

## Electronic Supplementary Information

# Hemoglobin-mediated synthesis of PEDOT:PSS: Enhancing conductivity through biological oxidants

*Joshua D. Morris,<sup>†</sup> Dipesh Khanal, Jessica A. Richey, and Christine K. Payne\**

School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, Georgia 30332, United States  
and Petit Institute for Bioengineering and Biosciences, Georgia Institute of Technology, Atlanta, Georgia  
30332, United States

<sup>†</sup> Current address: School of Science and Technology, Georgia Gwinnett College, Lawrenceville, Georgia  
30043, United States

\* Corresponding author: [christine.payne@chemistry.gatech.edu](mailto:christine.payne@chemistry.gatech.edu)

## Experimental

### Materials

3,4-ethylenedioxythiophene (EDOT, #483028), poly(styrenesulfonate) (MW = 70kDa, #243051), hemoglobin (Hb, from bovine blood, #H2500, Fig. S1a), catalase (CAT, from bovine liver, #C9322, Fig. S1b), and 30% hydrogen peroxide (#16911) were purchased from Sigma-Aldrich. All solutions were prepared in 18M $\Omega$  cm water generated by an EASYpure II water purification system (Thermo Fisher Scientific).

### Polymerization of PEDOT:PSS.

PEDOT:PSS was polymerized by combining the appropriate biomolecule (7  $\mu$ M by heme concentration), EDOT (50 mM), and PSS (MW = 70 kDa, 25 mM by monomer concentration) in an HCl-KCl buffer (pH ~2) while stirring. The polymerization was initiated by adding a stoichiometric quantity of hydrogen peroxide. The reaction was allowed to proceed for 6 hours at room temperature. The total reaction volume was 2.5 ml. Solutions were then centrifuged at 700g for 5 minutes to remove protein and insoluble material. The polymer mixtures were then dialyzed (cutoff MW = 1000 Da, #132636, Spectrolabs) against 400 ml of deionized water to remove the HCl-KCl buffer and any unreacted monomer. Water was exchanged at 1, 2, and 3 hours and again after dialysis proceeded overnight. This protocol was adapted from previous research using CAT as an oxidant.<sup>1</sup> For polymerizations carried out in the presence of EDTA, equimolar quantities of EDTA and heme groups were used.

### Characterization of PEDOT:PSS

X-ray photoelectron spectroscopy (XPS, k-alpha X-ray photoelectron spectrometer, Thermo Scientific) spectra were collected on PEDOT:PSS films cast on glass wafers and heated at 60 °C for 30 minutes (Table S1). The slightly greater carbon content of CAT-polymerized PEDOT:PSS suggests a slightly higher ratio of PEDOT to PSS.

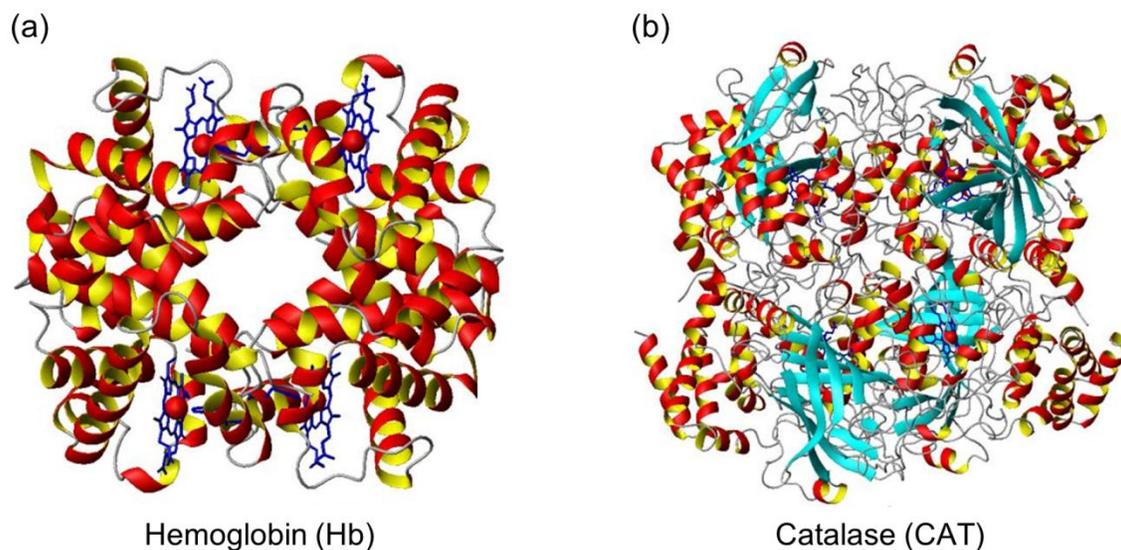
**Table S1.** Elemental analysis of PEDOT:PSS synthesized using Hb or CAT as an oxidant was obtained with XPS.

