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DISCUSSIONS

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Biological approaches to artificial photosynthesis: general discussion

Vivek Badiani, Mark Bajada, Matthias Beller, Andrew B. Bocarsly, Sylvestre Bonnet, Carlota Bozal-Ginesta, Peter Brueggeller, Julea N. Butt, Flavia Cassiola, Michael Grätzel, Leif Hammarström, Marta C. Hatzell, Lars J. C. Jeuken, Burkhard König, Moritz F. Kuehnel, Joshua Lawrence, Chong-Yong Lee, Marcelino Maneiro, Shelley D. Minteer, Esther Edwardes Moore, Samuel E. H. Piper, Nicolas Plumeré, Joost N. H. Reek, Erwin Reisner, Souvik Roy, Jeremy Shears, Sergii I. Shylin, Han Sen Soo, Andreas Wagner, Dominik Wielend, Jenny Zhang and Martijn Zwijnenburg

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Jenny Zhang opened the discussion of the paper by Shelley Minteer: Was the vision of this study to produce salinity adapted microorganisms in the lab and then release them into the environment? Or was it mainly to try to understand how salinity adaptation can occur in the natural environment, using RNA sequencing to facilitate this?

Shelley Minteer replied: A comprehensive answer is that we are interested in both aspects. Specifically, our vision is that a better understanding of the salinity adaptation strategies will pose the basis to develop photo-bioelectrochemical systems capable of stable and long-term operation in saline environments. Furthermore, elucidating bacterial metabolism and their influence on photo-bioelectrocatalysis will not only allow for more robust and stable devices, but also for the better correlation of current response and changes taking place in the environment. Accordingly, we consider that the deeper understanding of biological processes related to the metabolism of electroactive microorganisms is critical for their successful application in the environment.

Jeremy Shears commented: You mentioned the formation of biofilms. Is this a stress response to the pH of the medium the organisms are exposed to? Do you notice any effects of the biofilm on kinetics? Do you consider them "good" or "bad" from a process perspective? And can you say a little bit more about using alginates as a form of biofilm?

Shelley Minteer responded: In our experiments aimed to the study of salinity adaptation effects on bioelectrocatalysis, we are directly depositing *R. capsulatus* cells on the electrode surface. Since the electrodes are tested right after preparation, no particularly thick biofilm is developed, and we did not investigate biofilm effects on the kinetics of the reactions taking place. However, in view of the in-field application of the technology, biofilm development will play a critical role, as it will facilitate bacterial cells' attachment to the electrode and their stability over long-term operation. We are currently investigating the effects of different quorum sensing autoinducers on *R. capsulatus* biofilm development. Regarding the use of alginate to form artificial biofilms, first, it is important to specify that alginate is one of the three major components of natural biofilms. Based on that, we are unveiling the possibility to prepare the artificial biofilm to enhance *R. capsulatus* salt tolerance, allowing immediate exposure of bacterial cells to solutions characterized by different salinities. It is important to remark that, while this approach has the advantage to facilitate a "prompt adaptation to salinity", its efficacy over long-term operation remains to be validated, thus motivating our interests in elucidating the biological mechanisms of adaptation.

Andrew Bocarsly remarked: Although having a biofilm very close to the electrode interface facilitates electron transfer between the film and the electrode, the biofilm may generate a mass transport limitation on the solution side of the interface. Will this mass transport resistance be a concern?

Shelley Minteer answered: Yes, it is definitely a concern as we improve the performance of the bioelectrodes. Eventually, they will become mass transport limited.

Julea Butt commented: Some reports suggest that bacteria 'in the middle' of biofilms are metabolically inactive, perhaps in stationary phase or dead. I wondered if you have any information on the metabolic state of the cells in your *Ralstonia* biofilms as a function of distance from the electrode?

Shelley Minteer answered: Our biofilms are *Rhodobacter*, but this is an important aspect. As correctly stated, in thick biofilms the cells far from being exposed to the solution will face a scarcity of available substrates, potentially leading to their death. However, for our current experiments the amount of bacterial cells deposited on the electrode surface is quite limited (30 microliters of a 1 g mL^{-1} bacterial cells solution). Moreover, all the obtained electrodes are utilized within a 1–3 h time window from their preparation, and thus, we do not expect a particular influence of dead cells on the obtained bio-photocurrent results. This is indeed a very interesting aspect to be elucidated with future research, and we have ongoing studies aimed at developing new immobilization techniques and redox mediator systems for enhanced operational stability of the biophotoelectrochemical systems, since we have experience with alginate and redox polymers for these applications.[1–3]

1 B. Alkotaini, S. L. Tinucci, S. J. Robertson, K. Hasan, S. D. Minteer and M. Grattieri, Alginate-encapsulated bacteria for hypersaline solutions treatment in microbial fuel cell, *ChemBioChem*, 2018, **19**, 1162–1169.

2 K. Hasan, M. Grattieri, T. Wang, R. D. Milton and S. D. Minteer, Enhanced Bioelectrocatalysis of *Shewanella oneidensis* MR-1 by a Naphthoquinone Redox Polymer, *ACS Energy Lett.*, 2017, **2**, 1947–1951.
3 G. Pankratova, D. Pankratov, R. D. Milton, S. D. Minteer and L. Gorton, Following Nature: Bioinspired Mediation Strategy for Gram-positive Bacterial Cells, *Adv. Energy Mater.*, 2019, **9**, 1900215.

Marta Hatzell asked: How are you able to control for electrical conductivity differences with the variable salinity wastewaters investigated?

Shelley Minteer replied: Thank you for pointing out this issue. First of all, our experiments were always conducted using a 20 mM MOPS buffer (pH 7) + 10 mM $MgCl_2$ + 50 mM malic acid, adjusting salinity as required. Accordingly, good conductivity of the utilized electrolyte was ensured for all the experiments, despite the different salinities tested. It is correct that an increased conductivity is obtained for the experiments performed at higher salinity (*i.e.* 22 gL^{-1}NaCl). While this would result in an expected current generation increase for a classical electrochemical system, this is not the case for bioelectrochemical systems employing intact bacterial cells, due to the inhibiting effects of high salt content on bacterial cell activity. Finally, it should also be noted that, with the final goal of achieving in-field application for the developed system, our goal is not to strictly control the experimental conditions (which would be not possible during in-field application), but rather to understand how the system behaves when exposed to stressing and variable environmental conditions.

