Case Western Reserve University

Scholarly Commons @ Case Western Reserve University

Faculty Scholarship

4-27-2016

The Use of Engineered Protein Materials in Electrochemical Devices

Julie N. Renner
Case Western Reserve University, julie.renner@case.edu

Author(s) ORCID Identifier:

Follow this and additional works at: https://commons.case.edu/facultyworks

Recommended Citation

Renner, Julie N., "The Use of Engineered Protein Materials in Electrochemical Devices" (2016). *Faculty Scholarship*. 57.
https://commons.case.edu/facultyworks/57

This Article is brought to you for free and open access by Scholarly Commons @ Case Western Reserve University. It has been accepted for inclusion in Faculty Scholarship by an authorized administrator of Scholarly Commons @ Case Western Reserve University. For more information, please contact digitalcommons@case.edu.

The use of engineered protein materials in electrochemical devices

Julie N. Renner[1] and Shelley D. Minteer[2]

[1]Case Western Reserve University Department of Chemical & Biomolecular Engineering, Cleveland OH 44106

[2]University of Utah Department of Chemistry, Salt Lake City, UT 84112

Abstract

Bioelectrochemical technologies have an important and growing role in healthcare, with applications in sensing and diagnostics, as well as the potential to be used as implantable power sources and be integrated with automated drug delivery systems. Challenges associated with enzyme-based electrodes include low current density and short functional lifetimes. Protein engineering is emerging as a powerful tool to overcome these issues. By taking advantage of the ability to precisely define protein sequences electrodes can be organized into high performing structures, and enable the next generation of medical devices.

Keywords

Protein engineering; bioelectrodes; biocatalysts; bioelectrocatalysis; electrochemical biosensors; enzymatic biofuel cell

Motivation

Enzymatic bioelectrodes already play an important role in the medical community, with the glucose sensor being the most widely used bioelectrode. Approximately 25.8 million people, or 8.3% of the U.S. population, are affected by diabetes.[1] Diabetes is a metabolic disease where the patient cannot make enough insulin or does not respond normally to insulin, which causes blood glucose levels to be abnormal. Currently, diabetic patients must employ a rigorous self-care plan, which often includes self-monitoring of

blood glucose using an enzymatic bioelectrode for quantification of blood glucose in blood samples. One study found that almost one third of patients do not perform these routine tests.[2] Continuous monitoring and drug delivery systems are emerging, but many of these devices still require significant user input with sensor replacement, calibration, and pump management. Bioelectrochemistry can play a role in future technology where sensors, power sources and drug delivery can all be safely implanted and automated, minimizing patient input and giving tighter control over critical health parameters. In order for this vision to be realized for diabetic patients, and other patients who need continuous monitoring and delivery of therapeutics, stable, specific, and high performing enzymatic bioelectrodes need to be developed.

Introduction

Enzyme-based electrodes are increasingly important to the medical community. Many thorough reviews have covered enzyme-based electrodes generally,[3] as well as specifically for use as electrochemical sensors,[4] and as implantable power sources.[5] The use of enzymes in medically relevant electrochemical devices has many advantages because enzymes are biocompatible, highly selective, and efficient catalysts at physiological conditions. However, enzymes have naturally poor conductivity and low volumetric catalytic activity, resulting low current density. In the area of fuel cells, low current density means less power can be generated for a given area, making miniaturization difficult. In the area of sensors, many are amperometric, so low current results in low sensitivity to analytes. Additionally, enzymes often suffer from durability issues making implantation difficult and increasing user input and maintenance.

Nanomaterials and other ordered electrode techniques such as the use of graphene,[6] carbon nanotubes,[7] and other mesoporous materials[8] have significantly increased electrode performance, thus proving that controlling the electrode structure and microenvironment is critical. One specific example directly compared the graphene nanosheets with carbon nanotubes in a membraneless enzymatic biofuel cell showing the use of graphene resulted in 2X higher current density.[9] Recently, proteins[10] and nucleic acids[11] have emerged as a viable approach to engineering electrode structure, particularly for multi-enzyme

cascades, with significant (5 to >50-fold) increases in yield of the desired products[12] and a 50-100% increase in current density.[13]

While all of the tools above are promising, this review will specifically outline how protein engineering has been employed to improve enzyme-based electrode performance. As shown in Figure 1, protein engineering strategies for improving enzyme-based electrode performance are tailored depending on the specific enzyme, reaction and the type of electron transport occurring in the electrode. Electrons can be transported directly from the active redox center to the electrode surface, known as direct electron transfer. This is highly desirable, but difficult, because the active centers are often buried in the enzymes, and far away from the surface of the electrode. Electrons can also be transported via small chemical mediators which diffuse to the redox centers. The potential challenges associated with mediators are cost, toxicity and practical management of the mediator within the device.

