22.JUL - 23.JUL

TUHH
Hamburg
University of
Technology

KEYNOTE SPEAKERS

Prof. Jeffrey Gralnick
Prof. Mohamed El-Naggar
Prof. Julea Butt
Prof. Tom Clarke
Prof. Ricardo Louro
Dr. Catarina Paquete
Prof. Daniel Bond

4th INTERNATIONAL MEETING
HAMBURG, GERMANY

BIOCHEMICAL FOUNDATION OF MICROBIAL EXTRACELLULAR ELECTRON TRANSFER

ABSTRACT BOOK

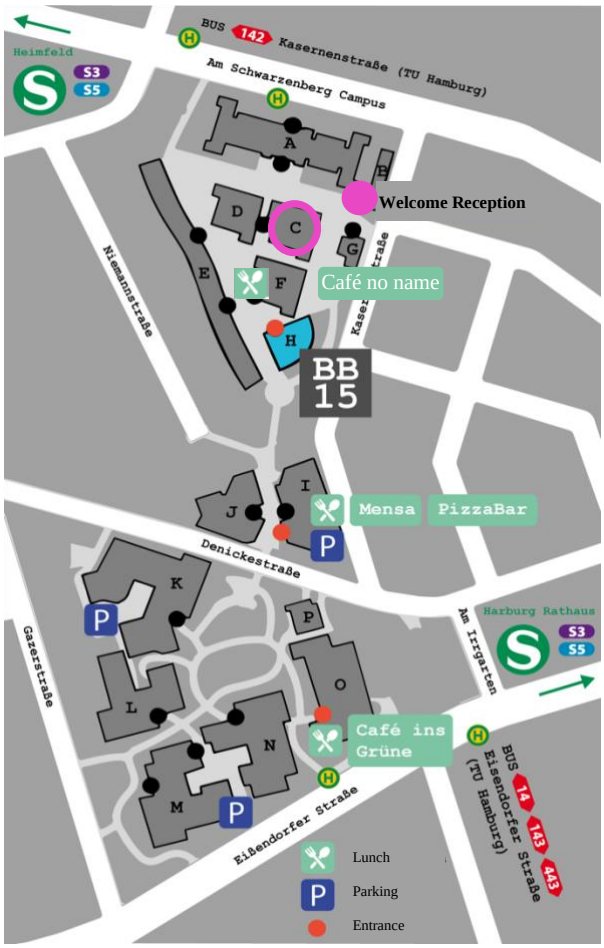
TECHNISCHE MIKROBIOLOGIE
BIOTECHNOLOGIE
TUHH

WWW.GESCHER-LAB.DE

Content

GETTING THERE

Hamburg University of Technology (TU Hamburg)
Am Schwarzenberg-Campus 1
DE-21073 Hamburg



You can reach the TUHH

- by the S 3/S 5 (as far as Harburg-Rathaus or Heimfeld, please follow the signs)
- by train, Harburg is an ICE and EC train station (From Harburg station you can either walk (30 min), go by Taxi or by S3/ S5 as mentioned above
- by car via the A1, A7, B 75 (see map)
- For visitors staying in Hotel Lindtner: please take the S3/ S5 to Heimfeld and then take Bus no. 142
- Registration, Talks and Poster session take place in bldg. C
- Technical Microbiology Lab is in bldg. F

TIMETABLE

Time (CET)	**Agenda: Monday, 22. July**
9:00 – 10:30	Registration (Posters can be put up, presentations must be transferred to the conference laptop during registration. Please bring a USB stick with your presentation)
10:30 – 12:15	**Session 1**
10:30	Welcome to BioCheMEET Johannes Gescher | TUHH
10:45	**Keynote K01:** From Microbes to Living Electronics: The Choreography of Extra- and Inter-cellular Electron Transport Moh El-Naggar | USC (USA)
11:30	**Keynote K02:** Rational re-design of the extracellular cytochrome MtrC from *Shewanella oneidensis* MR-1 Julea Butt | UEA (UK)
12:15 – 13:30	Lunch break Lunch selling spots are highlighted in the map
13:30 – 15:10	**Session 2**
13:30	S101: Investigating the Mechanism of Electrode Reduction in *Vibrio natriegens* Matthew D. Carpenter | Rice University (USA)
13:50	S102: Extracellular electron transfer in a new Gram-positive Microorganism, isolated around cable bacteria Kartik Aiyer | Aarhus University (Denmark)
14:10	S103: Chirality Induced Spin-Dependent Electron Transport through Bacterial Cell Surface Multiheme Electron Conduits Fnu Sukrampal |USC (USA)
14:30	S104: Electron transfer-coupled iron (fe) reduction by oral pathogen *Porphyromonas gingivalis* Divya Naradasu | University of Bristol (UK)
14:50	S105: Discovery of a minimal Extracellular Electron Transfer pathway in *Escherichia coli* Biki B. Kundu | Rice University (USA)
15:10 – 15:40	Coffee break
MiniMEEP *15:40*	S301: Upscaling endeavors of bioelectrochemical systems Ahmed Elreedy | TUHH (Germany)
16:00	**Keynote K03:** Unlocking the potential of electroactive microorganisms: a deep dive into the extracellular electron transfer mechanisms Catarina M. Paquete | ITQB Lisbon (Portugal)
16:45 – 18:00	**Poster session**
18:00 -	**Welcome reception** with Currywurst and french fries (incl. vegetarian and vegan options) Outside, in front of Building B on the TUHH Campus (see map)

Fundamentals of EET and Native Exoelectrogens
Mediated / Synthetic EET
Applied Bioelectrochemical Systems

Time (CET)	**Agenda: Tuesday, 23. July**
8:30 – 12:00	**Session 3**
8:30	**Keynote K04:** Extracellular electron transfer pathways in *Geobacter sulfurreducens* Tom Clarke, UEA (UK)
9:15	**Keynote K05:** The Flavin Awakens: Electron Shuttling in EET and Beyond Jeffrey Gralnick | University of Minnesota (USA)
10:00	S106: Centimeter-long electron conduction in cable bacteria Leonid Digel | Aarhus University (Denmark)
10:20	S107: Unraveling the structural basis of an electric metabolism: a tomographic journey into cable bacteria Anaísa Coelho | USC (USA)
10:40	S108: Cell surface appendages of cable bacteria Markéta Linhartová | Aarhus University (Denmark)
11:00	S201: Heterologous phenazine production in biotech hosts for bioelectrochemical applications Cole Carlson | Leibniz Hans Knöll Institute (Germany)
11:20	S202: Novel EET routes in *Cupriavidus necator* Elena Rossini | Technische Universität Berlin (Germany)
11:40	S203: Mediated extracellular electron transfer with *Cupriavidus necator* André Gemünde | KIT (Germany)
12:00 – 13:10	Lunch break
13:10 – 15:15	**Session 4**
13:10	**Keynote K06:** Exploration of the evolutionary history and application potential of multiheme cytochromes *c* Ricardo O. Louro | ITQB Lisbon (Portugal)
13:55	S302: Diving into the electrophysiology of *Clostridium ljungdahlii* – from understanding to engineering Miriam Rosenbaum | Friedrich Schiller University Jena (Germany)
14:15	S303: A covalent crosslinking approach to enlarge electroactive *Shewanella oneidensis* biofilms Lukas Kneuer | TUHH (Germany)
14:35	S304: Magnetic, conductive nanoparticles as building blocks for steerable micropillar-structured anodic biofilms René Wurst | TUHH (Germany)
14:55	S305: Salinity reduces the abundance and expression of genes involved in extra-cellular electron transfer in a MFC treating wastewater Antonio Castellano-Hinojosa | University of Granada (Spain)
15:15 – 16:15	Coffee break *Optional lab tour with Johannes Gescher*
16:15 – 17:15	**Session 5**
16:15	**Keynote K07:** Even nanowires need a little help sometimes Daniel R. Bond |University of Minnesota (USA)
17:00-17:15	Closing remarks Johannes Gescher | TUHH
18:00 – 23:30	**Harbour cruise and conference dinner** Harburg Harbour (see map) Joint walk from the TUHH (starting 17:15 in the foyer of bldg. C) Optional shuttle service is available. (For shuttle, please register on Monday during registration)

KEYNOTE

K01

From Microbes to Living Electronics: The Choreography of Extra- and Inter-cellular Electron Transport

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Abstract

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 and intercellular 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 electrochemistry, 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.

K02

Rational re-design of the extracellular cytochrome MtrC from *Shewanella oneidensis* MR-1

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Abstract

Recent years have seen significant advance in the opportunities for integrating abiotic functional groups with biology's natural suite of amino acids and cofactors. Examples include the genetic encoding of more than 200 non-canonical amino acids, introduction of metals that serve as novel catalytic sites, and site-selective labelling with synthetic light-harvesting materials. We have been exploring how such strategies can introduce novel functionality to multiheme cytochromes of *Shewanella oneidensis* MR-1. Our results provide fresh perspective on the fundamental properties of cytochromes involved in

extracellular electron transfer. They offer new prospects to probe, control, and re-design the function of these fascinating proteins.

K03
Unlocking the potential of electroactive microorganisms: a deep dive into the extracellular electron transfer mechanisms

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Abstract

Electroactive organisms perform extracellular electron transfer to exchange electrons with solid conductive materials, such as metal oxides in their natural environment or electrodes in bioelectrochemical systems. Given this ability, understanding the mechanisms by which these organisms perform these processes has been crucial for improving the technologies that use them for energy generation, bioremediation, biosensing, and production of added-value compounds. Over 100 electroactive organisms have been identified to date. Extracellular electron transfer can proceed either through direct contact between the cell and the extracellular electron acceptor or donor or through an indirect process where soluble redox mediators mediate electron transfer. Additionally, multiheme *c*-type cytochromes are key players in these processes, although not all electroactive organisms contain this type of protein. These proteins besides forming conductive pathways that transfer electrons from cellular metabolism to the cell surface, were also shown to be important for direct interspecies electron transfer.
In this presentation, the extracellular electron transfer pathway of several electroactive bacteria, including Gram-negative and Gram-positive bacteria, will be discussed. A specific focus will be placed on using this knowledge to optimize electroactive bacteria toward their practical application for the generation of current in microbial fuel cells, and hydrogen production from light.