Joshua Lawrence queried: Have you been able to analyse the RNA-Seq data you have collected? If the described horizontal gene transfer mechanism is involved it should be upregulated in salt-adaptation. RNA-Seq data available for other *Rhodobacter* species identified genes associated with salt stress, some of which slowly accumulate over time. Have you considered these as alternative explanations for your observed adaptation to salinity?

Shelley Minteer responded: We are still in the process of using bioinformatics to evaluate all of the data collected, so we can't confirm the mechanism yet.

Marcelino Maneiro commented: I found your approach very interesting. In my opinion, bioremediation of wastewater is crucial. Only 3% of the world's water is fresh water; a mere 0.014% of all water on Earth is both fresh and easily accessible. In this context it is necessary to develop systems which work in saline wastewater environments. My question is about the behaviour of the *R. capsulatus* bacteria in the presence of other living organisms that tend to be present in the wastewater media. How does it interact with them?

Shelley Minteer replied: While considerably fewer research efforts are focused on this particular topic, we consider it of the utmost importance to address the problem of contaminated saline solution release in the environment. The question is very important in view of the in-field application of *R. capsulatus*. Different reports of bioelectrochemical systems operated with mixed consortia biofilms have shown that the presence of various species can enhance both the removal of organic contaminants, as well as the current

output of the device,[1,2] as non-electrogenic bacteria can degrade complex molecules to easily degradable compounds that can be utilized by electrogenic bacteria. For the specific case of *R. capsulatus*, we have not yet investigated its performance and electrochemical behavior in the presence of other living organisms that tend to be present in wastewater media. We consider the study of possible interaction between these species (such as members of the *Geobacteraceae* family that has been found on electrode-respiring biofilms formed in wastewater media) and *R. capsulatus* very important, and our future research efforts will be focused in this direction.

1 N. S. Malvankar, J. Lau, K. P. Nevin, A. E. Franks, M. T. Tuominen and D. R. Lovley, Electrical Conductivity in a Mixed-Species Biofilm, *Appl. Environ. Microbiol.*, 2012, **78**, 5967–5971.
2 B. Virdis, D. Millo, B. C. Donose, Y. Lu, D. J. Batstone and J. O. Krömer, Analysis of electron transfer dynamics in mixed community electroactive microbial biofilm, *RSC Adv*, 2016, **6**, 3650–3660.

Jenny Zhang opened the discussion of the paper by Lars Jeuken: What are the main bottlenecks of your systems?

Lars Jeuken responded: The main bottlenecks is that haems in the transmembrane electron conduit, MtrCAB, have reduction potentials between −0.4 and 0 V *vs.* SHE (at neutral pH). Commonly used hydrogen-evolving catalysts like the DuBois-type nickel catalysts require an overpotential, which means that at neutral pH, a reduction potential below −0.4V is required to reduce the catalyst.

Long-term, bottlenecks can be identified that need to be solved before compartmentalised vesicle systems can be exploited for solar-fuel production. For instance, (1) the transmembrane electron conduit, MtrCAB, is laborious and expensive to purify and (2) MtrCAB requires specific conditions to incorporate into vesicles, which are not always compatible with conditions to encapsulate high levels of catalysts in the lumen of vesicles.

Leif Hammarström remarked: This is an interesting system. You did not mention how the transmembrane electron flow is charge-balanced to allow for continued electron transfer. Do you know what is the charge compensating ion transfer, *e.g.* proton leakage? Do you have any direct data on leakage rates?

Lars Jeuken answered: We hypothesize that charge-balance proceeds *via* proton leakage, but we do not have experimental proof for this. When the dye, Reactive Red 120, in the lumen of MtrCAB-containing vesicles is reduced with dithionite, no enhancement in reduction rates is observed upon the addition of protonophores. The latter suggests that ion or proton leakage is not rate limiting in this system.

Michael Grätzel commented: Transmembrane electron transfer will induce a build up of negative charges in the interior of the vesicle. This will arrest the electron flux unless it is accompanied by proton transfer. The question I have is what is the source of the protons ? Ultimately the source should be water. If the source is not well defined it is not possible to provide a solar to chemical conversion efficiency for the photoreaction.

Lars Jeuken replied: We propose that the transmembrane electron transfer is accompanied by proton transfer. The reduction rate of Reactive Red 120 is rate limited by reductive bleaching of the dye, which is relatively slow and takes place on the minutes to tens-of-minutes timescale. On this timescale, liposomes are somewhat permeable to protons.[1] Control experiments were performed with added protonophore, CCCP, to further increase the proton permeability into the lumen of the liposomes. However, this did not increase the observed rate of reductive bleaching, suggesting that 'base' proton permeability was sufficiently high.

We envision that in future applications, protonophores or other ion-selective channels might need to be added to prevent the build-up of transmembrane chemical or electrochemical gradients.

1 M. Rossignol, P. Thomas and C. Grignon, *Biochim. Biophys. Acta, Biomembr.*, 1982, **684**, 195–199.

Vivek Badiani asked: Are there any methods you have in mind to understand the protein/liposome orientation at the interface? Are there subsequent ideas on how to control this orientation?

Furthermore, do you envision artificial materials to replace the transmembrane; if so, what types are you considering?

Lars Jeuken responded: Similar questions were raised elsewhere and are more fully answered as part of those discussions. In brief, we currently do not control the orientation of MtrCAB in proteoliposomes, although it is likely that one of the orientations is more favourable as this is often observed when reconstituting membrane proteins in liposomes. Orientation can be monitored with various methods, for instance, protease treatment followed by mass spectrometry analysis.