General protein engineering approaches for enzyme-based electrodes have included directed evolution and rational design (Figure 2). Table 2 outlines strategies not disused in this review, with relevant references. Enzyme modification has employed both strategies,[14] resulting in more active enzymes, enzymes with expanded reactive capabilities or higher durability. More recently scaffolding techniques have been employed to either decrease the distance for direct electron transfer, manage mediators, or affect the reactivity of the enzymes. Specifically, in this review, recent rational protein engineering strategies for organizing, scaffolding, and metalizing electrode structure will be investigated. The effects of these organized structures on current density and durability will be discussed, since both parameters are important to the success of sensors and fuel cells.

Self-Assembling Protein Systems

Self-assembling protein scaffolds for enzymatic electrodes have the potential to be promising, because enzymes are maintained in a native solution-like environment. In addition, using self-assembly as a strategy

could reduce electrode processing steps and could be envisioned as a potential mechanism for electrode replacement. This section will highlight some self-assembling protein systems used to organize electrode structures.

In 2008, a bioelectrocatalytic hydrogel was designed which self-assembled from bi-functional protein building blocks.[15] Two engineered protein building blocks were used. One was a metallopolypeptide and consisted of physical crosslinking functionality and an electron conducting functionality for transferring the electrons between the enzyme and the electrode. The physical crosslinking occurred via coil-coil association of lucine zipper α-helices, a previously developed system dubbed HSH. In this system, HSH corresponds to the domains such that H represents two α-helices separated by S, a random coil domain. The electron conducting functionality occurred via attachment of osmium bis-bipyridine to strategically placed histidine residues in the HSH-derived polypeptide. The osmium acts as a redox mediator in the hydrogel. The second protein building block used was a modified enzyme, a polyphenol oxidase or small laccase (SLAC), with an H and S domain. Thus, it had duel functionality with enzymatic activity as well as ability to crosslink via the α-helices. The laccase catalyzes the reduction of dioxygen to water, and therefore has applications as an oxygen sensor or as a catalyst for fuel cell cathodes.

Rheology confirmed the formation of hydrogels and three modes of crosslinking were identified which included coil-coil formation, dimer formation of the laccase enzymes, and osmium mediated associations. A mixed hydrogel of the two proteins was deposited on glassy carbon electrodes and electrochemically characterized as a cathode for oxygen reduction. The mixed gel showed catalytic current while no current was observed when nitrogen gas was used, or when an osmium-free protein was used. This showed that the system supported enzyme activity, as well as functional mediators. Importantly, this study established proof-of-concept for this system as a tool for electrode design. The system was recently extended to support a synthetic metabolic pathway involving a cascade of three NAD(H)-dependent dehydrogenase enzymes.[16] This system consisted of three components, two tetrameric enzymes and one dimeric, each expressed with

a physical crosslinking functionality facilitated through an H and S domain. When the enzymes were incorporated together in this system they facilitated the complete oxidation of methanol to carbon dioxide in a biobattery. The device achieved current densities equivalent to other similar systems in literature, with the potential to be further optimized and tuned because of the precise molecular control of engineered proteins. One of the advantages of the protein hydrogel that became apparent in this biobattery was that the ability to load high concentrations of enzymes improves bioelectrode performance. The hydrogel also has the advantage of maintaining the enzymes in a hydrated state, similar to the native environment where the enzymes are active.

Recently, another two-component self-assembling protein hydrogel system was developed and demonstrated using SLAC.[17] This system is different in that mediators were not used and direct electron transfer was observed. Also, each building block contained a physical crosslinking functionality derived from a subunit of the system called CutA, a small (12 kDa) trimeric protein with high temperature stability (up to 150°C). Each building block also contained either a sequence derived from PDZ, a structural domain found in signaling proteins which facilitates protein-protein recognition, or a peptide ligand which recognizes and binds to the PDZ domain. The two functionalities encourage two forms of interaction in the base hydrogel. The PDZ interaction with the peptide ligand could also be reinforced with a disulfide bond. One more form of interaction was inserted into the system, by incorporating a peptide docking station into the components. This docking station allows the hydrogel to be functionalized with docking protein tagged proteins, like SLAC. The hydrogel was mixed with carboxylated multiwalled carbon nanotubes, and the catalytic current generated exceeded that of the previous example with the mediator, and was comparable to other equivalent direct electron transfer examples in literature.

