

Drop-casted Photosystem I/Cytochrome c Multilayer Films for Biohybrid Solar Energy Conversion

Long Viet Than¹, Kody D. Wolfe², David E. Cliffl³, G. Kane Jennings¹ ()*

¹ Department of Chemical and Biomolecular Engineering, Vanderbilt University, Nashville, Tennessee 37235-1604, United States

² Interdisciplinary Materials Science and Engineering Program, Vanderbilt University, Nashville, Tennessee 37235-0106, United States

³ Department of Chemistry, Vanderbilt University, Nashville, Tennessee 37235-1822, United States

(*) Corresponding author; email: kane.g.jennings@vanderbilt.edu

1. Cyclic voltammetry of protein films immediately after drop-casting

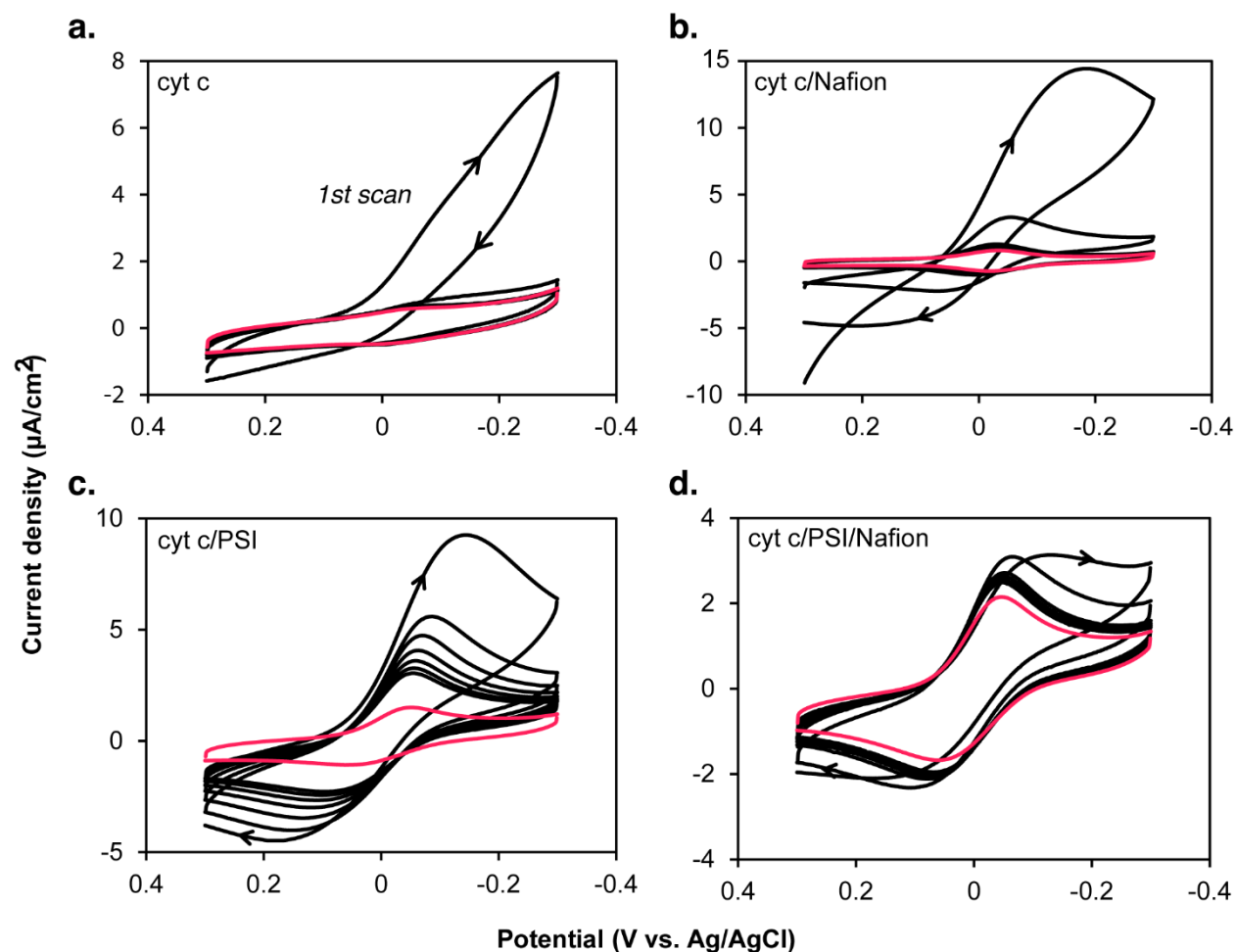


Fig. SI.1 Cyclic voltammograms of (a) cyt c, (b) cyt c/Nafion, (c) cyt c/PSI, and (d) cyt c/PSI/Nafion multilayer films taken continuously over 720 s (30 cycles at 50 mV/s). The 1st cycle and every 5th cycle are shown, as well as the CV after rinsing the film (red line). The CV after rinsing was repeated three times with nearly identical peak positions and intensities. This consistency shows that the retained or secured cyt c in each film remains electrochemically active for periods well longer than those shown in photochronoamperometry.

