Electronic Supplementary Material (ESI) for Journal of Materials Chemistry A. This journal is © The Royal Society of Chemistry 2020

Improved quantum efficiency in an engineered light harvesting/photosystem II super-complex for high current density biophotoanodes

Volker Hartmann,^{a‡} Dvir Harris,^{bc‡} Tim Bobrowski,^d Adrian Ruff,^d Anna Frank,^a Thomas Günther Pomorski,^f Matthias Rögner,^a Wolfgang Schuhmann,^d Noam Adir^{*bc} and Marc M. Nowaczyk^{*a}

Electronic Supplementary Information (ESI)

^{a.} Plant Biochemistry, Faculty of Biology & Biotechnology, Ruhr-University Bochum, 44780 Bochum, Germany.

^{b.} Grand Technion Energy Program, Schulich Faculty of Chemistry, Technion, Haifa 32000, Israel.

^c Schulich Faculty of Chemistry, Technion, Haifa 3200003, Israel

^d Analytical Chemistry - Center for Electrochemical Science (CES), Faculty of Chemistry and Biochemistry, Ruhr-University Bochum, 44780 Bochum, Germany

e. Present address: PPG Deutschland Business Support GmbH, PPG Packaging Coatings, Erlenbrunnenstr. 20, 72411 Bodelshausen, Germany

^f Molecular Biochemistry, Faculty of Chemistry and Biochemistry, Ruhr-University Bochum, D-44780 Bochum, Germany

[‡] Equal contribution

^{*} Corresponding authors

Supplementary Methods

Negative stain TEM of crosslinked AmPBS-PSII shows various formation of super-complexes. Since the crosslinking procedure is uncommon for proteins the resulting AmPBS-PSII complexes were depicted by negative stain TEM. Various sub-species of AmPBS-PSII super-complexes were found. Besides the natural interaction which was identified also complexes with a non-in vivo interaction were found. They differ in the binding angle, form AmPBS dimers in interaction with PSII or grow aggregates with a head-to-tail assembly of PBS and PSII.

The sample was non-specifically stained by uranyl acetate (UA, aq. 2% [w v⁻¹]). An electron microscopy grid, coated with formvar-carbon continuous thin film, was placed on a 10 μ l drop of a sample for 2 min. Then the reminders of the sample were soaked with filter paper, and the grid was placed on 10 μ l drop of UA for rinsing, and immediately transferred to another 10 μ l drop of UA for 2 min, followed by soaking the reminders of the stain and air-drying. Specimens were examined in a Tecnai G2 TEM operating at 120 kV (FEI, Netherlands). Images were recorded on a Gatan US1000 2k x 2k high-resolution cooled CCD camera using Digital Micrograph software (Gatan, U.K.) (Figure S5).

Verification of PBS-PSII super-complex formation by free flow electrophoresis. For the isolation of PBS-PSII supercomplexes a free flow electrophoresis (FFE Nextgen, FFE-Service GmbH, Feldkirchen, Germany) was performed according to Eichacker *et al.* 2015⁵¹. The elution profile of PBS-PSII super complexes show an additional elution peak in fraction 48-54 with fluorescence at 680 nm, as compared to the elution profiles of free PBS and free PSII complexes (Figure S8). This indicates a novel complex formed by the crosslinking procedure of modified PBS with PSII. Future studies will focus on the optimization of the separation procedure by free flow electrophoresis to achieve a highly purified super-complex.

Setup of the FFE instrument was performed according to manufacturer's instructions. Briefly, the media pump was set to 120 ml/h and the system equilibrated for 15 min with the buffers to establish a permanent media flow. The sample was applied for 70 s with a flow rate of 4 ml/h (~80 µl) with various protein concentrations. After 20 s a voltage of 1,600 V (~150 mA, ~100 W) was applied orthogonal to the flow direction. The media pump was set to 40 ml/h and complexes were separated for 4 min followed by a raise of the media pump flow rate to 240 ml/h. The samples were collected in a 96-well plate via micro-tubes. The collected samples were analysed in a fluorescence plate reader (CLARIOstar, BMG Labtech, Ortenberg, Germany). Excitation wavelength was set to 430 nm and fluorescence detection to 680 nm, respectively.

