few molecular layers above the film of gold nanoparticles. Therefore, it is uniquely suited to probe water molecules in the sub-membrane region and provides unique information concerning the structure of the hydrogen bond network of water surrounding the lipid bilayer. The IR spectra indicated that water with a strong hydrogen network is separating the membrane from the gold surface. This water is more ordered than water in the bulk. When alamethicin, a peptide forming ion channels is inserted into the membrane the network is only slightly loosened. The addition of amiloride-an ion channel blocker results in a significant decrease in the amount of water in the sub-membrane region. The remaining water has a significantly distorted hydrogen bonds network. This study provides unique information about the effect of the ion channel on water transport across the bilayer. The electrode potential has a relatively small effect on water structure in the sub-membrane region. However, the IR studies demonstrated that water is less ordered at positive transmembrane potentials. The present results provide significant insight into the nature of hydration of floating lipid bilayer on the gold electrode surface.

Oral presentations

S4O1: The Effect of Drug Lipophilicity on the Interactions with Model Cell Membranes – Surface and Electrochemical Studies of Anthracyclines and Statins.

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We have recently employed phospholipid monolayers prepared at the air-water interface by the Langmuir technique to study the influence of drug lipophilicity on the interactions with such simple models of cell membranes. Investigation of anticancer drugs (anthracyclines) showed that their incorporation into model membrane leads to the changes in the membrane properties and strongly depends on both the lipophilicity of the anthracycline derivative and the composition of a model membrane, specifically the net charge of the membrane, which may vary for healthy and cancer cell membranes [1]. The presence of electrochemically active quinone-hydroquinone group in the structure of anthracyclines allowed us to calculate surface concentrations of the drugs within the supported membranes based on cyclic voltammetry. The increase in the drug lipophilicity allowed for a more efficient penetration of the negatively charged model membrane resulting in the higher surface concentration obtained for more lipophilic idarubicin compared to less lipophilic daunorubicin. The results of Langmuir and electrochemical studies were also compared with the results of spectroscopic and neutron reflectivity studies [2]. We have also investigated the influence of lipophilicity of drugs lowering cholesterol levels (statins) on their interactions with simple models of lipid rafts. It has been shown that the most lipophilic statin, cerivastatin is able to penetrate the model raft layers at the air-water interface. The exposure of the supported layers to this drug leads to the decrease in the defectiveness of the model raft layer and increase in the barrier properties of the film as proved by electrochemical impedance spectroscopy [3].

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S4O2: Tailoring Algae-Based Vesicles for Drug Delivery

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Traditionally used model drug delivery systems have been constructed from simple phospholipids or phospholipid membranes with anchored and embedded components, albumin microspheres, soluble synthetic polymers, DNA complexes, protein-drug conjugates, or exosomes. None of these systems approach the complexity of cell membranes, while the molecular mechanism of their formation is not fully understood and detection and analysis are challenging. Screening of alternative bioinspired drug delivery systems such as isolated algal membranes is limited and membrane isolation methods are not routinely used. Our goal is to identify and mechanically characterize unicellular microalgal species (1,2) that can serve as a sustainable source for the isolation of plasma membrane vesicles (3). Using a comprehensive biophysical approach, the algal-based vesicles were characterized in terms of structure-properties and the ability to encapsulate fluorescent dyes was monitored. The observed differences in transport are attributed to differences in the structural features of the membranes and the physicochemical properties of the dye. Increased knowledge of this unique membrane system would help design marine bioinspired carriers for advanced biotechnological applications.

Acknowledgments: This work is supported by the Croatian Science Foundation Projects (IP-2018-01-5840, IP-2018-01-6910).

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S4O3: The EcCLC Antiporter Embedded in the Lipidic Liquid Crystalline Films: Electrochemistry and Molecular Dynamics Simulations.

Renata Bilewicz, Ewa Nazaruk, Przemysław Miszta, Dorota Nieciecka, Mariusz Możajew, Paweł Krysiński Sławomir Filipek

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Lipidic-liquid crystalline nanostructures (lipidic cubic phases), which are biomimetic and stable in excess of water, were used as a convenient environment to investigate the transport properties of the membrane antiporter E.coli CLC-1 (EcCLC). The chloride ion transfer by EcCLC was studied by all-atom molecular dynamics simulations combined with electrochemical methods at pH 7 and pH 5. The cubic phase film was used as the membrane between chloride donor and receiving compartments, as well as it was placed on the glassy carbon electrode and immersed in the chloride solution. Structural characterization of the lipidic mesoscopic systems with and without incorporated EcCLC was done using small-angle X-ray scattering. The EcCLC transported chloride ions more efficiently at more acidic pH, and the resistance of the film decreased at lower pH. The 4,4-diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS) employed as an inhibitor of the protein was shown to decrease transport efficiency upon hydrolysis to DADS both at pH 7 and pH 5. The molecular dynamics simulations, done for the first time in lipidic cubic phases for EcCLC, allowed to study the collective movements of chloride ions which can help to elucidate the mechanism of transporting the ions by EcCLC antiporter. The protein modified lipidic cubic phase film is a convenient and simple system for screening potential inhibitors of integral membrane proteins, as demonstrated by the example of the EcCLC antiporter. The use of lipid cubic phases may also be important in the further development of new electrochemical sensors based on membrane proteins and enzyme electrodes.

S4O4: Isolation, Characterization and Redox Properties of Lipid Rafts from HeLa Cells

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Lipid rafts are submicroscopic membrane microdomains enriched in phospholipids, sphingolipids, glycolipids, cholesterol and specific proteins [1]. This contribution is focused on the isolation of detergent-resistant lipid rafts from HeLa cells. The isolation procedure is performed using Triton X-100 treatment followed by 5-20% density OptiPrep gradient separation. The purified lipid rafts were subsequently characterized by using western blot with mono/poly-clonal antibodies against the protein markers caveolin and raftlin. The lipid rafts diam. of approx. 100 nm was determined by dynamic light scattering. We evaluated total protein level and cholesterol content in the rafts using electrochemical (chronopotentiometry and voltammetry) and spectrophotometric methods (Bradford, total cholesterol concentration is determined by a coupled enzyme assay, which results in a colorimetric product, proportional to the cholesterol present) [3]. The content of SH (thiol) groups was estimated based on Ellman's assay [4]. The redox properties and adsorption-desorption behavior of the lipid rafts were investigated at mercury and carbon electrodes [2]. The approach developed was used for a study of the stability, reactivity and antioxidant properties of the lipid rafts, including a study of their intermolecular interactions with other membranes or fatty acids.

The authors wish to thank the Czech Science Foundation (GACR 19-21237Y).

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S4O5: Activity of reconstituted HMG-CoA reductase in lipid rafts model

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Hypercholesterolemia is a condition of elevated cholesterol blood levels that are often dangerous to human health [1]. It promotes diseases of the cardiovascular system, causing heart attack or stroke. The key protein in the process of cholesterol synthesis is 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA). It is a liver enzyme located in the membranes of the endoplasmic reticulum (ER) which are rich in cholesterol and sphingolipids forming lipid rafts [2]. In order to control the increased production of cholesterol, the inhibitors of HMGR reductase, statins are used. The HMGR protein has been reconstituted in the model lipid membrane. For this purpose, the DOPC:Chol:SM system, typical for lipid rafts [3, 4], was selected. HMGR was reconstituted into liposomes, which resulted in obtaining proteoliposomes with a diameter of about 800 nm. The proteoliposomes were characterized using the dynamic light scattering, electrochemical impedance spectroscopy (EIS) and microscopic methods. Additionally, the activity of the reductase located in the model membrane has been also measured. The reaction catalyzed by HMGR is the 4e- reduction of HMG-CoA and oxidation of NADPH to NADP+ [1], which allowed us to measure the activity of reductase using the UV-Vis spectrophotometry and cyclic voltammetry. The inhibitory effect of the selected statins differing in their hydrophobicity (cerivastatin> fluvastatin> pravastatin) on the catalytic site of the reductase was also followed [5].

The conducted experiments allowed us to determine the optimal method of protein incorporation into the model lipid rafts and to confirm its activity after reconstitution. It has been shown that the reconstituted protein can be also inhibited by selected statins. The degree of HMGR inhibition depends both on the degree of hydrophilicity of the administered drug, and on its concentration.

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Posters

S4P1: Effect of the cholesterol on electroporation of planar lipid bilayer

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Electroporation is characterized by structural changes within the cell membrane, which are caused by the presence of strong electric field. These changes increase the plasma membrane permeability that are caused by rearranging of lipid molecules in lipid bilayer structure named also pores; if so, planar lipid bilayer is a good model for experimental and theoretical studies. Lipid bilayers of different compositions have different electrical as well as mechanical properties that are, due to their influence on membrane stability in electric field, important for the understanding and use of electroporation. Cholesterol is one of major components of biological membranes and plays a crucial role in its organisation, dynamics, and function. Therefore, its role in cell membrane electroporation is of great interest. Electroporation threshold depends on the membrane composition, with cholesterol as its key component being already studied in the past, but the results were inconclusive.

The aim of our study was to determine behaviour of planar lipid bilayers with varying cholesterol concentrations under electric field. This would give us a better insight into cholesterol effect on membrane properties during electroporation process. Planar lipid bilayers were prepared from phosphatidylcholine lipids with 0, 20, 30, 50 and 80 mol% cholesterol. Capacitance was measured using the discharge method. Results show no statistical difference of cBLM between the cholesterol concentrations. Breakdown voltage Ubr of planar lipid bilayers was measured by means of linear rising voltage with seven different slopes. Obtained results were fitted to a strength-duration curve, where parameter Ubrmin represents minimal breakdown voltage, and parameter tRC represents the inclination of the strength-duration curve. Adding cholesterol to planar lipid bilayer gradually increased its Ubrmin until 50 mol% cholesterol concentration. Afterwards at 80 mol% Ubrmin does not further increase, in fact it reduces by 20% of the Ubrmin at 50 mol% cholesterol concentration.