In the future we are looking to replace the lipid membrane with a polymer membrane or a hybrid polymer–lipid system. Together with a collaborator, we have published hybrid systems which show a much enhanced endurance compared to liposome systems.[1]

1 S. Khan, M. Li, S. P. Muench, L. J. C. Jeuken and P. A. Beales, *Chem. Commun.*, 2016, **52**, 11020–11023.

Chong-Yong Lee asked: In your previous study, you have employed decahaem MtrC which contains 10 haems. Your current work reported 20 haem MtrCAB. Could you please comment on the difference between both systems, and whether the number of haems influences the electron relay functionality?

Lars Jeuken answered: MtrC is a peripheral membrane protein, while MtrCAB is a transmembrane complex. The compartmentalised system requires a transmembrane complex to transfer electrons across the membrane. Thus, the number of haems does not influence the functionality of the system, but the fact that the haems in MtrCAB transverse the membrane is key. Electron transfer between the haems is known to be much faster (see ref. 1) than the kinetics measured for reduction of Reactive Red 120. Thus, we propose that the number of haems also does not influence the kinetics of the electron conduit MtrCAB.

1 X. Jiang, B. Burger, F. Gajdos, C. Bortolotti, Z. Futera, M. Breuer and Jochen Blumberger, *Proc. Natl. Acad. Sci. U. S. A.*, 2019, **116**, 3425–3430.

Andreas Wagner remarked: I was curious about the future outlook and general concept presented in your paper. If product is produced inside the membrane, it will somehow need to be released as it would otherwise accumulate. As soon as the reduced/oxidised product species would diffuse out, one would expect the same problems with re-oxidation/reduction outside the membrane by an oxidation/reduction catalyst as in a non-separated design approach. What would be the advantage of the membrane in this case?

Lars Jeuken responded: The advantages of compartmentalisation in this system are: (1) to separate oxidation and reduction catalysts, (2) create different chemical environments for reduction and oxidation, and (3) to stabilise the charge separated state by spatial separation of the hole and electron.

Flavia Cassiola commented: Your work is a clever illustration of how the control over organization and assembly should be considered in the design of photosynthetic systems. The transmembrane protein complex MtrCAB forms a long conductive molecular wire, (20 haem in bacteria outer membrane), which was estimated to have a transmembrane electron transfer of 10^3–10^4 electrons per second for the reaction used in your system, according to your paper. Synthesizing such a long molecular wire is not an easy task. Heinz Frei's group has proposed an interesting design (see ref. 1). Do you think the "volume" (as a way to look at MtrCAB as a long conductive molecular wire) that MtrCAB has as a protein can be matched in a synthetic system (not based on isolated proteins)?

1 W. Kim, B. A. McClure, E. Edri and H. Frei, *Chem. Soc. Rev.*, 2016, **45**, 3221–3243.

Lars Jeuken responded: The estimate of 10^3–10^4 electrons per second was determined by White *et al.* (ref. 1). In our work, the transmembrane electron transfer rate is much slower as it is limited by the relatively slow reductive bleaching rate of the dye, Reactive Red 120.

I think the 'length' or 'volume' of the molecular wire can be matched by synthetic systems, although it remains to be seen if synthetic systems can match the electron transfer rate of MtrCAB and the ability of MtrCAB to transfer electrons through a membrane of a vesicle (*i.e.* from a hydrophilic solvent, through a hydrophobic medium and back to a hydrophilic solvent). Besides the work of Frei, interesting work on synthesis conducting wires (some of them transmembrane) is being done by other groups. Some examples are the work of Bazan (ref. 2) and Albinsson (ref. 3). Both conducting polymers and redox polymers have been synthesized, while nanomaterials like carbon nanotubes are also considered.

1 G. F. White, Z. Shi, L. Shi, Z. Wang, A. C. Dohnalkova, M. J. Marshall, J. K. Fredrickson, J. M. Zachara, J. N. Butt, D. J. Richardson and T. A. Clarke, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, 6346–6351.
2 J. Du, C. Catania and G. C. Bazan, *Chem. Mater.*, 2014, **26**, 686–697.
3 M. Gilberta and B. Albinsson, *Chem. Soc. Rev.*, 2015, **44**, 845–862.

Sylvestre Bonnet remarked: Electrons in molecular wires usually follow a gradient of Gibbs free energy. What about these MtrCAB proteins? Related to the directionality of electron transfer through the membrane, is the Gibbs free energy curve flat, or does it go down from outside to inside?

Lars Jeuken responded: We note that this issue was also addressed in a comment made by Prof. Julea Butt.

The reduction potentials of the MtrCAB haems lie roughly between 0 and −0.4 V *vs.* SHE, but the reduction potentials of the 20 haems in MtrCAB have not been experimentally determined for the individual haems. Earlier simulations (ref. 1) indicated that the free energy profile for electron flow along MtrC has 'up and downs' and no directionality. In recent electronic structure calculations by the group of Blumberger (ref. 2) it was concluded that MtrC can shuttle electrons in both directions with similar efficiency.

1 A. Barrozo, M. Y. El-Naggar and A. I. Krylov, *Angew. Chem., Int. Ed.*, 2018, **57**, 6805–6809.
2 X. Jiang, B. Burger, F. Gajdos, C. Bortolotti, Z. Futera, M. Breuer and Jochen Blumberger, *Proc. Natl. Acad. Sci. U. S. A.*, 2019, **116**, 3425–3430.

Sylvestre Bonnet remarked: How do you control the orientation of the MtrCAB protein when reconstituting the liposomes? What will happen if one does not control this orientation? Does it have a role on the efficacy/rate/occurrence of electron transfer?

Lars Jeuken replied: We do not control the orientation of MtrCAB in proteoliposomes and it is expected that both orientations are adapted, although it is likely that one of the orientations is more favourable as this is often observed when reconstituting membrane proteins in liposomes. We hypothesize that the orientation of MtrCAB will not influence its ability to shuttle electrons into the liposomes. In a recent electronic structure calculations by the group of Blumberger (ref. 1) it was concluded that MtrC can shuttle electrons in both directions with similar efficiency. Furthermore, although the physiological function of MtrCAB is to shuttle electrons out of the bacterial cell, studies (*e.g.* ref. 2) have shown that the electron transfer direction can be reversed in the bacterial cell.