K04
Extracellular electron transfer pathways in *Geobacter sulfurreducens*

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Abstract

Extracellular electron transfer processes in Gram negative bacteria require electron conduits in the outer membrane. These conduits facilitate exchange between periplasmic electron shuttles and extracellular reductases that catalyse the reduction of terminal electron acceptors in the environment surrounding the cell. The outer membrane electron transfer conduits of the model electrogen *Shewanella oneidensis* are characterized, and contain a decaheme cytochrome enclosed within a hydrophobic porin that positions the cytochrome within the outer membrane so that the termini are exposed to opposite sides of the membrane. This porin-cytochrome is heavily conserved within the *Shewanellacea* family, but functionally homologous protein complexes within other bacteria share little sequence conservation. Another electrogen, *Geobacter sulfurreducens,* contains the genes that encode for 5 different outer membrane electron conduits, but share almost no sequence similarity to the *Shewanella* conduits. In particular the conduit that is

encoded by the *extABCD* gene cluster is reported to increase current production in *G. sulfurreducens* grown on electrodes. To better understand the properties of this cluster we generated recombinant forms of the genes encoding the cytochrome components ExtA, ExtC and ExtD. Biophysical and spectroscopic analysis, coupled with structural predictions through Alphafold-2 and -3 allowed the generation of an new electron conduit model that requires only 5 hemes to pass electrons through the outer membrane, and is capped by an extracellular reductase that allows for the reduction of terminal electron acceptors that can include soluble metals, electrodes or soluble extracellular cytochromes such as the triheme PgcA.

K05
The Flavin Awakens: Electron Shuttling in EET and Beyond

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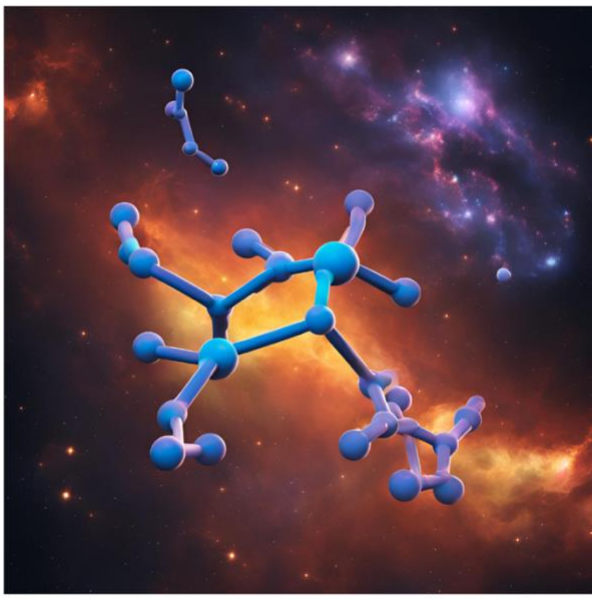
Abstract

Flavins are essential to all life on Earth and seem to be quite important for extracellular electron transfer in some bacteria. Here I will provide a historical perspective on the discovery, genetics, biochemistry and physiology related to flavins and electron shuttling, emphasizing the molecular mechanism of secretion and maturation. I will then discuss the physiological ramifications of electron shuttling and describe where flavin secretion has been quantified in bacteria capable of metal reduction. We will then consider unpublished research from my lab regarding flavins in extracellular electron transfer in *Shewanella*, *Aeromonas* and *Vibrio*. I will also describe potential roles for flavins in chemotaxis and in promoting association with zebrafish.



K06
Exploration of the evolutionary history and application potential of multiheme cytochromes *c*

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Abstract

Multiheme cytochromes *c* (MHC) are recognized key players in numerous anaerobic metabolic processes, some of which can be traced back to very ancient activities in the biogeochemical cycles of iron, sulfur and nitrogen. Some of these metabolic capabilities are nowadays being co-opted for the development of microbial electrochemical technologies that rely on the role of MHC for connecting the microbial metabolism to solid conducting surfaces. However, MHC were isolated and studied in detail only from a limited number of species. To obtain a broader view of the diversity of MHC, we employed bioinformatics tools to study the *cytochromome* encoded in the genomes of the *Desulfuromonadia* class. We found that MHC predicted to be extracellular are the least conserved and present higher diversity. Although the most prevalent MHC have

homologues already characterized, nearly half of the MHC families in the *Desulforomonadia* class have no known homologues, and this begs the questions of what do they look like and how did the MHC evolve to their current form? Up until now, the evolution of MHC was proposed to occur by gene fusion events. We combined structural and character-based phylogenetic analysis with an unbiased root placement method to refine the evolutionary relationships between these multiheme cytochromes. The evidence shows that NrfA and cytochrome c_{554} belong to different clades, which suggests that these two multiheme cytochromes are products of truncation of ancestral octaheme cytochromes related to extant ONR and MccA, respectively. From our phylogenetic analysis, the last common ancestor is predicted to be an octaheme cytochrome with nitrite reduction ability. Evolution from this octaheme framework led to the great diversity of extant multiheme cytochromes analyzed here by pruning and grafting of protein modules and hemes. This appears to have led to a scenario where MHC with up-to 10 hemes appear to be very common. However, the detailed functional characterization of such complex proteins is compounded by the fact that most spectroscopic techniques do not provide sufficient discrimination of the individual hemes. I will show that paramagnetic nmr spectroscopy has the potential to discriminate the individual redox states of the multiple hemes and in combination with stopped-flow kinetics and electrochemistry provide a detailed description of the electron transfer properties of MHC. I will also describe how these properties have been manipulated to modulate the rates of electron uptake and release of electrons, and how these properties imprint control of catalysis performed by cytochromes that perform 'chemistry' in addition to electron transfer.

K07

Even nanowires need a little help sometimes

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Abstract

Three extracellular *c*-type cytochrome filaments from *Geobacter sulfurreducens* are known. These nanowires are polymers of OmcS, OmcE, or OmcZ protein subunits, each containing a core of closely spaced hemes, but lacking similarity in sequence, fold, glycosylation, subunit size, or diameter. The conductivity of isolated nanowires wires attracts significant attention. But cell-free assays fail to explain why cells produce multiple expensive extracellular proteins if all of them can nonspecifically carry electrons away from the membrane. Further, the isolated structures do not address if nanowires need aid in assembly, surface anchoring, or obtaining electrons from the electron transport chain.

We utilized a markerless deletion approach to construct new single, double, and triple-deletion nanowire subunit mutants in an isogenic background. When soluble Fe(III) or fumarate were electron acceptors, all mutants grew similar to wild type. When Fe(III) oxide was the electron acceptor, only single mutants lacking *omcE* were strongly affected. Double mutants containing only *omcS* or *omcZ* (Δ*omcEZ* or Δ*omcES*) were unable to reduce Fe(III) oxide, while the mutant containing only *omcE* (Δ*omcSZ*) reduced Fe(III) after a short lag. The triple (Δ*omcESZ*) mutant was also unable to reduce Fe(III) oxide. In contrast, mutants lacking *omcS* were severely defective

in Mn(IV) oxide reduction. These defects could be eliminated by additions of micromolar concentrations of chelators or electron shuttles.

A series of genes for multiheme cytochromes, porin-like proteins, putative chaperones, and glycosylation-domain proteins are conserved next to most *omcE* homologs. A panel of Δ*omcSZ* mutants lacking each of these *omcE*-linked genes produced distinct phenotypes. Some, such as the porin gene mutants, failed to reduce Fe(III) oxide and failed to produce any OmcE wires, based on antibody detection. Others, such as the cytochrome mutants, failed to reduce Fe(III) but produced wild-type levels of extracellular OmcE filaments. Mutants lacking putative glycosylases failed to reduce Fe(III) oxide, yet some produced filaments comprised of lower molecular-weight OmcE.

These data show different nanowires are utilized by *G. sulfurreducens* depending on the electron acceptor, and that display of OmcE alone is not sufficient for its function. The requirement for two additional multiheme cytochromes suggests a possible interface linking the OmcE nanowire to the extracellular electron transport chain.

ORAL PRESENTATION

Session 1: Fundamentals of Extracellular Electron Transfer and Native Exoelectrogens

S101

Investigating the Mechanism of Electrode Reduction in *Vibrio natriegens*

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Abstract

Vibrio natriegens is an exoelectrogen with exciting biotechnological potential due to its rapid growth, easy genetic modification, and ubiquity in diverse marine environments. However, much is not yet understood about its extracellular electron transfer (EET) mechanism, particularly concerning electron transfer to electrodes. Electrode reduction by *V. natriegens* can be hypothesized to function similarly to electrode reduction by *Shewanella oneidensis* or *Aeromonas hydrophila*, given that *V. natriegens* possesses genes homologous to the multi-heme cytochrome-based direct electron transfer pathways of those exoelectrogens [1]. Recently, these genes, homologs of *cymA*, *mtrA*, *mtrB*, and *mtrC* of *S. oneidensis* and *pdsA* of *A. hydrophila*, were shown to be required for iron reduction [1]. However, their role in electrode reduction has yet to be tested.