Another emerging technology is self-assembling peptide systems. In one study, ionic-complementary peptides were used to modify highly ordered pyrolytic graphite (HOPG).[18] Atomic force microscopy indicated that the self-assembled peptide layer supported native glucose oxidase whereas the glucose

oxidase adsorbed to the unmodified HOPG appeared to be denatured. Comparing the anodic current between the two samples revealed the peptide-modified surface had higher response. These data suggest that protein systems can improve electrochemical device performance by providing a more enzyme-friendly micro-environment. Another prevalent self-assembly peptide system, which has been extensively reviewed,[19] is a diphenylalanine (FF) peptide inspired by motifs found in Alzheimer's b-amyloid proteins. This peptide can form nanotubes and other structures in aqueous solution, which can give them remarkable electronic and electrochemical properties. They have been used in combination with enzymes (e.g. glucose oxidase, microperoxidase-11, NADH) and have shown improved performance because of the non-mediated electron transfer.

Comparing the current density in these studies, we find a direct comparison can be made between the two systems which used SLAC. In the first system, mediators were used and a current density of 12 $\mu A/cm^2$ at 0.2 V vs. SHE was achieved.[15] In the second SLAC system, direct electron transfer occurred with current density measured over the course of 7 days.[17] An increase in current was observed between 2-3 days from −39.5 to −51.8 $\mu A/cm^2$, attributed to possible swelling of the hydrogel and influx of the reactant, and a decrease in current was observed, stabilizing to about −36.5 $\mu A/cm^2$ at 0.2 V vs. SHE. While many factors can contribute to observed current densities in a device, it is worth noting that equivalent and even higher current densities could be achieved without the use of mediators. In the second SLAC system, the authors attribute the decrease in current density to enzyme leaching. This is corroborated by erosion studies on the same hydrogels, showing the effect of pH, temperature and concentration. Loss from the hydrogels began to stabilize after 7 days showing good temperature stability and stability within a pH range of 6-8. Studies such as these and longer-duration electrochemical tests will be helpful in designing future protein scaffolds for electrochemical devices.

While durability was not characterized, the maximum current density in the biobattery example was 577 ± 26 $\mu A/cm^2$, much higher than the previously discussed examples. This supports the notion that none of

these hydrogel systems were fully optimized, meaning they could be tuned for optimum pore structure, mechanical properties or enzyme loading. Protein engineering will likely be an important tool in understanding the effect of these properties on device performance, because it allows such precise control over molecular architecture.

Metalized Protein Engineered Systems

By organizing enzymes near metal nanoparticles, the effective surface area of the electrode can be increased. In addition, orienting conductive surfaces near the active centers of enzymes can result in lower-tunneling distances. Finally, depending on the electrocatalytic moieties and the metal nanoparticles, hybrid bioelectrocatalysis may occur where both the enzyme and the metal nanoparticle are catalyzing redox reactions.[20] Therefore, a range of metal nanoparticles can be utilized, including gold, which is the most common because it is highly conductive and biocompatible and therefore suitable for use with proteins. However, it should be noted that more active materials such as metal oxides, palladium and platinum have also been utilized. This section outlines protein engineering tools to organize enzyme-based electrode structure through metallization. Strategies not discussed here include modifying enzymes with metal binding sequences, using directed evolution to develop metal binding motifs, and using proteins as destructible templates for inorganic structures.[21] Additionally, peptides and biomolecular catalysts as sensors are not covered since this review is aimed at covering strategies for enzyme-based electrodes.

