Supplementary Information for "Optical properties of Bacteriorhodopsin-Gold Bionano Interfaces"

Subhabrata Das",[†] Teguh Citra Asmara",[‡,¶] Abhirup Patra,[§] Zhaoning Song,[‖] Sandip Singh Bista,[‖] Ponisseril Somasundaran,[†] Andrivo Rusydi,[*,⊥] Bernardo Barbiellini,[*,#,@] and Renugopalakrishnan Venkatesan[*,△,∇]

†*Langmuir Center of Colloids and Interfaces, Columbia University in the City of New York, New York, NY 10027, USA*

‡*Department of Physics, National University of Singapore, 21 Lower Kent Ridge Rd, Singapore 119077*

¶*Photon Science Division, Paul Scherrer Institute, CH-5232 Villigen PSI, Switzerland*

§*School of Materials Science and Engineering, Georgia Institute of Technology, Atlanta, GA 30332*

‖*Department of Physics and Astronomy,University of Toledo, OH 43606, United States*

⊥*Department of Physics, National University of Singapore*

#*Department of Physics, LUT University, FI-53851 Lappeenranta, Finland*

@*Department of Physics,Northeastern University, Boston, Massachusetts 02115, USA*

△*Department of Chemical Biology, Center for Renewable Energy, Northeastern University, Boston,Massachusetts 02115, United States*

∇*Boston Children's Hospital, Harvard Medical School, Boston, MA 02115, USA*

E-mail: phyandri@nus.edu.sg; bernardo.barbiellini@lut.fi; v.renugopalakrishnan@northeastern.edu

Preparation of bR mutants

An extensive suite of Bacteriorhodopsin (bR) mutants with each mutant designed for specific technological application in bioelectronics have been made possible by recent advances in protein engineering. At least two classes of these bR mutants of several single and multiple mutants of the four negatively-charged glutamate side chains (Glu9, Glu74, Glu194 and Glu204) located in the extracellular (EC) region of bR are suited for development of excitonic solar cell. In fact, mutations E9Q/E194Q/E204Q in bR (3Glu BR) introduce biophysical interactions that alter the wells and barriers in the energy landscape of the protein.[1] Both residues (Glu194 and Glu204) are recognized as essential elements of the proton release mechanism[2] which is pivotal in solid-state solar cell development. We have targeted residue 36 in the sequence of bR as a strong candidate for mutating a Ser 36 to Cys towards the extracellular region.

Cloning, Expression, and Purification of Cys 36 bR

Mutant D36C of bR was obtained by the common plasmid transfection method of a bop- strain of *Halobacterium salinarum* by applying Mevoline resistance on the plasmid for the selection of transfected strains. During cell lyses and purple membrane (PM) isolation, a reductive environment was maintained by mercapto-ethanol solvent. Purified material was stored in distilled water with a 50-fold molar excess of 2-mercaptoethanol solution, in order to prevent irreversible auto-oxidation of the thiol functional group, at 2^0C to employ a genetically engineered cysteine for serine in the 36 amino acid position of bR compared to reports in literature.[2,3]

Surface Characterization of Bacteriorhodopsin-Gold interface

The bR within its natural PM can be visualized with a confocal microscope and atomic force microscope (AFM), as illustrated in Figure S1 (a,b) and (c, d), where the PM for Cys and WT

