Recent advances in surface chemistry of electrodes to promote direct enzymatic bioelectrocatalysis

I. Mazurenko, V.P. P Hitaishi, E. Lojou

Recent advances in surface chemistry of electrodes to promote direct enzymatic bioelectrocatalysis

I. Mazurenko, V. P. Hitaishi and E. Lojou*

Aix Marseille Univ, CNRS, BIP, Bioénergétique et Ingénierie des Protéines, 31 chemin Aiguier, 13402 Marseille, France

Abstract

Redox enzymes catalyze major reactions in microorganisms to supply energy for life. Their use in electrochemical biodevices requires their integration on electrodes, while maintaining their activity and optimizing their stability. In return, such applicative development puts forward the knowledge on involved catalytic mechanisms, providing a direct electrode connection of the enzyme is fulfilled. Enzymes being large molecules with active site embedded in an insulating moiety, direct bioelectrocatalysis supposes strategies for specific orientation of the enzyme to be developed. In this review, we summarize recent advances during the past three years in the chemical modification of electrodes favoring direct electrocatalysis. We present the different methodologies used according to the electrode materials, including metals, carbon-based electrodes or porous structures, and discuss the gained insights into bioelectrocatalysis. We especially focus on enzyme engineering which appears as an emerging strategy for enzyme anchoring. Remaining challenges will be discussed with regards to these last findings.

Keywords: Enzyme; catalysis; direct electron transfer; electrode chemical modification; enzyme engineering

*Corresponding author : Dr Elisabeth Lojou lojou@imm.cnrs.fr

Introduction

Redox enzymes are key elements in microorganisms where they catalyze crucial reactions at active sites composed of metals (Cu, Ni, Fe, Mo, W, etc.) or organic compounds (flavin adenine dinucleotide as an example). In addition, cofactors are maturated in the way to act as electronic relays enabling fast electron transfer between the active site, which can be buried in the insulating protein moiety, and the physiological partner or the substrate. Use of these enzymes as bioelectrocatalysts in biodevices (biosensors, bioreactors or enzymatic fuel cells), imposes their immobilization on electrodes, while maintaining their activity. When the enzyme is electrically connected to the electrode without the help of redox mediators acting as shuttles, the direct electron transfer (DET) process will allow not only the fundamental study of enzymatic catalysis, but also will decrease the overvoltages, increase the interfacial electron transfer (ET) rate, and simplify the future device [1]. Because enzymes are usually in the diameter range 5-10 nm, direct wiring supposes the orientation of the protein that places the active center or an electronic relay at a tunneling distance of the electrode (typically less than 2 nm). Within this objective, electrode chemical functionalization aims: i) to adsorb or graft the enzyme specifically or preferentially via the surface anchors thanks to suitable interactions, hence to succeed in DET, ii) to enhance the loading of enzymes, hence to enhance the catalytic current and eventually get the knowledge of the electroactive enzyme amount, iii) to narrow the orientation, hence to enhance the ET rate, iv) to maintain enzyme conformation, hence to enhance the electrocatalytic activity and stability. Generally speaking, three main ways are followed for DET-favored enzyme attachment: physical adsorption (electrostatic, hydrophobic, π-π interactions), chemical linkage (amide, imine, maleimide, click chemistry), and host-guest interactions [2]. Many works are still devoted to the effect of electrostatic interactions on DET, which is one of the main factor controlling protein-protein recognition, hence protein-electrode interactions. Although the existence of a strong dipole moment in the protein may narrow down the distribution of orientations to a set of prevailed ones [3], it is unlikely to achieve a 100% ordering of adsorbed proteins in a desired orientation. Furthermore, immobilization through electrostatic forces should not be sufficient for long term stability of enzyme based bioelectrodes, and hence, more specific attachment should be searched.

Previous reviews on enzyme/electrode interactions provided some key elements [4,5]. The current review focuses on the development and emerging concepts reported during the last 3 years. We will divide our discussion depending on the type of electrode which is used and the

corresponding available modifications. We will extend our discussion on engineering of enzymes allowing specific attachment on electrodes, an emerging domain in bioelectrocatalysis. We intend to provide the reader with the most relevant elements required to succeed in DET with all the enzymes newly identified.