One protein engineering strategy is to genetically modify the enzymes to place them in close proximity to nanoparticles. While not extensively reviewed here, a few examples are outlined. In one simple example of metallization, a glucose oxidase enzyme was modified to display a free thiol group near the active site.[22] Maleimide-modified gold nanoparticles could then be attached to the enzyme via covalent linkage between the thiol group and the maleimide group. This protein engineering strategy decreased the catalytic activity of the enzyme, but, enabled direct electrical communication from the enzyme to the nanoparticles, and thus the electrode surface. A few recent examples include the strategy of genetically engineering the enzyme to

express a metal-binding peptide (e.g. gold, ZnO and TiO_2), immobilizing the enzymes near a conductive electrode surface.[23]

Metalized peptides have also been used without modifying the enzymes. Efficient electron transfer was shown using a metallized peptide linked to a gold nanoparticle and the enzyme NADH peroxidase.[24] The system consisted of the enzyme joined to a leucine zipper with strategically introduced histidine residues as metal-binding sites. Cobalt was incorporated into this design. This cobalt-binding peptide was attached to a gold nanoparticle and the whole assembly was immobilized onto a gold electrode for characterization. The results suggested electrons are conducted through the cobalt chain, and the electron transfer rate was enhanced by the assembly. In another similar study, the assembly was linked to a carbon nanotube array electrode, and it was proposed that the linker also serves to separate the enzyme from the surface of the carbon nanotubes, preventing unwanted denaturation.[25]

Attempts have been made utilize protein engineering to organize metal structures while simultaneously linking enzymes to these structures. In one example, glucose oxidase was fused to a ring-shaped protein with a functional domain that binds gold nanoparticles in the inner pore via adsorption.[26] The ring-shaped protein can self-assemble into three-dimensional (3D) nanostructures. The design has the advantage of building layers of electrically connected enzymes off of the electrode surface. The 3D organization that took place on the surface facilitated electronic wiring between multiple functional enzymes.

Recently, a “nanomesh” was constructed using bacteriophages in a glucose oxidase fuel cell.[27] The bacteriophages featured an amine-rich shaft, for binding carboxylic acid-functionalized gold nanoparticles via a covalent bond. The glucose oxidase co-factor, flavin adenine dinucleotide (FAD) was covalently bound to the gold nanoparticles, which linked the glucose oxidase to the nanomesh. The phage was engineered to express proteins with a high histidine content, allowing the nanomesh to be anchored to a flat gold electrode. The performance of this electrode was compared to relevant glucose oxidase direct electron

transfer examples, and it was found that the mesh contributed to higher enzyme surface coverage and current densities.

Conclusions and Outlook

Self-assembled protein networks and metallization strategies are promising techniques to increase enzyme-based electrode performance and durability, protecting enzymes from denaturation and bringing conductive and reactive elements in close proximity to their active sites. Scientists and engineers have only begun to explore protein engineering tools to control electrode structures. Many peptide tools for metallization[28] and hydrogel systems[29] exist, allowing great flexibility in the control of properties for enzyme-based electrodes. Understanding and controlling parameters such as pore structure, metallization, mechanical properties, hydrophobicity/hydrophilicity and spatial organization will lead to long-lasting, specific, and miniaturized electrochemical devices.

Acknowledgments

SDM would like to thank the Air Force Office of Scientific Research for funding.

Author Contribution Statement

All authors participated in the drafting, critical revision, and final approval of this article.

REFERENCES

1. Centers for Disease Control and Prevention. National diabetes fact sheet: national estimates and general information on diabetes and prediabetes in the United States. 2011.

2. Hansen, M. V.; Pedersen-Bjergaard, U.; Heller, S. R.; Wallace, T. M.; Rasmussen, A. K.; Jorgensen, H. V.; Pramming, S.; Thorsteinsson, B., Frequency and motives of blood glucose self-monitoring in type 1 diabetes. *Diabetes Research and Clinical Practice* **2009,** *85* (2), 183-188.

3. (a) Sarma, A. K.; Vatsyayan, P.; Goswami, P.; Minteer, S. D., Recent advances in material science for developing enzyme electrodes. *Biosensors & Bioelectronics* **2009,** *24* (8), 2313-2322; (b) Rasmussen, M.; Abdellaoui, S.; Minteer, S. D., Enzymatic biofuel cells: 30 years of critical advancements. *Biosensors & Bioelectronics* **2016,** *76*, 91-102.

4. Ispas, C. R.; Crivat, G.; Andreescu, S., Review: Recent Developments in Enzyme-based Biosensors for Biomedical Analysis. *Anal. Lett.* **2012,** *45* (2-3), 168-186.

5. (a) Barton, S. C.; Gallaway, J.; Atanassov, P., Enzymatic biofuel cells for Implantable and microscale devices. *Chem. Rev.* **2004,** *104* (10), 4867-4886; (b) Willner, I.; Yan, Y. M.; Willner, B.; Tel-Vered, R., Integrated Enzyme-Based Biofuel Cells-A Review. *Fuel Cells* **2009,** *9* (1), 7-24; (c) Minteer, S. D.; Liaw, B. Y.; Cooney, M. J., Enzyme-based biofuel cells. *Curr. Opin. Biotechnol.* **2007,** *18* (3), 228-234.