S4P2: Electrochemical adhesion-based differentiation of algal cells

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The structural features of algae are important for the fundamental understanding of cell-cell and cell-substrate interactions. Most work on algal cell-substrate interactions focuses on substrate properties, while structural features and surface properties of algae are neglected. The goal is to investigate the effects of algal structural features on adhesion and spreading at a charged liquid-liquid interface. Upon impact of the algal cell on the mercury interface, rapid deformation occurs, followed by membrane rupture and spreading of the intracellular contents on the interface, resulting in a double-layer charge displacement and registration of the amperometric signal of a single cell. Four unicellular algal species with different barrier structures were examined. The results show that algal cells with a thin elastic plasma membrane and with a cellulose amphiesma adhere and spread at species-specific potential ranges that are registered as well-defined amperometric signals. In contrast, algal cells with a thin theca encrusted with biocalcite and an organosilicate cell wall do not adhere to the charged interface and therefore do not generate an amperometric signal because they do not deform at the interface (1). These differences in adhesion behavior of algal cells determined amperometrically are consistent with reported mechanical properties of cells determined by AFM (2,3). The ranges of surface charge density for algal adhesion at the mercury interface become broader with increasing complexity of the plasma membrane structure, so that the critical interfacial tension can be considered as a parameter for adhesion differentiation of soft algal cells. The electrochemical approach enables direct, rapid, high-throughput differentiation of algal cells, which could be used to monitor stressed algal cells in a disturbed aquatic environment.

Acknowledgments: This work is supported by the Croatian Science Foundation Projects (IP-2018-01-5840).

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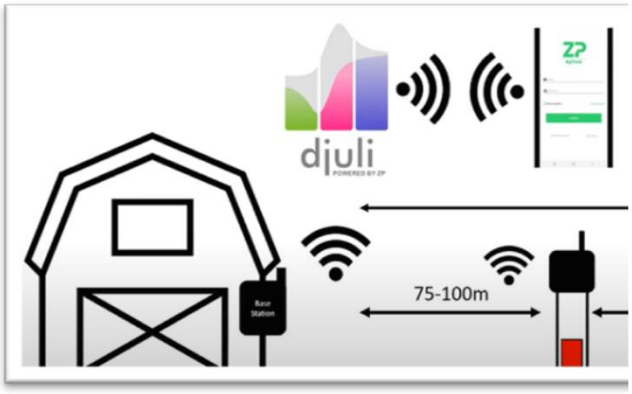

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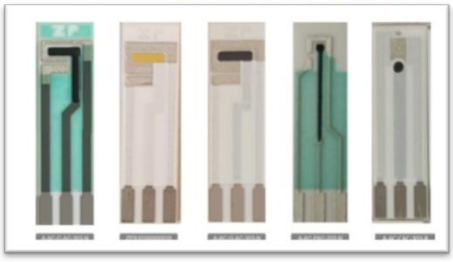

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S5: Nano technologies & architectures for bio-electrochemistry

Keynote speakers

S5K1: Nanoscale Imaging of Bioelectrocatalytic Viral Particles

Telmo O. Paiva,[1] Kristian Torbensen,[1] Angela Schneider,[2] Laure Bataille,[3] Agnès Anne,[1] Kristina Wege[2] Thierry Michon,[3] Arnaud Chovin,[1] Christophe Demaille[1]

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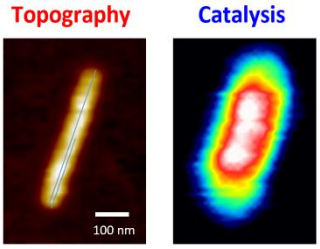
This work focuses on the scaffolding of complex enzymatic nanosystems onto different classes of virus capsids, in order to explore the effect of spatial organization on their electrocatalytic activity. A fully integrated system, consisting of the glucose-oxidizing quinoprotein glucose-dehydrogenase enzyme, and its elastically attached ferrocene mediator, is immunoassembled onto fd bacteriophage or Tobacco Mosaic Virus (TMV) particles.1-3 The enzymatic activity of the resulting electrocatalytic virions is probed at the single entity level by AFM-SECM microscopy, a correlative electrochemical imaging with exclusive biomolecular resolution.4 We analyze precisely how the catalytic current depends on the type of viral scaffold and its topological characteristics. We also report the first electrochemical measurement of the enzymatic activity of a few tens of enzyme molecules, site-selectively positioned on the 40 nm-long terminal domain of a reprogrammed TMV-like particle.2



Bioelectrocatalytic TMV particle

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S5K2: Bioelectroanalytical (nano)tools: new horizons for detecting immunity, predisposition and triggering of prevalent and unexpected diseases

Susana Campuzano[1], Rodrigo Barderas[2], Ana Montero-Calle[2], Rebeca M. Torrente-Rodríguez[1], Verónica Serafín[1], Maria Gamella[2], María Pedrero[1], José M. Pingarrón[1]

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These times have highlighted more than ever the need for bio-tools minimizing as much as possible the impact of both prevalent and unexpected diseases, preferably independently of the health care institutions.

In this sense, features offered by electrochemical bioplatforms, such as versatility to profile multiple or multiomics biomarkers at the point of care, simplicity, affordable cost and remarkably shorter analysis time and smaller sample quantity for the analyses, compared to conventional and latest generation technologies, make them suitable alternatives to address this tremendous challenge. The great advances demonstrated by electrochemical biosensors in the last years have gone hand in hand with the development of new electrochemical substrates, nanotechnology, attractive surface chemistries, bioassay formats and amplification strategies but also with the production and application of new bioreceptors (such as phage-displayed and in-house expressed viral antigens), which has allowed to generate these devices and demonstrate pioneering applications.

Bearing all this in mind, this keynote will discuss bioelectroanalytical tools recently developed in our research group, by exploiting advantages of latest generation bioreceptors, smart bioassay formats and multiplexed amperometric transduction, for detecting immunity, predisposition and triggering of prevalent and unexpected diseases. The most relevant aspects of electroanalytical bioplatforms potentially transferable to the clinic due to their simplicity, cost, testing time, versatility, multiplexing capability, and decentralized character, will be discussed. In particular, those which have shown pioneering applications to decisively assist in personalized early diagnosis of Alzheimer's Disease by targeting dysregulated autoantibodies, cancer diseases through the analysis of methylation events in nucleic acids, and infection and immunity to SARS-CoV-2 by interrogating total and isotype N- and S-specific serum immunoglobulins.

Invited speakers

S5I1: Bioelectrocatalytic Films for H2 Evolution and CO2 Fixation

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Implementation of biocatalysts in devices cannot proceed without solutions that mitigate their intrinsic fragility (1). Protection strategies using a redox-active matrix can effectively stabilize hydrogenases and CO2 reducing enzymes and significantly increase their operational lifetime. Novel methods for formation of homogeneous thin films (2, 3) that enable high catalyst utilization can theoretically provide O2 resistance quasi-infinitely even when using highly fragile hydrogenases (4). Different protection mechanisms can be exploited depending on matrix dimensions and intrinsic catalyst properties pushing the turnover lifetime of hydrogenases up to weeks (5). The use of hydrogenase reactivation also simplifies the preparation of the catalytic films (6), possibly bypassing entirely the need for anaerobic conditions. Finally, engineering catalytic reversibility into the redox-active films embedding the hydrogenase enables H2 oxidation and H2 evolution at minimal overpotential, making the protected hydrogenase energy efficient in fuel cell and electrolyzers (7). The same redox-active films were also successfully applied to construct energy efficient biohybrid systems based on CO2 fixating enzyme (8).

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Angew. Chem. Int. Ed., 2021, 60, 21056

Oral presentations

S5O1: 3D-printed Microneedles-based Potentiometric Sensors for pH Monitoring in Skin

Marc Parrilla,[a] Andres Vanhooydonck,[b] Karolien De Wael[a]

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Wearable analytical devices have been extremely attractive for non-invasive diagnostics and therapeutics owing to the ability to provide continuous on-body information.[1] Particularly, microneedle-based sensing can access the interstitial fluid (ISF) in the dermis layer of skin to carry out on-body transdermal detection.[2] This approach is interesting because: (i) it avoids the use of painful methods for blood extraction; (ii) it provides equivalent values of the analytes from blood; (iii) ISF matrix is less complex in terms of composition than blood; and (iv) it offers high body availability. On the other hand, 3D-printing technology allows for rapid and versatile prototyping reaching micrometer resolution. Herein, we explore 3D-printed hollow microneedle patches which are functionalized with conductive inks and polyaniline to develop a potentiometric sensor for pH monitoring. First, the hollow microneedles are filled with conductive inks to engineer a set of microelectrodes. Thereafter, the working and reference electrodes are properly modified tailoring toward the application. Once the microneedle sensor is ready, a full in vitro characterization is performed within a broad range of pH showing the analytical features of the sensor. The performance of the microneedle sensor is assessed in agarose gel to mimic the environment in the skin. Finally, the microneedle sensor is pierced on pork skin soaked in different pH to evaluate the ability to monitor changes in pH. Overall, 3D-printed microneedle-based sensing brings a versatile and affordable technology to monitor key physiological parameters in the body, and particularly in this work, the surveillance of pH changes in wounds to avoid potential infections during skin recovery.

