Electronic Supporting Information

Titel: „Advanced unidirectional photocurrent generation via cytochrome *c* as reaction partner for directed assembly of photosystem I“

Kai R. Stieger, Sven. C. Feifel, Heiko Lokstein and Fred Lisdat

(I) Assembly Study of photosystem I on cytochrome *c* modified electrodes

1A) Dependence of protein deposition on pH

Assembly studies have been performed to verify the optimal conditions to deposit photosystem I (PSI) on cytochrome *c* (cyt *c*). First, SPR-experiments have been done showing the influence of pH on the assembly process. Cyt *c* has been injected for 120 s at a flow rate of 1 µL min^{-1} in a concentration of 30 µM in phosphate buffer (5 mM) with different pH (6.5-8) on a prior modified mercaptoundecanoic acid / mercaptoundecanol (MUA/MU, 1:3) gold-chip. Afterwards PSI has been injected for 120 s at a flow rate of 1 µL min^{-1} in a concentration of about 1.5 µM in phosphate buffer (5 mM) with corresponding pH. In Fig. s1A, the surface concentration after the injection step is plottet against the used pH. Here, the cyt *c* assembly exhibits a strong reduction in surface concentration at pH 6.5 compared to the other pH values. For the PSI adsorption a clear maximum was found at pH 7. For further studies, all experiments have been carried out at pH 7.

1B) Dependence of PSI deposition on concentration

In a next set of SPR experiments PSI has been injected for 120 s at a flow rate of 1 µL min^{-1} at different concentrations to a surface with an adsorbed monolayer of cyt *c*. Prior to this injection the cyt *c* monolayer has been formed by injecting cyt *c* in a concentration of 30 µM for 120 s at a flow rate of 1 µL min^{-1} in phosphate buffer (5 mM, pH 7) to a MUA/MU-modified gold chip. Fig. s1B shows the concentration dependence of mass deposition of PSI. The maximum surface concentration within this time frame can be achieved at a concentration of approximately 0.2 to 0.3 µM. Higher or lower concentrations decrease the rate of protein adsorption. For further experiments PSI have been used at a concentration of 0.2 µM.

2A) Time dependence of PSI deposition

In this SPR experiment the time dependent adsorption of PSI to a cyt *c* monolayer was studied for different time intervals. Here, a 0.2 µM PSI solution have been flushed over the surface from 2 until 120 mins. The cyt *c* monolayer has been prepared by injecting cyt *c* to a MUA/MU-modified gold chip for 120 s at a flow rate of 1 µL min^{-1} in phosphate buffer (5 mM, pH 7). The result is shown in Fig. s2A. The maximum surface concentration is reached at about 60 mins. The one-site-binding equation has been used to fit the curve, which exhibits a saturated surface concentration of 1085 ng cm^{-2} (~1 pmol cm^{-2}).

2B) Dependence of the photocurrent output of an Au-SAM/cyt *c*/PSI electrode on the light power

Photocurrent densities have been measured under various light intensities of a white light LED lamp (Zahner) ranging from either 0 – 20 mW cm^{-2} and 0 – 60 mW cm^{-2}, without any additional electron acceptor in solution. This experiment has been performed under aerobic conditions in phosphate buffer (5 mM, pH 7). To fit the curves the Michaelis-Menten equation have been used considering PSI as photon converting enzyme and photons as substrate as previously proposed by Badura *et al.* 2011.[7] The light intensity is proportional to the photon concentration. In Fig. s2B the data and the fit are shown. At low light power the photocurrent densities do not follow the kinetic model.

