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SUPPLEMENTARY INFORMATION

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Optimized *E. coli* expression, secretion and assembly of *T. elongatus* recombinant cyt c_6

The SDS-PAGE analysis of typical purification is shown in Supplemental Fig. 1A. This purification first involves the use of IMAC followed by anion exchange chromatography. The combination of these two steps can be used to produce purified protein as is shown in **Supplemental Fig. 1a, lane 2.** The native environment of heme is confirmed for this recombinant protein by the reduced minus oxidized difference spectra shown in **Supplemental Fig. 1b**. This spectra indicates a β-peak at 552.1 nm which is identical to published values for native cyt c_6 isolated from a variety of related cyanobacteria[1]. Finally, the purity and heme coordination of this protein is also evident from the ratio of the A552/A274 of the reduced form of cyt c_6. We observed a ratio of 2.1 for the purified protein, considerably higher than the best reported value [2] indicating both high purity and uniform heme insertion

Thermal stability of *T. elongatus* cyt c_6

The structural stability of the recombinant cyt c_6 was determined by monitoring the secondary structure using circular dichroism Aviv 202 (AVIV Biomedical, Lakewood, NJ). The temperature was ramped from 20°C to 94°C at 1° per min. At each temperature, the sample was equilibrated for 5 min and the spectra was recorded and baseline corrected, the corrected spectra was deconvoluted using the CDPRO software with the IBASE3 reference set of 37 soluble proteins (http://lamar.colostate.edu/~sreeram/CDPro). The these results are shown in **Supplemental Fig. 2c.** Since the cytochrome is primarily alpha helical and since this is the most accurately predicted secondary structure, we used the helical content do determine the T_m for denaturation.

Evaluation of temporal sustainability of hydrogen evolution

The initial platinization of PSI was conducted using a 10:1 ratio of cyt c_6 to P_{700} in the presence of NaAsc and hexachloroplatinate for 60 h at 25°C; three representative L/D cycles are shown on the left of Fig. 4A. Upon termination of the reaction, the PSI preparation was dialyzed overnight (indicated as S1, for Segment 1) to remove small reactant molecules and by-products (Fig. 4A). There was no visible precipitation of platinum in the reaction. The dialyzed PSI preparation was re-introduced to the reaction vessel, fresh NaAsc was added; H_2 production was observed without the addition of hexachloroplatinate further demonstrating that the Pt catalyst was stably associated with PSI. After 170 h of hydrogen evolution at 25°C, shown schematically as S2, the temperature was increased to 60°C for 20 h to determine if the system was thermally stable, as expected for thermophilic cyanobacterial proteins. The H_2 evolution rate at 60°C was not only stable but actually increased by 80%. Following this temperature increase the platinized Pt-PSI biomimetic catalyst was stored at 4°C for an extended period of 64 days

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(S3) and the rate of hydrogen evolution was re-measured at 25°C for 29 L/D cycles and observed to be comparable or possibly a bit higher than the rates observed earlier at 25°C, however this may be due to fresh NaAsc being added at the beginning. Finally, the sample was stored another 5 days (S4) and then retested, showing only a slight decrease from the initial values.

Methods:

Purification of cyt c_6 by IMAC & HPLC. IMAC was performed using Ni-Sepharose (Pharmacia, GE Healthcare) chromatography column. The column was rinsed with 3 column volumes ddH_2O and then equilibrated with 3 column volumes of 50 mM Tris (pH 8.0). The total periplasmic fraction was passed over the column at a maximum rate of 3 ml/min. The column was washed with 3 column volumes of 50 mM Tris and then eluted with 50 mM Tris (pH 8.0) and 300 mM imidazole. The IMAC purified cyt c_6 was then purified using anion exchange chromatography using POROS HS resin and a BioCAD 202 Chromatography workstation.

SDS/PAGE and chemiluminescent heme stain. Purified cyt c_6 was subjected to Tris-Tricine-SDS as described by Schagger & Von Jagow[3] and stained with Coomassie Brilliant Blue R250. In order to further confirm the presence of the heme-containing holoprotein, cyt c_6 was additionally subjected to Tris-Tricine-SDS followed by electroblotting and chemiluminescent heme staining as described previously

Energy Dispersive Spectroscopy. Energy levels of the emitted X-rays at 2.05, 11.07/11.25, and 9.36/9.44 KeV indicate the presence of platinum. The presence of carbon (0.28 KeV), nitrogen (0.39 KeV), oxygen (0.52 KeV), magnesium (1.25/1.30 KeV), sulphur (2.31 KeV), chlorine (2.62 KeV) and iron (6.4 KeV) were also indicated by the energy levels of the emitted X-rays.