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S5O2: Enzyme-Driven Autonomous Swimmers for the Direct Detection and Quantification of Enantiomeric Excess

Serena Arnaboldi[1,2], Gerardo Salinas[1], Aleksandar Karajić[1,3], Patrick Garrigue[1], Tiziana Benincori[4], Giorgia Bonetti[4], Roberto Cirilli[5], Sabrina Bichon[3], Sébastien Gounel[3], Nicolas Mano[3] and Alexander Kuhn[1]

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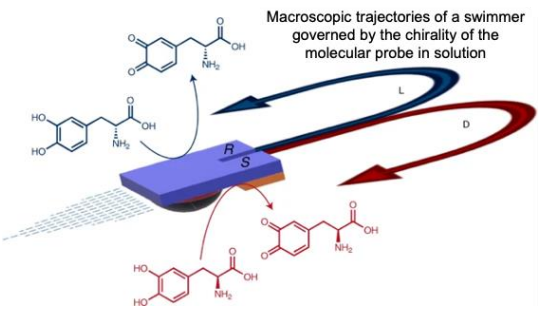
Chirality is found throughout nature, and it occupies also a special place in chemistry, perhaps for historical reasons, but mainly as a result of the beneficial properties of chiral molecules across a diverse range of areas, from medicine to materials science. The artificial systems presented herein can be considered as models for the transmission of chiral information across different length scales. This ambitious aim has been achieved by developing autonomous enzyme-driven swimmers based on chiral conducting polymers. The combination of three main ingredients namely electrical conductivity, driving force originating from the enzyme and enantiodiscrimination capability, makes these novel miniaturized bipolar objects perfect candidates for a multipurpose detection of enantiomeric excess of chiral analytes, by correlating the dynamic output signal, in terms of trajectories, with the concentration of the molecular antipodes present in solution. [1]



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S5O3: Au Nanoparticles decorated Nano-Graphene Oxide based Hybrid Nanocomposite electrochemical sensor for estrone determination

Serena Laschi[1], Paolo Cammarota[1], Fabio Vischio[2], Nicoletta Depalo[2], M. Lucia Curri[2,3], Chiara Ingrosso[2], Ilaria Palchetti[1]

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In this work, disposable carbon-based screen-printed electrodes (C-SPEs), modified with a hybrid nanocomposite formed of Nano Graphene Oxide (NGO) flakes decorated with Au nanoparticles (NGO/Au NPs) were fabricated and applied for the assembling of an immunosensor for estrone detection. The use of multifunctional NGO/Au NPs is an interesting innovative approach in sensor applications, as it improves electrocatalytic effect, electrochemical efficiency, sensitivity, and electron transfer rate with respect to conventional carbon-based sensors. C-SPEs modified by NGO/Au NPs nanocomposites, synthesized with different NGO:Au w/w ratio, were electrochemically characterized by using different techniques in the presence of redox probes. The NGO/Au NPs nanocomposite modified sensors were used in the development of an immunomagnetical assay for the detection of estrone, an emerging contaminant of environmental interest.

S5O4: Redox cycling in bottom-up designed porous coaxial twin-electrodes for efficient bioelectroanalysis

Tatjana Šafarik[1,2],Aleksandar Karajic[2], Stephane Reculusa[2], Nicolas Mano[1] and Alexander Kuhn[2]

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Macroporous electrodes are used for the elaboration of bioelectrochemical devices due to their unique structure which provides higher surface areas than their flat homologues while maintaining the geometric surface as small as possible. Here, we report the electrochemical elaboration and a characterization of a three-dimensional co-axial macroporous twin-electrode obtained by following a low-cost and bottom-up approach. As illustrated in Figure 1, the nanoengineered device is based on two threaded cylindrical porous gold microelectrodes for which the porosity, the thicknesses of the gold layers and the space between the inner and outer electrode can be tuned at will.

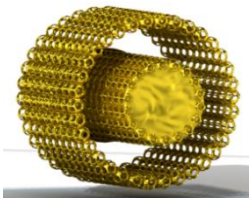

Figure 1. Schematic representation of a co-axial macroporous twin-electrode

Due to the confined architecture and the small distance between both inner and outer electrodes, such design can be used for signal amplification in analytical chemistry by redox cycling. A molecule can participate several times in the electron exchange reaction by diffusing between both electrodes. In addition, by using an architecture with a cylindrical geometry, it is possible to improve the mass transport of the electroactive species towards the electrode surface, which is one of the main problems to solve for electrochemical devices, especially in the field of implantable biofuel cells and biosensors. The resulting signal amplification, combined with a straightforward synthesis strategy of the architecture allows envisioning numerous (bio)analytical applications.

S5O5: Functionalized carbon nanotubes and glyconanoparticles for glucose oxidation in enzymatic biofuel cells and biosensors

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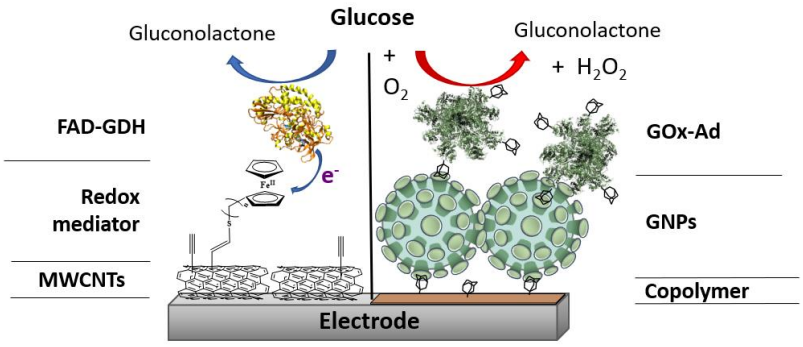
Two types of functionalized nano-objects, multiwalled carbon nanotubes (MWCNTs) and glyconanoparticles (GNPs), have been used as matrices combined with glucose oxidizing enzymes for the development of biofuel cells and for the design of an amperometric biosensor. A photoinduced thiol-yne reaction has been employed to immobilize redox thiol derivatives onto alkyne-modified MWCNTs for the mediated catalytic oxidation of glucose by FAD-GDH at the bioanode of an enzymatic glucose fuel cell. GNPs with an outer shell of cyclodextrin [1,2] have been fixed onto an electrogenerated copolymer film bearing adamantane groups and post-functionalized with an adamantane-tagged glucose oxidase (GOx-Ad) through cyclodextrin host-guest interactions resulting in an enhanced glucose biosensor performance both in terms of enzyme loading and sensitivity.



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S5O6: Biofuel cell based on hollow bioelectrodes containing enzymes in solution

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One of the important characteristics of batteries frequently used in disposable devices is their small size, low mass, and preferably low price. Biofuel cells constitute an interesting alternative for increasing the power or self-powering miniaturized portable or implantable devices [1, 2]. Enzymatic biofuel cells face two major technological obstacles their short lifespan and their low power output. To solve the present issues a recently developed strategy proposes to use the elements of the bioelectrode in a liquid medium. Hammond et al. have proposed bioanodes comprising discs placed in an aqueous suspension comprising enzymes and redox nanoparticles [1]. Li et al. also describe a bioanode comprising a system in the form of an aqueous suspension, or "slurry" [2]. However, these devices have increased volume and expensive components.

We propose a new concept of bioelectrodes based on free enzyme for the biofuel cell allowing its use in devices of small dimensions, inexpensive, easy to store and use. Besides an increase biofuel cell power, this system allows the easy combination of enzymes and catalysts with improved activity. For example, bioelectrode based on bilirubin oxidase presents a catalytic current of 2.3 mA at 0.3 V and exhibits 15 % of this initial activity after 5 months of storage in phosphate buffer. As for the bioanode based on FAD dependent glucose dehydrogenases and PLQ as mediator a catalytic current can reach 5mA and retain 18% of its activity after 1 month.

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Posters

S5P1: Infrared spectroscopy of the Old Yellow enzyme from Leishmania braziliensis under electrochemical control

Jessica C. Pacheco, Lucyano J. A. Macedo, Graziela C. Sedenho, Silvia H. Libardi, Júlio C. Borges, Frank N. Crespilho

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Old Yellow Enzymes (OYEs) are oxidoreductases present in several microorganisms, including the pathogenic protozoa Leishmania braziliensis and Trypanosoma cruzi, which are responsible for the transmission of Leishmaniasis and Trypanosomiasis, listed as neglected tropical diseases. Although menadione is used as a standard substrate in studies with OYEs, physiological substrates are not known for most OYEs. Thus, the study of the catalytic activity of these enzymes and their interaction with menadione and other possible substrates can provide important information for the development of drug design against parasitic diseases1. In this context, the goal of the present work was to study the redox behavior of the OYE from Leishmania braziliensis (LbOYE) immobilized on a high-edge-density graphite electrode (HEDGE). Protein-film voltammetric measurements showed a quasi-reversible redox pair with E1/2 = -0.36 V vs Ag/AgClsat in phosphate buffer, pH 5.8, evidencing a direct electron transfer (DET) between the FMN cofactor and the electrode surface. The α (electron transfer coefficient) and the k0 (rate constant at zero overpotential) were determined by Laviron's method2,3, being 0.52 ± 0.01 and 20.1 ± 0.8 s-1, respectively. By operando FTIR spectroelectrochemical measurements with LbOYE immobilized on HEDGE, we monitored the main vibrational bands related to the FMN cofactor (νC=N in 1547 cm-1) and menadione as substrate (νC=O in 1589, 1630, 1664 cm-1), with the applied oxidation and reduction potential. It was observed a downshift in the FMN signal attributed as νC=N to 1540 cm-1 when menadione is present and its reduction is being catalyzed. Thus, the molecular interactions observed through FTIR spectroelectrochemistry between the enzyme cofactor and the organic substrate in this bioelectrochemical system, as well as the redox behavior of the enzyme, can help future investigations about enzyme-substrate interaction to elucidate the physiological role of LbOYE in pathological parasites.

S5P2: Impact of Plasma-induced Surface Functionalization on Performance of Microfabricated Pyrolytic Carbon as Electrode Material: An Electrochemical Study

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Pyrolytic carbon is a relatively new electrode material finding a wide application in chemical sensing,1,2 biosensing3,4 and monitoring of cell growth and differentiation.5-8 It possesses a wide potential window, good mechanical strength, high chemical stability, reasonable electrical conductivity and, importantly, offers highly tailored and hierarchically structured morphologies of various customized designs. Plasma treatment is a widely used approach for cleaning of such pyrolytic structures, but also activating carbon by generating various polar functional surface groups, the composition of which depends on gas selection.

In the current study, we have investigated the impact of plasma processes on microfabricated pyrolytic carbon obtained by pyrolysis of SU-8 photoresist film. The surface chemistry and geometry of carbon before and after plasma activation were characterized by X-ray photoelectron spectroscopy and atomic force microscopy. The electrochemical performance of the carbon treated with various plasma gases (air, Ar, O2, N2) was studied in respect to Ru(NH3)63+/2+, Fe(CN)63-/4- and dopamine/dopaminequinone redox systems and compared based on the information of heterogeneous electron transfer rates determined by cyclic voltammetry and electrochemical impedance spectroscopy.

This work was financially supported by the European Research Council under the Horizon 2020 Framework Programme grant no. 772370-PHOENEEX and the European Union's Horizon 2020 research and Innovation Program under the Marie Sklodowska-Curie grant agreement no. 713683-AMPHIBIAN (COFUND fellows DTU).