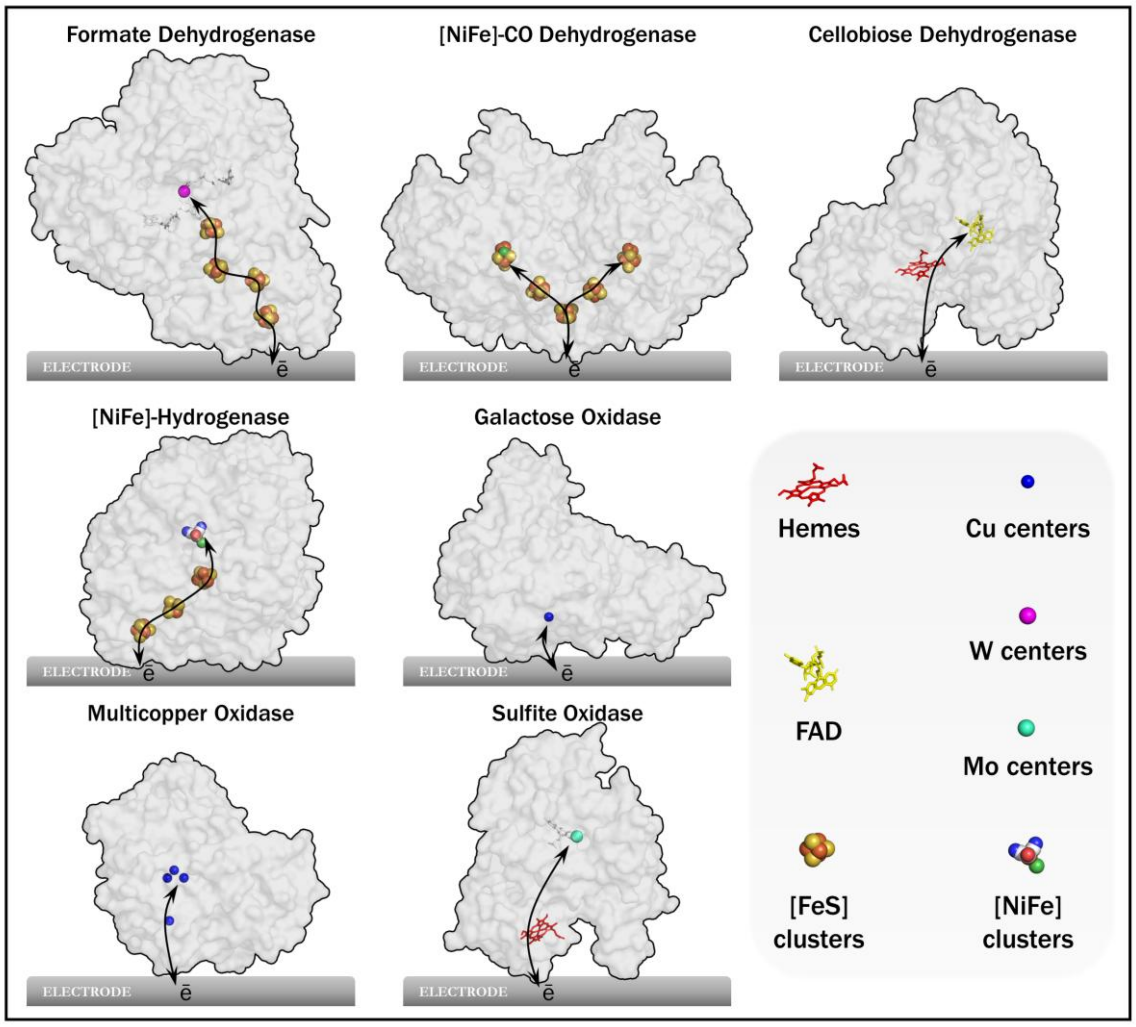

Figure 1. Examples of redox enzymes and their active centers addressable by DET.

Modification of metallic surfaces

Metallic electrodes used for bioelectrocatalysis are mainly made of Au, although Ag, Cu, Ni, Fe and metal oxides or alloys are also reported. Metal modification is expected to prevent change in enzyme conformation induced by certain amino acids interaction with the bare metal [6]. Self-assembled-monolayers (SAMs), most often based on Au-S bonds, on metal surfaces are ideal tools to understand the role of surface hydrophobicity or charges on the

efficiency of the electrocatalysis as highlighted by studies on copper efflux oxidase [7], cellobiose dehydrogenase [8], or human sulfite oxidase [9]. The additional advantage is the possibility to couple electrochemistry with analytical surface methods, such as quartz crystal microbalance (QCM), surface plasmon resonance (SPR), ellipsometry, surface enhanced infrared absorption (SEIRA), polarization modulation-infrared reflection-adsorption spectroscopy (PMIRRAS), surface-enhanced Raman spectroscopy (SERS) etc. Hitaishi et al. provided an in-depth analysis of bilirubin oxidase (BOD) immobilization on various SAMs by such coupling [10]. Varying the pH of *M. verrucaria* BOD adsorption or the pH of electroactivity, this study allowed a full correlation between enzyme loading, conformation and electroactivity. New fundamental concepts driving enzyme immobilization were demonstrated: i) the global charge of the enzyme drives its loading on the interface; ii) the dipole moment of the enzyme induced by anisotropy of charges, and the environment charge around the first electron acceptor (Cu T1), drive the enzyme orientation on the surface; iii) higher enzyme coverage does not necessarily translate into higher specific activity; iv) change in local pH induces dynamics of reorientation of enzymes on electrodes. All-atom molecular dynamic simulation confirms the electrostatic model for BOD, and highlights preserved conformation of the enzyme in the immobilized state [11]. Mixed SAMs can be used to tune the number of surface functionalities and their distribution [12]. Covalent immobilization can be realized by amide coupling between corresponding carboxylic and amine functions on the enzyme and the SAM. Discrepancy exists concerning induced stabilization of the catalytic signal, mainly depending on the way the grafting is carried out [13].

Diazonium salt reduction is another strategy to functionalize gold electrode, leading to even more stable modification [14]. Enzymes can then be either physically adsorbed or covalently attached by methods described for SAMs. The main challenge is to achieve a strict monolayer formation enabling fast DET between enzymes and the electrode [15]. Using a radical scavenger to decrease multilayer formation, and coupling SEIRA to electrochemistry, the O_2-tolerant hydrogenase from *Ralstonia eutropha* was shown to adsorb on the surface while directly oxidizing H_2 [16].

Chemical modification of metal nanoparticles (NPs)

NPs are ideal matrixes for enzyme immobilization as their size and shape may tune enzyme adsorption, loading and wiring [17]. Functionalization of NPs in the way similar to chemical modification carried out on flat metal electrodes serves to specifically tune the adsorption of enzymes for DET. Electrostatic, hydrophobic, π-π interactions, and eventually combination of

them help in narrowing the distribution of orientations [18]. As in the case of flat metal, AuNP functionalization allows covalent binding of the enzymes [19]. Interestingly, by coupling electrochemistry to SERS it was demonstrated that different ET pathway induced by a different orientation of *Didymocrea* sp. J6 Lac could be obtained depending on whether AuNPs are modified by SAMs or not [20].

NPs other than AuNPs shed new lights on bioelectrocatalysis. TiO_2 NPs coated with carboxylic-functionalized Ag nanoclusters wired carbon monoxide dehydrogenase via surface Cys residues for efficient photoelectrochemical conversion of CO_2 to CO [21]. Magnetic NPs (MNP), such as those based on iron oxides, served as a platform for direct bioelectrocatalysis. In addition MNPs enabled disassembling of enzymes through external magnetic stimulus, allowing the refreshment of the bioelectrodes [22]. Multidisciplinary analysis of the conformation of the *Aspergillus* sp. Lac [23] on MNPs revealed structural changes in the environment of Cu T1. Electrocatalysis was not hampered, but spatial hindrance between Cu sites and MNPs were reduced instead, suggesting importance of structure flexibility contrary to the admitted rule that change in conformation induces lost of activity.