PEDOT:PSS	%C	%O	%S	%Na
Hb	70	19	9.0	1.03
CAT	73	18	8.0	0.93

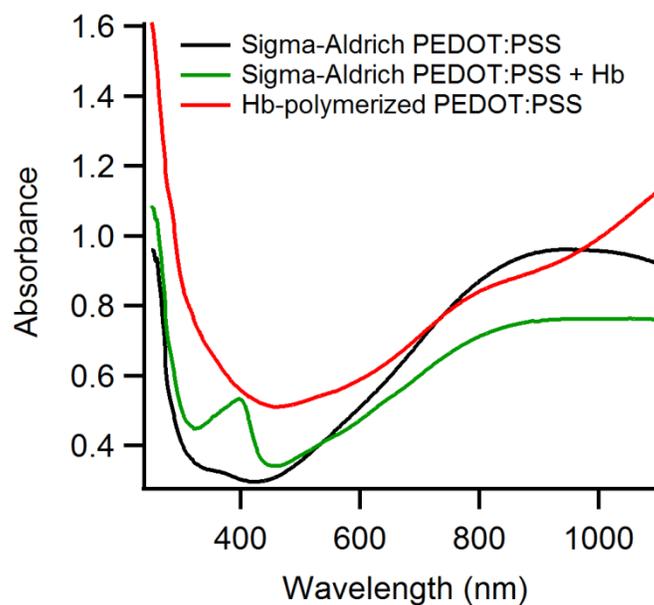
Visible and near IR (DU 800 Spectrophotometer, Beckman Coulter) spectra were recorded of PEDOT:PSS solutions in deionized water. FTIR (Alpha FTIR, Bruker) spectra were taken on films drop cast on germanium wafers (#160-1191, Pike Technologies, Madison, WI). Films were dried in ambient atmosphere for 30 minutes at 80 °C. X-band ESR (Bruker) spectra were collected using PEDOT:PSS powder freeze dried (FreeZone, Lanconco) at -50 °C overnight. ESR spectra were collected at a fixed frequency of 9.878 GHz at a power of 1 mW. Signal intensity was integrated for 10 scans of 20 seconds each and the total signal intensities were normalized by PEDOT:PSS weight. All spectroscopy measurements were collected in triplicate at minimum. Conductivity was recorded using a four point probe (CPS-05 probe station, Cascade Microtech) on films of PEDOT:PSS drop casted on glass. Films were allowed to dry at room temperature overnight and then heated at 60 °C for 1 hour. Film thickness was measured from the average of three scans with a profilometer (P15, Tencor). Conductivity was measured for a minimum of three separate polymerizations. In each case, a minimum of four spots on twelve films were measured.

It is possible that small amounts of protein, below levels detectable by XPS, are not removed during centrifugation. To ensure that the spectral changes observed in Fig. 1 did not result from residual protein, we measured the visible and near-IR absorption of commercial PEDOT:PSS (#739332, Sigma-Aldrich) with the addition of Hb (7  $\mu$ M by heme concentration) following polymerization (Fig. S2). The absorption spectrum was unchanged showing even undetectable residual protein would not lead to a bipolaron absorption signature.