1 X. Jiang, B. Burger, F. Gajdos, C. Bortolotti, Z. Futera, M. Breuer and Jochen Blumberger, *Proc. Natl. Acad. Sci. U. S. A.*, 2019, **116**, 3425–3430.
2 D. E. Ross, J. M. Flynn, D. B. Baron, J. A. Gralnick and D. R. Bond, *PLoS One*, 2011, **6**, e16649.

Julea Butt commented: I would like to contribute some information relevant to the earlier discussions relating to redox properties of MtrCAB and how its orientation in the vesicle bilayers can be probed. As Lars has indicated, cyclic voltammetry reveals MtrCAB is redox active between approx. 0 and −400 mV *vs.* SHE (ref. 1). Experimental resolution of reduction potentials for individual hemes is challenging. They have very similar optical properties as all hemes are c-type with His/His ligation. In favourable cases information is provided by EPR monitored potentiometric titration as sub-sets of hemes are distinguished by signals determined by the dihedral angle between the ring planes of the axial ligands (ref. 2). Crystal structures are available for the extra-cellular cytochrome, MtrC and its homolog MtrF, which show the hemes as a staggered cross, rather than linear

chain. Multiple possible sites of electron exchange between redox partners and the proteins can be identified. The structures also provide a basis for calculation of heme reduction potentials (ref. 3 and 4) and those studies indicate a thermodynamic landscape more akin to a roller-coaster, than a slide. To define the orientation of MtrCAB in the vesicles a number of methods are possible. For example, the externally facing proteins are more accessible to antibodies, protease digestion or dye labelling (ref. 5).

1 R. S. Hartshorne *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 22169.
2 T. A. Clarke *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 9384.
3 X. Jiang *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, 2019, **116**, 3425.
4 H. C.Watanabe *et al.*, *Proc. Natl. Acad. Sci. U. S. A.,* 2017, **114**, 2916.
5 G. F. White *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, 6346.

Jenny Zhang opened the discussion of the paper by Nicolas Plumeré: I read in your paper that you've developed an app for others to use; is this ready for you to show us? Also, can you tell us how this model can be extended to other systems (*e.g.* synthetic systems)?

Nicolas Plumeré responded: Yes, the app to run simulations of the photocurrents and of the concentration gradients can be downloaded from the link given on our webpage. Four different models are always included to choose from. Additional models can be developed on request.

Jenny Zhang asked: When applying the model to synthetic systems, redox polymers are not needed. Is it easier to model this? How does the model differ for a synthetic system that uses a linker to adsorb the photo-harvester to the surface?

Nicolas Plumeré replied: This is correct. If the photosynthesizer can accept electrons directly from the electrode the model is simplifed. The electron mediation by hopping can be omitted. The charge transfer between the photosensitizer and the electrode could be accounted for based on the Butler–Volmer model.

Jenny Zhang remarked: In your model, there are several assumptions. For example, one of the assumptions is for the diffusion coefficient for *Y* (electron accepter for PSI) to be the same when diffusing through the redox polymer compared to just electrolyte. To what extent is this a valid assumption? What are some other assumptions that still need validation for your model?

Nicolas Plumeré answered: Indeed, the model is based on assumptions. The value of the diffusion coefficient of the electron acceptor in the electrolyte *vs.* its value in the film is a good example. The validity of this particular assumption is true in hydrogel films, which are mostly composed of the electrolyte itself. However if a film with significantly different properties than the solvent itself is used, the model must be adapted with the possibility to use different diffusion coefficients. Other assumptions are, for example, that there is no partition coefficient between the film and the electrolyte, the diffusion in the electrolyte is semi-infinite, and that the photoactive and electron mediating components are homogeneously distributed in the film.

Joshua Lawrence asked: Do you plan on experimentally confirming your model? For example, testing whether differences in diffusion coefficients have the predicted effect described in the paper.

Nicolas Plumeré replied: Agreements between experimental observation and predictions from the model are on-going in our lab. We have already demonstrated that modulation of the kinetic constants of the recombination between the charge carrier and the electrode lead to experimental photocurrents that match both qualitatively and quantitatively the predicted currents. The corresponding manuscript will be submitted soon.

The effect of the diffusion coefficient of the charge carrier is also a very interesting parameter to modulate. We intend to use electrolytes with increasing viscosity to confirm this prediction.

Souvik Roy remarked: I am curious about polymer matrices, can you have a silent matrix in the model where you have direct electron transfer?

Nicolas Plumeré answered: If the redox matrix were silent and if only direct electron transfer was possible, the system would simplify to a monolayer of photosynthetic proteins (since multilayers are not accessible to direct electron transfer). The model does not account for this specific case at the moment but the needed modification for this purpose would be straightforward since it consists of a simplified case of the current model.

Esther Edwardes Moore returned to the discussion of the paper by Lars J. C. Jeuken: Have you considered what catalysts, whether artificial or enzymatic, you might use inside your liposome for fuel synthesis?

Lars Jeuken answered: This question was partly answered in response to an earlier question. The main consideration when selecting appropriate catalysts is that haems in the transmembrane electron conduit, MtrCAB, have a reduction potential between −0.4 and 0 V *vs.* SHE (at neutral pH). −0.4 V *vs.* SHE is thermodynamically sufficient to reduce protons to hydrogen at neutral pH and hence we are focusing on hydrogen-evolving catalysts. Commonly used hydrogen-evolving catalysts like the DuBois-type nickel catalysts require an overpotential, which means that at neutral pH, a reduction potential below −0.4V is required to reduce the catalyst. We are currently considering the use of enzymes (*e.g.* hydrogenases) or Pt nanoparticles, which typically require low overpotentials, although their large size (compared to molecular catalysts) requires technical development to encapsulate them in the lumen of liposomes.

Martijn Zwijnenburg asked: Would leakage of products such as hydrogen out of the liposomes become an issue when using the envisaged biomimetic system for fuel production?