Chemical modification of carbon nanostructures

Carbon nanostructures, such as carbon particles, carbon nanofibers (CNF), carbon nanotubes (CNTs), eventually arranged as buckypaper films, or graphene have been the subject of intensive research toward efficient redox enzyme immobilization. Many recent reviews are available that the reader can look at [24-26]. The interest in carbon structures is the wide range of available chemical surface functionalities, from aromatic to carboxylic ones, and their easy to tune chemistry thanks to wall reactivity, allowing physical or covalent attachment of enzymes [27]. Atomic oxygen/carbon ratio of CNF [28], or length of CNTs [29] can be varied to modify the surface charge, hence the orientation of the enzyme through electrostatic interactions. Integral membrane proteins can directly exchange electrons with the electrode when incorporated in hydrophobic CNF networks [30]. Carbon surfaces can also be modified through deposition of polyelectrolyte as thin films [31]. Further, diazonium salt reduction is the method of choice to graft different functions on the carbon surface to drive specific interactions with regions of enzymes involved in DET [32-34]. Different ET pathways respectively through Cu T1 or TNC of *M. verrucaria* BOD were suggested according to the charge of the function generated by diazonium salt reduction [35]. Functionalities on the carbon surface can alternatively be induced by amine electrooxydation generating a nitrogen-carbon bond [36]. Finally, modified-CNT and NP-based electrodes can

be combined, as demonstrated by Minteer and coworkers for laccase and BOD-based O_2 reduction [37], and by Leopold et al. for galactose oxidase [38].

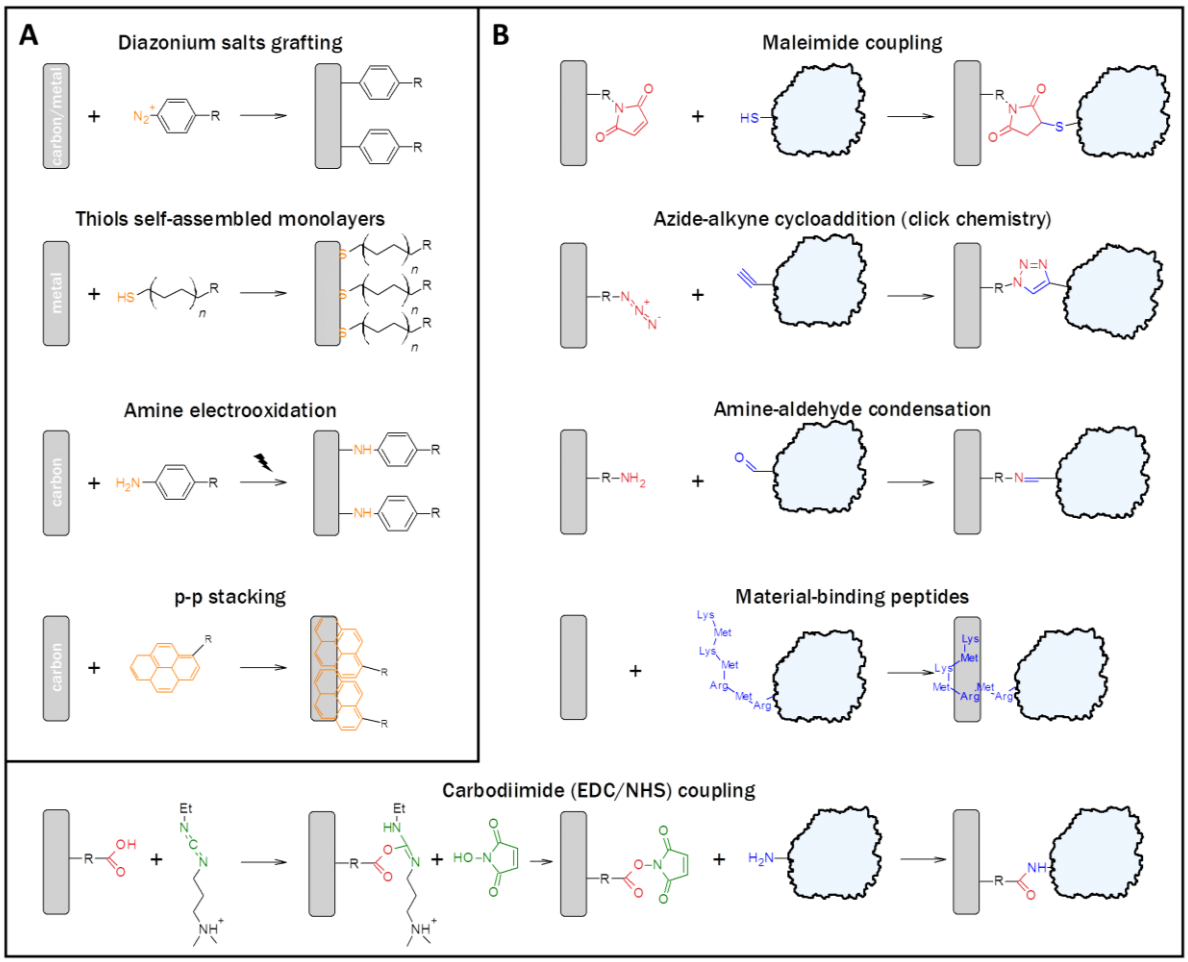

Figure 2. Most used electrode modification methods to promote direct electron transfer. (A) Reactions used to introduce the required functionalities to the electrode surface; (B) Reactions used to couple enzymes to the electrode surface

Chemical modification of porous structures

There is a tremendous interest in the design of hierarchical porous materials, including carbon- and Au-based ones, due to their use in a wide range of applications [39]. Porous structures enhance enzyme loading and may alleviate the orientation requirement by providing multipoint attachment. The pore size is one of the main factor in that case, and it was formalized that pores with size close to enzyme size were the most suitable for high ET rate [40,41]. However, in most reported cases, pore size is significantly larger that enzyme dimension, and suitable chemistry of the pore walls is required for desired orientation [42]. A full analysis has been provided by Kano' group underlining that, in addition to pore curvature and edge effects, electrostatic modulations induced by chemical modification of pores of Ketjen Black affect the efficiency of the bioelectrocatalysis [40]. Further, potential below and

above the pzc of the electrode can be applied not only to force penetration of the enzyme in the pores, but also to tune the charge of the pore walls for favored enzyme orientation [43].