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S5P3: Innovative targeted drug delivery systems based on magnetic and gold nanoparticles

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Hepatocellular carcinoma (HCC) is the most common type of liver cancer and the fifth cause of cancer-related mortality worldwide. Sorafenib (SOR), the treatment of choice for advanced HCC, presents several limitations, such as poor water solubility (1). These limitations can be overcome by encapsulating SOR into different drug carriers whose specificity can be enhanced by functionalizing them with tumor cell-specific aptamers.

In this study, two types of carriers, gold (AuNPs) and magnetic nanoparticles (MNPs) were used for the encapsulation of SOR. The commercially available carboxyl PEG-ylated AuNPs were functionalized with amino-terminated aptamers via NHS/EDC coupling and the functionalization was confirmed by UV-Vis spectrophotometry. Two types of carboxyl-functionalized MNPs were synthetized and functionalized with the aptamer using the same method. Electrochemical techniques (cyclic voltammetry and electrochemical impedance spectroscopy) were used to confirm aptamer immobilization onto the MNPs. The aptamer-functionalized carriers were then loaded with SOR and loading and release studies were performed via UV-Vis spectrophotometry. The results confirmed the successful encapsulation and release of SOR from the carriers.

Acknowledgements: Alexandra Pusta acknowledges UMF internal grant no. 1032/57/13.01.2021.

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S5P4: Bacterial Imprinting with Electro-active Bacteria for Next Generation Microbial Electrochemical Technologies

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Microbial Fuel Cell (MFC) performance strongly depends on the anode, moreover the attachment of electro-active bacteria to the electrode surface. Expensive carbon electrodes, associated with poor bacterial adhesion can be modified by a polymer, employing a technique referred to as molecular imprinting creates artificial cavities complementary to the template molecule [1]. The binding sites generated can be engineered to selectively extract the bacteria of interest (in this case Geobacter)[2] from wastewater, improving colonisation and MFC power output. However, a balance needs to be struck between polymers that can adhere to bacteria whilst promoting cell growth.

Bacterial binding sites are generated on the polymer, complementary to the size, shape and chemical functionality of the target bacteria as shown in Fig. 1. It is possible to discriminate bacterial species, due to different antigens on the surface (e.g., glycans) that interact with the binding sites in different ways [3].

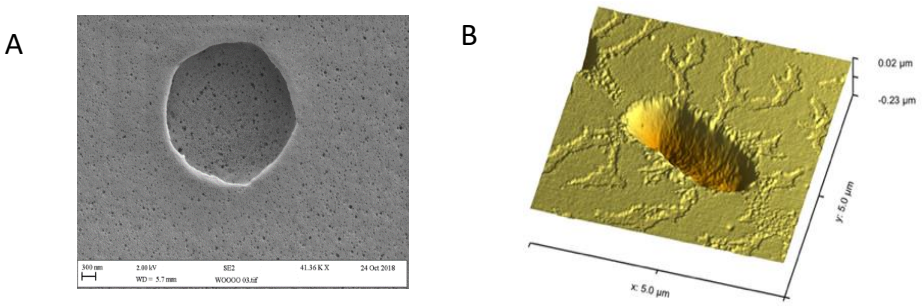

Figure 1. (A) A typical binding site for S. aureus (visualised by scanning electron microscopy). (B) Profile analysis of an E. coli cavity (visualised by 3D atomic force microscopy) [4].

S5P5: Biofunctionalization of neural electrodes with electroactive organic monolayers

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Brain-computer interfaces (BCI) are used as a communication pathway between the nervous system and an external device. In order to properly serve their application, whether it is stimulation or recording of neural signals, they need to meet several criteria. It is required for BCI to be highly conductive, prevent signal noise with low impedance, provide safe charge injection, and ensure integration with neural tissue during long-term implantation. However, conventional materials for the construction of neural interfaces, i.e. noble metals, lack chronic biocompatibility, which leads to inflammation, glial scar formation, or corrosion.

In this work, our objective was to functionalize the surface of neural electrodes by means of a diazonium salt electrografting (4-nitrobenzenediazonium tetrafluoroborate). Organic monolayer deposition process was optimized through electrogravimetry. A subsequent reduction step allowed for the formation of a chemical anchor for further biofunctionalization with a model biomolecule (heparin). The obtained surfaces were characterized by electrochemical methods with a focus on the electroactive surface area, impedance, and charge injection safety. We analyzed the surface properties with the use of atomic force microscopy and water contact angle measurements. Chemical analysis was performed with Raman spectroscopy. Finally, biological studies on the SH-SY5Y cell line confirmed the beneficial influence of surface modifications on the electrode biointegration with neural cells.

Acknowledgements: This research was supported by the National Science Centre, Poland (OPUS 2019/35/B/ST5/00995).

S5P6: Facile Modification of Carbon Electrodes with ITO Nanoparticles for H2 Production by [FeFe]-Hydrogenase

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The demand for renewable fuels such as H2 continues to rise during the climate crisis. Metalloenzymes such as [FeFe]-hydrogenase offer an attractive option for electroenzymatic H2 evolution under mild conditions. Enzymatic electrocatalysis requires either direct or mediated electron transfer to the biocatalyst, which can be immobilized on electrode surfaces. Here we present our efforts to develop an electrode functionalized with In:Ti oxide (ITO) nanoparticles, onto which [FeFe]-hydrogenase is directly immobilized for high current density H2 production. We report on the modification of glassy carbon and pyrolytic edge plane graphite electrodes with ITO nanoparticles and [FeFe]-hydrogenase, where H+ reduction was observed with current densities of up to 8 mA cm−2 at −0.8 V vs SHE. Rotating ring disk electrochemistry was also employed alongside gas chromatography for H2 quantification and to calculate the faradaic efficiency of the system.

Importantly, the ITO-modified electrode is prepared by a simple single step prior to enzyme immobilization. We therefore anticipate the application of this “nanoITO”-electrode to be diverse, possibly in combination with a range of other (metallo)enzymes of interest to electroenzymatic biotechnologies.

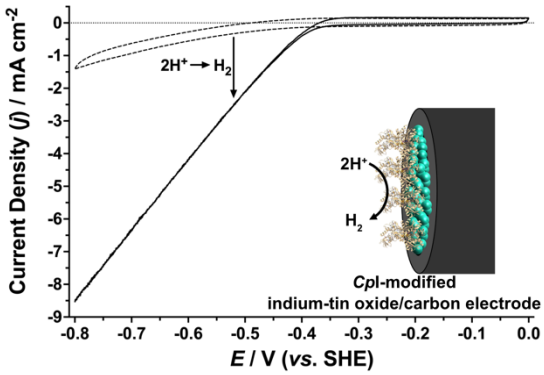

FIGURE 1. CYCLIC VOLTAMMOGRAM OF H_2 FORMATION BY CPI [FEFE]-HYDROGENASE ON A CARBON ELECTRODE MODIFIED WITH ITO NANOPARTICLES. THE DASHED LINE REPRESENTS A CONTROL ELECTRODE PREPARED WITH O_2-DENATURED ENZYME. SCAN RATE = 10 MV S^{-1}, MOPS BUFFER (PH 7, 100 MM).

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S6: Bio(photo)electrochemistry & bio-energetics

Keynote speakers

S6K1: Whole cell and enzymatic catalysis for production of biofuels

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Developing sustainable alternative processes to produce H2 and reduce the levels of CO2 is one of the most urgent and challenging issues facing our society. Our lab studies anaerobic bacteria and redox enzymes as biocatalysts for H2 production and the reversible reduction of CO2 to formate, which is an added value compound and hydrogen storage material. Using whole cell studies we have identified highly active bacteria in H2 production as well as CO2 reduction [1,2]. We have characterized the enzymes involved, a NiFeSe-hydrogenase [3] and W/Sec-formate dehydrogenase [4], both of which are highly active and show remarkable oxygen stability, and produced variants with improved properties [5]. The O2 stability and robustness of these enzymes has also been exploited in the development of efficient semi-synthetic photo- and electrocatalytic systems [6,7], and also whole-cell photocatalysis [8].

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Invited speakers

S6I1: Electro-driven production of substituted N-heterocycles via an artificial enzymatic cascade

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We developed a scalable platform that employs electrolysis for an in vitro synthetic enzymatic cascade in a continuous flow reactor. Both H_2 and O_2 were produced by electrolysis and transferred via a gas permeable membrane into the flow system. The membrane enabled the separation of the electrolyte from the biocatalysts in the flow system, where H_2 and O_2 served as electron mediators for the biocatalysts. We demonstrated the production of methylated N-heterocycles as building blocks for pharmaceuticals with an artificial enzymatic cascade composed of an engineered putrescine oxidase (PuOE203G) [1], a designed NADH-dependent imine reductase (IRED) and the NAD+-reducing hydrogenase (SH) from Ralstonia eutropha [2]. The O2-dependent PuOE203G oxidizes diamines to the corresponding imines, which are subsequently reduced by the NADH-dependent IRED to the saturated N-heterocycles. The O2-tolerant SH catalyses the H2-driven recycling of NADH [2].

Through powering the cascade with electricity, substituted pyrrolidines and piperidines were obtained with up to 99% product formation in a one-pot reaction directly from the corresponding diamine substrates [3]. We extended the applicability of the system towards performing regioselective isotopic labeling providing useful insights into the enzyme mechanism of SH. This platform represents an important advance in the field for biocatalytic synthesis, and it can be expanded for powering various cofactor-dependent oxidoreductases.

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Oral presentations

S6O1: Spectroelectrochemical Studies of Oxygen-Tolerant [NiFe] Hydrogenase Immobilized on Transparent Conducting Oxides for Hydrogen Oxidation

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[NiFe] hydrogenases are a class of metalloenzymes that catalyze the reversible heterolytic splitting of molecular hydrogen (H2) into protons and electrons. While most hydrogenases are catalytically inhibited in the presence of oxygen, the membrane-bound [NiFe] hydrogenase from Ralstonia eutropha exhibits a remarkable tolerance to oxygen. Its ability to maintain catalytic activity in aerobic conditions makes this enzyme suitable to incorporate into hydrogen-based devices. To utilize its catalytic processes in technological applications, it is necessary to successfully immobilize the enzyme on desirable substrates and concomitantly have an in-depth understanding of the enzyme-surface interaction.