Lars Jeuken answered: In the approach presented in this paper, it is important that the fuel can be exported (either by design or as leak) out of the nanocompartment to prevent a build-up of fuel. In other words, the envisioned role of compartmentalisation in this system is (1) to separate oxidation and reduction

catalysts, (2) create different chemical environments for reduction and oxidation, and (3) to stabilise the charge separated state by spatial separation of the hole and electron.

Han Sen Soo commented: The photoreduction rates involving the different light harvesting nanoparticles are fairly different. Are the different rates due to thermodynamic reasons or due to the kinetics of charge transfer? Or could it be due to the charge extraction kinetics? Although the nanoparticles in principle have sufficient potential for the photoreduction, Marcus theory suggests that a larger driving force would increase the rate up to a certain extent and the dye-sensitized TiO_2 systems may happen to have larger driving forces?

Lars Jeuken responded: Our interpretation of the data is that photoreduction rates are determined by kinetics of charge transfer between the light-harvesting nanoparticles and MtrCAB and between MtrCAB and Reactive Red 120 (RR120). The reduction of RR120 by amorphous carbon dots (aCD) is faster in the compartmentalised systems (with MtrCAB) compared to the direct reduction of RR120 by aCD. Additionally, we see that for graphitic carbon dots with core nitrogen doping (g-*N*-CDs) and dye-sensitised TiO_2, MtrCAB is reduced at a higher rate compared to RR120, indicating that RR120 reduction is limited by charge transfer from MtrCAB to RR120.

Jenny Zhang addressed Nicolas Plumeré and Lars Jeuken: One of the biggest issues of using biological components in artificial photosynthesis is stability.

Nicolas, can you extend your model to help us understand, predict or perhaps overcome the issue of stability?

Lars, how is stability an issue for your vesicles? Will the artificial membrane breakdown before the proteins?

Nicolas Plumeré responded: The model could in principle be extended to take into account deactivation processes. If a mechanism for deactivation and the related kinetic parameters are known, they could be integrated into the kinetic scheme to predict the change in photocurrent overtime accordingly. Agreement between experiment and predicted current response would support a hypothesized mechanism.

Lars Jeuken answered: Liposomes and membrane proteins are typically stable on the timescale of hours to days. For future applications, however, stability needs to be extended to weeks or months. Phospholipids with double bonds (such as used in this work) are prone to oxidation, disrupting the hydrophobic core of the lipid bilayer. To stabilise liposomes, it is possible to use (phospho)lipids without double bonds and a typical example is the lipid 1,2-diphytanoyl-sn-*glycero*-3-phosphatidylcholine, which has high chemical and physical stability. An approach we have adapted to stabilise both the lipid membrane and proteins inside the membrane is to make hybrid vesicles of phospholipids and amphilic polymers. In an example with the polymer PBd_{22}-*b*-PEO_{14}, we have recently shown that the functional durability of an oxygen-reducing enzyme can be extended from days/weeks to over a year.[1,2]

1 S. Khan, M. Li, S. P. Muench, L. J. C. Jeuken and P. A. Beales, *Chem. Commun.*, 2016, **52**, 11020–11023.
2 R. Seneviratne, S. Khan, E. Moscrop, M. Rappolt, S. P. Muench, L. J. C. Jeuken and P. A. Beales, *Methods*, 2018, **147**, 142–149.

Carlota Bozal-Ginesta returned to the discussion of the paper by Shelley D. Minteer: How long do you expect bacteria on the electrode to survive? Do you expect them to grow?

Shelley Minteer replied: There are examples of microbial bioelectrocatalysis in literature continuing for years, but we have only studied them for months, but during that time, they do grow and reproduce.

Sergii I. Shylin asked: In Fig. 5 of the paper (DOI: 10.1039/c8fd00160j), you show a clear difference in biophotocurrent for *R. capsulatus* in fresh water and saline media. In the case of the highest salinity (20 g L^{-1} NaCl), concentration of NaCl is *ca.* 10 times higher than concentration of the supporting electrolyte used in the experiments (10 mM $MgCl_2$). Where does the difference come from? Is it the effect of the electrolyte concentration (*i.e.*, conductivity of the solution) or adaptation of bacteria to saline environments?

Shelley Minteer replied: As correctly noted, there was an increase in bio-photocurrent generation obtained at 20 gL^{-1} NaCl when cells fully adapted to salinity were utilized for the study. It should be noted that when performing the experiments at 20 gL^{-1} NaCl using bacterial cells not adapted to salinity, the bio-photocurrent response was consistently lower (or null) compared to the result obtained in fresh water. Based on this consideration, the response obtained at 20 gL^{-1} NaCl with cells fully adapted to salinity must account for the adaptation of bacterial cells to the increased salinity. It is true, however, that a contribution of increased solution conductivity could also account for part of the enhanced biophotocurrent response obtained with cells fully adapted to salinity.

Souvik Roy asked: Have you tested the substrate scope for your system? Instead of malic acid, can you oxidize a different substrate, such as benzyl alcohol or amines?

Shelley Minteer answered: This is a very important aspect. In our current experiments, we focused on understanding the influence of different salinities on the bioelectrocatalytic properties of *R. capsulatus*, and thus, we performed our study keeping substrate conditions constant, and the substrate scope in the photo-bioelectrochemical system was not investigated. However, the possibility of utilizing a different carbon source as a substrate is of extreme interest, and will be the objective of future research. It is important to note that *R. capsulatus* has a wide substrate scope, being able to utilize lactate, butyrate, propionate, succinate, and malate as a carbon source.[1,2] Furthermore, photocatabolism of nitrophenol and other aromatic compounds has been reported for *R. capsulatus*, opening up its application for the light-driven remediation of contaminated environments in photo-bioelectrochemical systems.[3,4] Since the influence on

bioelectrocatalysis of the broad substrate scope has not yet been determined, future research will focus on this aspect.