Complex architectures are nowadays designed to provide new insight in direct bioelectrocatalysis. Functionalized AuNPs embedded in mesoporous carbon matrixes were shown to induce productive interactions between pyridine moiety on AuNPs and FeS site on formate dehydrogenase favoring direct formate/CO_2 conversion [44]. Porous graphite electrode was used as a platform for amorphous carbon nitride deposition (a-CNx) [45] presenting a versatile surface chemistry. *Trametes versicolor* Lac was bound to such electrode surface either through amide or imine coupling, and the two binding methods were combined. 4-fold enhancement of the catalytic current was observed. Despite a full coverage of the surface by Lac, electrochemistry coupled to AFM and XPS demonstrated that less than 10% of immobilized enzymes exchanges electrons with the electrode. Such a low percentage of enzymes participating to the catalysis was also calculated in the case of both hydrogenase and BOD on functionalized CNTs [46]. These data demonstrate that enzyme immobilization is far from being optimum, and novel immobilization strategies are required.

As an example of one promising direction, an original construction was proposed to get effective direct bioelectrocatalytic oxidation of glucose. It is based on the enzymatic reduction of platinum nanoclusters by glucose oxidase inside carbon mesopores, allowing inside-out electrical wiring of FAD active site for direct glucose oxidation at a potential of -80 mV vs Ag/AgCl [47]. A biofuel cell was constructed based on this new concept at the anode, displaying a power density around 50 $\mu W.cm^{-2}$ and retaining 80% original power after several days. However, considering enzymes are used as alternatives to platinum catalysts, this concept, although an elegant idea, has to shift toward the use of non noble metals.

Protein engineering for specific recognition of functionalized electrodes

Protein engineering is a growing research direction toward efficient and controlled DET for bioelectrocatalysis. Such procedure takes benefit of surface modification strategies, in such a way the engineered protein must specifically recognize chemical functions on the electrode. His-Tags are classical tools initially developed to facilitate protein purification that can be also used to anchor enzymes on electrodes [48]. Such modification may however decrease DET because of the length of the tag and its almost exclusive positioning on C-ter or N-ter sequences, thus restricting the modulation of orientation [49]. Alternatively, coupling between single amino acid residues present or introduced on the surface of proteins and corresponding

chemical functions on electrodes can be realized [2]. In most cases, cysteine (Cys) is the targeted residue. Al-Lolage et al. introduced a single Cys residue by site-directed mutagenesis at different locations in the flavodehydrogenase domain of cellobiose dehydrogenase [50-52]. After covalent attachment of the various mutants through maleimide groups on the electrode, it was demonstrated that DET occurs through the cytochrome domain, whose mobility is utmost crucial for electron transfer. The coverage of maleimide functions was restricted to 10% of the surface to favor the access and the coupling of enzymes. A ratio of 4-5 was however still obtained between the deposited enzymes and the electroactive ones. A similar protocol was carried out in *Magnaporthe oryzae* BOD, highlighting the key role of enzyme orientation and distance between the Cu T1 and the electrode on DET [53]. In a similar work, engineered *M. oryzae* BOD was immobilized by Au-S bond formation in a macroporous gold electrode [54]. The stability of the bioelectrode was enhanced compared to the non-engineered enzyme. Interestingly, the occupation of the whole internal pore volume by the enzyme was demonstrated using confocal fluorescent microcopy. A single Cys residue was also introduced in *E. coli* [NiFe] hydrogenase close to the surface FeS cluster, after removal of the naturally existing Cys. Specific attachment to Ag nanoclusters allowed enhanced photoproduction of H_2 [55].

Non canonical amino acids (NAA) can be incorporated in any place of a redox enzyme, contrary to His-tag [56]. NAA library offers a great versatility already largely exploited in protein-protein interaction biological studies. However, although prone to provide new insight on DET pathways, the use of NAA for enzyme anchoring is currently scarce in bioelectrocatalysis. Schlesinger et al. wired copper efflux oxidase through propargyl-L-lysine NAA incorporated at different distances from the Cu T1 [57]. The carbon electrode was chemically modified by pyrene-azide derivative for π-π stacking on the electrode on one hand and for Huisgen copper catalyzed azide-alkyne click reaction on the other hand. Effect of the length of the linker and positions of the NAA on the ET rate was discussed. A similar strategy was employed to wire tryptophan oxidase [58]. An alternative close strategy is to introduce peptides in enzymes for specific binding to electrodes [59]. The influence on DET of distances between actives sites and electrodes, but also of enzyme-enzyme neighboring that could induce steric hindrance or conformation change were reported. Lee et al. [60] designed NP nanopatterns with NP sizes between 10 and 80 nm, enabling to tune their special organization on the surface. An engineered glucose dehydrogenase (GDH) was bound to NPs through a fused specific peptide. Although no clear catalytic waves of glucose oxidation were

visible on the voltammograms,, this strategy should be relevant in the future to investigate the effect of coverage on other enzymatic systems bioelectrocatalysis.