To investigate the mechanism of electrode reduction, we generated single knock-out and complementation mutants for each of *cymA*, *pdsA*, *mtrA*, *mtrB*, and *mtrC*. We found that the loss of these genes dramatically reduced current production in bioelectrochemical systems, demonstrating that these genes are involved in EET to electrodes. Furthermore, we used cyclic voltammetry to investigate the molecular species involved in *V. natriegens* EET. Although the full EET pathway was required to reduce the insoluble electrode, we were curious whether all components of the pathway would be necessary to reduce soluble electron acceptors. By quantifying the iron reduction ability of our single gene knock-out strains when supplied with iron (III) oxide nanoparticles versus iron (III) citrate as electron acceptors, we learned that MtrC is not required to support the reduction of iron (III) citrate.

This study of the mechanism of electrode reduction by *V. natriegens* advances fundamental understanding of EET in *V. natriegens*. We show that *cymA*, *pdsA*, and *mtrCAB* are required for electrode reduction, as they are for iron reduction. By examining single gene deletions within the MtrCAB complex, we were able to elucidate the conditional importance of MtrC in *V. natriegens* EET. By demonstrating the relationship between EET pathway gene expression level and current production, our results will accelerate the application of *V. natriegens* for microbial bioelectronic sensing in diverse marine environments.

[1] Conley, B. E., *et al*. (2020). *Appl. Environ. Microbiol.*, *86*(19). doi:10.1128/AEM.01253-20.

S102

Extracellular electron transfer in a new Gram-positive Microorganism, isolated around cable bacteria

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Abstract

Microbacteriaceae are a class of gram-positive, soil microbes present commonly in cable bacteria enrichments. We report extracellular electron transfer (EET) in the genus *Microbacterium*, which was isolated from close proximity to a cable bacteria filament. Electrochemical experiments with pure *Microbacterium* cultures in three-electrode cells revealed the presence of soluble mediators diffusing through the cell wall, confirmed by HPLC to be riboflavin. Genomic analysis revealed the presence of the complete riboflavin synthetic pathway, with enhanced flavin production under oxygen-limiting conditions promoting EET. Interestingly, *Microbacterium* was able to grow and perform EET in pH 9. The presence of cable bacteria in sediments create pH gradients, which may induce unique selection pressures. Our experiments indicate that Microbacterium can potentially use the cable bacterial filament as an ´electrode´ to perform interspecies electron transfer. These findings may potentially be relevant to understand metabolic changes in oxic-anoxic interfaces and understand biogeochemical cycling in sediments.

S103

Chirality Induced Spin-Dependent Electron Transport through Bacterial Cell Surface Multiheme Electron Conduits

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Abstract

Extracellular electron transport (EET) is a critical metabolic strategy for bacteria to access insoluble electron acceptors or donors in anoxic environments, primarily mediated through multiheme cytochromes (MHCs) spanning cellular membranes and forming nanowires. In *S. oneidensis* MR-1, it has been previously shown that the electron transport through various MHCs, including the periplasmic tetraheme STC, decaheme MtrA, and cell surface decaheme OmcA, exhibits the chirality-induced spin selectivity (CISS) effect. The CISS phenomenon enhances the electron transfer of a preferred electron spin orientation over the other by coupling electron velocity to spin, based on the chirality and length of the MHCs.

However, despite the critical role of MtrC in *S. oneidensis*, the CISS effect in this MHC had not been previously investigated. MtrC is a cell surface bound, decaheme (branched heme chain arrangement), highly helical MHC, and is the primary terminal EET conduit that allows *S. oneidensis* to respire solid-state electron acceptors including ferric oxides and electrodes. Here we present preliminary studies of charge transport and spin filtering through MtrC. For studying CISS in MtrC, conductive probe atomic force microscopy measurements (CP-AFM) are pursued to quantify the conductivity of MtrC molecules as a function of the magnetization of underlying ferromagnetic substrates. These results, and comparison to spin polarization measurements in a range of MHCs, reveal fundamental insight into how molecular properties (helicity, optical activity, size, and heme chain arrangement) impact both electron transport and spin filtering through the CISS effect.

Moreover, CISS has not been directly investigated in the intact micron-scale cytochrome nanowires of *Geobacter*. Our current research aims to fill this knowledge gap, and shed light on both electron and spin

transport along micron length scales by isolating cytochrome nanowires and measuring CISS as a function of length using CP-AFM. These studies explore the relationship between spin selectivity and conduction channel length, providing an opportunity to understand spin filtering at inter-cytochrome transfer steps and its role in enhancing electron transfer efficiency and extracellular respiration.

Keywords: Spintronics, Extracellular electron transport, Electroactive microorganisms, Outer membrane cytochrome, Atomic Force Microscopy, *Geobacter* nanowires.

S104
Electron transfer-coupled iron (Fe) reduction by oral pathogen *Porphyromonas gingivalis*

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Abstract

Porphyromonas gingivalis (PG) is an asaccharolytic bacterial pathogen associated with the chronic, debilitating oral disease of periodontitis and other inflammatory disorders such as Alzheimer's. Iron is an essential transition metal crucial for the survival of human and microbial cells, and exists in an insoluble, oxidised state (Fe^{3+}) or a soluble, reduced state (Fe^{2+}). The latter, however, is highly reactive and can cause cell damage by generating reactive oxygen species (ROS) through Fenton reactions. To avoid cytotoxicity, cells must therefore tightly regulate their iron acquisition and storage. PG pathogenesis has been linked with its versatile iron acquisition capabilities, but its iron regulation and export mechanisms are not fully understood.

Metal reduction is well studied in environmental bacteria *Shewanella* and *Geobacter*, and iron has been shown to aid biofilm metabolism for the gut pathogen *Enterococcus faecalis* through extracellular electron transfer (EET)-associated iron reduction. Our studies have demonstrated that PG also exhibits EET capability. If PG can couple EET with iron reduction/export, to maintain intracellular iron homeostasis, the resultant extracellular Fe^{2+} may drive the inflammatory diseases associated with this pathogen.
Electrochemical analyses combined with ferrozine assay have demonstrated that the presence of iron as hemin/Fe^{3+} can enhance the growth of PG and lead to localised ROS generation, supporting a potential mechanism of mammalian cell cytotoxicity. It is anticipated that deciphering the EET capability of PG could ultimately be applied to the development of novel approaches to combat PG- and other bacteria-associated inflammatory diseases.

Keywords: Extracellular electron transfer, *P. gingivalis*, Iron reduction, Inflammation, Pathogenicity, Drug discovery.

S105
Discovery of a minimal Extracellular Electron Transfer pathway in *Escherichia coli*

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Abstract

Escherichia coli is conventionally classified as a facultative anaerobe that respires on

electron acceptors that are soluble and intracellular. Intriguingly, *E. coli* is known to reduce insoluble and extracellular electrodes when it incurs anaerobic reductive stress. This gain of the extracellular electron transfer (EET) phenotype has been linked to the biosynthesis and secretion of a naphthoquinone (NQ) redox mediator. Astonishingly, the NQ-mediated respiratory-like function in *E. coli* has remained unexplored for decades. In this work, we have investigated the NQ-mediated EET in *E. coli* to unravel its molecular mechanism and implications on cellular energetics.

Since several cellular enzymes could potentially reduce redox mediators, identifying the mechanism of the mediated EET pathway has been seen as an intractable problem. To address this challenge, we leveraged high-fidelity multiplexed genome editing to target anaerobic respiration, fermentation, redox homeostasis, and xenobiotic metabolism. With this library of strains, we probed the roles of cellular electron carriers, i.e., NAD(P)H, quinones, and biothiols, in NQ-mediated EET using chronoamperometry. These experiments revealed that cytoplasmic reductases selectively reduce NQ. We further show that NQ-mediated EET drives catabolism and growth in *E. coli*. The discovered EET pathway is a minimal and generalizable mode of respiration that has never been described before. Notably, this study provides the only blueprint to investigate the mechanism of mediated EET in any bacteria with any redox mediator *in vivo*.

S106

Centimeter-long electron conduction in cable bacteria

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Abstract

Cable bacteria are centimeter-long, electrically conductive filamentous bacteria. Using two single-strain enrichments of genera Electrothrix and Electronema we systematically investigated variations and similarities in morphology and electrical properties across these genera. Electrical conductivity of different cable bacteria

samples showed a range of values covering three orders of magnitude indicating trade-offs with other adaptations. With different kinds of electron microscopy and elemental analyses, we showed that the two cable bacteria genera had a similar cell envelope architecture, cell-cell junctions, and elemental composition of the periplasmic conductive fibers. Our data indicates that these fibers are organized as stranded rope-like structures with strand components of 3 nm in diameter. We showed co-localization of nickel, sulfur, and iron with the periplasm of cable bacteria in both genera, indicating key functional roles for these elements. Put together, our study provided supporting evidence that electrically conductive fibers of different cable bacteria have a similar composition, but somehow can display tunable conductivity.

S107

Unraveling the structural basis of an electric metabolism: a tomographic journey into cable bacteria

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Abstract

Cable bacteria are multicellular filaments of the deltaproteobacterial family *Desulfobulbaceae*, composed of thousands of end-to-end cells, and are found worldwide in both marine and freshwater sediments. Remarkably, these organisms accomplish centimeter-scale electron transport through the entire filaments, allowing the cells to gain energy by coupling sulfide oxidation in the deeper sediment layer to the reduction of oxygen near the sediment-water interface. Recent studies suggest that electron transport in cable bacteria proceeds along a network of parallel longitudinal periplasmic protein nanofibers that run along the entire length of each cable bacteria filament. However, the structure of the electron transport network, its biomolecular identity, and that of the external cable conduits that allow electron exchange with electrodes, remain enigmatic.
To overcome the knowledge gap between structure and function in the conductive network of cable bacteria, a combination of cryo-electron and soft X-ray tomographic tools is being used to study the structural basis of the nanofiber network and to image the elemental composition of the (metallo)proteins involved. These tomographic techniques provide new insights into the overall organization of cell components and cell division in cable bacteria. Combined with biochemical characterization of the proteins, these integrative structural biology studies promise to reveal the structure-function link underlying conductivity in cable bacteria. The results provide new insights into how biology exploits the principles of physics to achieve an 'electric metabolism', enabling the understanding and development of new renewable energy technologies and bioelectronics.