1 A. Dupuis, M. Chavallet, E. Darrouzet, H. Duborjal, J. Lunardi and J. P. Issartel, The Complex I from *Rhodobacter capsulatus*, *Biochim. Biophys. Acta, Bioenerg.*, 1998, **1364**, 147–165.
2 M. A. Tichi and R. Tabita, Interactive Control of *Rhodobacter capsulatus* Redox-Balancing Systems during Phototrophic metabolism, *J. Bacteriol.*, 2001, **183**, 6344–6354.
3 R. Blasco and F. Castillo, Light-Dependent Degradation of Nitrophenols by the Phototrophic Bacterium Rhodobacter capsulatus E1F1, *Appl. Environ. Microbiol.*, 1992, **58**, 690–695.
4 C. Sasikala and C. V. Ramana, Biodegradation and Metabolism of Unusual Carbon Compounds by Anoxygenic Phototrophic Bacteria, *Adv. Microb. Physiol.,* 1998, **39**, 339–377.

Souvik Roy queried: How much current enhancement is observed in the absence of malic acid?

Shelley Minteer responded: Due to the current aim of our research, where salinity influence on bioelectrocatalysis was being investigated, we did not focus on optimizing substrate concentration for our experiments. The utilized concentration of 50 mM malic acid was based on recently published works where the influence of different substrates concentrations was studied. Furthermore, it was determined that when no malic acid is present in solution, no photocurrent generation is obtained.[1,2]

1 K. Hasan, K. V. R. Reddy, V. Eßmann, K. Górecki, P. Ó. Conghaile, W. Schuhmann, D. Leech, C. Hägerhäll and L. Gorton, Electrochemical Communication Between Electrodes and *Rhodobacter capsulatus* Grown in Different Metabolic Modes, *Electroanalysis*, 2015, **27**, 118–127.
2 K. Hasan, S. A. Patil, K. Górecki, D. Leech, C. Hägerhälla and L. Gorton, Electrochemical communication between heterotrophically grown *Rhodobacter capsulatus* with electrodes mediated by an osmium redox polymer, *Bioelectrochemistry*, 2013, **93**, 30–36.

Dominik Wielend said: I have a question regarding the benzoquinone you use as electron mediator: In one of your recent publications (ref. 1) you identified benzoquinone to be the best candidate upon several other halogenated benzoquinones as well as one naphthaquinone-derivative.

As benzoquinone is toxic to humans, as well as apparently also to the purple bacteria you mentioned in your Faraday Discussions paper, I wonder if you also considered or tested similar less-toxic compounds like for examples substituted naphtha- or anthraquinone derivatives?

1 M. Grattieri, Z. Rhodes, D. P. Hickey, K. Beaver and S. D. Minteer, *ACS Catal.*, 2019, **9**, 867–873.

Shelley Minteer answered: This is a very important question. As correctly mentioned, p-benzoquinone is a toxic compound, and our initial choice to utilize monomeric quinone-based redox mediators was motivated by the need to clarify the extracellular electron transfer (EET) process for purple bacteria, which was not deeply understood. The monomeric mediators allowed a simple system to study the EET, correlating it to the chemical-physical properties of the utilized mediators; however, the use of these monomeric mediators for the development of the technology on field is not suitable. In view of developing

a system where no toxic compounds are introduced in solution, we are currently investigating the photo-bioelectrochemical response of *R. capsulatus* in the presence of a redox polymer. The use of less toxic mediating systems is providing us with the possibility to expand our studies to long-term applications.

Julea Butt remarked: A great thing about using bacteria as a (photo)electrocatalyst is that they can be self sustaining and renewable. *Ralstonia* is a great chassis organism for developing related biotechnology while an alternative, for operating in sea water, might be to use halophilic or halotolerant bacteria. What do you see as the challenges of this alternative approach?

Shelley Minteer responded: The aspect pointed out in this question is critical and of great relevance. First of all, it is correct that, thanks to the capability of halophilic or halotolerant bacteria to grow in a broad range of salinities, they could be applied in bioelectrochemical systems operating in saline conditions. As a matter of fact, we are currently using a halotolerant strain of *Salinivibrio* isolated from the Great Salt Lake to develop bioelectrochemical systems operating in hypersaline environments (salinity higher than 35 g L^{-1}, and for our specific case, higher than 100 g L^{-1}), where the extracellular electron transfer happening at the interface of bacteria–electrode surface allow enhancing the removal of contaminants while the obtained electrical current can be used to monitor the degradation process.[1–3] Accordingly, the application of these organisms is of high relevance for decontamination and monitoring of extreme environments; however, the major challenge of this alternative approach is that these organisms utilize a consistent amount of energy obtained from the oxidation of organic substrates to sustain their metabolism and to grow in such extremely saline conditions. As a result, lower current outputs are obtained, leading to the scientific challenges of enhancing the extracellular electron transfer while ensuring the stability and the capability to grow the microorganisms in such saline conditions. On the contrary, the possibility to apply the photosynthetic organisms *Rhodobacter capsulatus* in a salinity range up to 35–40 g L^{-1}, common for sea and ocean waters, would provide us with the advantage of using sunlight as the energy source, enhancing the amount of electrons available for the extracellular electron transfer with an electrode surface.

1 M. Grattieri, M. Suvira, K. Hasan and S. D. Minteer, Halotolerant Extremophile Bacteria from the Great Salt Lake for Recycling Pollutants in Microbial Fuel Cells, *J. Power Sources*, 2017, **356**, 310–318.
2 M. Grattieri, N. D. Shivel, I. Sifat, M. Bestetti and S. D. Minteer, Sustainable Hypersaline Microbial Fuel Cells: Inexpensive Recyclable Polymer Supports for Carbon Nanotube Conductive Paint Anodes, *ChemSusChem*, 2017, **10**, 2053–2058.
3 M. Grattieri, D. P. Hickey, B. Alkotaini, S. J. Robertson and S. D. Minteer, Hypersaline microbial self-powered biosensor with increased sensitivity, *J. Electrochem. Soc.*, 2018, **165**(5), H251–H254.