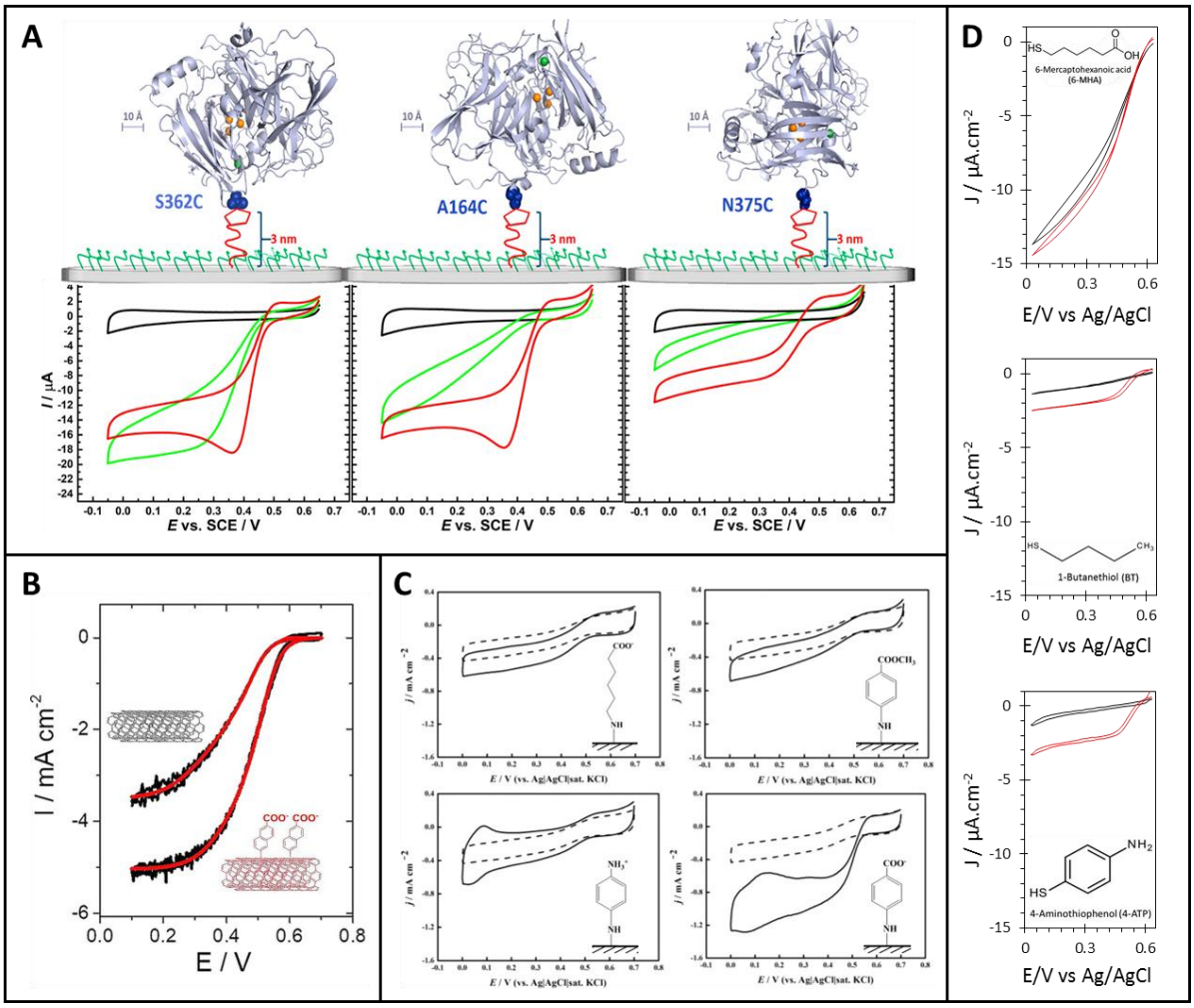

Figure 3. Examples of surface modification to promote direct electron transfer of multicopper oxidases: (A) *Magnaporthe oryzae* BOD attached to the electrode surface through maleimide coupling at different positions and corresponding (green) direct and (red) mediated catalytic currents of oxygen reduction. Adapted from [53]; (B) Catalytic oxygen reduction by *Magnaporthe oryzae* BOD adsorbed on non-modified and modified by diazonium salt grafting MWCNTs. Adapted from [33]; (C) Catalytic oxygen reduction by *Myrothecium verrucaria* BOD adsorbed on GCE modified by different functions using amine electrooxidation. Dashed lines correspond to non-modified GCE. Adapted from [36]; (D) (black lines) Direct and (red lines) mediated catalytic oxygen reduction by *Myrothecium verrucaria* BOD adsorbed on negatively (6-MHA), neutral (BT) or positively (4-ATP) charged SAMs. Adapted from [10].

Conclusion and Future directions

Attractive features exhibited by redox enzymes in solution, such as selectivity and high activity of catalysis, have prompted numerous attempts to convert enzymes into heterogeneous catalysts. Gold and carbon seem to be the most suitable electrode materials and even simple adsorption on them have often allowed to address electrochemically many redox enzymes. However, if one wants to strictly control enzyme immobilization, to perform more

precise enzymatic studies or to unveil the applied potential of enzymatic catalysts, an additional functionalization is required.

In the quest for the optimum bioelectrode in terms of catalytic efficiency and stability, chemical modification of the electrochemical interface has been the mostly investigated. Although, the general rules have been now identified to succeed in promoting DET, many enzymes are still not addressable by direct electrochemistry or display a very low interfacial ET rate. New approaches are further required for such enzymes, e.g. for some carbon monoxide dehydrogenases or [MoFe] nitrogenase [61] which would be very attractive enzymes to convert CO_2 or N_2. Extended interdisciplinary collaboration between chemist and biochemist communities should allow in the future to take advantage of the tremendous possibility offered by genetic enzyme engineering, as illustrated in this review by the recent results obtained with non natural amino acids inserted in specific location of an enzyme. As one of the examples, specific enzyme recognition by surface groups with controlled density might be used for enzyme patterning or cascades on the surface and to study the effect of molecular crowdedness. Furthermore, careful control of the immobilization chemistry will allow the development of the renewable bioelectrodes that, while still in its infancy, may help to overcome the inherent instability of redox enzymes.

Advanced methods to get new insights into enzyme amount, conformation and activities distribution are required especially coupling electrochemistry to spectroscopies or microscopies [62,63]. Ultimately, control of enzyme binding on electrodes will help the development of single-enzyme electrochemistry [64].

Acknowledgement

This work was financially supported by ANR (ENZYMOR-ANR-16-CE05-0024), and by Aix Marseille University for funding V.P. Hitaishi.

References

1. Milton RD, Minteer SD: **Direct enzymatic bioelectrocatalysis: differentiating between myth and reality**. *Journal of the Royal Society Interface* 2017, **14**.
2. Lalaoui Nm, Rousselot-Pailley P, Robert V, Mekmouche Y, Villalonga R, Holzinger M, Cosnier S, Tron T, Le Goff A: **Direct electron transfer between a site-specific pyrene-modified laccase and carbon nanotube/gold nanoparticle supramolecular assemblies for bioelectrocatalytic dioxygen reduction**. *Acs Catalysis* 2016, **6**:1894-1900.
3. Mazurenko I, Monsalve K, Rouhana J, Parent P, Laffon C, Goff AL, Szunerits S, Boukherroub R, Giudici-Orticoni M-Trs, Mano N: **How the intricate interactions between carbon nanotubes and two bilirubin oxidases control direct and mediated O2 reduction**. *ACS applied materials & interfaces* 2016, **8**:23074-23085.

