Integrating a photosynthetic protein into a bio-solar-photovoltaic for electricity production

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BACKGROUND

Cyanobacteria perform oxygenic photosynthesis, in which electrons generated from water splitting by photosystem II (PSII) are transferred to the cytochrome b_6f complex via a plastoquinone pool, to a soluble cytochrome, to photosystem I (PSI).

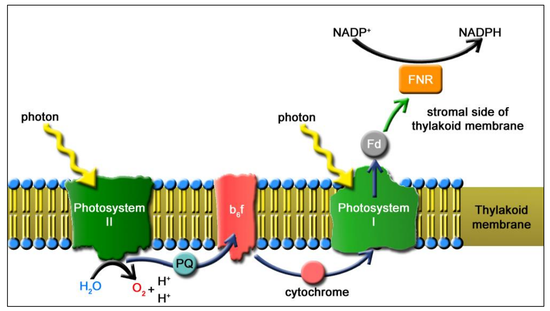

Figure 1. Electron transport chain in thylakoid membrane.

PSI transfers electrons to ferredoxin (Fd) where it is used by Fd-NADP$^+$ reductase (FNR) to reduce NADP$^+$ to NADPH for CO_2 fixation in the Calvin cycle. Light-induced charge separation in PSI allows for electron transfer through the complex. The quantum efficiency of PSI is near unity, and the power conversion efficiency is much higher than typical semi-conductor-based solar materials. By replacing the diffusion-limited electron transfer to Fd with a direct linkage to a conductive surface, the rate of electron turnover can be doubled, in comparison to natural photosynthesis.

Sortases are enzymes found in Gram-positive bacteria, used to attach proteins to the cell wall. Sortase A from *Staphylococcus aureus* cleaves the threonine-glycine peptide bond in a cell wall "sorting tag" (amino acid sequence LPXTG) to form a thioacyl intermediate. The amine group of a $(Gly)_n$ unit attacks the complex, resulting in an LPXT$(G)_n$ link. This technique can be used to couple proteins modified to contain the recognition sequence to a surface decorated with glycines, n ≥ 3.

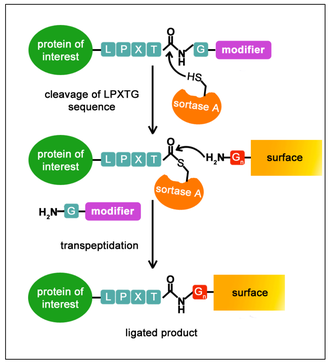

Figure 2. Generalized sortase-mediated linkage of protein with desired modifier peptide. The thiol group of the active site cysteine residue cleaves the amide bond between T and G of LPXTG motif, forming an acyl-enzyme intermediate. The thioester intermediate is attacked by an amino group of an oligo-G peptide forming a protein-surface ligated product.

PROJECT OVERVIEW & RATIONALE

The goal of this work is generate a system for photovoltaic applications using the PSI protein complex from *Synechocystis* sp. PCC 6803. The interdisciplinary nature of this work employs knowledge of biomolecular engineering, biochemistry, and electrochemistry to develop a biosolar nanodevice as a solar-based alternative to fossil fuels for electricity production, while considering the economic, environmental, and social impacts, the fundamentals of sustainability, which are integral to STAIR projects.

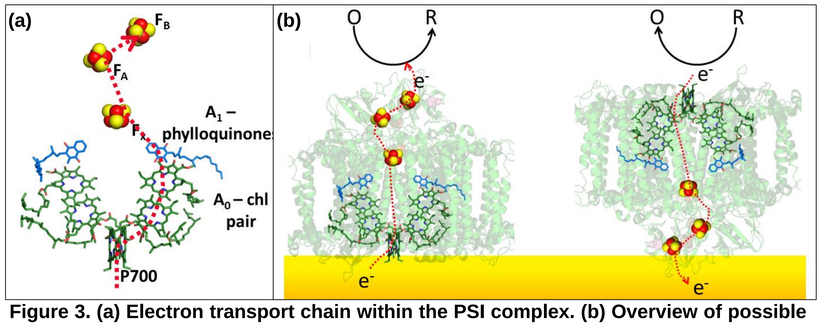

Figure 3. (a) Electron transport chain within the PSI complex. (b) Overview of possible orientation and directionality of electron transport in PSI-gold system. Electrons are transferred from the P700 special chlorophyll pair to an intermediary chlorophyll pair (A_0), to the phylloquinone (A_1), and finally to the proximal, medial (F_A), and distal (F_B) iron-sulfur clusters.

We have used the highly specific sortase-mediated ligation (SML) reaction to link PSI to a conductive surface to produce electrical current when exposed to light. The purpose for using a highly specific reaction is to increase the net current produced, which is lowered by mixed orientation of PSI, where anodic and cathodic currents are competing. From the perspective of the working gold electrode an anodic current is one where an e^- is supplied by the surface and is donated to the P700 special chlorophyll pair through the electron transport chain of PSI to the distal iron-sulfur cluster (F_B) and ultimately to an oxidized species which becomes reduced. Conversely, a cathodic current occurs when a redox mediator donates its e^- to P700 through the iron-sulfur clusters, and the e^- is accepted by the gold surface.