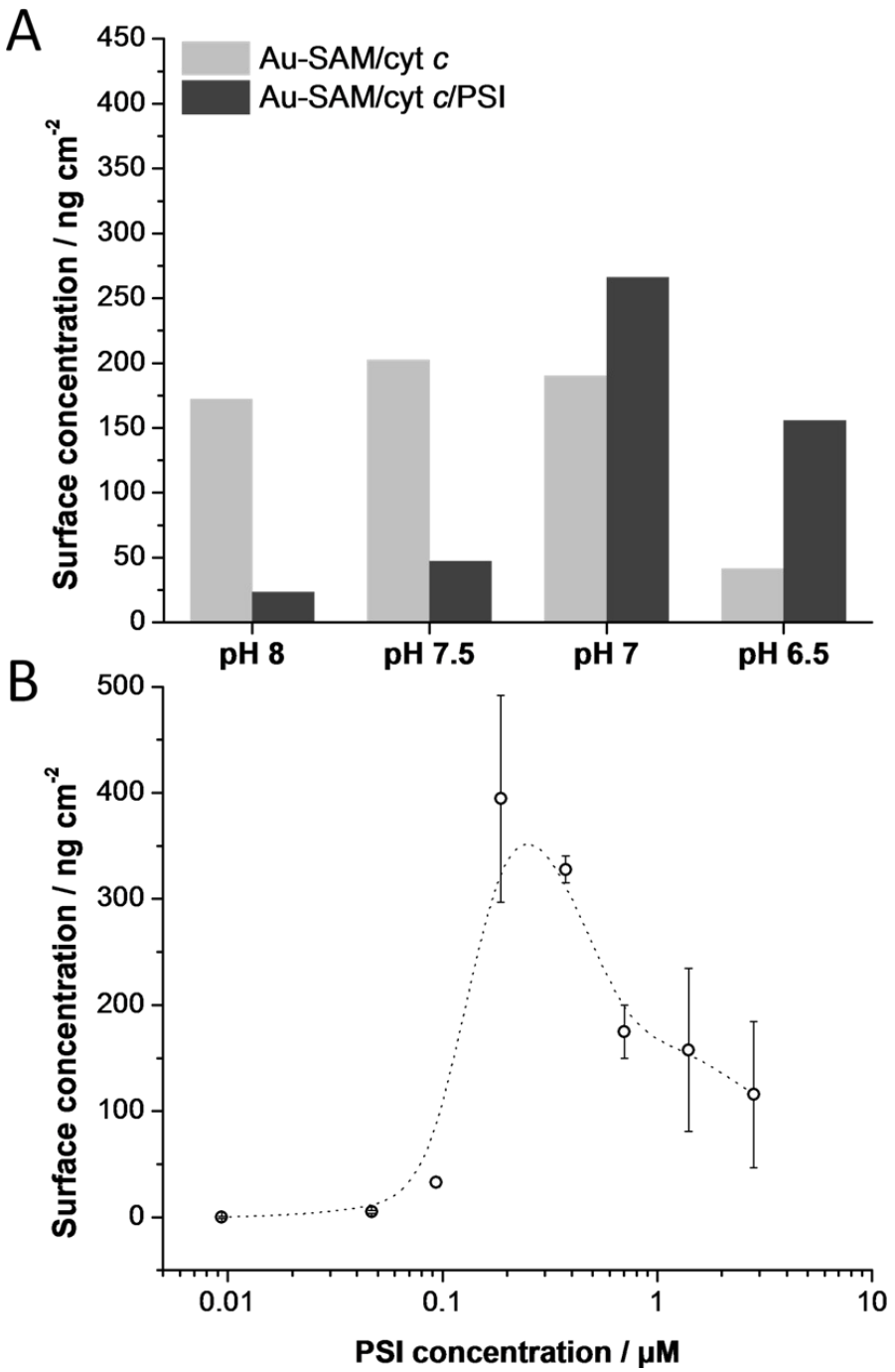

Fig s1 Surface plasmon resonance (SPR) experiments to elucidate the assembly conditions of photosystem I (PSI) on a cytochrome *c* (cyt *c*) monolayer. Here, the pH of the assembly buffer was changed from pH 8 to pH 6.5 to verify the best interaction between cyt *c* and PSI. At pH 7 PSI concentration was changed to investigate the optimal protein concentration for the highest mass deposition. (A) Dependence of cyt *c* assembly (gray) on a mercaptoundecanoic acid / mercaptoundecanol (MUA/MU) 1:3 modified gold-surface and photosystem I assembly (black) on a MUA/MU/cyt *c* surface according to the pH of the assembly buffer. (B) Concentration dependence of PSI assembled on a MUA/MU/cyt *c* modified gold-surface. Surface concentration is calculated from responsive units (RU). For experimental details see text.