The use of transparent conducting oxide (TCO) thin films has gained attention due to their conductivity and optical transparency. As an electrode for enzyme immobilization, it is a protein compatible platform that is ideal for spectroelectrochemical analysis. In this work, the membrane-bound [NiFe] hydrogenase from Ralstonia eutropha was equipped with either a His- or Strep-tag and immobilized on different TCO films. The hydrogenase-TCO surface interactions were studied with a modified ATR-IR setup to examine the absorption and desorption processes, as well as the catalytic mechanism by probing the hydrogenase Ni/Fe active site. Both in-situ and operando electrochemical measurements were conducted to investigate the changes in enzyme orientation on the electrode surface. Electrocatalytic behaviour, including changes in current density, enzyme activation/deactivation, catalytic bias, and stability were studied and discussed in detail.

S6O2: Studying Enzymatic Catalysis by Fluorescence Microscopy – Electrochemistry Coupling

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Redox enzymes have an array of distinctive properties such as high catalytic turnovers, low overpotentials, and high affinity for their substrates, that can be exploited in bio-electrochemical devices. In such cases, enzymes are immobilised on the electrode surface, and the reaction requires an efficient electron transfer. Using electrochemistry, the kinetic and thermodynamic parameters of the enzymatic reactions can be investigated. However, as an electrochemical measurement is an integration of all currents over the entire electrode surface, spatially resolved/local information at the surface is still lacking. To investigate such heterogeneities locally, a multitude of microscopic techniques have been coupled with electrochemistry1 which enables the simultaneous collection of global and local data. In the frame of bioelectrochemistry, it would also allow for the mapping of enzymatic reactivity at the electrode surface. We demonstrate here that the coupling of confocal laser scanning fluorescence microscopy with electrochemistry allows for the characterisation of electro-enzymatic catalysis.2 We chose a model reaction of 4 H+/4 e- oxygen reduction to water, catalysed by the enzyme bilirubin oxidase from Myrothecium verrucaria (MvBOD). Local pH variations occurring at the vicinity of the bioelectrode throughout catalysis can be visualised via a pH-dependent fluorophore (fluorescein). Proton depletion profiles are rebuilt in 3D images, and the influence of buffer or ionic strength of the local environment are probed. The enzymes are also labelled with a fluorescent dye (Alexa Fluor 430), allowing to map local heterogeneities of the enzyme distribution at the electrode surface.

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S6O3: Rational Design and Optimization of a Photosystem I-Based Light-Driven Bioanode

Panpan Wang,[1] Fangyuan Zhao,[1] Anna Frank,[2] Sarra Zerria,[1] Anna Lielpetere,[1] Adrian Ruff,[1] Marc M. Nowaczyk,[2] Wolfgang Schuhmann,[1] Felipe Conzuelo[1,3]

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Photosystem I (PSI) is an attractive building block for the fabrication of semi-artificial energy conversion devices. However, one of the major challenges in the development of PSI-based biophotoelectrodes is the fabrication of well-defined structures able to provide unidirectional electron flow. As we have shown recently, the amphiphilic nature of PSI can be exploited for the fabrication of bioelectrodes by means of the Langmuir-Blodgett (LB) method, leading to densely packed PSI films that exhibit a preferential orientation of PSI over the entire electrode surface.1,2 Consequently, PSI-based bioelectrodes, in which an anisotropic electron flow is ensured, can be readily fabricated. On this basis, we present an optimized bioelectrode able to operate as a light-driven bioanode. A highly efficient electrode architecture is obtained after rational integration of the PSI-LB film using specifically designed redox polymers for electrical wiring of the terminal redox centers that are located at opposite sides of the protein complex. Particular attention is given to minimize charge recombination and short-circuiting processes, which conventionally limit the performance of PSI bioelectrodes. As a result, substantial photocurrents can be achieved even at relatively low applied potentials, surpassing the performance of previously reported bioelectrodes and paving the way towards the development of advanced biophotovoltaic devices.

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S6O4: Instilling New Characteristics in Whole-cell Biohybrids Using Novel Inorganic Nano-Organelles

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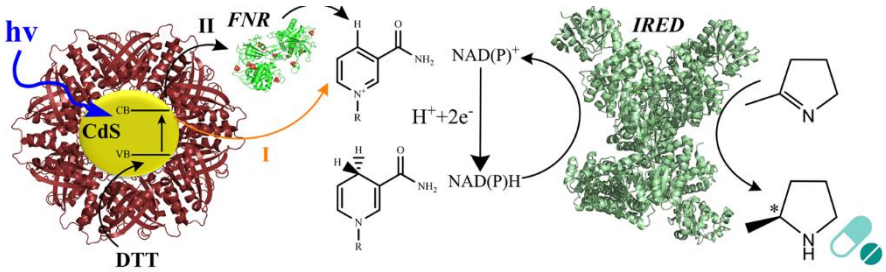
In this work, we present the use of the globular protein stable protein 1 (SP1) for the synthesis of various optically and electronically active nanoparticles (NPs). We demonstrate the biosynthetic processes and their catalytic applications both with isolated SP1 and within whole cells expressing the protein. Using genetic engineering tools, we modify the SP1 with metal binding peptides (MBPs) for the selective biosynthesis of metal or semiconductor NPs.

We show the ambient biosynthesis of CdS NPs stabilized by a predesigned SP1 variant1. The sized controlled crystalline NPs are utilized for NADPH regeneration that is subsequently used for the activation of the imine reductase (IRED) enzyme (as shown in the scheme). The presented nano-bio hybrid system enables the generation of a single enantiomeric product, (R)-2-methyl pyrrolidine, which is required for the pharmaceutical industry. Additionally, in our recent published work, we demonstrated the biosynthesis of palladium NPs in whole cells, characterized by multiple methods2. These biohybrids were used for the enhancement of desired hydrogenation reactions.



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S6O5: Nitrogenase Based Nano-Bio-Hybrid Systems for Photo-biocatalytic Processes

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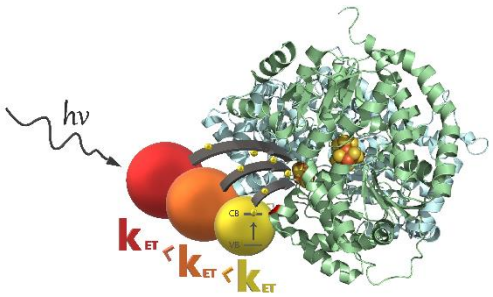
Due to its unique ability to generate ammonia by atmospheric nitrogen fixation, nitrogenase enzyme is considered a promising alternative to chemical fertilizers production. While extensive research has been performed for several decades, only recently have researchers found its aptness toward artificial fixation by coupling the nitrogenase to semiconductor nanoparticles (NPs) or by immobilization on electrode surfaces.

Here we present methodologies for the artificial activation of mutated or wild-type nitrogenase variants using various NPs.1,2 The effect of the CdS NPs ligands and the redox mediators on the hydrogen or ammonia generation will be presented.1 We will discuss how the NP sizes affect the CdSe NPs' ability to activate the nitrogenase enzyme. We will show the use of advanced spectroscopic and electrochemical methods to elucidate the electron transfer rate and the nano-bio hybrid photo-induced catalytic activity.2



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S6O6: Electrochemical Analysis of the Redox State of Mitochondrial Quinones to Study the Warburg Effect

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Cellular energy metabolism mostly relies on glucose metabolism and in the production of adenosine triphosphate (ATP). ATP is generated through glycolysis in the cytosol and by oxidative phosphorylation (OXPHOS) at the inner membrane of mitochondria. Normal differentiated cells primarily rely on mitochondrial OXPHOS to aerobically produce ATP whereas cancer cells display a preference for a fermentative metabolism, regardless of the presence of oxygen. This metabolic reprogramming of cancer cells, called the “Warburg effect,” is characterized by an OXPHOS repression and an enhanced glycolysis proportional to their growth rate 1. The yeast Saccharomyces cerevisiae is able to switch its metabolism from OXPHOS to alcoholic fermentation under oxygen, making it a good model to study the Warburg effect2. Since glycolysis generates reduced equivalents that are then oxidized by mitochondria, the cellular redox pressure increases. Lipophilic quinones are embedded in the inner mitochondrial membrane (Q9 ; Q10) to transfer electrons from dehydrogenases to complex III. Their redox state depends on availabilities of substrates and dioxygen, and then hypothetically reflect the cellular redox state.

Based on early works on plants 3,4, we developed an indirect electrochemical method to monitor in real-time the redox state of quinones of yeast mitochondria. An exogenous short-length chain species (Q2) is used as a redox mediator. Q2 permeates across mitochondrial membranes and reaches a redox equilibrium with the internal quinones. The reduced form Q2H2 then diffuses back to the solution and is oxidized at a glassy carbon electrode. Respiratory chain and ATP synthase substrates are added to monitor in real-time the modulation of the quinones’ redox state. Variations are correlated with the oxygen consumption detected by a Pt Clark electrode. The method will be further applied to intact yeasts to study their cellular redox state in the context of a Warburg effect induction.

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S6O7: Using electric fields to manipulate and assess the mechanics of photoswitchable membranes

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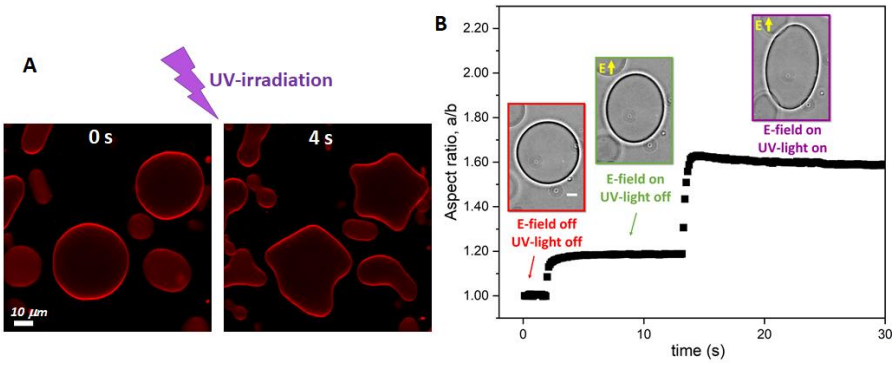
Over the years, light has been widely used as an effective trigger in biotechnology to interrogate biological systems and provide a conditional control over complex cellular processes. Understanding light-triggered changes in bio-systems is crucial for cell viability and optimization of clinical applications in optogenetics and photopharmacology. In this study, we employed artificial cells, i.e. cell-sized giant unilamellar vesicles (GUVs), doped with azobenzenephosphatidylcholine (azoPC) as a photoswitch, and quantified light-induced changes of the membrane material properties. The vesicles were exposed to electric fields to examine light-triggered vesicle area change (see figure), reversibility and kinetics of photoswitching, bending rigidity and interleaflet interaction in the bilayer. We show that light and electric fields can be used to manipulate the shape and mechanics of these synthetic cells. The experimental results are supported by molecular dynamics simulations. The combined results illustrate that the mechanics of azoPC-doped membranes can be finely controlled by light.