2. Summary of cyt c areal density measured via integration of CV peaks

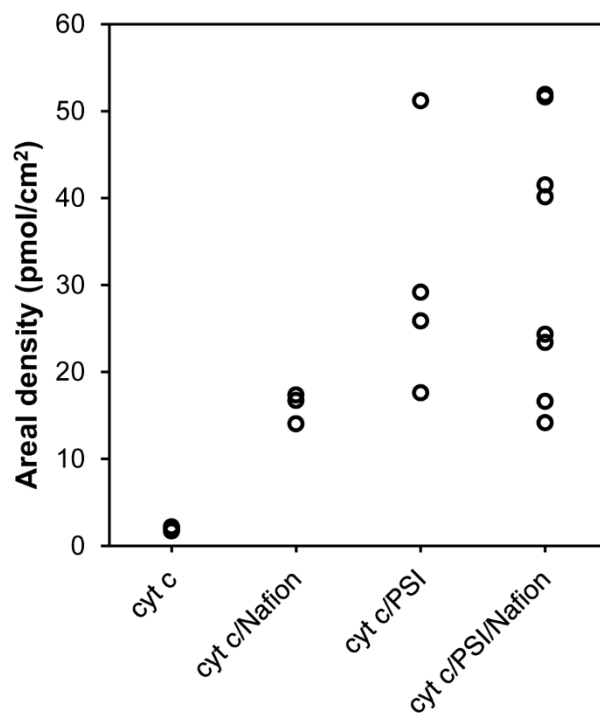


Fig. SI.2 Cyt c areal density in multilayer films estimated by the integration of cyt c Faradaic peaks in cyclic voltammograms, assuming a Gaussian peak shape.

3. CV scan rate study

Further electrochemical evidence for the existence of both surface-adsorbed and retained but freely diffusing cyt c in the cyt c/PSI film can be obtained by a scan rate study. The peak current in the cyclic voltammogram of a Faradaic process varies with scan rate for a surface-adsorbed redox species (Bard & Faulkner, 2000a), or the square root of scan rate for a freely-diffusing redox species (Bard & Faulkner, 2000b). Figure SI.3 shows that the linear fit with the square root of scan rate ($v^{1/2}$) has a lower uncertainty than the linear fit with scan rate (v), indicating that cyt c is likely to be behaving mostly as a diffusing redox species.

