Improved biosensing through engineered microbe-electrode interfaces

by
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Abstract

Electroactive microorganisms (EAMs) have the unique ability to generate electricity by exchanging free electrons with their environment, either abiotic substrates or other microbes. As a result, EAMs can interface directly with electronic devices, enabling their application in biohybrid technologies with unique and useful properties. In particular, the model EAM *Shewanella oneidensis* has been harnessed for a number of systems, including portable sensors and bioreactors that utilize electricity as an energy input or output. Unfortunately, the commercial viability of these technologies remains limited by several factors, including poor kinetics of microbial electron transfer, high device complexity, and limited tools for engineering EAMs. **In this thesis, I address these limitations by developing electrocatalyst materials, device architectures, and novel approaches to EAM engineering that improve the performance, function, and versatility of microbial electronics.**

Electrochemical current generation by *S. oneidensis* is slow, a critical issue that contributes to slow response times and poor device sensitivity for sensors, as well as low conversion in catalytic processes. I show that current generation is significantly improved by interfacing *S. oneidensis* with an electrocatalyst that mimics biological electron transfer. Specifically, I show that the imidazolium-functionalized poly(3-hexyl)thiophene (P3HT-Im^+) improves per-cell electricity generation more than five-fold by catalyzing electron transfer from flavin mononucleotide (FMN), the mediator used by *S. oneidensis* for electron shuttling. P3HT-Im^+ promotes concerted two-electron transfer from FMN, a reaction which was previously restricted to enzymes. To enable first principles-based engineering of improved electrocatalysts, I elucidated the mechanism underlying the P3HT-Im^+-derived improvements, showing that P3HT-Im^+ promotes biosimilar electron transfer by sequestering FMN in molecular pockets that alter its thermodynamic landscape. To connect the physical properties of P3HT-Im^+ to this observed mechanistic change, I demonstrate that the ionic conductivity of P3HT-Im^+ enables it to envelop reacting molecules. The achievement of rapid microbial electron transfer to P3HT-Im^+ improves the prospects of electroactive microbes for both sensing and catalysis and establishes a novel catalytic strategy for electron transfer across biotic-abiotic interfaces.

Building on lessons from studying the microbe-electrode interface, I develop a microbial sensor device for point-of-use molecular detection that is substantially less complex than state-of-the-art designs. Using a series of simple yet robust signal transduction steps, I integrate electroactive microbes into a resonant frequency identification (RFID) tag, enabling wireless communication between microbes engineered to sense a particular compound and a user’s smartphone. This microbial RFID sensor is more than 80-fold less costly than leading alternatives and can be operated without training, eliminating major barriers to the commercialization of microbial electronic devices for point-of-need molecular detection.

Finally, I improve upon these microbial sensors by developing tools to engineer *S. oneidensis* strains to detect an arbitrary molecule of interest. Transcription efficiency has been identified as a key challenge when using foreign genetic parts in *S. oneidensis*, which I solve through tuning translation efficiency. I have also developed an efficient strategy for selecting and screening exogenous sensor proteins and demonstrate its effectiveness by engineering *S. oneidensis* to detect several agriculture-relevant soil nutrients. By developing a protein degradation system to continuously remove reporter proteins produced in response to an analyte, I further enable cycling of on/off sensing. These cell engineering principles enable the application of microbial electrochemical sensors to a wider range of analytes, enabling their use in previously unexplored applications such as agriculture.

Collectively, the findings described in this body of work substantially improve the prospects of microbial electrochemical technologies for commercialization by enhancing microbial electricity generation, simplifying the architecture of microbial electronic devices, and developing tools to accelerate the engineering of electroactive microbes for sensing. These advances set the stage for microbial electrochemical technologies to address diverse technical challenges including point-of-need molecular detection, renewable energy generation, and carbon dioxide fixation.

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