4. Yates NDJ, Fascione MA, Parkin A: **Methodologies for "Wiring" Redox Proteins/Enzymes to Electrode Surfaces**. *Chemistry-a European Journal* 2018, **24**:12164-12182.
5. Jenner LP, Butt JN: **Electrochemistry of surface-confined enzymes: Inspiration, insight and opportunity for sustainable biotechnology**. *Current Opinion in Electrochemistry* 2018, **8**:81-88.
6. Pankratov D, Sotres J, Barrantes A, Arnebrant T, Shleev S: **Interfacial behavior and activity of laccase and bilirubin oxidase on bare gold surfaces**. *Langmuir* 2014, **30**:2943-2951.
7. Chumillas S, Maestro B, Feliu JM, Climent V: **Comprehensive Study of the Enzymatic Catalysis of the Electrochemical Oxygen Reduction Reaction (ORR) by Immobilized Copper Efflux Oxidase (CueO) From Escherichia coli**. *Frontiers in Chemistry* 2018, **6**.
8. Lamberg P, Hamit-Eminovski J, Toscano MD, Eicher-Lorka O, Niaura G, Arnebrant T, Shleev S, Ruzgas T: **Electrical activity of cellobiose dehydrogenase adsorbed on thiols: Influence of charge and hydrophobicity**. *Bioelectrochemistry* 2017, **115**:26-32.
9. Kalimuthu P, Belaidi AA, Schwarz G, Bernhardt PV: **Chitosan-Promoted Direct Electrochemistry of Human Sulfite Oxidase**. *Journal of Physical Chemistry B* 2017, **121**:9149-9159.
10. Hitaishi VP, Mazurenko I, Harb M, Clement R, Taris M, Castano S, Duche D, Lecomte S, Ilbert M, de Poulpiquet A, et al.: **Electrostatic-Driven Activity, Loading, Dynamics, and Stability of a Redox Enzyme on Functionalized-Gold Electrodes for Bioelectrocatalysis**. *Acs Catalysis* 2018, **8**:12004-12014.
11. Yang SJ, Liu J, Quan XB, Zhou J: **Bilirubin Oxidase Adsorption onto Charged Self-Assembled Monolayers: Insights from Multiscale Simulations**. *Langmuir* 2018, **34**:9818-9828.
12. Lacour V, Moumanis K, Hassen WM, Elie-Caille C, Leblois T, Dubowski JJ: **Formation Kinetics of Mixed Self-Assembled Monolayers of Alkanethiols on GaAs(100)**. *Langmuir* 2019, **35**:4415-4427.
13. Gutierrez-Sanchez C, Ciaccafava A, Blanchard PY, Monsalve K, Giudici-Orticoni MT, Lecomte S, Lojou E: **Efficiency of Enzymatic O2 Reduction by Myrothecium verrucaria Bilirubin Oxidase Probed by Surface Plasmon Resonance, PMIRRAS, and Electrochemistry**. *ACS Catalysis* 2016, **6**:5482-5492.
14. Guo L, Ma L, Zhang Y, Cheng X, Xu Y, Wang J, Wang E, Peng Z: **Spectroscopic Identification of the Au–C Bond Formation upon Electroreduction of an Aryl Diazonium Salt on Gold**. *Langmuir* 2016, **32**:11514-11519.
15. Lopez I, Dabos-Seignon S, Breton T: **Use of Selective Redox Cross-Inhibitors for the Control of Organic Layer Formation Obtained via Diazonium Salt Reduction**. *Langmuir : the ACS journal of surfaces and colloids* 2019, **35**:11048-11055.
16. Harris T, Heidary N, Kozuch J, Frielingsdorf S, Lenz O, Mroginski MA, Hildebrandt P, Zebger I, Fischer A: **In Situ Spectroelectrochemical Studies into the Formation and Stability of Robust Diazonium-Derived Interfaces on Gold Electrodes for the Immobilization of an Oxygen-Tolerant Hydrogenase**. *Acs Applied Materials & Interfaces* 2018, **10**:23380-23391.
17. Bollella P, Hibino Y, Conejo-Valverde P, Soto-Cruz J, Bergueiro J, Calderon M, Rojas-Carrillo O, Kano K, Gorton L: **The influence of the shape of Au nanoparticles on the catalytic current of fructose dehydrogenase**. *Analytical and bioanalytical chemistry* 2019.
18. Takahashi Y, Kitazumi Y, Shirai O, Kano K: **Improved direct electron transfer-type bioelectrocatalysis of bilirubin oxidase using thiol-modified gold nanoparticles on mesoporous carbon electrode**. *Journal of Electroanalytical Chemistry* 2019, **832**:158-164.
19. Ratautas D, Tetianec L, Marcinkeviciene L, Meskys R, Kulysa J: **Bioanode with alcohol dehydrogenase undergoing a direct electron transfer on functionalized gold nanoparticles for an application in biofuel cells for glycerol conversion**. *Biosensors & Bioelectronics* 2017, **98**:215-221.
20. Dagys M, Laurynenas A, Ratautas D, Kulys J, Vidziunaite R, Talaikis M, Niaura G, Marcinkeviciene L, Meskys R, Shleev S: **Oxygen electroreduction catalysed by laccase wired to gold nanoparticles via the trinuclear copper cluster**. *Energy & Environmental Science* 2017, **10**:498-502.