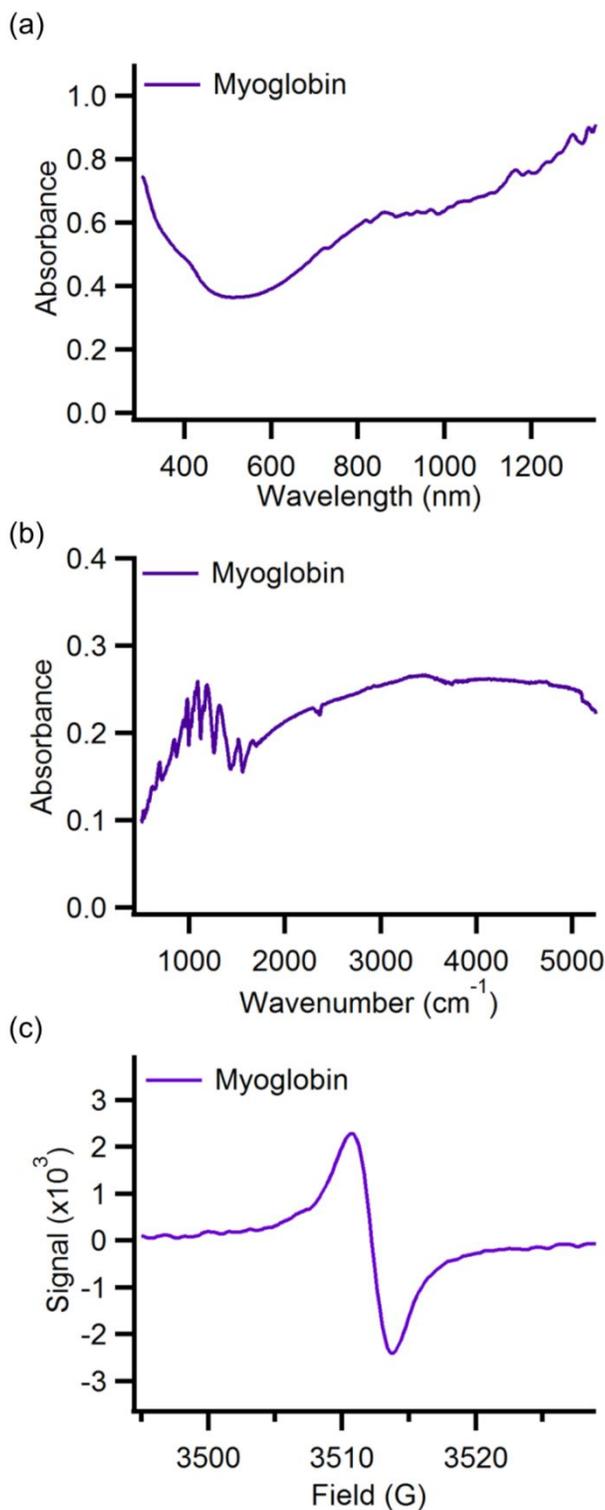
## Supporting Figures



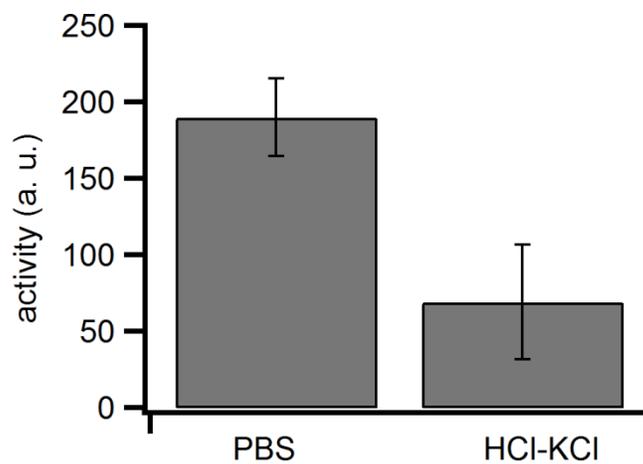
**Figure S1.** Structures for Hb (PBD ID: 1HDA) and CAT (PBD ID: 4BLC) were obtained from the heme protein database.<sup>2</sup> (a) Hb (MW = 65 kDa). (b) CAT (MW = 240 kDa). Alpha-helices are red and yellow, beta sheets are light blue, random coils are grey, heme groups are dark blue, and the iron centers are represented by red spheres. Hb and CAT each have 4 identical heme B groups.