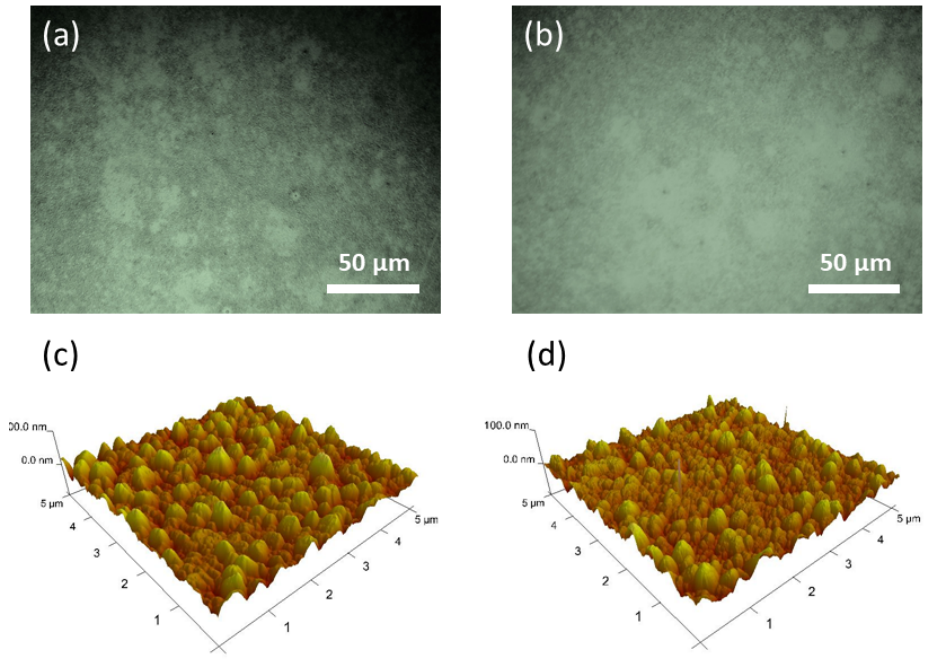

Figure S1: (a, b) Confocal microscopic and (c, d) AFM images of Cys and WT bR deposited on Au coated glass substrates.

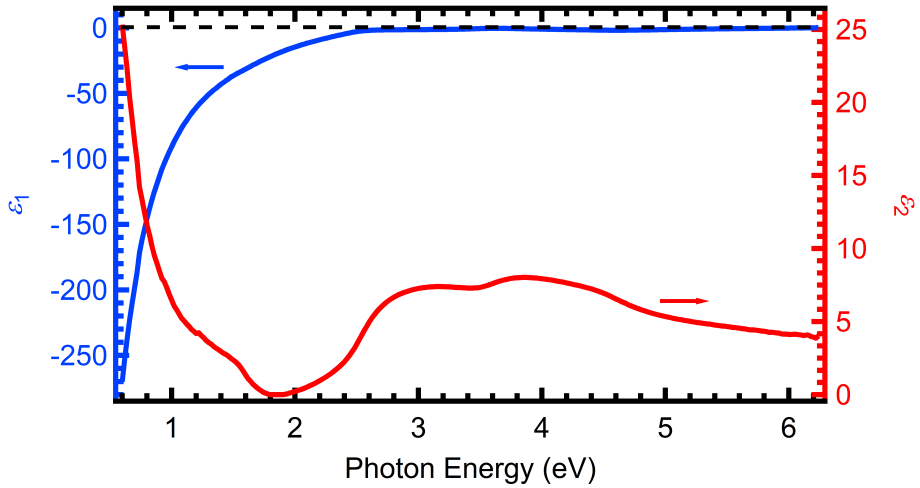

Figure S2: Ellipsometry Data fitting for Au substrate.