Erwin Reisner opened a general discussion of the papers by Shelley Minteer, Nicolas Plumeré and Lars Jeuken: You have presented elegant examples of bacterium- and protein-based systems, which rely on the controlled flow of electrons at the bio-electrode interface or through vesicle membranes. However,

natural photosynthesis does not only generate low potential electrons, but also produces proton gradients, in particular the 'proton pump' Photosystem II, for the synthesis of chemical energy carriers such as ATP. What would be the prospects and potential merits in your point of view of producing proton gradients in compartmentalized artificial or semi-biological hybrid systems for the synthesis of chemical energy carriers?

Shelley Minteer answered: This is an aspect of extreme relevance. Our point of view is that the possibility to use semi-biological, or engineered hybrid systems, for the synthesis of chemical energy carriers would have the prospect to rethink classical synthetic routes. Specifically, new bio-electrosynthetic routes could open up for the preparation of important chemicals. An example is the bio-electrocatalytic production of ammonia without the need for an external ATP supply, or costly ATP regeneration systems. Such approaches would have the potential merit of overcoming cost-limitations of current synthetic and biosynthetic routes, achieving cost-effective light-driven bioelectrosynthetic systems. While several challenges remain to be solved to fully implement such technologies, the positive outcome of such research efforts would drastically foster the field of more sustainable and green chemical synthesis.

Lars Jeuken answered: There are potentials for utilising proton (or electrochemical) gradients, but this depends on the envisioned application of the light-driven system. Two examples can be given to illustrate this. In the first, electrochemical gradients formed by transmembrane electron transfer could be utilised for, for instance, ATP formation and recycling (from ADP) if the lipid vesicles also include F0F1-ATPase. The latter system might find applications in chemical syntheses that use ATP-driven biocatalytic steps. In a second example, light-driven proton pumps (*e.g.* bacteriorhodopsin) could be incorporated in these lipid vesicles and light energy could be used to actively lower the pH inside the liposomes to enhance the formation of solar fuels such as hydrogen.

We note, however, that in both these (and other) examples, the complexity of the system increases, potentially reducing the feasibility of these systems in future devices.

Samuel E. H. Piper returned to the discussion of the paper by Shelley D. Minteer: What is the species specificity of the gene transfer agent discussed in your work? Can you imagine a system where *Rhodobacter capsulatus* carries out adaptation to a particular environment and then transfers that adaptation to a different species of microorganism?

Shelley Minteer replied: Previous studies have shown the Rhodobacter capsulatus gene transfer agent (rcGTA) to be species specific.[1] Since the discovery of the rcGTA, many other alphaproteobacteria have been seen to have similar gene transfer agents.[2] One such example from the Rhodobacterales family marine bacteria Roseovarius nubinhibens and Ruegeria mobilis, produce gene transfer agents that are capable of transferring genetic material among other bacteria and even in different phyla.[3] Regarding the possibility to use R. capsulatus to transfer the adaptation to a different species of microorganisms, unfortunately the R. capsulatus GTA is not known to be capable of interspecies gene transfer.

However, a better understanding of rcGTA role in adaptation to salinity and its influence on bioelectrocatalysis could be impactful for other GTAs and their organisms''' adaptions to high salinity, as well as other environmental stress factors.

1 J. D. Wall, P. F. Weaver and H. Gest, Gene Transfer Agents, Bacteriophages, and Bacteriocins of *Rhodopseudomonas capsulata*, *Arch. Microbiol.*, 1975, **105**, 217–224.
2 A. S. Lang, O. Zhaxybayeva and J. T. Beatty, Gene transfer agents: phage-like elements of genetic exchange, *Nat. Rev. Microbiol.*, 2012, **10**, 472–482.
3 L. D. McDaniel, E. Young, J. Delaney, F. Ruhnau, K. B. Ritchie and J. H. Paul, High Frequency of Horizontal Gene Transfer in the Oceans, *Science*, 2010, **330**, 50.

Peter Brueggeller returned to the discussion of the paper by N. Plumeré: Could you imagine that your kinetic model is also suitable for very fast electron transfer? Especially in the case of dyads and triads, where the electron transfer occurs intramolecularly, the velocity is no longer diffusion controlled. This means that the algorithm changes and has to be adapted to effects like "superexchange" in the presence of a suitable bridge.

Nicolas Plumeré replied: In our kinetic scheme, the electron mediation from electrode to the photosynthetic protein occurs by electron hopping through redox relays such as metal complexes or small redox proteins. Such electron hopping is modelled based on Fick's laws because electron transfer (defined by an apparent diffusion coefficient of the electron) is governed by a redox gradient in analogy to the transport of a freely diffusing molecule. If the photosensitizer is directly immobilized on the electrode surface and intramolecular electron transfer is delivering the charges, the model needs indeed to be adapted.

Mark Bajada asked: If you are aiming for the software to be open source, and to be used by other scientists in the field, why did you make use of MATLAB rather than Python? Also, the solver used (ode15s) is quite slow, did you encounter any difficulties when running the calculations?

Nicolas Plumeré replied: We chose to use MATLAB because it is a widely used commercial software, and it has many ready-to-use, built-in functions, which reduce the development time. Although we agree that using MATLAB is unlikely to result in the fastest possible simulation times, it would nevertheless take a significantly longer time to develop the code when compared to other approaches, and both considerations are important. For applications which are extremely computationally demanding, for example, the use of another approach (such as coding in C++) might be required, and the additional time and effort required in implementing the code would be justified. For the numerical solutions generated in this work, however, the simulation times achieved using MATLAB were satisfactory. Since it is possible to convert the simulation script into a stand-alone app that can be used without having to purchase MATLAB (which was done in this case), the ability to run simulations according to this model is widely accessible despite the fact that the app itself was produced using commercial software.

Jenny Zhang asked: How do you expect students to use the app to enhance their research? Can it give us feedback on what components to change to improve their systems?

Nicolas Plumeré answered: The first versions of the app have been used by students in their research projects as well as in teaching exercises since mid-2018. The initial feedback shows that the app is beneficial in three ways:

1. Simulations yield not only the photocurrent but also the concentration gradients of all components involved in current generations. This means one can identify the reason for bottlenecks in current generation. The time and space dependent observations of the film behavior are of high pedagogic value since they enable to "visualize" the meaning of the equation defining the photoelectrochemical processes.