We favor the orientation with the iron-sulfur clusters proximal to the gold surface (Figure 3b, right) because of the potential for using a molecular wire to enhance electron transfer and the distance between the iron sulfur cluster to the surface would be more favorable. The iron sulfur clusters are more accessible compared to the P700 special chlorophyll pair buried within the complex, making it more difficult for a soluble electron carrier to access the P700, unless a mediator was directly bound to the reaction center.

METHODS

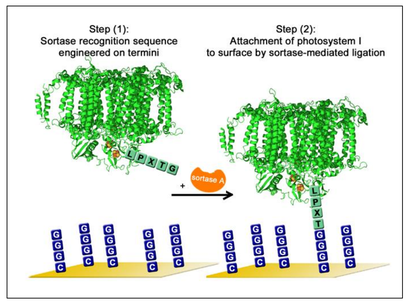

Figure 4. Sortase-mediated ligation of PSI to a surface scheme for enhanced e^- transfer.

- PSI engineered by basic molecular cloning techniques and homologous recombination in *Syn*. 6803 to contain sortase recognition sequence (LPXTG) at exposed C-terminus
- Gold surfaces decorated with tri-glycine peptide by incubating with peptide solution forming thiol bonds with surface
- Surfaces incubated with solution containing modified PSI and sortase to attach the PSI to the surface via SML

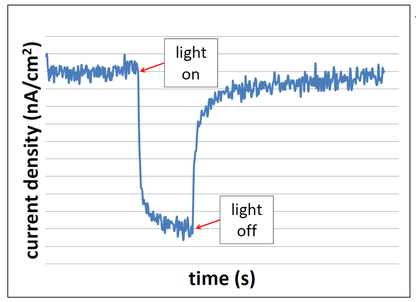

Figure 5. Example of photochronoamperometry data of PSI-gold system.

- Electrochemically characterized surface using photochronoamperometry
- Applied constant potential and measured current density when light turned on, as a function of time
  - Protein is photoactive; response produced only in presence of light
- Probed at open circuit potential, current = zero, system at equilibrium before exposure to light

RESULTS

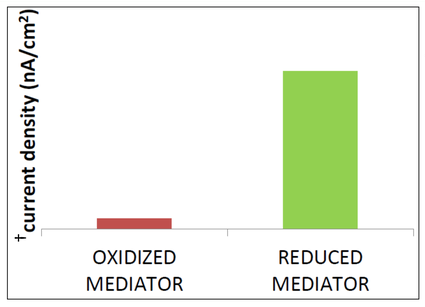

Figure 6. Current density for sortase-mediated ligated PSI-gold system in oxidized mediator vs. reduced mediator.

- Preliminary experiment to determine orientation of protein using photochronoamperometry in oxidized or reduced redox mediator yielded expected results, assuming PSI in desired orientation
  - In presence of reduced redox mediator, significantly higher current density (10X) compared to oxidized mediator

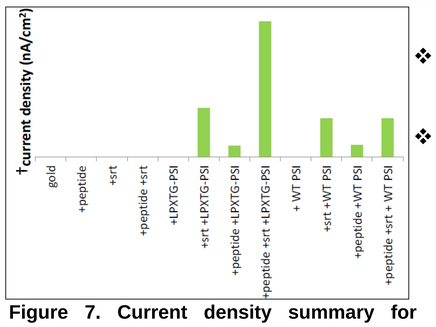

Figure 7. Current density summary for possible controls for sortase-mediated ligation system.

- Bare gold, peptide alone, sortase alone produce 0 current, as expected
- LPXTG-PSI and wild-type PSI produce 0 current in absence of sortase and peptide
  - No non-specific binding of PSI or non-specific binding occurred – anodic and cathodic currents cancel → 0 net current

CONCLUSIONS

- Photochronoamperometry in presence of reduced redox mediator suggests desired and uniform orientation is dominant due to 10X higher current density when compared to oxidized mediator; electrons could only travel from P700 to F_B to surface, minimal electron donation from surface occurred
- LPXTG-PSI, peptide, and sortase must be present to achieve increased current density, for this system, producing highest current compared to non-specific binding controls; addition of sortase and peptide with PSI mutant and WT make surface conditions more prone to non-specific binding
- Sortase-mediated ligation of PSI to a surface is a useful tool for orienting proteins to surfaces for enhanced protein orientation and electricity production by producing higher current densities than comparable systems to date
- Highest current density produced in presence of sortase-recognition sequence containing PSI, tri-glycine peptide, and sortase
  - System produces current density on order of 150–250 nA/cm^2, un-oriented comparable systems produce 80–100 nA/cm^2

ACKNOWLEDGMENTS

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†Full data not disclosed due to upcoming publication.