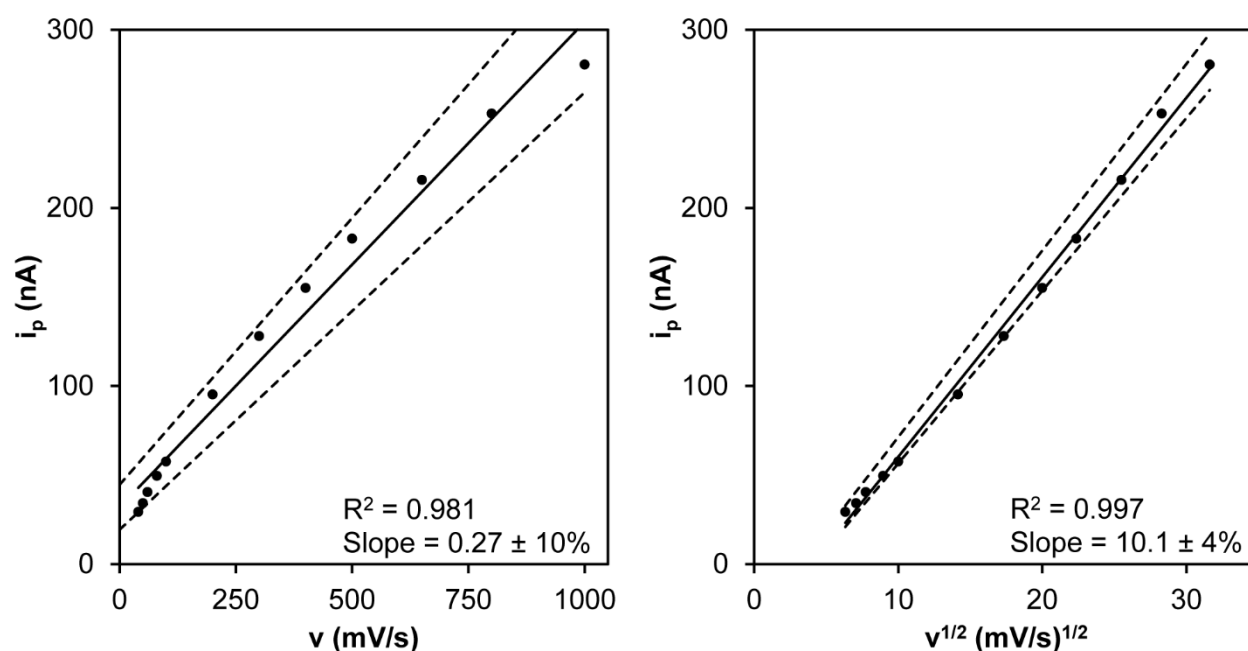


Fig. SI.3 Linear dependence of Faradaic peak current i_p on (A) scan rate v or (B) square root of scan rate $v^{1/2}$ in cyclic voltammograms of the Au/MU:MUA/cyt c/PSI/Nafion electrode. Cyclic voltammograms were performed between 300 and -300 mV at 40-1000 mV/s scan rate in 5 mM phosphate buffer. Dotted lines represent the 95% confidence interval for the slope of the linear fit.

Supplementary Information

4. Comparison of cyt c areal density estimates

Table SI.1 Comparison of cyt c areal density estimates via integration of CV Faradaic peaks vs. integration of PCA residual current density after illumination.

cyt c areal density via CV integration (pmol/cm ²)	17	52	24	23	42
cyt c areal density via PCA integration (pmol/cm ²)	29	45	45	43	22

5. ATR-FTIR characterization of protein films

ATR-FTIR was used to probe the chemical composition of the cyt c/PSI/Nafion film, in addition to the other mixed films (cyt c/Nafion, PSI/Nafion, and cyt c/PSI) and drop-casted pure component films, as this technique has the capability to survey surfaces with a penetration depth on the scale of microns and thus is capable of accessing the entire thickness of the composite film.(Kazarian & Chan, 2013) The primary features of both cyt c and PSI are the Amide I and Amide II vibrational modes at 1650 and 1540 cm^{-1} , respectively, making it difficult to distinguish the two species within the composite film based on IR peak intensity. Nafion, on the other hand, has distinctive sharp bands at 1245 cm^{-1} and 1158 cm^{-1} due to contributions from C-F, $-\text{SO}_3^-$, and C-O-C vibrational modes.(Singh et al., 2016) When Nafion is co-deposited with either protein, the Nafion bands broaden and shifts to 1210 and 1150 cm^{-1} , caused by strong electrostatic interactions between the sulfonate groups of Nafion and positively charged amino acid side chains.