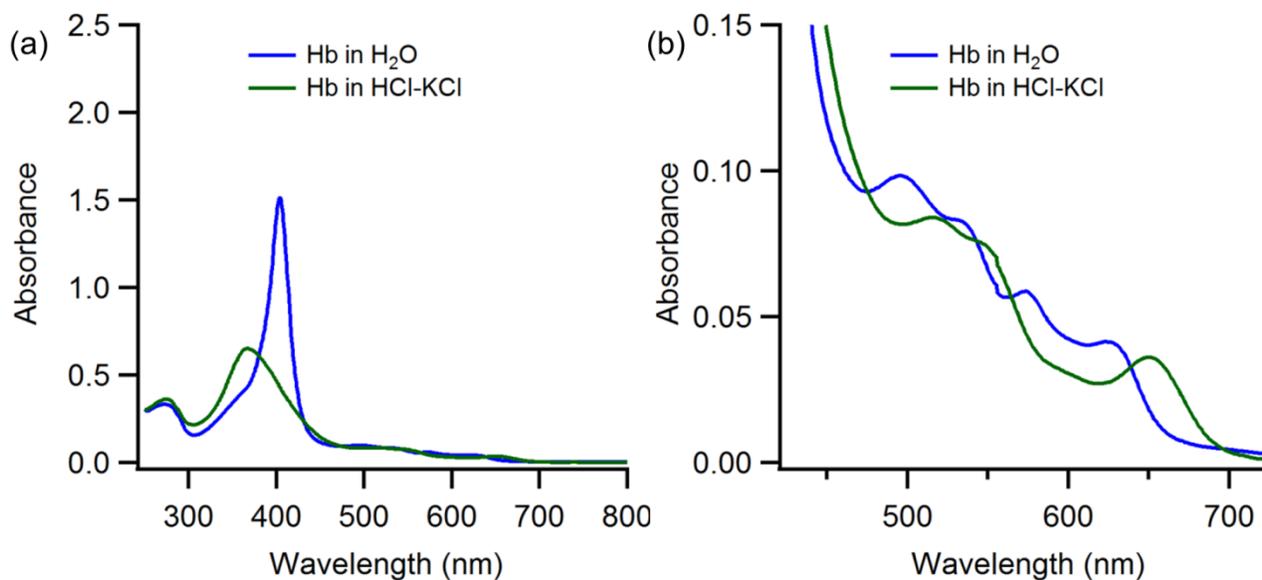
**Figure S2.** Visible and near IR spectra of Sigma-Aldrich PEDOT:PSS (black), Sigma-Aldrich PEDOT:PSS with Hb added ( $7 \mu\text{M}$  by heme concentration) (green, peak at 400 nm shows the presence of protein), and Hb-polymerized PEDOT:PSS. The presence of Hb does not alter the dominant charge carrier species of PEDOT:PSS.