(A) Confocal screenshots of UV-induced complex shape transformations of azoPC-doped GUV. (B) The membrane area change due to UV irradiation can be quantified by exposing the GUVs to electric field which deforms the vesicles into well-defined ellipsoidal shapes (see phase-contrast snapshots) from the aspect ratio of which one can precisely measure the area changes associated with UV irradiation. The scale bars are 10 mm.

S6O8: 3D ITO as electrode material for DET of photosystemI

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Photobioelectrodes combine photoactive components with inorganic electrode materials for energy conversion and application in catalysis and sensing. When isolated photoactive proteins such as photosystem I are used, then one faces problems in electrode-protein communication since these membrane protein complexes are supramolecular structures of significant size. Thus, often mediators are applied for electron transfer. 3D electrode materials became a popular research area since they allow the immobilisation of large amounts of the biocomponent. When transparent materials are applied then also sufficient light interaction can be ensured. Thus, we have reported on different systems using ITO as electrode material and a template-based approach for easy preparation exploiting a simple spin coating procedure [1,2].

Here we report on direct electron transfer between photosystem I and such 3D ITO. It can be shown that the buffer concentration significantly influences the electrical communication of PSI with the electrode surface. This is based on the interaction of the luminal side of the protein with the ITO material since only cathodic photocurrents have been detected. Different ions can further influence the situation of the protein complexes on the ITO surface. Interestingly high KCl concentrations have been found particularly advantageous. Furthermore, it can be shown that such hybrid structures operate in a rather wide window from pH 5 to 9. Another attractive feature is the rather high onset potential of the cathodic photocurrent of ~ 0.4V vs Ag/AgCl. Since the 3D electrode preparation is scalable with respect to the thickness of the structure and the accessibility of the surface for PSI immobilisation one can enhance the photocurrent output. With electrodes prepared from 12 spin coating steps one can reach current densities of more than 10µA/cm2 at -0.1V vs Ag/AgCl.

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S6O9: Bridging Homogeneous and Electro- Catalysis: A Story of High-Potential Multicopper Oxidases

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In nature, oxygen activation and reduction are performed by several enzymes. A particularly interesting class of oxygen-reducing enzymes are high potential multicopper oxidases (MCOs). The active sites of MCOs include at least four copper ions, arranged in a mononuclear type 1 (T1) Cu, and a trinuclear Cu cluster (TNC), where oxygen reduction (ORR) occurs. High-potential MCOs exhibit astonishing ORR electroactivity at potentials approaching 1.2 V (vs RHE), which leads to their high importance as catalysts for the construction of efficient biofuel cells and as model systems for the design of biomimetic catalysts. The oxygen reduction mechanism for the low potential MCOs in solution is well-characterized; however, O2 reactivity of high-potential MCOs is not well understood, especially for the case of electrocatalysis.

This presentation will discuss the mechanism of the ORR by high-potential MCOs in solution and for proteins immobilized on the electrode surfaces. Particularly, the implications of the low driving force for electron transfer (ET) from the T1 site to the TNC on the ORR mechanism will be considered.1 Moreover a central question in MCO electrocatalysis, whether the T1 Cu is the primary electron acceptor site for ET from the electrode, or whether electrons can be transferred directly to the TNC, bypassing the rate-limiting intramolecular ET step will be addressed.2,3

Findings that will be presented provide insight into factors controlling the ET mechanism in homogeneous and electrocatalysis by high-potential MCOs and can contribute to the design of more efficient MCO (MCO mimic)-based cathodes for the ORR.

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S6O10: Electrocatalytic Studies on Cytochrome bd Oxidase, a Bacterial Defense Factor

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The selective reduction of oxygen to water is crucial to life and a central process in aerobic organisms. It is catalyzed by several different enzymes, including cytochrome bd oxidases (cyt bd) that are solely present in prokaryotes, including several pathogens. In addition, these enzymes play a crucial role in protection against oxidative stress, in virulence, adaptability and antibiotics resistance. The reduction of O2 occurs at the high spin D-type heme in all cyt bd oxidases, that is also the binding site for several ligands from signaling processes, including CO, H2S and CO.

Here we present the electrocatalytic study of the cytochrome bd I and bd II oxidases from Escherichia coli. (1,2) Structural parameters that are crucial for the reactivity towards oxygen are analyzed. The pH dependency of the binding and release of NO, an important signaling factor is presented. Finally, the question why E. coli comprises two highly comparable cytochrome bd oxidases is discussed.

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S6O11: Electrochemical Investigation of Cu+ Oxidation by Multicopper Oxidases

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Multicopper oxidases (MCOs) are redox enzymes present in all domains of life that contain a couple of Cu-centres: mononuclear T1 and trinuclear T2/T3. Although the coordination and environment of these copper centres are highly conserved, the overall protein structure may present significant disparities responsible notably for various physiological roles MCOs can play. Accordingly, electron donors accepted by MCOs vary from small organic molecules to metal ions, while electron acceptor is always oxygen [1]. Together with terminal cytochrome c oxidases, MCOs are the only enzymes capable of four-electron oxygen reduction. But, unlike the former, MCOs can perform this reaction at higher potentials reaching 0.78 V (NHE) which is beneficial for different enzymatic and biohybrid fuel cells.

Earlier, we showed that the MCO from Thermus thermophilus, which exhibits direct electron transfer of oxygen reduction, gives rise to a new catalytic wave upon Cu2+-addition in certain conditions [2,3]. We rationalised and ascribed this wave to a cuprous oxidase activity displayed by this enzyme thus allowing us to propose its physiological role in copper detoxification process [4]. The only other well-studied MCO with confirmed cuprous oxidase activity, CueO from E.coli, has a large methionine-rich domain proposed to play a role in the copper binding. This domain is significantly shorter in the MCO from T.thermophilus raising a question about its true role. Here we present the utility of electrochemistry and importance of electrode surface chemistry in study and quantification of the cuprous oxidase activity of MCOs related notably to the presence of an additional Met-rich domain.

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S6O12: Photobio-Electrocatalytic Production of H2 Using Fluorine-doped Tin Oxide (FTO) Electrodes Covered With CuGaS2 Semiconductor and NiFeSe Hydrogenase

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Clean energy vectors are needed towards a fossil fuel-free society, diminishing both greenhouse effect and pollution. Water splitting is a clean route to obtain hydrogen, the cleanest fuel; although even after decades of research it is not yet efficient enough to overcome its high overpotentials.

Some recent advances have considered the combination of inorganic catalysts with biocatalysts searching for new advantages and alternative approaches to this problem. Sulfides like In2S3 have been demonstrated as good candidates to transfer light energy to biocatalysts like hydrogenase and reduce protons to H2 upon a sacrificial electron donor [1]. However, as n-type semiconductor it is not feasible its direct immobilization on an electrode to accomplish the same reaction, as the photo-excited electrons flow towards the electrode and only favor oxidation reactions [2]. We have explored the p-type material CuGaS2 to deposit on the surface of a FTO electrode and produce H2 in a photoelectrochemical biocatalytic system in absence of sacrificial reagents. The optimization of the deposition of CuGaS2 was critical due to its role as n-type semiconductor. The need for a regular and homogeneous distribution of the CuGaS2 led to 3 different methods to settle it on the surface: spin coating, electro-dip coating and in situ growth in the solvothermal reactor. The amount of CuGaS2 layers deposited had an important impact in the performance of the electrode and was optimized. The electrodes were characterized through SEM, AFM and XRD. Photoelectrocatalysis with hydrogenase-loaded electroactive surfaces was performed and characterized.

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S6O13: A Foldable Assembly of Photosystem I Monolayers Enables Improved Incident-Photon-to-Electron Conversion Efficiency in Biophotovoltaic Devices

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Photosystem I (PSI), one of the main protein complexes driving photosynthesis, can achieve charge separation upon absorption of visible light, leading to a final state consisting of two redox centers of opposite charge with a potential difference of about 1 V. As we have shown previously, integration of PSI monolayers with electrodes using the Langmuir-Blodgett technique enables a preferential anisotropic orientation of PSI in a closely packed structure, which minimizes short-circuiting processes and aids to improve the performance of PSI-based biodevices.[1] Although the obtained photocurrent was among the highest reported for a PSI monolayer on semiconductor-free electrodes, the practical application of PSI monolayer-based biodevices is limited due to the comparatively small loading of immobilized PSI molecules, leading to an overall low incident-photon-to-electron conversion efficiency. In terms of multilayered PSI architectures, the challenge is to overcome the large photoinduced potential difference between the two terminal redox centers that are located at opposite sides of PSI, which translates into a driving force for charge recombination resulting in underperformance of the system. In addition, the blocking of light by multiple PSI units in parallel needs to be taken into consideration.

Inspired by the highly ordered arrangements of PSI in native thylakoid membranes, we demonstrate improved light utilization using multiple PSI monolayers assembled into a foldable architecture.

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Posters

S6P1: Bioelectrochemical CO2 conversion using carbon mono-xide dehydrogenase on gas-diffusion electrodes

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The implementation of devices for CO2 conversion offers the possibility to lower the amounts of this greenhouse-effect gas present in the atmosphere while at the same time it allows for the use of CO2 as carbon feedstock, in alternative to the use of fossil fuels. As a result, the electrochemical reduction of CO2 has become a field of major importance over the course of the last years. Due to the broad range of possible products, the use of an efficient and selective catalyst is crucial for enabling the synthesis of valuable products. In this regard, the ability of redox enzymes to effectively convert substrates towards specific products while operating under mild conditions makes them highly promising catalysts for the envisaged purpose.