Upon rinsing the electrode to remove weakly bound material and simulate the aqueous environment of the electrochemical cell, all peak intensities decreased as is expected for a thinner film. However, a qualitative comparison between the amide bands of the cyt c and cyt c/Nafion spectra suggests that more cyt c has been retained in the cyt c/Nafion film compared to the unprotected cyt c film, supporting the conclusion that Nafion assists in cyt c retention within the composite film. Furthermore, in the cyt c/Nafion spectrum, the bands at 1210 and 1150 cm^{-1} partially shifted back towards the original positions in the pure Nafion spectrum after rinsing; this supports the argument that these bands occur due to the red-shifting of Nafion bands, as the local environment around the sulfonate moieties of Nafion within the rinsed cyt c/Nafion film becomes cyt c-deficient and more closely resembles a pure Nafion film.

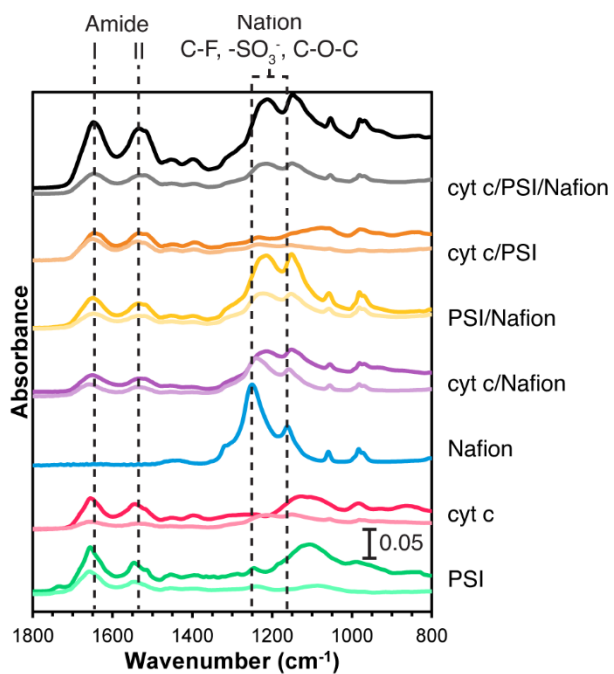


Fig. SI.4 ATR-FTIR spectra of multilayer films immediately after vacuum-assisted drop-casting (darker line) and after rinsing to remove weakly adsorbed material (lighter line).

References

- Bard, A. J., & Faulkner, L. R. (2000a). *Electrochemical Methods: Fundamentals and Applications* (D. Harris, E. Swain, C. Robey, & E. Aiello, Eds.; 2nd ed., pp. 590–593). John Wiley & Sons, Inc.
- Bard, A. J., & Faulkner, L. R. (2000b). *Electrochemical Methods: Fundamentals and Applications* (D. Harris, E. Swain, C. Robey, & E. Aiello, Eds.; 2nd ed., pp. 231–232). John Wiley & Sons, Inc.
- Kazarian, S. G., & Chan, K. L. A. (2013). ATR-FTIR spectroscopic imaging: recent advances and applications to biological systems. *Analyst*, *138*(7), 1940–1951. <https://doi.org/10.1039/C3AN36865C>
- Singh, R. K., Kunimatsu, K., Miyatake, K., & Tsuneda, T. (2016). Experimental and Theoretical Infrared Spectroscopic Study on Hydrated Nafion Membrane. *Macromolecules*, *49*(17), 6621–6629. <https://doi.org/10.1021/acs.macromol.6b00999>