Buffer compositions: E1 (anode) – 100 mM HCl, 50 mM formic acid, 50 mM isobutyric acid, 250 mM sucrose, adjusted to pH 3.83 with BisTris, conductivity of 7.06 mS/cm; E2-E4 – 10 mM α -hydroxyisobutyric acid (HIBA), 250 mM sucrose, 0.03% [w v⁻¹] β -dodecyl-D-maltoside (β -DM) adjusted to pH 5.58 with BisTris, conductivity of 1.07 mS/cm; E5+E6 – 10 mM HIBA, 250 mM sucrose, 0.03% [w v⁻¹] β -DM adjusted to pH 6.24 with BisTris, conductivity of 0.7 mS/cm; E7 – 10 mM HIBA, 250 mM sucrose, 5 mM NaCl, 0.03% [w v⁻¹] β -DM adjusted to pH 6.79 with BisTris, conductivity of 1 mS/cm); E8 – 10 mM HIBA, 250 mM sucrose, 0.03% [w v⁻¹] β -DM adjusted to pH 6.85 with BisTris, conductivity of 0.51 mS/cm); E8 – 10 mM HIBA, 250 mM sulfurce, 0.03% [w v⁻¹] β -DM adjusted to pH 6.85 with BisTris, conductivity of 9.25 mS/cm); Anode buffer – 100 mM sulfuric acid, pH 1, conductivity of 44.3 mS/cm; Cathode buffer – 100 mM NaOH, 200 mM glycine, pH 10, conductivity of 7.72 mS/cm; Counter flow – 250 mM mannitol.

Supplementary figures and tables



Figure S1 77K fluorescence spectra of an *Am*PBS-PSII mixture. A same volume mixture of PSII solution (50 mM MES pH 6.5, 10 mM MgCl₂, 10 mM CaCl₂, 0.03% [w v¹] β -DM) and *Am*PBS (0.9 M phosphate buffer, pH 7.5) was incubated in dark at 4 °C for 60 min. The sample was measured undiluted in a capillary tube system (approx. 10 μ I) with excitation at 580 nm (average of 5 repetitions).



Figure S2 Stability of stored AmPBS-PSII super-complex by means of low temperature fluorescence spectra. The excitation wavelength is 580 nm and the sample were diluted 40-times prior to measurement. Spectra of decorated phycobilisomes with PSII after 24 h (solid line) stored either on ice (dotted line) or frozen in liquid nitrogen and stored at -80 °C (dashed line).



Figure S3 Representative raw measurement of PSII/Os-P under monochromatic light, where each illumination cycle (10 s) represents a wavelength from 400-700 nm (cf. Table S1 for light intensities) and is followed by a dark cycle (10 s). A 5 μ l droplet containing 5 mg mL⁻¹ P-Os, 3 mg mL⁻¹ PSII and 0.02 mg mL⁻¹ PEGDGE dissolved in distilled water was drop-casted on the electrode surface. After 4 h incubation at 4 °C in dark, the electrodes were rinsed carefully with buffered electrolyte solution (50 mM MES, pH 6.5, 10 mM MgCl₂, 10 mM CaCl₂, 100 mM KCl) and a potential of +0.4 V vs. Ag/AgCl (3 M KCl) was applied.

 Table S1
 Lamp intensity calibration of monochromatic light source.

400	410	560	421
410	427	570	419
420	365	580	418
430	382	590	410
440	410	600	379
450	456	610	372
460	534	620	379
470	585	630	357
480	527	640	332
490	505	650	319
500	455	660	296
510	436	670	297
520	431	680	303
530	430	690	312
540	435	700	255
550	430		



Figure S4 Cyclic voltammetry of P-Os modified electrode and structure of P-Os. P-Os was immobilized on Au electrode surface and the measurement was performed in buffered electrolyte solution (50 mM MES, pH 6.5, 10 mM MgCl₂, 10 mM CaCl₂, 100 mM KCl) with a scan rate of 50 mV s⁻¹. Applied potential is given vs. Ag/AgCl (3.5 M KCl) reference electrode (E1/2 = 205 mV).