We present the use of the enzyme carbon monoxide dehydrogenase (CODH) from C. hydrogenoformans as a biocatalyst for the fabrication of gas diffusion bioelectrodes capable of the selective reduction of CO2 to CO. The use of a gas diffusion layer as electrode substrate enables to overcome mass transport limitations.[1,2] Moreover, to ensure immobilization and electrical wiring of the biocatalyst, the enzyme is embedded into a redox polymer deposited over the gas diffusion electrode. In this way, an efficient and selective bioelectrode for the conversion of CO2 into CO is obtained.

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S6P2: Spectroelectrochemistry of the Nitrogenase-like Dark-Operative Protochlorophyllide Oxidoreductase (DPOR)

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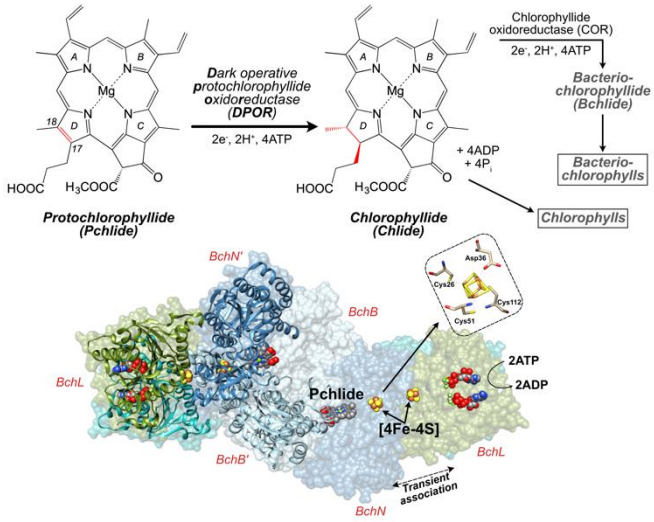
Dark-Operative Protochlorophyllide Oxidoreductase (DPOR) shares structural and mechanistic properties with the N2-fixing enzyme nitrogenase to participate in the biosynthesis of bacteriochlorophyll, thereby enabling photosynthesis by photosynthetic bacteria. Specifically, DPOR hydrolyzes ATP to catalyze the stereo-selective 2e− reduction of protochlorophyllide (Pchlide) to chlorophyllide (Chlide) in the dark. Similar to nitrogenases, the importance of ATP hydrolysis is not fully understood. Further, the thermodynamic properties of the iron-sulfur clusters of DPOR (i.e., their reduction potentials) have not been reported.

In this work, we investigate the enzymatic activity of DPOR and its redox properties. Considering the absorbance properties of the substrate (Pchlide) and product (Chlide), activity assays and redox characterizations have been performed using UV-visible spectroscopy, UV-visible spectroelectrochemistry and bioelectrocatalysis. We anticipate that the determination of thermodynamic and kinetic properties of DPOR will be informative to related metalloenzymes, such as nitrogenase.



S6P3: Hydrogel-Based Bioinspired Wearable Battery

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Hydrogels-based organic electrolytes have been shown as a promising alternative for the development of batteries(1) to power newly developed technologies, such as wearable devices, sensors, and smart clothes. Here, we describe a bioinspired organic/organometallic microbattery using hydrogel redox active-compounds electrolytes and flexible carbon electrodes. 4,4'-((9,10-anthraquinone-2-yl)oxy)butyrate (BEAQ) and potassium ferricyanide were applied in both anodic and cathodic compartments, respectively. BEAQ presented self-gelling property in alkaline medium, and the rheological data indicated a gel with high stability at room temperature and shear-thinning behavior. The measured BEAQ's half-wave potential and diffusion coefficient were -0.66 V vs Ag/AgCl/KClsat and 3.04 × 10-6 cm-2 s-1, respectively. Potassium ferricyanide was applied in a Xanthan gum hydrogel matrix and presented a half-wave potential of + 0.28 V vs Ag/AgCl/KClsat. When the two hydrogels were paired in a hard-battery configuration using a Nafion 212 membrane as a separator, a primary battery with open-circuit voltage (OCV) of 0.88 V and a maximum volumetric power of 11.2 µW µL-1 (state of charge of 50%) was obtained. As a proof-of-concept, we build up a wearable bioinspired microbattery (WBM) replacing the hard casing by commercial textiles. The WBM presented an OCV and maximum volumetric power of 1.3 µW µL-1 in twisted conformation, respectively. Due to the mechanical properties of the electrodes and the hydrogel, this battery is flexible and can be twisted and combined with wearable platforms, preserving its electrochemical properties for power generation.

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S6P4: How do Redox Polymers Enhance Photocurrents from Cyanobacteria?

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Semi-artificial photosynthetic technologies that incorporate whole photosynthetic microorganisms as catalysts offer a sustainable way of generating either electricity or fuels from solar energy.1 At present, the main bottleneck limiting the development of such technologies is the low level of extracellular electron transport from the cells to the electrode. A commonly used strategy to enhance the current from photosynthetic cells is the use of redox polymers. Redox polymers offer a range of advantages including enhancing extracellular electron transfer kinetics, lower toxicity, and increased cell loading by means of acting as an immobilisation matrix to encapsulate the microorganisms.[2] However, the cell-polymer-electrode interface is complex and the precise mechanism of enhancement is often not well understood, limiting rational interface design. Here, we tested the capacity of a common osmium-based redox polymer to act as a wiring tool for Synechocystis sp. PCC 6803 on hierarchically-structured inverse opal indium tin oxide (IO-ITO) electrodes. By systematically investigating the polymer-cell loading concentration that gave rise to optimal photocurrents using stepped chronoamperometry, and normalising this against cell loading, we could clearly identify at which conditions the polymer served only as an immobilisation matrix, enhancing the photocurrent by means of increasing the cell loading rather than by mediation. We also investigated the interaction of the polymer with a Synechocystis mutant lacking extracellular polymeric substances (EPS). Photocurrents of the EPS mutant in the presence of the polymer were not as large as for the wild type illustrating the importance of the cell-polymer interaction for effective wiring.

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S6P5: Draft Genome Sequence of Paenibacillus profundus YoMME, e new Gram-positive bacteria with exoelectrogenic properties

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The mixed bacterial society on the anode contributes to the current generation by the Sediment Microbial Fuel Cells (SMFC). The bacterial composition of the anodic biofilms, however, is specific for the different sediments and the participation of individual bacterial species in the anodic processes has to be evaluated. Recently, we identified new bacteria Paenibacillus profundus named YoMME by 16 S rDNA gene and established that it is capable of transferring electrons extracellular to the anode of MFC when used as a pure culture while oxidizing peptone as a carbon source and thus the strain became one of the few recognized exoelectrogenic Gram-positive bacteria [1]. Here we report for the first time the draft genome sequence of the new strain Paenibacillus profundus YoMME. The generated draft genome assembly has a size of approximately 6,92 Mb and a GC content of 48.68%. The Whole Genome Shotgun project for P. profundus YoMME has been deposited at DDBJ/ENA/GenBank under the accession JAJNBZ000000000.

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S6P6: A photobioelectrochemical cell feeded by light and sugar with rather high open cell voltage

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The combination of light-sensitive entities with electrodes has gained increasing interest in the last decade. Light gives access to multiplexing by spatially resolved illumination of sensing surfaces, but it can also be used to modulate the electrochemical reactions [1]. This is interesting with respect to different aspects. One direction of research is devoted to systems which can improve the potential behavior for electrochemical signal chains. This is relevant particularly in bioenergetic applications.

Thus, we have already demonstrated a photobioanode which allows the collection of electrons from the sugar oxidation at extremely low potentials under illumination. Here a combination of two semiconducting materials (TiO2 and PbS) with a biocatalytic reaction (FAD-GDH or PSII) has been exploited [2,3].

The present study is devoted to the investigation of a cathode material which can be prepared as a thin layer by a simple spin coating procedure. The material used here is BiFeO3. It is characterized by a rather high positive onset potential for cathodic photocurrents (~0.6V vs Ag/AgCl). When hydrogen peroxide is applied in solution the cathodic photosignal can be significantly enhanced - although there is no electrochemical H2O2 reduction in the dark. On this basis a photobiocathode has been constructed by coupling glucose oxidase to the photoactive electrode [4].

By combining these two electrodes a cell can be created with two light-sensitive electrodes and sugar as the substrate for both electrodes. Advantageously illumination of the anode is realized through the cathode and an OCV of about 1V is reached. These investigations may illustrate the potential of coupling suitable semiconductor structures with biocatalytic conversions on electrode surfaces for application in bioenergetics.

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S6P7: Bioelectrocatalytic CO2 Reduction by Formylmethanofuran Dehydrogenase

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Climate change is one of the most serious concerns facing our society, largely due to an increase in atmospheric CO2 concentrations. Therefore, efforts have increased towards decreasing the atmospheric CO2 level. In particular, using CO2 reducing enzymes has drawn great attention because of their outstanding catalytic properties under mild conditions.1 Formylmethanofuran dehydrogenases are multi-subunit enzyme complexes of around 800 kDa containing molybdenum- (named as Fmd) or tungsten- (named as Fwd) dependent cofactors, up to 46 iron-sulfur clusters and dinuclear Zn centers. These complexes catalyze the reduction of CO2 to formate at the Mo-cofactor, followed by formate transport along an internal substate channel prior to selective condensation with methanofuran by an amidohydrolase containing dinuclear Zn2+.2 Here, we present on the bioelectrochemical reduction of CO2 with Mo-containing formylmethanofuran dehydrogenase (Fmd) from the thermophilic archaeon Methermicoccus shengliensis adsorbed to graphite electrodes in the absence of electron mediators.

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S6P8: Ion potassium channel ROMK2 - studies of protein electrochemical properties.