S108

Cell surface appendages of cable bacteria

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Abstract

Cable bacteria form multicellular filaments in which cells have divided roles in electron transport chain. Majority of cells profit from sulfide oxidation in the anoxic sediment and only a minor portion reaches to aerobic surface where they reduce oxygen. The split of this energy-supplying reactions is bridged with conductive periplasmic nanowires interconnecting all cells in a filament up to a centimeter-long distance. Except for internal transport, cable bacteria are involved in extracellular electron transport attracting flocks of diverse bacteria by either secretion of reducible metabolites or working as an electron sink. Secretion capacity of cable bacteria is documented by polysaccharide trails left after gliding filaments. Indeed, their genomes contain several polysaccharide exporters and a full set of type IV pili gliding motility apparatus intertwined with type II secretion pathway.
We focused on cell surface characterization of cable bacteria and combined two electron microscopy methods to examine the gliding apparatus. Cable bacteria produce pili-like appendages which are very fragile and sensitive to shearing. They can be surrounded by a thick mesh of extracellular polysaccharides (EPS) as was detected on freshwater species but not very prominent on marine ones. Distribution of pili on cell surface resembles that of filamentous cyanobacterium *Nostoc* sp. Our negative contrast TEM images documented pili of approximately 3 nm in diameter along the whole cell surface while cryo-electron tomography provided clear 6 nm thick pili occurrence in vicinity of gap junctions and chemosensory arrays. Thus, it needs to be tested whether both types of extracellular appendages are variants of type IV pili or the 3 nm filaments are of different origin with a potential to be involved in extracellular electron transfer. Absence of genetic tools for transformation of cable bacteria hampers direct confirmation of pili subunits leaving only a possibility of specific antibody detection or non-direct fluorescence labelling. Abundance of major pili subunits in proteomic analysis of cable bacteria is of top ten among 800 identified proteins which is in accordance with other bacteria actively producing extracellular type IV pili. The presented data rule out type IV pili as a candidate for periplasmic conductive nanowires, instead, subunits of pili machinery may be involved in secretion and uptake of electron acceptors in the extracellular space.

Session 2: Mediated/ Synthetic Extracellular Electron Transfer

S201

Heterologous phenazine production in biotech hosts for bioelectrochemical applications

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Abstract

Mediated extracellular electron transfer (MET) offers to improve the efficiency of bioelectrochemical applications by allowing the use of the entire reactor volume via the diffusion of soluble redox mediators that act as electron shuttles. Phenazines are redox mediators that have gained attention for their potential in improving bioelectrochemical systems (BES) performance. *Pseudomonas aeruginosa* natively produces four phenazines: pyocyanin (PYO), phenazine-1-carboxylic acid (PCA), phenazine-1-carboxamide (PCN), and 1-hydroxy-phenazine (1-HP) and has been successfully utilized as an exoelectrogen in BES. However, upscaling bioelectrochemical production with *P. aeruginosa* is problematic due to its pathogenicity. As an alternative, we previously engineered non-pathogenic

P. putida for PCA and PYO phenazine production. However, this bacterium is very depended on oxygen availability for relevant metabolic activity. Because of this, we seek to express the PCA biosynthetic pathway from *P. aeruginosa* PA14 in a more biotechnologically flexible host, such as *Escherichia coli.* Specifically, we expressed the more active of two PCA-yielding operons from *P. aeruginosa*, *phz2* (genes *phzA2-G2*), as it has been shown to lead to the production of higher amounts of PCA than the other PCA operon (*phzA1-G1*) in heterologous expression in *Pseudomonas putida*. Additionally, we co-expressed *phzH*, the gene from *P. aeruginosa* responsible for synthesis of the derivative PCN. We want to compare the effects of PCN's slightly more electropositive formal potential than that of PCA on BES performance. Here, we evaluated the general growth performance of these strains with the non-fermentable substrate glycerol with varied levels of induction. Additionally, we investigated the bioelectrochemical activities of these new electroactive *E. coli* strains under passive and active aeration conditions. Finally, we will discuss the steps necessary for integrating this new trait with electrobiosynthesis.

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S202
Novel EET routes in *Cupriavidus necator*

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Abstract

Cupriavidus necator H16 (formerly *Ralstonia eutropha* H16) is an H_2-oxidizing chemolithoautotrophic bacterium able to produce chemicals and biopolymers from CO_2, H_2 and O_2. The protons and electrons derived from the H_2 oxidation serve to regenerate ATP and NADH, which drive the metabolism including the Calvin Benson Bassam cycle (CBB) for the assimilation of CO_2 into biomass and value-added chemicals.
In the context of a circular carbon economy, *C. necator* is gaining increasing interest in biotechnology. However, to make the H_2-driven metabolism of this bacterium attractive for industrial applications, the co-presence of H_2 and O_2 should be avoided, as dangerous mixtures of these two gases limit the applicability for safety reasons [1].
The ultimate goal of this project is to use an electrode to replace O_2 as the terminal electron acceptor. One advantage of using such an electrode is that both anodic respiration, as well as electrosynthesis can be established.
To achieve cathode-driven electrosynthesis, a membrane-bound formate dehydrogenase was overexpressed as soluble protein in the periplasm of *C. necator* to enable direct interaction with artificial redox mediators or native cytochromes. To improve the direct electronic contact between oxidoreductases and cytochromes, a post-translational coupling strategy in the cellular periplasm was also investigated using SpyCatcher/SpyTag technology. As proof of concept, the membrane-bound hydrogenase of *C. necator* was made soluble by removing the membrane-integral C-terminal anchor. The resulting variant was then fused to SpyTag and coupled *in vivo* with a cytochrome fused to SpyCatcher. The SpyTag-carrying hydrogenase remained catalytically active and showed an improved interaction with artificial redox mediators compared to the native membrane-bound hydrogenase. In fact, the strain overproducing the tagged hydrogenase was shown to produce much more current than the wild-type strain when tested in an electrochemical cell with H_2 as sole electron donor and ferricyanide as redox mediator.
Thus, the successful detachment of the hydrogenase from the membrane and the coupling with a cytochrome is an important

step towards the development of a novel (external) electron transfer pathway in *C. necator*.

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S203
Mediated extracellular electron transfer with *Cupriavidus necator*

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Abstract

Cupriavidus necator, despite lacking direct electron transfer capabilities, demonstrates efficient reduction of various redox mediators in oxygen-free cultivation within bioelectrochemical systems. The so far reached current densities of up to 260 µA cm^{-2} [1] may enable the substitution of O_2 in "Knallgas" fermentation on H_2 and CO_2 as sustainable feedstocks. Here, we investigated the reduction mechanics of ferricyanide, a proven viable redox mediator for anodic extracellular electron transfer with *C. necator* and other microorganisms [1–3]. Therefore, inhibition and expression rate analysis of oxygen and nitrate respiration chain complexes was performed, comparing aerobic cultivation conditions with fructose as carbon and electron donor to autotrophic (CO_2/H_2/O_2) and anodic cultivation conditions (fructose/anode). Azide inhibition identified cytochrome c oxidase as the primary complex facilitating electron transfer to ferricyanide, with a secondary role proposed for nitrite reductase (NirS), demonstrating a 3.9±1.1-fold higher expression when exposed to anodic conditions. The 2.9±0.6-fold increase in the expression of the natural porin OmpA under anodic conditions implies its potential involvement in ferricyanide uptake.

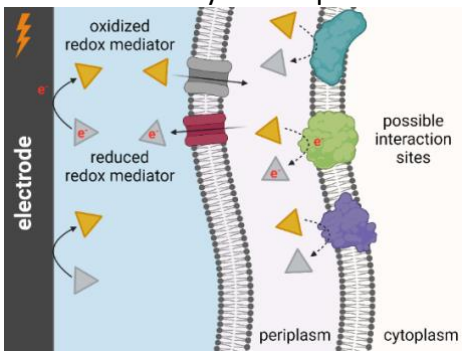

Figure 1: Mediated extracellular electron transfer from an unknown interaction site in the periplasm of *C. necator to* the anode.

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Session 3: Applied Bioelectrochemical Systems

S301
Upscaling endeavors of bioelectrochemical systems

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Abstract

The upscaling of bioelectrochemical systems (BES) presents a spectrum of opportunities and challenges that are critical to their

successful deployment in real-world applications. On the opportunity side, upscaling BESs holds the potential to revolutionize fields such as renewable energy generation, wastewater treatment, and bioremediation, contributing to a more sustainable and circular economy. BESs can harness microbial processes to convert waste into valuable resources like electricity, biofuels, platform chemicals and clean water, offering a promising alternative to traditional methods. However, these endeavors face significant challenges, including the need for cost-effective materials and components, maintaining system stability and efficiency at larger scales, and addressing the variability of environmental conditions. Key strategies include optimizing the design and materials of electrodes, membranes and reactors, integrating advanced microbial communities, and improving system configurations to boost performance and durability. The better understanding of anodic/cathodic biofilms development and robustness represents a key guidance to more reactor designs which play a crucial role among such endeavors. Still, overcoming these challenges demands interdisciplinary collaboration, innovative engineering solutions, and continuous research to optimize performance and scalability. Addressing these issues will be key to unlocking the full potential of BES technologies on a commercial scale.