2. Parameter screening enables to identify suitable parameter space for planning experimental strategies. In particular the screening of the effect of the parameters that contribute both to the photocatalytic processes and to the competing recombination processes are valuable since they are not accessible otherwise.

3. Simulations based on the app are also very useful to validate quantitatively a hypothesized mechanism based on the agreement between the predicted current and the experiment.

Jenny Zhang returned to the general discussion on the papers of Shelley Minteer, Nicolas Plumeré and Lars Jeuken: What should be the next steps for the field (biological approaches for artificial photosynthesis)? What is needed in your specific area that will help to push this field to a new level?

Shelley Minteer replied: Whether subcellular (proteins) or cellular biophotoelectrocatalysis, better materials are needed for interfacing biological entities with electrode surfaces.[1] However, bioengineering is also needed. Microbial cells and proteins were not designed to directly communicate with electrodes (for the most part), so further work is needed to engineer them for better interactions.[2–4] Finally, stability is always an issue with biological approaches,[4] so self-healing and regeneration systems are needed to improve the stability issues.

1 R. D. Milton, T. Wang, K. L.Knoche and S. D. Minteer, Tailoring Biointerfaces for Electrocatalysis, *Langmuir*, 2016, **32**, 2291–2301.
2 G. Güven, R. Prodanovic, and U. Schwaneberg, Protein engineering – an option for enzymatic biofuel cell design, *Electroanalysis*, 2010, **22**, 765–775.
3 N. Sekar, R. Jain, Y. Yan and R. P. Ramasamy, Enhanced photo-bioelectrochemical energy conversion by genetically engineered cyanobacteria, *Biotechnol. Bioeng.*, 2016, **113**, 675-679.
4 M. J. Moehlenbrock and S. D. Minteer, Extended lifetime biofuel cells, *Chem. Soc. Rev.*, 2008, **37**, 1188–1196.

Lars Jeuken answered: I agree with the answer of Prof. Shelley Minteer to this question. The field would need better materials to interface biological entities with inorganic materials, be it catalysts, nanomaterials or macro-electrodes. Similarly, the community needs a better understanding of how biological entities interact with materials; this knowledge is required to underpin the informed

engineering of microbes and biomacromolecules. Finally, stability of biological systems needs to be optimised. This can be done by using 'self-healing' or regenerative systems (*i.e.*, living microbes). Alternatively, the field of biotechnology is mature and stabilisation of biocatalysts by classical protein engineering is a well established discipline.

Leif Hammarström opened a discussion of the introductory lecture by Matthias Beller: Can you explain more on how these metal single site catalysts work? Should they be described as going through a sequence of oxidation states, and how does the matrix/surface help in stabilizing these transitions? Essentially, can they be described in the same way as molecular catalysts?

Matthias Beller responded: Yes, in principle single metal center catalysts on a surface can be regarded as supported molecular catalysts. Notably, no metal center exists in an isolated form (at least not at ambient conditions) on such a surface, although such materials are often called "single metal atom catalysts". Similar to classic homogeneous catalysts, the metal center is stabilized by coordinating functional groups. However, in contrast to most organometallic complexes, stabilizing functional groups include oxygen and/or nitrogen atoms, while in homogeneous catalysts P-based ligands still prevail.

Joost Reek remarked: Prof Beller started an interesting discussion on batteries *vs.* solar fuel. Of course energy density is a very important factor for mobile applications and as such solar fuels have a big advantage. The question is whether, for stationary functionalities such as households or energy farms, the energy density of the storage material is also important? You can imagine that cheap but large heavy batteries may be sufficient for households, but can we also imagine advantages for solar fuel approaches for these type of applications?

Matthias Beller responded: The energy density for stationary applications is also an important aspect for their practical implementation. In this respect, chemical storage materials such as hydrogen, methane, methanol, or solar fuels are advantageous compared to batteries. Obviously, current technologies for generating these storage materials from renewable energy have to be improved to become cost competitive.

Burkhard König remarked: The vision of a chemical industry collecting carbon dioxide as carbon starting materials directly from the atmosphere is fascinating. What are the big challenges in realizing this? Are the absorber techniques used by current start up companies a good way or do you see alternatives?

Matthias Beller responded: Fully agree. Apart from increasing the efficiency of CO_2 absorption, also performing reactions of CO_2 under ambient conditions could improve the efficiency. In this respect, the tolerance of present abundant gases (oxygen) is challenging. For example, the reduction of CO_2 in the presence of oxygen! For improved photo- and classic thermal reactions the use of multifunctional catalysts might be a solution.

Perhaps we might propose a Schwerpunktprogramm (SPP) of the DFG on this topic?

Moritz F. Kuehnel asked: There is considerable controversy around CO_2 reduction on TiO_2 in the literature, with concerns over observed CO_2 reduction products actually originating from organic contaminants. Can you comment on these concerns, and how you overcame the problems previously encountered in this field?

Matthias Beller replied: I fully agree, this is an important concern. Due to the small amount of reduction products "ultrapure" materials have to be used. We are confident that the observed carbon monoxide stems from carbon dioxide because of labeling studies.

Flavia Cassiola remarked: I agree with your observation that the majority of the work done on artificial photosynthesis has not considered mechanisms for CO_2 capture. The current assumption is that the photosynthesis system will be fed by a concentrated source of pure CO_2. Except for the biological approaches any reported systems have considered direct air capture of CO_2. What are your thoughts on how CO_2 capture from the environment could be considered in the current proposed devices for artificial photosynthesis? What might we be missing from nature and its ability to capture CO_2 from the environment, which is a very diluted and impure source of CO_2?

Matthias Beller responded: To realize carbon dioxide valorization on a large scale and/or to permit for decentralized usage of carbon dioxide, its capture from air is extremely important. In my opinion this problem can only be overcome by cooperative efforts from biology, chemistry and engineering. On the one hand we can be inspired by the natural RuBisCO system; on the other hand artificial receptors based on our understanding of supramolecular binding principles can be used.

Conflicts of interest

There are no conflicts to declare.