6. Karimi, A.; Othman, A.; Uzunoglu, A.; Stanciu, L.; Andreescu, S., Graphene based enzymatic bioelectrodes and biofuel cells. *Nanoscale* **2015,** *7* (16), 6909-6923.

7. Cosnier, S.; Holzinger, M.; Le Goff, A., Recent advances in carbon nanotube-based enzymatic fuel cells. *Frontiers in bioengineering and biotechnology* **2014,** *2*, 45.

8. (a) Catalano, P. N.; Wolosiuk, A.; Soler-Illia, G.; Bellino, M. G., Wired enzymes in mesoporous materials: A benchmark for fabricating biofuel cells. *Bioelectrochemistry* **2015,** *106*, 14-21; (b) Liu, Y.; Du, Y.; Li, C. M., Direct Electrochemistry Based Biosensors and Biofuel Cells Enabled with Nanostructured Materials. *Electroanalysis* **2013,** *25* (4), 815-831.

9. Liu, C.; Alwarappan, S.; Chen, Z. F.; Kong, X. X.; Li, C. Z., Membraneless enzymatic biofuel cells based on graphene nanosheets. *Biosensors & Bioelectronics* **2010,** *25* (7), 1829-1833.

10. (a) Caruana, D. J.; Howorka, S., Biosensors and biofuel cells with engineered proteins. *Mol. Biosyst.* **2010,** *6* (9), 1548-1556; (b) Guven, G.; Prodanovic, R.; Schwaneberg, U., Protein Engineering - An Option for Enzymatic Biofuel Cell Design. *Electroanalysis* **2010,** *22* (7-8), 765-+.

11. Nguyen, K. V.; Minteer, S. D., Investigating DNA hydrogels as a new biomaterial for enzyme immobilization in biobatteries. *Chem. Commun.* **2015,** *51* (66), 13071-13073.

12. Lin, J. L.; Palomec, L.; Wheeldon, I., Design and Analysis of Enhanced Catalysis in Scaffolded Multienzyme Cascade Reactions. *Acs Catalysis* **2014,** *4* (2), 505-511.

13. Nguyen, K. V.; Giroud, F.; Minteer, S. D., Improved Bioelectrocatalytic Oxidation of Sucrose in a Biofuel Cell with an Enzyme Cascade Assembled on a DNA Scaffold. *J. Electrochem. Soc.* **2014,** *161* (14), H930-H933.

14. Prabhulkar, S.; Tian, H.; Wang, X. T.; Zhu, J. J.; Li, C. Z., Engineered Proteins: Redox Properties and Their Applications. *Antioxid. Redox Signal.* **2012,** *17* (12), 1796-1822.

15. Wheeldon, I. R.; Gallaway, J. W.; Barton, S. C.; Banta, S., Bioelectrocatalytic hydrogels from electron-conducting metallopolypeptides coassembled with bifunctional enzymatic building blocks. *Proceedings of the National Academy of Sciences of the United States of America* **2008,** *105* (40), 15275-15280.

16. Kim, Y. H.; Campbell, E.; Yu, J.; Minteer, S. D.; Banta, S., Complete Oxidation of Methanol in Biobattery Devices Using a Hydrogel Created from Three Modified Dehydrogenases. *Angewandte Chemie-International Edition* **2013,** *52* (5), 1437-1440.

17. Guan, D.; Ramirez, M.; Shao, L.; Jacobsen, D.; Barrera, I.; Lutkenhaus, J.; Chen, Z., Two-Component Protein Hydrogels Assembled Using an Engineered Disulfide-Forming Protein-Ligand Pair. *Biomacromolecules* **2013,** *14* (8), 2909-2916.

18. Yang, H.; Fung, S. Y.; Pritzker, M.; Chen, P., Ionic-Complementary Peptide Matrix for Enzyme Immobilization and Biomolecular Sensing. *Langmuir* **2009,** *25* (14), 7773-7777.

19. (a) Kim, S.; Kim, J. H.; Lee, J. S.; Park, C. B., Beta-Sheet-Forming, Self-Assembled Peptide Nanomaterials towards Optical, Energy, and Healthcare Applications. *Small* **2015,** *11* (30), 3623-3640; (b) Adler-Abramovich, L.; Gazit, E., The physical properties of supramolecular peptide assemblies: from building block association to technological applications. *Chem. Soc. Rev.* **2014,** *43* (20), 6881-6893.

