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Investigating limitations of biohybrid photoelectrode using synchronized spectroelectrochemistry

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Abstract

The challenge in optimizing biohybrid photoelectrodes lies in identifying kinetic bottleneck and energy loss of photoinduced electron transfer. In this issue of *Joule*, Friebe's group describes a method for synchronized spectroscopic and electrochemical measurements of biophotoelectrode *in operando*, which indicates the electron transfer bottleneck steps and the energy loss processes.

Photosynthesis is a natural process presented in plants, algae, and bacteria that converts solar energy into chemical energy at a rate of of 100 terawatts.[1] In contrast to the relatively low solar-to-biomass efficiency of the overall photosynthesis in plants (~1%), the quantum efficiency in the compartmentalized light-harvesting reaction center, such as the reaction center in *Rhodopseudomonas spheroids* containing the bacteriochlorophyll pigment, could approach 100%.[2,3] The perfection of energy conversion of the light-harvesting reaction center has encouraged research into the configuration of biophotoelectrodes by integrating natural and artificial catalysts to achieve decent energy efficiency and product selectivity. However, the recombined biohybrids are mostly not comparable to their counterparts in nature in terms of energy efficiency. The reduced efficiency in the biophotoelectrode could be explained by changes in the operating environment of the reaction center, such as interfacing with foreign cofactors and separating from the membrane lipid bilayer, which used to be an insulator preventing short-circuiting electron transfer between electron carriers. Several studies on biophotoelectrodes have unraveled the mechanisms impacting the electron transfer kinetic and energy efficiency, including excitation energy loss, electron transfer bottlenecks between cofactors and reaction center, and short-circuiting events.[4–6] These mechanisms are impacted by a variety of factors, including electrode configuration, selection and concentration of cofactors, applied potentials, etc. Such multivariate dynamics make it challenging to identify kinetic bottlenecks and energy loss processes and leaves researchers in a maze when trying to optimize the system. There is a quest for analytical tools for characterizing electron transfer event kinetics and quantifying energy efficiency in sophisticated biohybrid photoelectrode systems.

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DECLARATION OF INTERESTS

The authors declare no competing interest.

In this issue of *Joule*, Friebe's group configured a system synchronizing the spectroscopic and electrochemical measurements of a biohybrid photoelectrode.[7] By using the spectroscopic characteristics of bacteriochlorophyll pigment, a quantitative description of the redox transition in the reaction center can be made. In combination with simultaneous electrochemical measurement of photocurrent, electron transfer bottlenecks were identified, and energy loss was quantified in the developed system.

Friebe's group configured the biohybrid photoelectrode by absorbing the reaction-center light-harvesting-1 (RC-LH1) complex purified from the photosynthetic bacterium *Rhodobacter sphaeroides* and cytochrome *c* (cyt *c*) from equine heart on a semi-transparent mesoporous indium tin oxide (mITO) electrode. The 2,3-dimethoxy-5-methyl-p-benzoquinone (Q_0) was provided as an electron acceptor to RC-LH1 complex for the charge separation (Figure 1A). The mITO|cyt c|RC-LH1 working electrode, reference electrode (3 M KCl Ag/AgCl), and counter electrode (platinum wire) were immersed in the electrolyte containing 5 mM Q_0 and 50 mM KCl in a custom spectroelectrochemical cell equipped with a ms/μs pump-probe spectrophotometer and excitation sources (i.e., LED at 590 nm and laser at 532 nm) (Figure 1B). The photocathodic current was controlled at an applied potential of +160 mV versus SHE (standard hydrogen electrode), which can reduce cyt *c* without reducing terminal electron acceptor (i.e., Q_0). The mITO|*c*yt c|RC-LH1 was excited at a pulsed single turnover laser flash (5 ns) using a saturating laser (532 nm), which was sufficient to induce the complete photochemical reaction in RC-LH1 for one charge separation. A pair of bacteriochlorophyll forms the primary electron donor (P_{870}) inside the RC-LH1 complex showing the maximal amplitude on the absorbance spectrum at a wavelength of 870 nm. The ratio between oxidized P_{870}^+ and reduced P_{870} could be quantitatively examined by spectroscopic analysis, based on their different signal responses. In a single turnover laser flash, the total amount of photoexcited P_{870}^+ was determined to be 95 pmol cm^{-2} by spectroscopic analysis in combination with absolute quantification of the loaded RC-LH1. The post-flash recovery of P_{870} occurred within μs to s and was accompanied by oxidation of cyt c^{2+} to cyt c^{3+}. Simultaneously, the electric current that could reduce cyt c^{3+} and regenerate cyt c^{2+} in a single turnover laser flash was measured electrochemically and converted to approximately 100 pmol cm^{-2} of cyt c^{3+} regeneration. The approximate 1:1 stoichiometry between the photoexcited P_{870}^+ and accumulative cyt c^{3+} regeneration indicated that the quantum efficiency in a single photochemical event per RC-LH1 complex is close to 100%. Such a high quantum efficiency can be maintained in laser flashes with 20 turnovers.