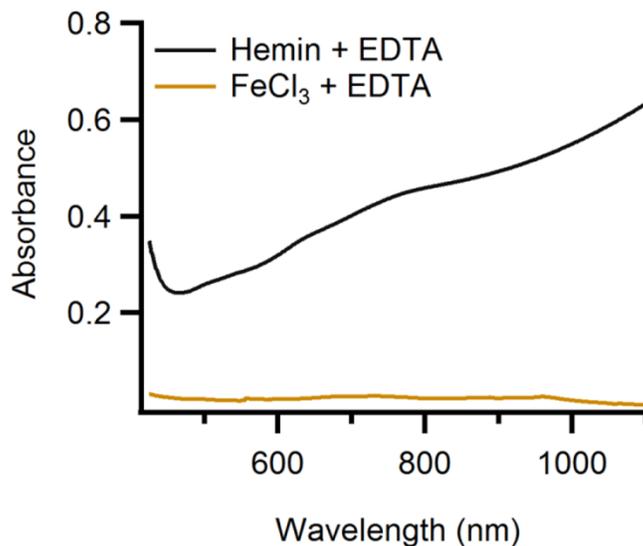
**Figure S3.** Spectroscopic and electrical characterization of myoglobin (Mb) shows that, like hemoglobin, bipolarons are the dominant charge carrier. (a) Representative visible and near IR spectrum of myoglobin (purple). (b) Representative FTIR spectrum. (c) Representative ESR spectrum.



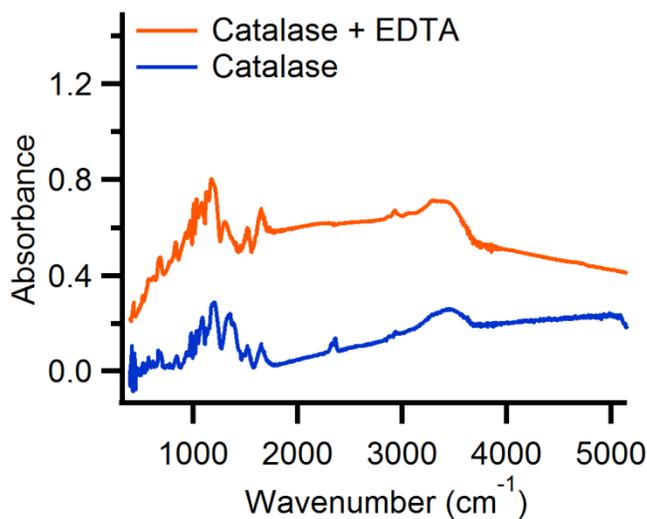
**Figure S4.** Catalase activity in PBS buffer (pH = 7) compared to catalase activity in the HCl-KCl reaction buffer (pH = 2) used for polymerization. Activity was measured with an Amplex Red assay (Invitrogen) following a 30 minute incubation of catalase in the buffer. Error bars represent the standard deviation of 3 measurements.