Spectral analysis of chlorophyll in PSI and supernatant. Hydrogen evolution was carried out for 14 L/D cycles w/ platinized PSI complexes (90 µg Chl/ml, 10 mM NaAsc, 10 molar excess cyt c_6 to PSI ratio, 20 mM MES, pH 6.4) at 30°C and illumination w/ 300 µE/m^2/s. Mock samples were treated similarly yet they were kept placed in a second reactor that was kept in the dark. After 56 h (equivalent to 14 L/D cycles) 1.5 ml samples were removed from both the mock treatment and the hydrogen evolving sample and centrifuged at 50,000 g. The supernatant was collected and the remaining green pellet was resuspended in 1 ml of 90% methanol, shaken using a MP Biomedical FastPrep-24 and spun at 21,000 g for 5 min. This methanol extraction was further diluted 10 fold and the spectra was collected on a Cary 50 Bio UV/Visible spectrometer using a 1 mm pathlength quartz cuvette. The 50,000 g supernatant was also measured yet this sample was not extracted in 90% methanol nor was it diluted (shown in **Supplemental Figure 3**).

References:

1 Cho, Y. S., Wang, Q. J., Krogmann, D. & Whitmarsh, J. Extinction coefficients and midpoint potentials of cytochrome c(6) from the cyanobacteria Arthrospira maxima, Microcystis aeruginosa, and Synechocystis 6803. *Biochimica Et Biophysica Acta-Bioenergetics* **1413**, 92-97 (1999).

2 Diaz, A. *et al.* Cloning and correct expression in Escherichia coli of the petJ gene encoding cytochrome c6 from Synechocystis 6803. *Febs Letters* **347**, 173-177 (1994).