The performance of mITO|cyt *c*|RC-LH1 was also tested under continuous actinic illumination while the quantum efficiency dropped to 11%. The efficiency loss in the developed system could be derived from, first, the loss of excitation energy when "inactive" P_{870} is present in the RC-LH1 complex; second, excitation quenching resulting from poor electron separation kinetics presented in the cyt *c*–RC-LH1–Q_0 electron transport chain; and third, short circuits between the interfaced electron carriers (Figure 1C). The methodological design of simultaneous characterization of the RC-LH1 working state and photocurrent intensity by using absorbance spectroscopy and electrochemistry, respectively, enables authors to understand the kinetic behavior and identify the electron transfer bottleneck *in operando*. P_{870} can present three states in the RC-LH1 complex, including oxidized, open,

and closed states. P_{870} in the open state is capable of conducting photochemical reactions, while P_{870} in the oxidized and closed state is considered to be “inactive” in mediating electron transfer by photoexcitation. The fraction of P_{870} in different states can be analyzed by absorption difference in spectroscopy. During the continuous actinic illumination, only 40% of the P_{870} stayed in the open state and resulted in an electron transfer rate (ETR) of 44 e^{-1} s^{-1} per RC-LH1, suggesting the substantial excitation energy loss may be due to the presence of a large fraction of “inactive” pigments (i.e., closed and oxidized P_{870}). Furthermore, the accumulation of closed P_{870} and oxidized P_{870}^{+} indicated that the electron transfer bottlenecks restrict the electron flow between RC-LH1 to an electron acceptor (i.e., Q_0) and to an electron donor (i.e., cyt c), respectively. Control experiments revealed that as Q_0 was reduced from 5 mM to 1 mM, the electron transfer bottleneck became increasingly severe, and the percentage of closed P_{870} increased from ~25% to ~70%. Multivariate studies demonstrated that applied potential, light intensity, and thickness of the electrode all play crucial roles in regulating P_{870} populations in different states and affecting efficiency.

In combination with the spectroscopic analysis, electrochemical analysis recorded the photocurrent intensity and converted it into a comparable electrochemistry-derived ETR, also known as turnover frequency. The spectroscopy-derived ETR reflects the electron turnover in RC-LH1 complex accounting for quenching, short-circuiting, and leaks, whereas the electrochemistry-derived ETR measures only the electron exchange occuring at the interface between the mITO electrode and the mobile electron acceptors (e.g., cyt c and Q_0). The electrochemistryderived ETR of 12 e^{-1} s^{-1} per RC-LH1 is significantly lower than spectroscopy-derived ETR of 44 e^{-1} s^{-1} per RC-LH1. The 73% loss in efficiency was predominantly caused by the short circuit between Q_0 and the mITO electrode.

Although mechanisms of efficiency loss have been theorized in previous studies,[4,5] it is challenging to identify the kinetic and efficiency limits of a desired electron transfer process under the influence of multiple variables in practice. The design of synchronizing spectroscopic and electrochemical analysis of the semi-transparent biohybrid mITO|cyt *c*|RC-LH1 system *in operando* provides a versatile platform to deconvolute the processes limiting photocurrent generation and can guide the optimization of biohybrid photoelectrodes to improve kinetics and efficiency. This approach is, in the meantime, applicable to the measurement of other biohybrid systems containing light-harvesting centers of photosystem I and II, which provides a practical tool for diagnosing and optimizing biophotoelectrodes that perform solar-to-chemical conversion.

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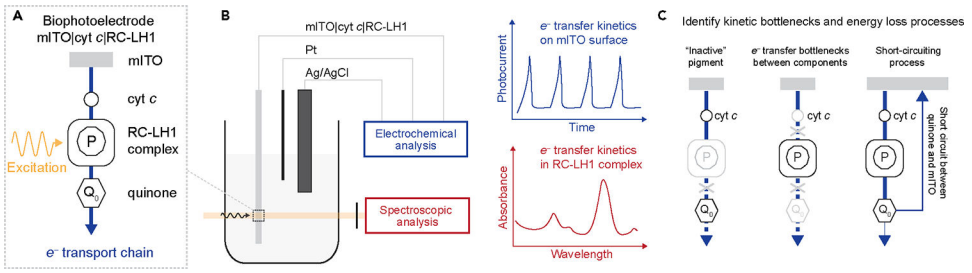

Figure 1. Application of synchronized electrochemical and spectroscopic analysis to study the mITO|cyt c|RC-LH1 biophotoelectrode

(A) Schematic diagram of the mITO|cyt *c*|RC-LH1 biophotoelectrode.

(B) Synchronized electrochemical and spectroscopic analysis of the biophotoelectrode during operation.

(C) Illustration of the identified electron transfer bottlenecks and energy loss processes. All figures have been redrawn from the recent publication of the Friebe group.[7]