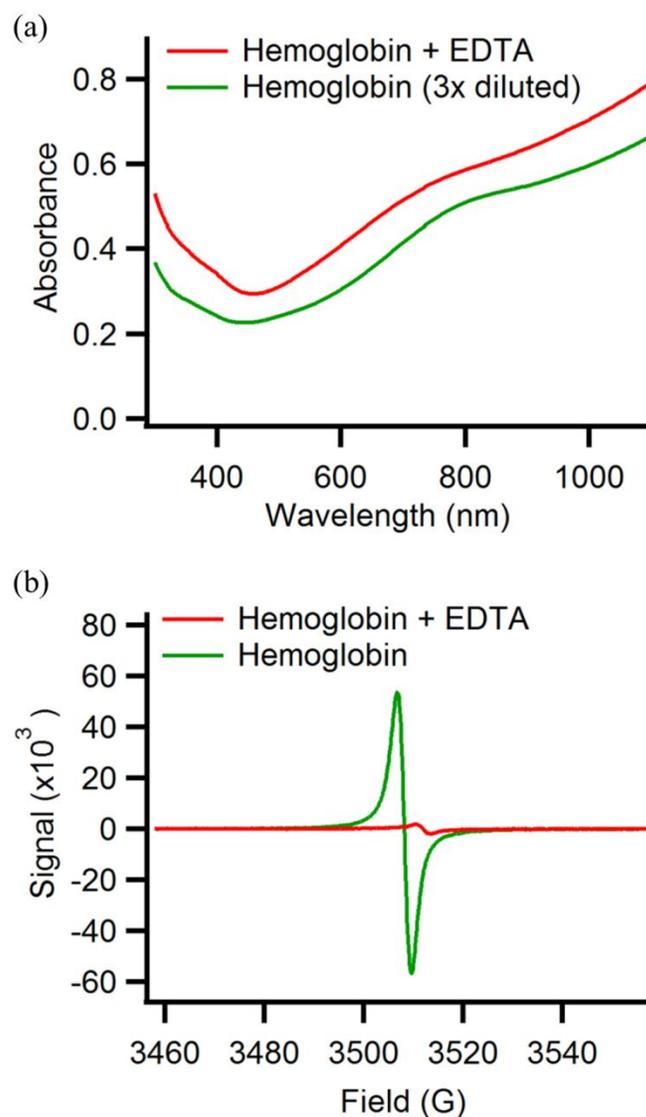
**Figure S5.** UV-vis absorption spectra of Hb in H<sub>2</sub>O (blue) and HCl-KCl (green) from (a) 250 to 800 nm and (b) an enlarged plot of the region from 425 to 725 nm. The Soret peak of Hb in HCl-KCl is blue shifted with respect to the Soret peak of Hb in H<sub>2</sub>O. This blue shift, and the observed broadening, are consistent with acid denatured Hb.<sup>3,4</sup> The spectrum of Hb in H<sub>2</sub>O shows the four peaks characteristic of ferric hemoglobin (496 nm, 536 nm, 575 nm, and 630 nm). Ferrous hemoglobin shows a strong peak at 560 nm, which is not observed.<sup>5-7</sup>



**Figure S6.** Near IR absorption spectra of  $\text{FeCl}_3$ - and hemin-polymerized PEDOT:PSS in the presence of EDTA.



**Figure S7.** Representative FTIR spectra of PEDOT:PSS synthesized with CAT in the presence (orange) and absence (blue) of EDTA. A red shift in the broad absorption feature is likely associated with a lower density of bipolarons due to the reduced quantity of heme B available for polymerization. This reduced charge density would reduce the confinement of bipolarons due to the columbic repulsion of adjacent charge carriers, perhaps red shifting this absorption feature.<sup>8</sup>



**Figure S8.** (a) Visible and near IR absorption spectra of Hb-polymerized PEDOT:PSS with and without EDTA. The Hb-polymerized PEDOT:PSS has been diluted by a factor of three relative to Hb-polymerized PEDOT:PSS with EDTA. (b) ESR spectra of Hb-polymerized PEDOT:PSS with and without EDTA.

## References

- 1 S. M. Hira and C. K. Payne, *Synth. Met.*, 2013, **176**, 104-107.
- 2 C. J. Reedy, M. M. Elvekrog and B. R. Gibney, *Nucleic Acids Res.*, 2008, **36**, D307-313.
- 3 L. Marmo Moreira, A. Lima Poli, A. J. Costa-Filho and H. Imasato, *Biophys. Chem.*, 2006, **124**, 62-72.
- 4 S. Kamimura, A. Matsuoka, K. Imai and K. Shikama, *Eur. J. Biochem.*, 2003, **270**, 1424-1433.
- 5 B. L. Horecker, *J. Biol. Chem.*, 1943, **148**, 173-183.
- 6 E. A. Rachmilewitz, J. Peisach and W. E. Blumberg, *J. Biol. Chem.*, 1971, **246**, 3356-3366.
- 7 M. F. Perutz, J. K. M. Sanders, D. H. Chenery, R. W. Noble, R. R. Pennelly, L. W. M. Fung, C. Ho, I. Giannini, D. Poerschke and H. Winkler, *Biochemistry*, 1978, **17**, 3640-3652.
- 8 R. K. Khanna, Y. M. Jiang, B. Srinivas, C. B. Smithhart and D. L. Wertz, *Chem. Mater.*, 1993, **5**, 1792-1798.