3 Schagger, H. & von Jagow, G. Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal Biochem* **166**, 368-379 (1987).

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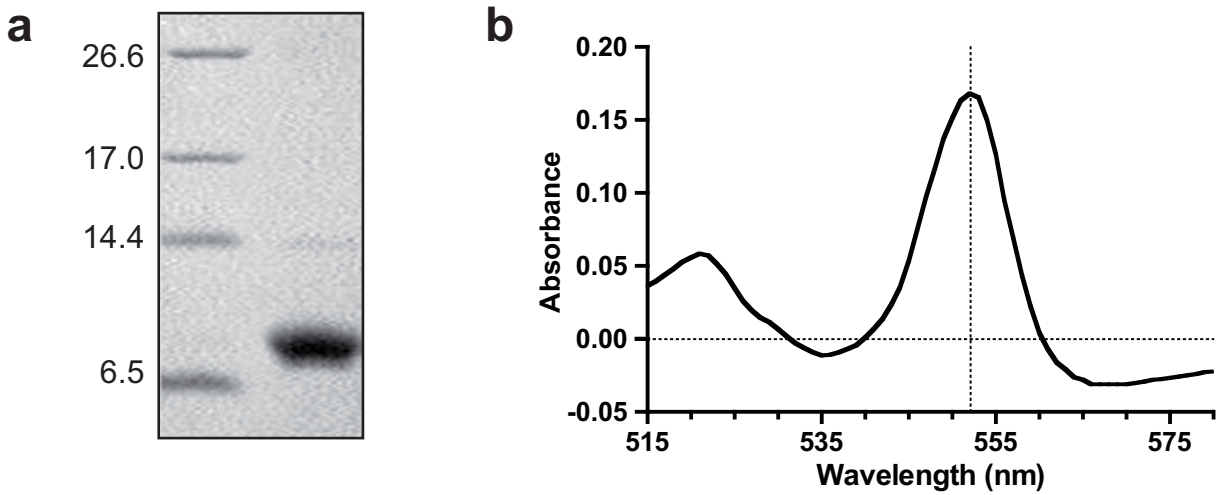
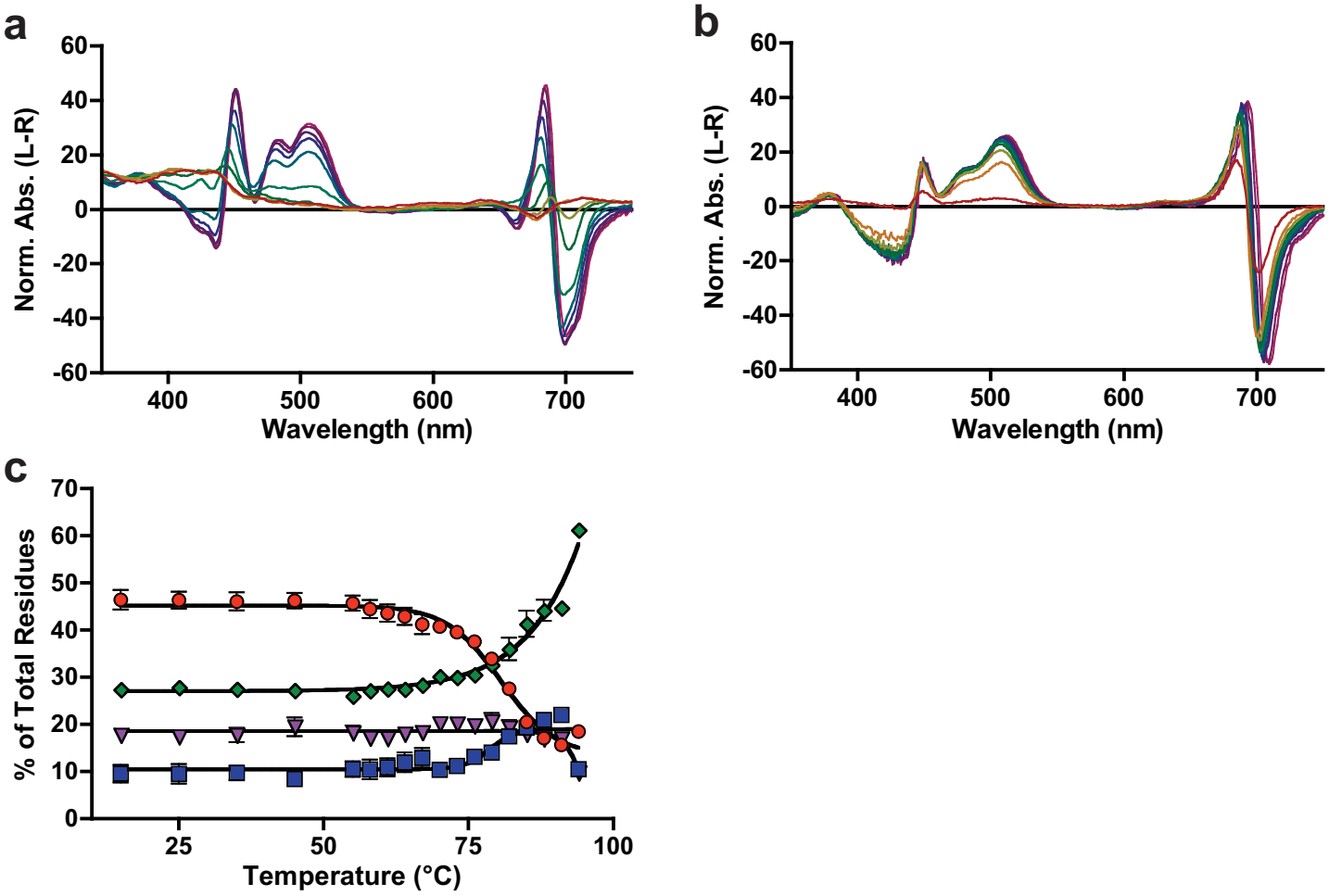


Supplemental Figure 1. Characterization of recombinant *T. elongatus* Cyt c_6. Cytochrome c6 was cloned into pET21d (Novagen) and used for expression in order to reduce the P700+ in this work, **(a)** is a Tris-Tricine SDS-PAGE of purified Cyt c_6 with no significant impurities. After much optimization, large amounts of cytochrome were obtained at this level of purity. **(b)** In order to test the reversible redox activity and estimate concentration, reduced-minus-oxidized difference spectroscopy was used in which Cyt c_6 demonstrates the expected maximum at 552.1 nm and the extinction coefficient of Synechocystis PCC6803 was used.