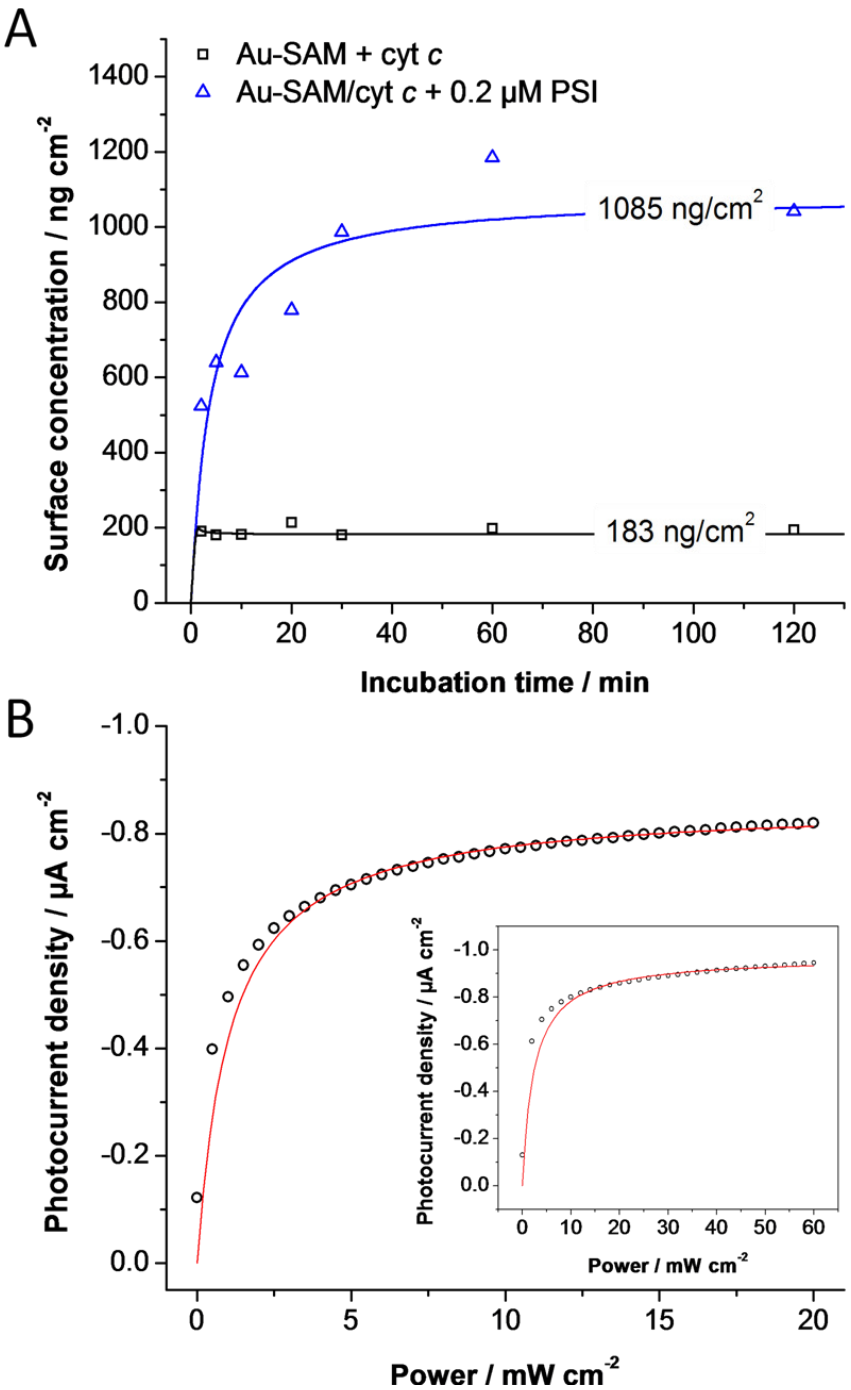

Fig. s2 (A) Surface plasmon resonance (SPR) experiment to elucidate the maximal mass deposition of photosystem I (PSI) on a mercaptoundecanoic acid / mercaptoundecanol (MUA/MU) 1:3 / cytochrome *c* (cyt *c*) modified gold-surface. Incubation time dependence of the assembled surface concentration of PSI on MUA/MU/cyt *c* modified gold-surface. The MUA/MU modified gold-surface had been in conatct with the cyt *c* solution (black squares) before the MUA/MU/cyt *c* modified gold-surface has been in contact with the PSI solution (blue triangles). Surface concentration has been calculated from responsive units (RU). (B) Light intensity dependence vs. photocurrent density of a Au-MUA/MU/cyt *c*/PSI electrode without additional mediator (MV) in solution for two different sets of light intensities (0 - 20, 0 - 60 mW cm^{-2}). The data has been fitted using the Michaelis-Menten-equation. For experimental details see text.