21. Zhang LY, Can M, Ragsdale SW, Armstrong FA: **Fast and Selective Photoreduction of CO2 to CO Catalyzed by a Complex of Carbon Monoxide Dehydrogenase, TiO2, and Ag Nanoclusters**. *Acs Catalysis* 2018, **8**:2789-2795.
22. Trifonov A, Stemmer A, Tel-Vered R: **Power Generation by Selective Self-Assembly of Biocatalysts**. *ACS nano* 2019, **13**:8630-8638.
23. Rodriguez-Padron D, Puente-Santiago AR, Caballero A, Balu AM, Romero AA, Luque R: **Highly efficient direct oxygen electro-reduction by partially unfolded laccases immobilized on waste-derived magnetically separable nanoparticles**. *Nanoscale* 2018, **10**:3961-3968.
24. Mazurenko I, de Poulpiquet A, Lojou E: **Recent developments in high surface area bioelectrodes for enzymatic fuel cells**. *Current Opinion in Electrochemistry* 2017, **5**:74-84.
25. Gross AJ, Holzinger M, Cosnier S: **Buckypaper bioelectrodes: emerging materials for implantable and wearable biofuel cells**. *Energy & Environmental Science* 2018, **11**:1670-1687.
26. Reeve HA, Ash PA, Park H, Huang AL, Posidias M, Tomlinson C, Lenz O, Vincent KA: **Enzymes as modular catalysts for redox half-reactions in H-2-powered chemical synthesis: from biology to technology**. *Biochemical Journal* 2017, **474**:215-230.
27. Gupta S, Murthy CN, Prabha CR: **Recent advances in carbon nanotube based electrochemical biosensors**. *International Journal of Biological Macromolecules* 2018, **108**:687-703.
28. Komori K, Huang J, Mizushima N, Ko S, Tatsuma T, Sakai Y: **Controlled direct electron transfer kinetics of fructose dehydrogenase at cup-stacked carbon nanofibers**. *Physical Chemistry Chemical Physics* 2017, **19**:27795-27800.
29. Xia HQ, Kitazumi Y, Shirai O, Ozawa H, Onizuka M, Komukai T, Kano K: **Factors affecting the interaction between carbon nanotubes and redox enzymes in direct electron transfer-type bioelectrocatalysis**. *Bioelectrochemistry* 2017, **118**:70-74.
30. Wang X, Clement R, Roger M, Bauzan M, Mazurenko I, de Poulpiquet A, Ilbert M, Lojou E: **Bacterial Respiratory Chain Diversity Reveals a Cytochrome c Oxidase Reducing O-2 at Low Overpotentials**. *Journal of the American Chemical Society* 2019, **141**:11093-11102.
31. Tang J, Werchmeister RML, Preda L, Huang W, Zheng ZY, Leimkuhler S, Wollenberger U, Xiao XX, Engelbrekt C, Ulstrup J, et al.: **Three-Dimensional Sulfite Oxidase Bioanodes Based on Graphene Functionalized Carbon Paper for Sulfite/O-2 Biofuel Cells**. *Acs Catalysis* 2019, **9**:6543-6554.
32. Bollella P, Hibino Y, Kano K, Gorton L, Antiochia R: **Enhanced Direct Electron Transfer of Fructose Dehydrogenase Rationally Immobilized on a 2-Aminoanthracene Diazonium Cation Grafted Single-Walled Carbon Nanotube Based Electrode**. *Acs Catalysis* 2018, **8**:10279-10289.
33. Gentil S, Mansor SMC, Jamet H, Cosnier S, Cavazza C, Le Goff A: **Oriented Immobilization of NiFeSe Hydrogenases on Covalently and Noncovalently Functionalized Carbon Nanotubes for H-2/Air Enzymatic Fuel Cells**. *Acs Catalysis* 2018, **8**:3957-3964.
34. Gentil S, Carriere M, Cosnier S, Gounel S, Mano N, Le Goff A: **Direct Electrochemistry of Bilirubin Oxidase from Magnaporthe orizae on Covalently-Functionalized MWCNT for the Design of High-Performance Oxygen-Reducing Biocathodes**. *Chemistry-a European Journal* 2018, **24**:8404-8408.
35. Lalaoui N, Holzinger M, Le Goff A, Cosnier S: **Diazonium Functionalisation of Carbon Nanotubes for Specific Orientation of Multicopper Oxidases: Controlling Electron Entry Points and Oxygen Diffusion to the Enzyme**. *Chemistry-a European Journal* 2016, **22**:10494-10500.
36. Xia HQ, Kitazumi Y, Shirai O, Kano K: **Enhanced direct electron transfer-type bioelectrocatalysis of bilirubin oxidase on negatively charged aromatic compound-modified carbon electrode**. *Journal of Electroanalytical Chemistry* 2016, **763**:104-109.
37. Neto SA, Da Silva RG, Milton RD, Minteer SD, De Andrade AR: **Hybrid Bioelectrocatalytic Reduction of Oxygen at Anthracene-modified Multi-walled Carbon Nanotubes Decorated with Ni90Pd10 Nanoparticles**. *Electrochimica Acta* 2017, **251**:195-202.
38. Wayu MB, Pannell MJ, Labban N, Case WS, Pollock JA, Leopold MC: **Functionalized carbon nanotube adsorption interfaces for electron transfer studies of galactose oxidase**. *Bioelectrochemistry* 2019, **125**:116-126.