20. Aquino Neto, S.; Almeida, T. S.; Palma, L. M.; Minteer, S. D.; de Andrade, A. R., Hybrid nanocatalysts containing enzymes and metallic nanoparticles for ethanol/O2 biofuel cell. *J. Power Sources* **2014,** *259*, 25-32.

21. Dickerson, M. B.; Sandhage, K. H.; Naik, R. R., Protein- and Peptide-Directed Syntheses of Inorganic Materials. *Chem. Rev.* **2008,** *108* (11), 4935-4978.

22. Holland, J. T.; Lau, C.; Brozik, S.; Atanassov, P.; Banta, S., Engineering of Glucose Oxidase for Direct Electron Transfer via Site-Specific Gold Nanoparticle Conjugation. *J. Am. Chem. Soc.* **2011,** *133* (48), 19262-19265.

23. (a) Yucesoy, D. T.; Karaca, B. T.; Cetinel, S.; Caliskan, H. B.; Adali, E.; Gul-Karaguler, N.; Tamerler, C., Direct bioelectrocatalysis at the interfaces by genetically engineered dehydrogenase. *Bioinspired Biomim. Nanobiomat.* **2015,** *4* (1), 79-89; (b) Simmerman, R. F.; Zhu, T.; Baker, D. R.; Wang, L. J.; Mishra, S. R.; Lundgren, C. A.; Bruce, B. D., Engineering Photosystem I Complexes with Metal Oxide Binding Peptides for Bioelectronic Applications. *Bioconjugate Chem.* **2015,** *26* (10), 2097-2105.

24. Yeh, J. I.; Zimmt, M. B.; Zimmerman, A. L., Nanowiring of a redox enzyme by metallized peptides. *Biosensors & Bioelectronics* **2005,** *21* (6), 973-978.

25. Yeh, J. I.; Lazareck, A.; Kim, J. H.; Xu, J.; Du, S., Peptide nanowires for coordination and signal transduction of peroxidase biosensors to carbon nanotube electrode arrays. *Biosensors & Bioelectronics* **2007,** *23* (4), 568-574.

26. Frasconi, M.; Heyman, A.; Medalsy, I.; Porath, D.; Mazzei, F.; Shoseyoy, O., Wiring of Redox Enzymes on Three Dimensional Self-Assembled Molecular Scaffold. *Langmuir* **2011,** *27* (20), 12606-12613.

27. Blaik, R. A.; Lan, E.; Huang, Y.; Dunn, B., Gold-Coated M13 Bacteriophage as a Template for Glucose Oxidase Biofuel Cells with Direct Electron Transfer. *ACS Nano* **2016,** *10* (1), 324-332.

28. Care, A.; Bergquist, P. L.; Sunna, A., Solid-binding peptides: smart tools for nanobiotechnology. *Trends in Biotechnology* **2015,** *33* (5), 259-268.

29. Banta, S.; Wheeldon, I. R.; Blenner, M., Protein Engineering in the Development of Functional Hydrogels. In *Annual Review of Biomedical Engineering, Vol 12*, Yarmush, M. L.; Duncan, J. S.; Gray, M. L., Eds. Annual Reviews: Palo Alto, 2010; Vol. 12, pp 167-186.

30. (a) Gilardi, G.; Fantuzzi, A.; Sadeghi, S. J., Engineering and design in the bioelectrochemistry of metalloproteins. *Curr. Opin. Struct. Biol.* **2001,** *11* (4), 491-499; (b) Campas, M.; Prieto-Simon, B.; Marty, J. L., A review of the use of genetically engineered enzymes in electrochemical biosensors. *Semin. Cell Dev. Biol.* **2009,** *20* (1), 3-9.

31. Pavan, S.; Berti, F., Short peptides as biosensor transducers. *Anal. Bioanal. Chem.* **2012,** *402* (10), 3055-3070.

32. Schoffelen, S.; van Hest, J. C. M., Chemical approaches for the construction of multi-enzyme reaction systems. *Curr. Opin. Struct. Biol.* **2013,** *23* (4), 613-621.

Figure Legends

Figure 1: Protein engineering is a promising tool for addressing the challenges associated with enzyme-based electrodes.

Figure 2: Protein engineering approaches for enzyme-based electrodes. Topics in grey are covered in this mini-review.