S302

Diving into the electrophysiology of *Clostridium ljungdahlii* - from understanding to engineering

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Abstract

In Microbial Electrosynthesis (MES), microbial biosynthetic reactions are driven by electron uptake from a cathode. In the best case, electrical energy is used by the microbes to fix carbon dioxide and produce, for instance, organic acids or alcohols. Thus, MES present a promising power-to-x technology platform for a circular bioeconomy driven by renewable electricity input and the quest for carbon dioxide reuse.
A model organism for MES is the autotrophic bacterium *Clostridium ljungdahlii*. However, compared to the closely related process of gas fermentation with hydrogen gas and carbon dioxide as the feed sources for acetate and ethanol production, the performance regarding rates of growth and acetate production in MES are very low.
Here, we present a systematic evaluation of potential stress factors in MES by a physiological comparison of the performance of *C. ljungdahlii* in MES versus gas fermentation. Indeed, our proteomics and transcriptomics analysis revealed a down regulation of many growth-related metabolic pathways and a strong upregulation of stress related reactions during MES. Electron

microscopy evaluation depicted highly damaged cell structures for MES grown bacteria. We predicted and evaluated three potential reasons for this stress in MES: 1) The cross-over of oxygen from the anode reaction, which is toxic for *C. ljungdahlii*; We show that an active oxygen removal from the anodic compartment increases MES performance. 2) A limitation in hydrogen availability and thus metabolic energy input from the cathode; In fact, increased cathode surface sizes directly correlated with increased hydrogen formation and increased metabolic activity. And 3) A general stress to the cells in the presence of an electrochemical potential; We anticipate that reasons 1 and 2 cannot be responsible for the drastic phenotypic appearance of the cells and are systematically investigating redox stress in our system. By tackling challenges 1-3, we derive information which directly feed into the design of new electrobioreactors, which are tailored to the needs of the microbial electrocatalysts and warrant a scalable electrosynthesis performance.

S303

A covalent crosslinking approach to enlarge electroactive *Shewanella oneidensis* biofilms

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Abstract

Geobacter sulfurreducens and *S. oneidensis* are the two model organisms for microbial extracellular electron transfer (MEET), each with its own unique advantages and disadvantages. While *G. sulfurreducens* produces superior biofilms, resulting in high current densities in bioelectrochemical systems, its oxygen sensitivity impedes cultivation and genetic engineering. In contrast, *S. oneidensis* forms only very thin electroactive biofilms potentially due to the lack of conductive pili and thus produces comparably low current densities. However, its ability to thrive on oxygen led to the development of a versatile genetic toolbox and a detailed understanding of the electron transport chain. Hence, multiple genetic and technical engineering approaches were conducted to increase the power output of *S. oneidensis* in bioelectrochemical systems. Still, the benchmark set by *G. sulfurreducens*, has yet to be matched[1].

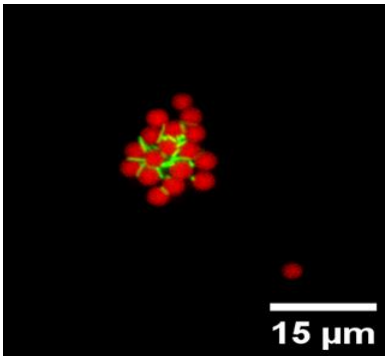

Figure 2: Fluorescence micro-scopy of *S. oneidensis ΔmtrC* pBAD_*mtrC-spytag* cells (green) encapsulated with SpyCatcher functionalized magnetic beads (red).

In this study, the surface exposed *c*-type cytochrome MtrC was genetically modified with the ultimate goal to simultaneously enable direct intraspecies electron transfer and covalent cell coupling to enlarge the electroactive *S. oneidensis* biofilms.
Therefore, the crosslinker pair *spytag* and *spycatcher*, which spontaneously form covalent bonds when they face each other, was integrated into almost every position of *mtrC* using a transposon mediated *in vitro* assembly. A high-throughput screen was then conducted to identify appropriate integration positions that consider protein functionality and crosslinker accessibility. Oxford nanopore sequencing revealed the three most suitable integration positions which were then selected for further analysis. Anoxic growth experiments with ferric citrate as the sole electron acceptor confirmed that all selected MtrC-crosslinker constructs remained functional. Moreover, it was demonstrated that all cells expressing MtrC-crosslinker

constructs resulted in a significantly higher number of cells adhering to counterpart-functionalized magnetic beads compared to cells expressing native MtrC.
The next step will be to use a microfluidic bioelectrochemical platform to evaluate the thickness, conductivity and productivity of the synthetic *Shewanella oneidensis* biofilms.

[1] Klein, E.M., Knoll, M.T. & Gescher, J. (2023) Microbe–Anode Interactions: Comparing the impact of genetic and material engineering approaches to improve the performance of microbial electrochemical systems (MES). *Microbial Biotechnology*, 16, 1179–1202. Available from: https://doi.org/10.1111/1751-7915.14236

S304
Magnetic, conductive nanoparticles as building blocks for steerable micropillar-structured anodic biofilms

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Abstract

In bioelectrochemical systems (BES), biofilm formation and architecture are of paramount importance, particularly in flow-through applications. The interface between electroactive microorganisms and the electrode surface plays a pivotal role, as the available surface area influences current generation, particularly for microorganisms that form thin anodic biofilms. To circumvent the constraints imposed by the limited surface area of the electrode, nanoparticles (NPs) comprising a magnetic iron core and a conductive, hydrophobic carbon shell were employed as building blocks to form conductive, magnetic micropillars on the anode surface. The formation of this dynamic three-dimensional electrode architecture was monitored and quantified *in situ* using optical coherence tomography (OCT) in conjunction with microfluidic BES systems. Cyclic voltammetry demonstrated that the assembled three-dimensional anode extensions were electrically conductive and increased the available electroactive surface area. The NPs were employed as steerable carriers for the electroactive model organisms *Shewanella oneidensis* and *Geobacter sulfurreducens*, resulting in a 5-fold increase in steady-state current density for *S. oneidensis*, which could be 22-fold increased when combined with poly(3,4-ethylene dioxy-thiophene)-poly(styrenesulfonate) (PEDOT:PSS) aggregates. In the case of *G. sulfurreducens*, the steady-state current density was not increased, but was achieved four times faster. The study presents a controllable, scalable, and easy-to-use method to increase the electrode surface area in existing BES by applying a magnetic field and adding conductive magnetic NPs. These findings can most likely also be transferred to other electroactive microorganisms.

S305
Salinity reduces the abundance and expression of genes involved in extra-cellular electron transfer in a MFC treating wastewater

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Abstract

Microbial fuel cell (MFC) system has received increased attention as a suitable treatment technology to mitigate the environmental impact of wastewater while producing electricity. However, few studies have examined the effect of salinity on current production and electroactive microorganisms using real saline effluents. We used a continuous-flow MFC system to treat real urban wastewater. Three salinity levels (low, LW; medium, ME; and high, HG) were tested and adjusted by adding NaCl at 3.5, 7, and 15 g/L, respectively, to the influent wastewater. A no-treatment was used as a control. The anode was inoculated with activated sludge taken from an urban wastewater treatment plant. Each treatment was tested for a month. Biomass samples from the anode biofilm were taken every 10 days and used for metagenomic and metatranscriptomic analyses. Our results showed that treatment with LW and CT produced significantly greater current production and organic matter removal % compared to ME and HG. Salt addition altered the taxonomical and functional profiles of the anode microbiome compared to CT. These variations were greater for the taxonomic than functional profiles based on metagenomic and metatranscriptomic data. Salt addition produced changes in the abundance and expression of pathways involved in carbon fixation, central metabolism, methane metabolism, fermentation, nitrogen metabolism, secretion systems, sulfur metabolism, phosphorus metabolism, and oxidative phosphorylation. For example, the abundance and expression of genes involved in the methanogenic and denitrifying pathways and fermentation were reduced with increased salinity. In addition, increased salinity reduced the abundance and expression of genes involved in extra-cellular electron transfer (EET). We identified 17 MAGs (Metagenome Assembled Genomes) involved in EET which mainly belonged to the Ignavibacteriaceae family. Treatment with LW and CT increased the abundance and activity of MAGs containing c-type cytochromes compared to ME and HG. This study shows that salinity determines variations in the anode microbiome of an MFC treating real wastewater with critical impacts on current production and treatment performance.

POSTER

P01
Towards Pairing Solar Chemical Production with Central Metabolism of *S. oneidensis* MR-1

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Abstract

Shewanella oneidensis MR-1 is capable of extracellular electron transfer (EET) as an alternative method of enabling continued central metabolic function in the absence of oxygen. A key component of this EET mechanism is the extracellular decahaem protein MtrC, bound to the outer membrane by lipid anchor. MtrC receives electrons from intracellular central metabolism via a redox active outer membrane spanning protein and channels those electrons to metal oxides in the surrounding soil.[1] Functionalization of the MtrC cytochrome wire with a blue-light sensitive ruthenium dye could enable the released electrons to be energised to power valorising reductive chemistry, for example proton or CO_2 reduction.[2] Site-directed mutagenesis of MtrC enables cysteine implantation for subsequent covalent bonding to Ru(4-bromomethyl-4'-methylbipyridine)(2,2'-bipyridine)$_2$ (RuMeBr).[3] Two sites on the surface of MtrC (S634 and Y657) have been chosen for replacement by Cys because this should result in minimal disruption of MtrC structure and a short distance (<10 Å) between the subsequently bound Ru-dye and outermost haem (haem 10) of the molecular wire. The S634C and Y657C MtrC proteins have been purified and photosensitised by reaction with RuMeBr. Rates of charge separation between the Ru-dye and MtrC haem wire have been determined using Time-Resolved Photo-luminescence (TRPL), as a steppingstone towards demonstrating advantageous photochemistry to bacterial internal metabolism.

