Supporting Information

Thylakoid direct photobioelectrocatalysis: utilizing stroma thylakoids to improve bio-solar cell performance

Michelle Rasmussen and Shelley D. Minteer*

Department of Chemistry and Materials Science and Engineering, University of Utah, 315 S

1400 E Rm 2002, Salt Lake City, UT 84112, United States

*Corresponding author email: minteer@chem.utah.edu

Fractionation of thylakoid membranes

Leaves were de-veined, washed, and blended in extraction buffer containing 0.35 M sucrose, 25 mM HEPES (pH 7.6), 2 mM ethylenediaminetetraacetic acid (EDTA), 2 mM ascorbic acid, 4 mM dithiothreitol (DTT), 10 mM $MgCl_2$, and 1 mM phenylmethanesulfonyl fluoride (PMSF). After filtering through cheesecloth, the mixture was centrifuged at 200×g for 10 min. The pellet was discarded and the supernatant was centrifuged at 2500×g for 10 min. The pellet was resuspended in 40 mL of the extraction buffer and centrifuged again at 2500×g for 10 min. This process was repeated one more time. Next, the pellet was resuspended in a hypotonic buffer (10 mM $MgCl_2$, 10 mM NaCl, 10 mM tricine, pH 7.8) to lyse the chloroplasts by osmotic shock. The mixture was centrifuged at 2500×g for 10 min. The pellet was washed with the hypotonic buffer and centrifuged again at 2500×g for 10 min. The pellet was then resuspended in a sorbitol buffer containing 0.1 M sorbitol, 10 mM $MgCl_2$, 10 mM NaCl, 10 mM tricine, pH 7.8) and the chlorophyll (Chl) content was determined. The thylakoid mixture was diluted with sorbitol buffering contining 1% (w/v) digitonin to final concentrations of 0.4 mg Chl/mL and 0.5% digitonin. The mixture was incubated at 4ºC with gentle shaking for 30 min. and then centrifuged at 1000×g for 3 min. The pellet was discarded and the supernatant was centrifuged at 40,000×g for 30 min. The resultant pellet (which contains the grana thylakoids) was resuspended in the hypotonic buffer. The supernatant was centrifuged at 115,000×g for 90 min. The pellet (which contains the stroma thylakoids) was resuspended in the hypotonic buffer. The thylakoid fractions were stored at -20ºC until further use.

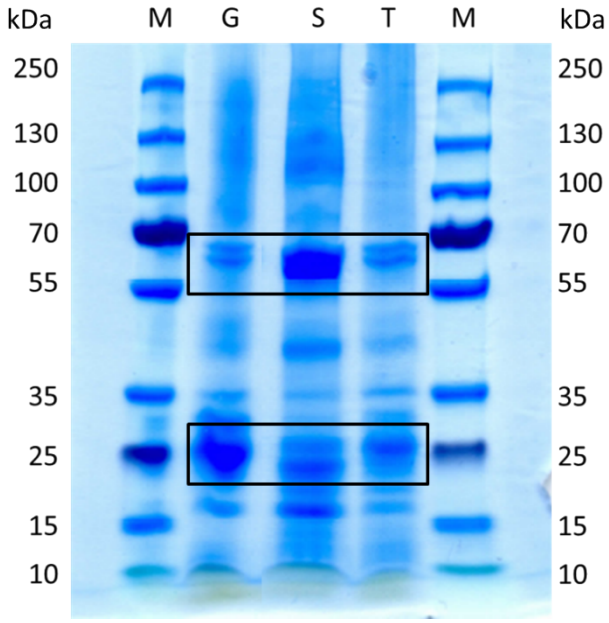

Fig. S1 SDS-PAGE analysis of thylakoid membranes isolated from spinach. Intact thylakoids (T), grana (G) thylakoids, and stroma thylakoids (S) are shown along with protein markers (M). The rectangle at ~60 kDa indicates the location of the two subunits, PsaA and PsaB, of the PS I core.[20] The rectangle at ~ 25 kDa indicates the location of the light-harvesting center (LHC) of PS II.