Supplemental Figure 2. Thermal Stability of PSI and Cyt c_6. **(a)** and **(b)** contain the visible CD absorbance data for PSI derived from Synechocysitis PCC6803 and Thermosynechococcus elongatus measured between 15 and 98°C, respectively. Lowest temperature scans are shown in violet and increasing temperatures proceed through blue, green yellow and finally red for the highest temperature scans. The visible CD absorbance for PSI is a function of pigment arrangement/environment and is a sensitive indicator of the functional state of PSI, the fine stucture of these spectra is lost as temperature is increased.
(c) The thermal stability of Cyt C6 was also investigated using UV-Vis CD spectroscopy. Using the CDPro software package the data was deconvoluted as indicated in the text and the resulting values are plotted as a function of temperature. Helical content plotted with red circles, unordered with green diamonds, turn with inverse purple triangles, and beta strand with blue squares. Using the helical content for fitting to a sigmoidal Boltzman distribution we estimate the T_m value to be 80.6±1°C.

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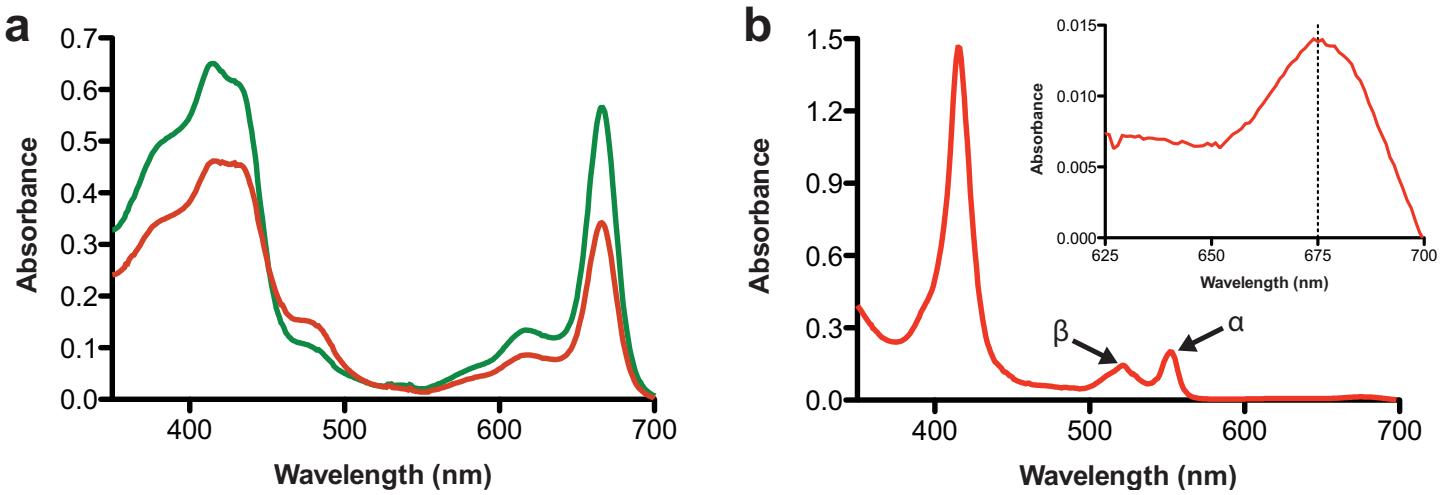
Supplemental Figure 3. TEM of individual PSI particles showing electron dense particles following platinization and staining with 0.5% potassium phosphotungstic acid, Images were collected on a Hitachi H800 TEM at 50,000x magnification. Seven individual particles are shown to reveal the somewhat irregular electron dense labeling that may represent the bound metallic platinum particles. Sample preparation was the same as described for Fig. 1.



Supplemental Figure 4. Spectral analysis of chlorophyll loss. **(a)** This represents the spectra of the mock PSI treatment (green lines) and the hydrogen evolving sample (red lines) after 56 h (14 L/D) after extraction with 90% methanol. **(b)** This is the spectrum of the 50,000 g supernatant following 14 L/D cycles of hydrogen evolution. The inset represents an enlargement of the chlorophyll absorbance at 675 nm. The arrows indicate the α- and β-peaks of the Cyt c_6 that also remains in the supernatant.