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P02
Electrical control of gene expression in *Shewanella oneidensis* MR-1 by Arc-regulated promoters

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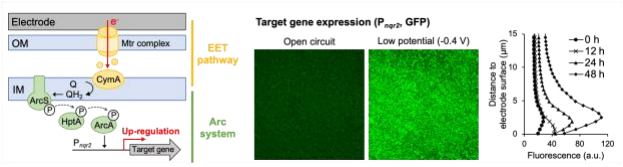
Abstract

Electrochemically active bacteria (EAB) interconnect biological and electrical modules by extracellular electron transfer (EET), leading to their applications in biotechnology such as wastewater treatment, biosensors, and toolkits for synthetic biology. Rational

engineering of EAB is crucial for improving their performance, however, there are limited genetic tools available. We have shown that the model EAB, *Shewanella oneidensis* MR-1, senses electrode potential via EET pathway and Arc regulatory system to alter gene expression [Hirose *et al*., 2018, Nat. Commun., 9: 1083]. Based on this, we have proposed the concept of electrogenetics as a novel methodology for directly controlling gene expression in electrode-associated living cells. Here we searched for compatible transcriptional promoters that respond to potential shifts for the practical use of electrogenetics.

Because the target DNA sequences of Arc systems in MR-1 and *Escherichia coli* are highly conserved, we explored Arc-dependent promoters in the genomes of these bacteria to identify electrode potential-responsive promoters differentially activated in MR-1 exposed to low- and high- potential electrodes (-0.4/+0.7 V vs. SHE). In LacZ reporter assays, we found that the activities of promoters located upstream of MR-1 *nqrA2* (P_{nqr2}) and *E. coli feoA* were increased under low and high potential conditions, respectively. These responses were lost in Δ*arcA* mutant. In addition, both promoter regions bound to the phosphorylated ArcA protein in gel shift assay, suggesting that these promoters were directly regulated by the Arc system in MR-1. The spatiotemporal response of the identified promoters to electrode potential was analyzed by inoculating a reporter strain expressing GFP from P_{nqr2} into an electrochemical system equipped with a confocal laser scanning microscope. The induction of GFP expression from P_{nqr2} under the low potential condition first occurred in cells localized near the working electrode surface and was sustained for 48 h. In summary, electrode-attached biofilms of MR-1 sense extracellular redox state by a combination of EET and Arc system, and this system was successfully applied to electrogenetics.



P03

Activation of electrochemically active biofilms by c-di-GMP signaling in *Shewanella oneidensis* MR-1

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Abstract

Microbial electrochemical technology (MET) has attracted attention as a sustainable biotechnology. In MET, biofilms formed by electrochemically active bacteria (EAB) on electrodes (electrochemically active biofilms; EABF) facilitate electron transfer between microbial cells and electrodes, contributing to the enhancement of target bioelectrochemical reactions. We have previously reported that a diguanylate cyclase, DgcS (SO_1646), plays an important role in EABF formation by a model EAB, *Shewanella oneidensis* MR-1 (Matsumoto et al., 2021, AEM 87:e00201-21). In the present study, we constructed a strain overexpressing *dgcS* (named *dgcS*-OE) and showed that increased intracellular c-di-GMP levels in *dgcS*-OE led to enhanced EABF formation and current generation in electrochemical flow cells (EFCs) (Fig. 1). Transcriptome analysis revealed that overexpression of *dgcS* resulted in the upregulation of genes associated with type IV pili synthesis (e.g., *mshA* and *mshB*) and extracellular electron transfer (e.g., *mtrA* and *omcA*) (Table 1). These results suggest that c-di-GMP-associated signaling cooperatively activates the expression of

genes involved in attachment and electron transfer to electrodes.

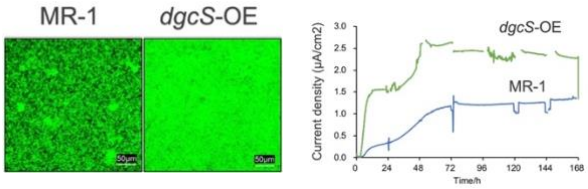

Fig. 1. CLSM images of electrode-associated biofilms (A) and current generation (B) in EFCs inoculated with wild-type MR-1 and *dgcS*-OE

Table 1. List of biofilm and EET genes upregulated in *dgcS*-OE

Gene name	Description	log2 FC
dgcS	diguanylate cyclase	5.45
mshA	MSHA major pilin subunit MshA	2.69
mshB	MSHA minor pilin protein MshB	2.42
mtrA	extracelllular iron oxide respiratory system periplasmic decaheme cytochrome c component MtrA	2.27
omcA	extracelllular iron oxide respiratory system surface decaheme cytochrome c component OmcA	3.32

P04

ALE of *C. necator* H16 to improved process conditions for electroactivity: electrolytes

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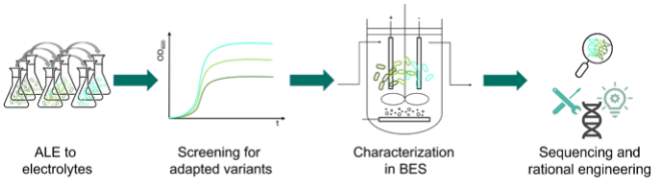
Abstract

Adaptive Laboratory Evolution

A rising earth population having a comfortable industrial lifestyle is causing climate change, environmental pollution and accordingly created a huge need for renewable, sustainable and environment friendly resources. Involvement of microorganisms in bio-electrochemical processes show their importance in the context of sustainable energy resources but also mark the vulnerability of these systems. Bacterial growth works best under specific conditions, which don't always match the ideal process-parameters. Growth inhibition by high concentration of electrolytes or changing pH cause a huge stress for bacteria. Genetic modification is one way to engage these problems, however it cannot be used on every strain due to a lack of knowledge about genetic fundamentals. A more natural way of adaptation to ideal process parameters, is adaptive laboratory evolution – ALE, which can result in more stable naturally modified strains.

Salt adaptation in order to enhance electron transfer

One basic need for electron transfer in liquid medium is the presence of electrolytes. For microorganisms on the other hand an elevated osmotic pressure, as it comes along with the needed amount of electrolytes, can lead to major growth inhibition. For the compensation of osmotic pressure multiple intracellular systems are known e.g., accumulation of trehalose, glutamate and glycine betaine, therefor there isn't one simple target for genetic engineering. ALE however offers the possibility to modify the whole cell catalyst with all involved systems. To this end *C. necator* strains H16 and H16ΔPHB were adapted in shake flasks to rising concentrations of NaCl and Na_2SO_4 (100 – 200 mM) over a period of 30 days.



P05

Desulfuromonas acetoxidans: multifunctional marine bacterium conquering water desalination and electricity production

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Abstract

According to the United Nations, freshwater scarcity is a pressing issue that affects nearly 40% of the world's population. Therefore, desalinating the abundant salty water available on the planet is crucial for addressing the global scarcity of safe drinking water, as well as meeting irrigation and industrial demands. The desalination methods available to date are thermal desalination and membrane-based technologies. These methods, besides requiring a great amount of energy, either to heat the water or to pump it through reverse osmosis, also create an environmental issue due to brine discharging, costliness, and potential harm to marine ecosystems.
Bioelectrochemical systems (BES) can tackle both these issues, by harnessing the metabolism of microorganisms capable of utilizing solid electron acceptors or donors and using them for energy production in microbial fuel cells (MFC), chemical synthesis in microbial electrosynthesis (MES), and water desalination in microbial desalination cells (MDC). The interest in MDCs has increased in the last decade, given the ability to desalinate water while producing electricity from renewable and carbon-neutral materials under ambient temperature and pressure.
Although many microorganisms have been successfully applied in BES, most of them are not suited to operate under the conditions required for MDC, since they do not tolerate high salt concentrations. Therefore, marine microorganisms are much better suited for desalination purposes. In experiments where the anode was inoculated with marine wastewater, the marine anaerobic bacterium Desulfuromonas acetoxidans thrives, making this an interesting candidate for successfully running MDCs. The main goal of this study is to elucidate the best condition to grow D. acetoxidans on electrodes to use them in MDCs, showing that this is a good target for larger-scale uses. Our data demonstrated that this bacterium can grow on MFC at a salt percentage corresponding to nearly 1M, which makes it a moderate halophilic organism. Other conditions, such as different applied potentials and stirring, were also tested to obtain the highest and most stable current production, with the goal of using this bacterium for water desalination coupled with electricity generation from wastewater treatment.

P06

Microbial electrochemical fluidized bed reactors for bioconversion of CO_2: a sustainable approach for bioelectrosynthesis.

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Abstract

BIOCO$_2$MET is an ambitious project that seeks to revolutionize the use of carbon dioxide (CO_2) by developing a Microbial Electrosynthesis Fluidized Bed Reactor (ME-FBR) for the bioconversion of CO_2 into valuable chemicals. Its objectives include the design and operation of 5L pilot scale reactors operated for the bioelectrosynthesis of value-added products such as volatile fatty acids (VFA), alcohols and biotechnological hydrogen by using different electroconductive materials as fluidized bed (activated carbon, glassy carbon, and biochar) as well as under a wide range of potentials (from −0.3 V to −1.0 V vs. Ag/AgCl). In addition, the project aims to explore the use of redox mediators as energetic vectors for the decoupling of energy accumulation reactions (obtained from oxidation of organic matter) to be used for CO_2 fixation, though the design and development a scalable laboratory system for wastewater treatment and accumulation of redox compounds. The ME-FBR also includes a video surveillance system and an electric signal analysis in order to monitor continuously the bioreactor performance and bacterial activity. The ad-hoc video surveillance system allows the development of algorithms to extract a set of descriptors related to the characterization of the fluidization regime, pressure drop in bed, the liquid circulation velocity, and the distribution of particles inside the reactors. Finally, a life cycle assessment analysis will allow the evaluation of environmental impact associated with products or activities associated with the project.