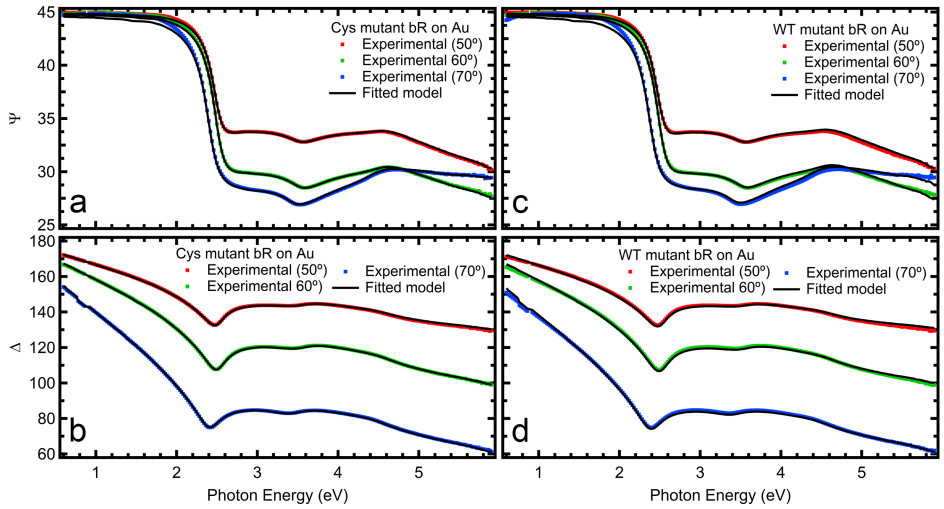

Figure S3: Ellipsometry Data fitting for Cys-bR and WT bR.

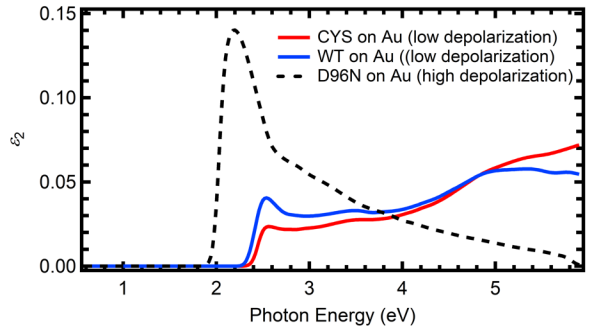

Figure S4: Fitted imaginary part of dielectric function, $\varepsilon_2(\omega)$, of D96N mutant bR film on Au compared with those of CYS and WT bR films on Au.

bR were deposited on Au coated glass substrates. Both images showed uniform deposition of the bR with formation of highly oriented monolayers of bR in PM. The microscopic images were obtained by a confocal microscope (Olympus) with a digital camera under green light illumination. The AFM measurements were carried out using a Veeco Nanoscope IIIA Multimode Atomic Force Microscope with a scan area of 5μm$\times5\mu$m. Surface roughness determined by AFM are 9.9 and 11.8 nm for Cys and WT bR, respectively, indicating smooth surface of bR films.

Spectroscopic Ellipsometry of pure Gold Substrate

At low energies, the optical dielectric function of monovalent metals like gold is associated with electronic intra-band transitions within the conduction band. In this spectral range, the optical response is dominated by free-electron behavior and provides information about the electron scattering rate and mean free path. Precise knowledge of the frequency dependence of its dielectric function is required for successful nano-optical device engineering. The fitted complex dielectric function, $\varepsilon(\omega) = \varepsilon_1(\omega) + i\varepsilon_2(\omega)$, obtained from spectroscopic ellipsometry analysis[4,5] is shown in Fig. S2. The fitted $\varepsilon(\omega)$ is in good agreement with a previous study.[6]

Sensitivity of Spectroscopic Ellipsometry to sample quality

Each measurement was repeated (using automatic scripts) for 150-300 times to ensure good statistics on the data. Then, each set of measurements was also measured at 3 different incident angles (Fig. S3) for both Cys-bR and WT-bR to separate the effects of thickness and dielectric function. These measurements were taken to ensure the robustness of the ellipsometry experiments. Aside from the samples presented in the main text, we also attempted to measure several other bR film samples. However, since the quality of these samples are poorer than the current samples that we presented in the paper, we could not model and fit them properly to extract the thickness and dielectric functions. As a representative example, in Fig. S4, we present the fitted imaginary part of dielectric function, $\varepsilon_2(\omega)$, of another

bR mutant specimen called D96N (also deposited on Au), compared with those of Cys and WT mutant bR films on Au that we present in the main text. If some samples have high depolarization effects, for instance from high surface roughness, thickness modulation, or simply low quality, these effects will deteriorate the accuracy of ellipsometry measurements. The raw ellipsometry data of both Cys and WT bR films have very low depolarization (1%) ensuring the reliability of the fitting analysis. Meanwhile, the D96N mutant bR film has high depolarization ($> 4\%$) making the fitted $\varepsilon_2(\omega)$ shown in Fig. S4 unreliable since the depolarization effects massively overshadow the actual $\varepsilon_2(\omega)$.

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