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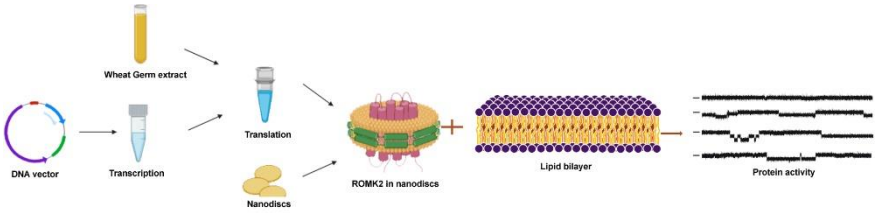
The primary function of mitochondrial potassium channels is to regulate the potential of the mitochondrial membrane, which allows them to play an important role in cytoprotection, especially in the case of cell hypoxia. The following project focuses on obtaining the ROMK2 protein (ATP-regulated potassium channel) using cell free expression system, incorporation of the channel protein into the lipid bilayer and studying the influence of voltage changes and molecular modulators on channel activity (Fig). The ROMK2 transcription / translation reactions is carried out in the presence of protein nanodiscs. It allows the protein to be more stable and facilitates their incorporation into the membrane. Channel activity is measured using two biomimetic membranes models - Black Lipid Membrane and Solid Supported Black Lipid Membrane. After carrying out BLM measurements in an asymmetric system, it was observed that the channel shows activity only at negative applied potentials, which may indicate the rectifying properties of the protein. In the second method, the lipid bilayer is created on the gold electrode, which makes it more stable and durable, allows for the incorporation of several channels simultaneously, and the use of techniques such as alternating current voltammetry, cyclic voltammetry or electrochemical impedance spectroscopy (EIS). The EIS technique that we mainly focus on in our research interprets the charge transfer and storage processes in terms of electrical equivalent circuits consisting of resistances, capacitances and pseudo-capacitances and assigning these values to the appropriate processes related in our case to ion transport.



S6P9: Carbon nanograss electrodes for miniaturized microbial solar cells

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Pyrolytic carbon is a favourable electrode material due to its properties such as conductivity, biocompatibility, good chemical resistance and a wide range of possibilities for surface functionalization and modification. In particular, these properties identify pyrolytic carbon as a suitable material for bioelectrochemical systems such as microbial solar cells. In the so-called CMEMS process microelectrodes are fabricated by pyrolysis of photoresist precursor structures at high temperature and in an inert atmosphere. The resulting pyrolytic carbon has a very low surface roughness, which is unfavourable for the good attachment of cells and bacteria. Therefore, we here focus on developing pyrolytic carbon electrodes with nanostructured surfaces potentially allowing improved attachment of cyanobacteria Synechocystis sp. PCC6803 enhancing electron transport between the cells and the electrode in microbial solar cells.

Anisotropic reactive oxygen plasma etching of crosslinked layers of SU-8 polymer creates grass-like nanostructures on the surface of the resist. Pyrolysis of the structures at high temperature converts the polymer into pyrolytic carbon, which retains the form of nanograss. The carbon nanograss (CNG) structures add roughness to the surface and significantly increase the electroactive area of the electrode. For characterization, we developed a miniaturized test system based on 3D printed components. Electrodes covered with a biofilm of cyanobacteria in BG11 media are characterized by amperometric measurements with alternating periods of light illumination and darkness. In the study, we observe the effect of the length of CNG compared with flat carbon electrodes.

The preliminary results suggest excellent attachment of the cyanobacteria on the electrodes. Furthermore, we can observe increasing current densities with increasing length of CNG, which correlates well with the increasing surface area of the electrodes. which suggests better attachment of cyanobacteria on the electrode.

S6P10: Can NADPH serve as an electron shuttle in extracellular electron transport in Synechocystis PCC6803?

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Extracellular electron transport is the export of energetic electron carriers out of a cell, and is one of the least well characterised photosynthetic electron transport pathways in both cyanobacteria and unicellular algae. The unravelling of this phenomenon has implications for renewable energy and biotechnology applications [1]. In the model cyanobacterium Synechocystis PCC6803, extracellular electron transport is understood to proceed via the secretion of a small, soluble redox-active mediator[2]. Recently, NADPH was reported to be the endogenous mediator in Synechocystis[3]; however, while we do observe NADPH in the extracellular environment using 3D fluorescence spectroscopy, we question the likelihood of this conclusion using a series of considerations. Firstly, we model the LogD value of NADPH to understand its cell permeability and find that at cellular pHs it is too hydrophilic to diffuse across membranes. Secondly, we characterise the electrochemical properties of NADPH using cyclic voltammetry and observe that it does not reversibly oxidise non-enzymatically, as the mediator is expected to. Thirdly, we test NADPH and NADP+ as exogenous redox mediators, and find they do not increase the photocurrent of a Synechocystis biofilm. Altogether, we propose that NADPH is present in the extracellular space due to cell rupturing, but it is a weak candidate as a diffusional mediator for cyanobacterial cells.

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S6P11: On the improvement of the cathode for enzymatic biofuel cells

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Enzymatic biofuel cells (EBFCs) typically comprise enzymatic anodes and cathodes[1]. The performance of EBFCs, especially the power density, is typically limited by the enzymatic cathode, in which the dioxygen reduction reaction (ORR) is the core process. The concentration of dissolved dioxygen in aqueous solution is, however, low, and even lower *in vivo* (0.14 mM in arterial blood and 0.08 mM in intestinal tissue), posing substrate supply constraints to the biocathode. The stability of the enzymes used, predominantly multi-copper oxidases such as laccases and bilirubin oxidase (BOx), is another recurrent problem.

Herein, we will overview several strategies to overcome the limitations of the cathode, such as using air-breathing electrodes to improve the supply of dioxygen[2] and the employment of consuming type cathodes such as MnO_2 based electrodes[3]. We will also discuss the utilization of O_2 binders[4] and abiotic, non-noble metal based ORR catalysts[5].

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S6P12: Biotic/Abiotic Interfaced Systems for Biosensing and Enhanced (Bio)Catalysis

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The development of biotic-abiotic interfaced systems extracts increasing attention in recent years. Enzymes have great catalytic capabilities, nevertheless, have limitations in terms of stability and the ability to interface with abiotic materials. In the featured talk, I will present different methodologies to interface biotic materials like enzymes and bacteria with abiotic materials. I will show the use of these biotic/abiotic systems for amperometric biosensing, biofuel cells, and photo(bio)electrochemical cells. Furthermore, I will present a synthetic route for the formation of a cyborg organism that consists of an integrated nanoelement that expends the known biological building blocks and enables unique photo-bio catalytic reactions.

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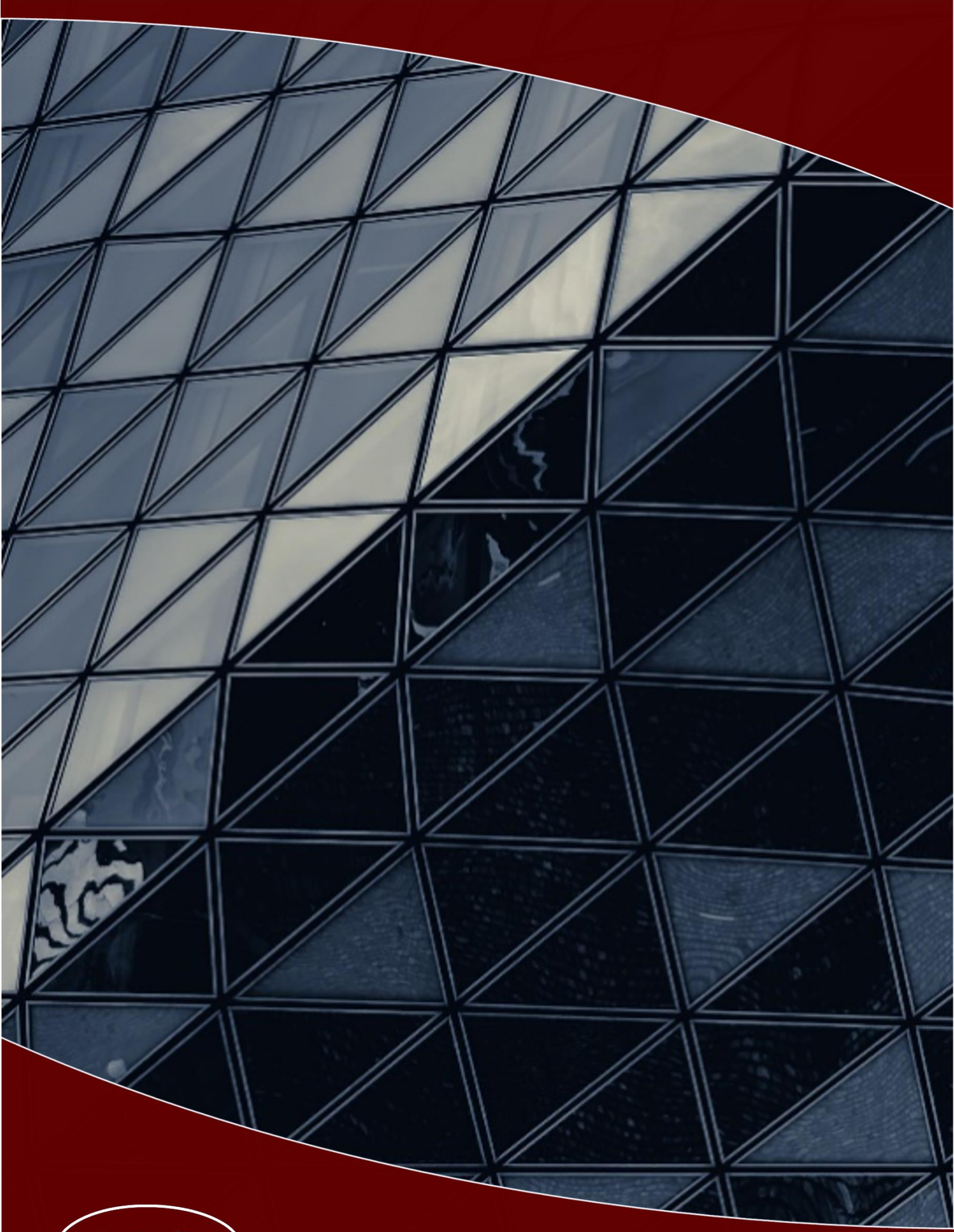

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