Acknowledgement:

BIOCO$_2$MET Project has receiver financial support from Grant TED2021-132870B-I00 funded by MCIN/AEI/10.13039/501100011033 and by the European Union NextGenerationEU/PRTR.

P07

Exploring the effects of modifying outer-membrane cytochromes on extracellular electron transfer rates in *Shewanella oneidensis*

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Abstract

Electroactive bacteria can respire using insoluble substrates as their terminal electron acceptors. In the metal-reducing bacterium *Shewanella oneidensis* MR-1, a porin *c*-type cytochrome complex (PCC) called **MtrCAB** facilitates electron transfer across the outer membrane. This is comprised of **MtrA**, a periplasmic decaheme cytochrome; **MtrB**, an outer membrane porin; and **MtrC**, a cell-surface localised outer membrane decaheme cytochrome. MtrC can be subdivided into four domains: two α-helical domains (II and IV) which contain ten bis-His coordinated hemes arranged in a “staggered cross”, and two split β-barrel domains (I and III). To investigate the significance of having the ten hemes in MtrC divided into two sets of five, a premature stop codon was inserted in a plasmid-based *mtrC.* This yielded a soluble, truncated variant of the protein that only contained domains I and II ($MtrC_{DI,II}$). X-ray crystallography, analytical ultra centrifugation, and LCMS were used to confirm that the tertiary structure of domains I and II in $MtrC_{DI,II}$ was unchanged by the removal of domains III and IV. Subsequently, a plasmid-based lipid-anchored variant of

$MtrC_{DI,II}$ was introduced into a Δ*mtrC/omcA* strain for whole cell assays. Our results suggest that cells containing the $MtrC_{DI,II}$ were not able to transfer electrons onto flavin mononucleotide, ferric citrate, amaranth, or OmcA (another *S. oneidensis* outer membrane decaheme cytochrome). However, they were able to transfer electrons onto reactive black 5 and methyl orange. We hypothesise that all four domains of MtrC are necessary for electron transfer onto the physiological electron acceptors or *S. oneidensis.* However, some synthetic acceptors will take the electrons from domain II, indicating functional electron transfer system remains.

P08
Identification of genetic elements for transplantation of extracellular electron transfer chains

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Abstract

Research in electromicrobiology provides new unique opportunities to study and exploit microbial physiology. In this work individual modules of the extracellular electron transfer (EET) chain of the native exoelectrogenic model organism *Shewanella oneidensis* were individually analyzed and the influence of associated factors was determined by the stepwise transplantation into the host organism *Escherichia coli*. For this purpose, the suggested minimal protein set, consisting of the *c*-type cytochromes CymA, STC, MtrA and MtrC as well as the *β*-barrel protein MtrB, were heterologously expressed in different expansion/ configuration stages. These stages were subsequently compared to corresponding *S. oneidensis* strains in terms of anthraquinone-2,6-disulfonate (AQDS) / Fe-citrate reduction rates. This revealed that transplantation of heterologous EET chains is associated with tremendous losses in electron transfer rates compared to those of native exoelectrogens. Based on these findings, additional genetic elements, suggested to be indirectly involved in EET in terms of protein maturation, localization, and functionality, were identified. In particular, this involved transplantation of the cytochrome *c* maturation system, proteins necessary for menaquinone-7 biosynthesis, as well as the type II secretion system. The latter allowed the secretion of the terminal reductase MtrC onto the cell surface and therefore the correct localization of MtrC in *E. coli* for first time. Nevertheless, the resulting increased reduction rates were not competitive with reduction rates in *S. oneidensis*. The acquired data suggest that EET processes are far more complex than previously thought and that correct folding and localization of MtrB might be the bottleneck.

P09
Unraveling factors necessary for MtrB incorporation into the outer membrane

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Abstract

The extracellular electron transport chain of *S. oneidensis* has been extensively studied, leading to the most detailed understanding of an electron transfer cascade across the outer membrane. However, more than a decade ago, several research groups identified a co-dependence on the stability of the c-type cytochrome MtrA and the ß-barrel protein MtrB in the outer membrane. Although a

better understanding of the assembly mechanisms of the MtrABC complex would be of great importance, no deeper investigations have been carried out, probably due to the complexity of the process.

Western blots with *S. oneidensis* strains lacking MtrA showed that MtrB is degraded in the absence of MtrA. Experiments conducted with *E. coli* suggest that MtrA is the only chaperone necessary for MtrB transport through the periplasm. Still experiments with *S. oneidensis* are so far missing. We set out to generate a chaperone null mutant to dissect the role of MtrA and the canonical chaperones SurA, Skp and DegQ in MtrB folding. Therefore, we generated a *S. oneidensis* Δ*mtrAB* Δ*degQ*::P_{tac}*surA-skp* skp* strain. The next step will be to knock out the last remaining chaperone, *surA*, followed by Western blot experiments with strains expressing only MtrB or MtrAB. This will clearly deepen the understanding of MtrB maturation in *S. oneidensis*.

The complex process of ß-barrel protein folding and insertion into the outer membrane obviously requires several factors. However, it is rather complicated to identify the factors that are crucial for the correct assembly of the mtrABC complex, since the function of MtrB can only be detected indirectly via the reduction of extracellular electron acceptors. In particular, it does not seem possible to transfer electrons across the outer membrane in an *E. coli* CymA STC MtrABC strain without tremendous losses in electron transfer rates compared to *S. oneidensis*.
Here, we provide a new tool for a high-throughput screening towards outer membrane protein folding factors. We integrated a cross-linking peptide spytag into each individual position of MtrB and functionalized magnetic beads with the corresponding spycatcher. These magnetic beads were used to capture cells with a surface exposed spytag integrated into MtrB and expressed it in *S. oneidensis* as well as in an *E. coli* attTn7::*mtrA* pEC86_*ccm* strain. Only with *S. oneidensis* it was possible to capture cells with a surface-accessible spyTag, indicating that MtrB is not properly incorporated into the outer membrane of E. coli. In the next step, we will express a fosmid library of the *S. oneidensis* genome in the *E. coli* strain and again search for colonies with a surface exposed spytag. The re-sequencing of the fosmids will reveal the missing folding factors for MtrB and therefore has the potential to significantly improve the performance of *E. coli* in bioelectrochemical systems.

P10
SMART continuous bioelectrochemical processes: Advancing Sustainable Biotransformation

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Abstract

The development of efficient bioelectro-chemical systems is crucial for advancing sustainable biotechnological processes. The objective of this research project is to gain insight into the dynamics of continuous bioelectrochemical reactions and to establish a foundation for the operation of SMART reactors by developing a versatile framework for various biotechnological processes. The model reaction involves the biotransformation of glycerol (GL) to 1,3-propanediol (1,3-PD) using a bioelectro-chemical system with biocatalysts on the cathode. The electroautotrophic bacterium *Cupriavidus necator*, which is able to grow on CO_2, O_2 and cathodically produced hydrogen, is cultivated as a biofilm on the cathode. By

decoupling GL from central metabolism, the objective is to achieve near 100% carbon efficiency. A GL kinase and membrane-bound hydrogenase mutant strain of *C. necator* will be developed while a gene cluster consisting of GL dehydratase, its activator, and 1,3-PD dehydrogenase from *Clostridium butyricum* will be integrated into a pBBR1-based plasmid resulting in a strain for the whole cell biotransformation. The mutation of GL kinase genes reduces anabolic glycerol use, while the membrane-bound hydrogenase deletion renders the strain reliant on soluble cytoplasmic hydrogenase for lithotrophic growth, producing NADH from hydrogen. This dependence is of critical importance for maintaining process stability, which is dependent on the availability of electron acceptors. In the absence of oxygen, the reductive conversion of GL to 1,3-PD maintains reducing equivalent homeostasis, thereby supporting a viable biotransformation pathway. The introduction of oxygen enables the production of ATP and facilitates cell growth, thereby ensuring the sustainable renewal of the biocatalyst.

P11

Novel autoproteolytic-secretion mechanism for export of extracellular enzymes revealed by G. sulfurreducens PgcA

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Abstract

Secretion of proteins is a ubiquitous process biology, allowing organisms to influence the conditions of their extracellular environment, and extract resources from it. Many such secreted enzymes incorporate autoproteolytic processes as mechanisms of regulation or translocation. *Geobacter sulfurreducens* like other microorganisms that participate in extracellular electron transfer (EET), is adept at the secretion of cytochrome proteins which contribute to EET.

Here we report describe characterisation of the non-heme domain from the triheme *G. sulfurreducens* protein PgcA which has been reported to exist in an extracellular soluble form. Through a series of mutants and crystallographic structures, we demonstrate it to contain a unique autoproteolytic activity via two conserved aspartate residues which might help to reconcile seemingly inconsistent observations in the literature regarding the localisation, lipidation and truncation of this protein. Though this activity invokes aspartic proteases, it appears mechanistically and evolutionarily distinct from those enzymes. This novel mechanism is conserved across homologues of this domain and likely supports the secretion of diverse extracellular hydrolytic enzymes and extracellular matrix proteins, by strains distributed amongst the gamma and beta proteobacteria.