39. Yang XY, Chen LH, Li Y, Rooke JC, Sanchez C, Su BL: **Hierarchically porous materials: synthesis strategies and structure design**. *Chemical Society Reviews* 2017, **46**:481-558.
40. Sakai K, Xia H, Kitazumi Y, Shirai O, Kano K: **Assembly of direct-electron-transfer-type bioelectrodes with high performance**. *Electrochimica Acta* 2018, **271**:305-311.
41. Mazurenko I, Clément R, Byrne-Kodjabachian D, de Poulpiquet A, Tsujimura S, Lojou E: **Pore size effect of MgO-templated carbon on enzymatic H2 oxidation by the hyperthermophilic hydrogenase from Aquifex aeolicus**. *Journal of Electroanalytical Chemistry* 2017.
42. Xiao XX, Siepenkoetter T, Conghaile PO, Leech D, Magner E: **Nanoporous Gold-Based Biofuel Cells on Contact Lenses**. *Acs Applied Materials & Interfaces* 2018, **10**:7107-7116.
43. Lopez F, Siepenkoetter T, Xiao XX, Magner E, Schuhmann W, Salaj-Kosla U: **Potential pulse-assisted immobilization of Myrothecium verrucaria bilirubin oxidase at planar and nanoporous gold electrodes**. *Journal of Electroanalytical Chemistry* 2018, **812**:194-198.
44. Sakai K, Kitazumi Y, Shirai O, Takagi K, Kano K: **Direct electron transfer-type four-way bioelectrocatalysis of CO2/formate and NAD(+)/NADH redox couples by tungsten-containing formate dehydrogenase adsorbed on gold nanoparticle-embedded mesoporous carbon electrodes modified with 4-mercaptopyridine**. *Electrochemistry Communications* 2017, **84**:75-79.
45. Blout A, Billon F, Calers C, Methivier C, Pailleret A, Perrot H, Jolivalt C: **Orientation of a Trametes versicolor laccase on amorphous carbon nitride coated graphite electrodes for improved electroreduction of dioxygen to water**. *Electrochimica Acta* 2018, **277**:255-267.
46. Mazurenko I, Monsalve K, Infossi P, Giudici-Orticoni MT, Topin F, Mano N, Lojou E: **Impact of substrate diffusion and enzyme distribution in 3D-porous electrodes: a combined electrochemical and modelling study of a thermostable H-2/O-2 enzymatic fuel cell**. *Energy & Environmental Science* 2017, **10**:1966-1982.
47. Trifonov A, Stemmer A, Tel-Vered R: **Enzymatic self-wiring in nanopores and its application in direct electron transfer biofuel cells**. *Nanoscale Advances* 2019, **1**:347-356.
48. Amano Y, Koto A, Matsuzaki S, Sakamoto H, Satomura T, Suye S: **Construction of a biointerface on a carbon nanotube surface for efficient electron transfer**. *Materials Letters* 2016, **174**:184-187.
49. Takamura E, Suzuki H, Nakamura T, Sakamoto H, Satomura T, Sakuraba H, Ohshima T, Suye S: **Improvement in Electron Transfer Efficiency Between Multicopper Oxidase and Electrode by Immobilization of Directly Oriented Enzyme Molecules**. *Journal of Fiber Science and Technology* 2019, **75**:47-51.
50. Meneghello M, Al-Lolage FA, Ma S, Ludwig R, Bartlett PN: **Studying Direct Electron Transfer by Site-Directed Immobilization of Cellobiose Dehydrogenase**. *Chemelectrochem* 2019, **6**:700-713.
51. Ma S, Laurent C, Meneghello M, Tuoriniemi J, Oostenbrink C, Gorton L, Bartlett PN, Ludwig R: **Direct Electron-Transfer Anisotropy of a Site-Specifically Immobilized Cellobiose Dehydrogenase**. *Acs Catalysis* 2019, **9**:7607-7615.
52. Al-Lolage FA, Meneghello M, Ma S, Ludwig R, Bartlett PN: **A Flexible Method for the Stable, Covalent Immobilization of Enzymes at Electrode Surfaces**. *Chemelectrochem* 2017, **4**:1528-1534.
53. Al-Lolage FA, Bartlett PN, Gounel S, Staigre P, Mano N: **Site-Directed Immobilization of Bilirubin Oxidase for Electrocatalytic Oxygen Reduction**. *Acs Catalysis* 2019, **9**:2068-2078.
54. Zhang L, Carucci C, Reculusa S, Goudeau B, Lefrancois P, Gounel S, Mano N, Kuhn A: **Rational Design of Enzyme-Modified Electrodes for Optimized Bioelectrocatalytic Activity**. *Chemelectrochem*.
55. Zhang LY, Beaton SE, Carr SB, Armstrong FA: **Direct visible light activation of a surface cysteine-engineered NiFe -hydrogenase by silver nanoclusters**. *Energy & Environmental Science* 2018, **11**:3342-3348.

56. Yanez-Sedeno P, Gonzalez-Cortes A, Campuzano S, Pingarron JM: **Copper(I)-Catalyzed Click Chemistry as a Tool for the Functionalization of Nanomaterials and the Preparation of Electrochemical (Bio)Sensors**. *Sensors* 2019, **19**.
57. Schlesinger O, Pasi M, Dandela R, Meijler MM, Alfonta L: **Electron transfer rate analysis of a site-specifically wired copper oxidase**. *Physical Chemistry Chemical Physics* 2018, **20**:6159-6166.
58. Xia L, Han M-J, Zhou L, Huang A, Yang Z, Wang T, Li F, Yu L, Tian C, Zang Z, et al.: **S-Click Reaction for Isotropic Orientation of Oxidases on Electrodes to Promote Electron Transfer at Low Potentials**. *Angewandte Chemie (International ed. in English)* 2019.
59. Zernia S, Frank R, Weisse RHJ, Jahnke HG, Bellmann-Sickert K, Prager A, Abel B, Strater N, Robitzki A, Beck-Sickinger AG: **Surface-Binding Peptide Facilitates Electricity-Driven NADPH-Free Cytochrome P450 Catalysis**. *Chemcatchem* 2018, **10**:525-530.
60. Lee H, Lee YS, Lee SK, Baek S, Choi IG, Jang JH, Chang IS: **Significant enhancement of direct electric communication across enzyme-electrode interface via nano-patterning of synthetic glucose dehydrogenase on spatially tunable gold nanoparticle (AuNP)-modified electrode**. *Biosensors & Bioelectronics* 2019, **126**:170-177.
61. Milton RD, Abdellaoui S, Khadka N, Dean DR, Leech D, Seefeldt LC, Minteer SD: **Nitrogenase bioelectrocatalysis: heterogeneous ammonia and hydrogen production by MoFe protein**. *Energy & Environmental Science* 2016, **9**:2550-2554.
62. Kornienko N, Ly KH, Robinson WE, Heidary N, Zhang JZ, Reisner E: **Advancing Techniques for Investigating the Enzyme-Electrode Interface**. *Accounts of Chemical Research* 2019, **52**:1439-1448.
63. Zigah D, Lojou E, de Poulpiquet A: **Micro- and Nanoscopic Imaging of Enzymatic Electrodes: A Review**. *ChemElectroChem* **0**.
64. Baker LA: **Perspective and Prospectus on Single-Entity Electrochemistry**. *Journal of the American Chemical Society* 2018, **140**:15549-15559.