(II) Surface potential calculations at monomeric photosystem I

Surface potential calculations of monomeric PSI (*T. elongatus*, PDB: 4FE1) has been done at pH 7 to verify the excess charge distribution of PSI and thus to support the basic idea of electrostatic assembly on cyt *c*. A similar calculation has been done by Mukherjee *et al.* 2011.[6] Here, the surface potential has been calculated from the crystal structure of Brunger *et al.* 2012[1] (PDB: 4FE1) using the PyMOL Molecular Graphics System (PyMol, Version 1.7, Schrödinger, LLC.). Using PDB2PQR 1.8, pKa calculations have been performed by PROPKA at pH 7, while using PARSE as standard forcefield.[3-4] The adaptive Poisson-Boltzmann Solver[2] (APBS) was used with standard parameters (Temperature: 298.15 K, protein dielectric = 2.0, solvent dielectric = 78.0). For simplicity ionic strength and concentrations have been assumed to be 5 mM K^+ (ionic radius = 133 pm) and 5 mM $H_2PO_4^{-1}$ (ionic radius = 213 pm). Fig. s3 shows the surface potential distribution in units of K_BT/e (from -5 to 5) of monomeric PSI in three different views (luminal, side, stromal) with the cytochrome *c6*/plastocyanin docking site[5] (black circle) at the luminal side of the PSI. This calculations verify the negative (red, luminal) and positive (blue, stromal) potential situation in PSI at pH 7 in a low ionic buffer system. It supports the idea of an orientated assembly of PSI on cyt *c* with a positive excess net-charge.

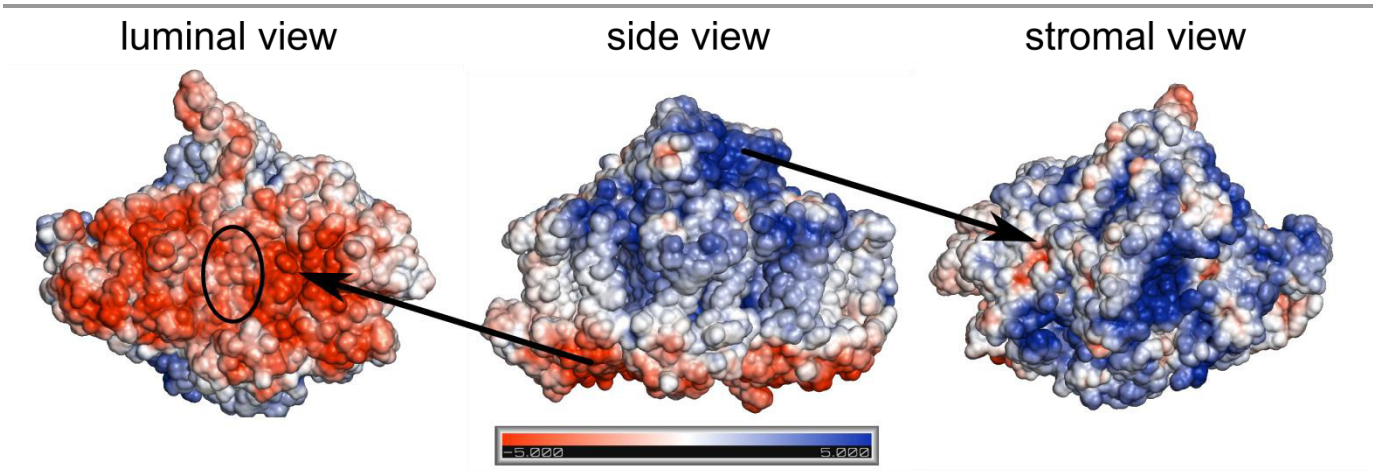

Fig. s3 Surface potential calculation of monomeric PSI (PDB: 4FE1) from three different view points (luminal, side, stromal) in phosphate buffer (5 mM, pH 7). Color bar range from a negative potential of -5 K_BT/e (red) to a positive potential of 5 K_BT/e (blue). The black circle shows the cytochrome *c6*/plastocyanine docking site. Calculations have been done with the use of PyMol V. 1.7, PDB2PQR V. 1.8, APBS V. 1.3. For citation see text.

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