P12

Analysis of BES-BioH$_2$ System for Sustainable Hydrogen Production using Waste Water

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Abstract

Bioelectrochemical systems (BES) combine electrochemistry with the metabolism of electroactive microorganisms for energy production. In Microbial Electrolysis Cells (MECs), electroactive bacteria grow building a biofilm on the surface of a conductive anode, which acts as electron acceptor. The electroactive microorganisms oxidize organic matter to CO_2 under anaerobic conditions and the electrons obtained in the process are transferred from the anode to the cathode through an electrical circuit **[1]**. The cathodic reaction is the H_2 formation through H_2O reduction under alkaline conditions. The reduction of water to hydrogen is a non-spontaneous process, so the application of an external potential is required.
In this work, which is part of the Regenera Project (MISIONES 2019, CDTI) we present the BES-BioH$_2$ system, a reactor based on a MEC system **[1]** with the purpose of obtaining biohydrogen with lower energy requirements using the energy produced by electroactive microorganisms, being the main objective of this study is to determine the operational conditions for production of hydrogen with energy cost below 20 kWh/kg H_2.

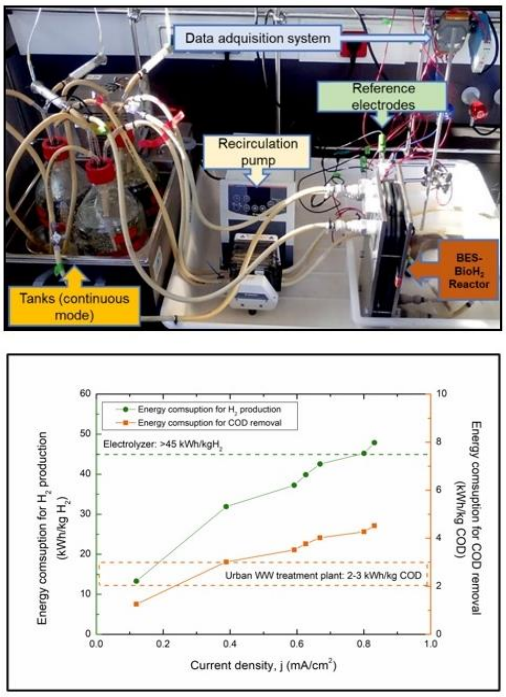

Figure 1. Experimental setup for BES-BioH$_2$ system. Bottom: energy cost for H2 production and energy consumption for COD removal (synthetic waste water).

[1] Ramírez-Moreno, M., Esteve-Núñez, A., Ortiz J.M. (2021). Desalination of brackish water using a microbial desalination cell: Analysis of the electrochemical behaviour. Electrochim. Acta, 388.
Acknowledgement: REGENERA project has been funded by the CDTI within the framework of the MISIONES 2019 program with the support of the Ministry of Science and Innovation (Funded by the European Union – NextGenerationEU).

P13

Tailored functional electrode structures for SMART bioreactors

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Abstract

In biotechnological production processes, continuous biofilm-based processes can be advantageous due to characteristics such as natural retentostate and self-optimization

over time. This project aims to develop and optimise tailor-made electrodes as a substratum for hydrogenotrophic biofilms that catalyse and adapt to continuous biotechnological processes in SMART reactors. A suitable electrode design with a homogeneous distribution of relatively large pores is required for reliable medium flow and as little dead volume as possible. For this purpose, graphite has been chosen as the preferred electrode material to ensure good electrical conductivity and durability. Different graphite forms were evaluated regarding porosity and electrochemical activity. So far, globugraphite seems to have several superior characteristics, especially regarding surface area per volume. *Cupriavidus necator*, a model knallgas bacterium is the organism of choice for the envisioned microbial electrosynthesis application. Microfluidic channels have been designed to house a complete bio-electrochemical system, to establish a steady, predictable fluidic regime, and to promote reproducible continuous biofilm experiments. First experiments were conducted using plain graphite as benchmark electrode material. *C. necator* was grown first under mixotrophic conditions with fructose as a heterotrophic growth substrate and a cathode poised to a potential of -100 mV against standard hydrogen electrode. After confluent biofilm development on the electrode was observed, using optical coherence tomography, the process conditions were changed to electro-autotrophic. There were no biovolume losses in conjunction with this modification. The experiments were so far conducted with plain graphite and will further be performed using globugraphite. In addition, the study of the key genes associated with biofilm growth, using transcriptomic analysis, followed by laboratory evolution experiments is planned to better understand the fundamental principles governing cathodic biofilm formation.

P14

Cathodic production of platform chemicals with *Cupriavidus necator* H16

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Abstract

The biotechnological production of bio-based platform chemicals combined with the chemical production of various end-products based on the platform molecules is a promising way to replace petrochemistry-based processes within a sustainable bioeconomy framework. Metabolic engineering of *Cupriavidus necator* H16 was employed to develop a strain capable of producing the platform chemicals acetoin and 2,3-butanediol with a carbon yield of up to 89% from fructose, organic acids or carbon dioxide and hydrogen.

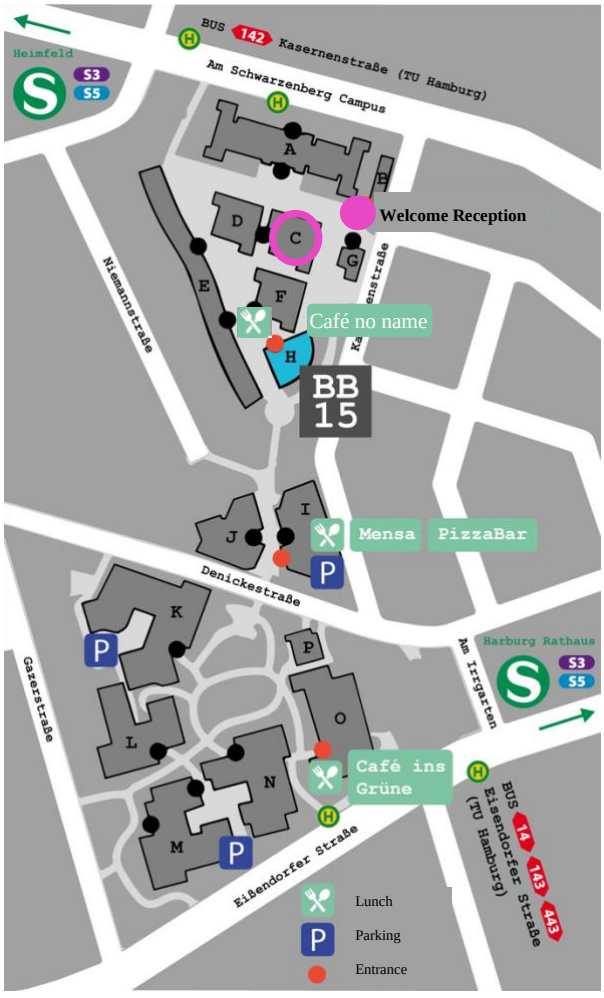
This was accomplished by the strategic deletion of the *phaC1*, *phaC2*, *acoABC* [1], and *adh* genes, which are implicated in competing pathways and the addition of the production genes *alsS, alsD* and *budC*. The resulting engineered strain was characterized in a microfluidic membrane biofilm system with the objective to optimize parameters for biofilm formation and applications of this strain in membrane biofilm bioreactors operated with carbon dioxide as carbon source. This system is supported by a robotic platform enabling multiparallel experiments showing promising results of up to 10 times higher cell numbers per reactor volume compared with planktonic cultivations.
Moreover, recent findings from our institute indicate that this strain may be suitable for cathodic electrosynthesis processes. This

approach would enable the delivery of hydrogen via the cathode and the provision of carbon dioxide and oxygen through the medium phase, thereby effectively mitigating the challenges posed by explosive gas mixtures and the low solubility of hydrogen in the bulk phase in gas fermentations.

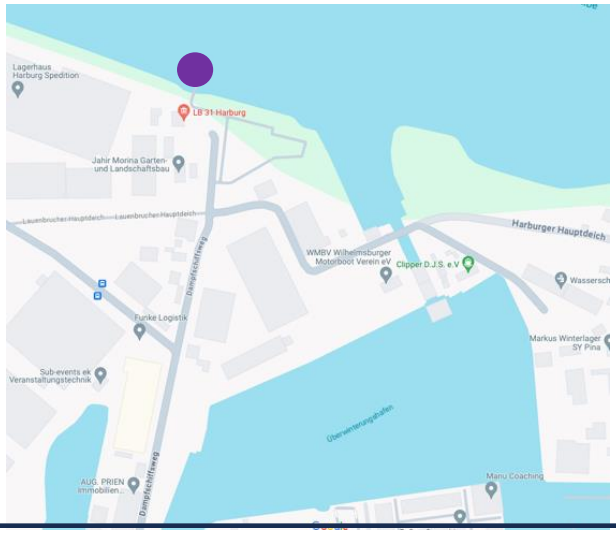
SOCIAL EVENTS

Welcome Reception:
Starting from 6pm Monday 22.07.24
Catering from https://bruzzelhütte.de
"Currywurst" (also vegetarian and vegan option), drinks (non-alcohol, beer)

Harbour boat cruise and Conference Dinner:
Starting from 6pm Tuesday 23.07.24
Boarding: 6-6:15pm
30 min walk from TUHH. We will start a group-walk at 5:15pm in front of building C.
If you do not join the walk, please let us know at the registration on Monday so that we can book taxis.



Harbour boat cruise:
2hrs boat tour through the port of Hamburg including sights like the Elbphilharmonie and the historic warehouse district.
Drinks available
Dampfschiffsweg pier,
21079 Hamburg



Conference Dinner:
Starting approx. 8:30pm (the boat will drop us there)
Ending 11:30pm (shuttle bus to S-Bahn Wilhelmsburg, TUHH, Hotel Lindtner)
Zum Anleger
Vogelhüttendeich 123,
21107 Hamburg