Figure S5 Negative stain TEM of cross-linked *Am*PBS-PSII super-complex following staining and dilution, without or with docked molecular models. Micrographs of negative-stained cross-linked *Am*PBS-PSII super-complex in different orientation (panels A,B,C,D and H). Below each of these panels, molecular models (ChimeraX) of the *A. marina* PBS (shades of blue) and PSII (shades of green) were docked into complexes density (panels D,E,F,J and K). In panels B and C the oligomeric state of the PBS is inconclusive, as the cylinder is facing upright (cylinder hole is visible). (I,L) Cross-linked aggregate demonstrates head-to-tail assembly of *Am*PBSs and PSII.



Figure S6 Fluorescence detection of fractions eluted during free flow electrophoresis. Fluorescence of free *Am*PBS (blue), free PSII (green) and crosslinked PBS-PSII super complex (red) was normalized to the maximum fluorescence peak of each sample. Samples were excited at 430 nm and fluorescence detected at 680 nm. Fractions containing the PBS-PSII super complex are indicated by dashed lines.



Figure S7 SEM images of MP-ITO. a and b: top view. c and d cross-section with layer indication for pure indium tin oxide (ITO) and macro-porous ITO (MP-ITO). b indicates the diameter of the pore opening on top of the MP-ITO based electrode. d shows the diameter of some pores in the cross-section. Note that the absolute pore diameter and the pore opening can differ depending on the position where the hollow pore is epode to the surface. Cross sections (c and d) were prepared by using the FIB (focused ion beam) technique, for details see experimental section.

Publication	IPCE [%]	Ref.
Maly <i>et al.</i> 2005	0.013	13a
Badura <i>et al.</i> 2008	3.1	14
Kato <i>et al.</i> 2013	0.008	S2
Hartmann et al. 2014	0.052	6
Mersch et al. 2015	0.37	23
Sokol <i>et al.</i> 2016	4.4	15
Hartmann et al. 2018	0.37	30
This study	10.9	

 Table S2
 IPCE values of reported direct electron transfer PSII-based biophotoanodes

100 mM KCl) a potential of +0.4 V vs. Ag/AgCl (3 M KCl) was applied (n = 2). Light intensities of 370 μ W cm⁻² (607 nm) and 300 μ W cm⁻² (685 nm) were used in 30 s light off/on cycles.

Flastrada madification	T _{1/2} with standard deviation [min]		
Electrode modification	607 nm	685 nm	
PSII/P-Os	182 ± 23	81 ± 6	
AmPBS-PSII/P-Os	189 ± 11	62 ± 5	
SynPBS-PSII/P-Os	177 ± 35	72 ± 19	
MLPBS-PSII/P-Os	141 ± 5	75 ± 13	



Figure S8 Monochromator measurement of MP-ITO electrodes without PSII. (A) P-Os without addition of PBS and (B) with addition of *Am*PBS (light blue), *ML*PBS (purple) or *Syn*PBS (blue). A 5 µl droplet containing (A) 5 mg mL⁻¹ P-Os and 0.02 mg mL⁻¹ PEGDGE or (B) 5 mg mL⁻¹ P-Os, 3 mg mL⁻¹ PBS and 0.02 mg mL⁻¹ PEGDGE dissolved in distilled water was drop-casted on the electrode surface. After 30 min incubation at 4 °C in dark, the electrodes were rinsed carefully with buffered electrolyte solution (50 mM MES, pH 6.5, 10 mM MgCl₂, 10 mM CaCl₂, 100 mM KCl) and a potential of +0.4 V vs. Ag/AgCl (3 M KCl) was applied.

S1 – L. Eichacker, G. Weber, U. Sukop-Köppel and R. Wildgruber, Methods Mol. Biol., 2015, 1295, 415-25.

S2 – M. Kato, T. Cardona, A.W. Rutherford and E. Reisner, J. Am. Chem. Soc., 2013, 135, 10610-10613.