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<td>As Published</td>
<td><a href="http://dx.doi.org/10.1038/srep00234">http://dx.doi.org/10.1038/srep00234</a></td>
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<tr>
<td>Publisher</td>
<td>Nature Publishing Group</td>
</tr>
<tr>
<td>Version</td>
<td>Final published version</td>
</tr>
<tr>
<td>Accessed</td>
<td>Thu Jan 03 06:24:36 EST 2019</td>
</tr>
<tr>
<td>Citable Link</td>
<td><a href="http://hdl.handle.net/1721.1/97441">http://hdl.handle.net/1721.1/97441</a></td>
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Self-assembled photosystem-I biophotovoltaics on nanostructured TiO$_2$ and ZnO

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The abundant pigment-protein membrane complex photosystem-I (PS-I) is at the heart of the Earth’s energy cycle. It is the central molecule in the “Z-scheme” of photosynthesis, converting sunlight into the chemical energy of life. Commandeering this intricately organized photosynthetic nanocircuitry and re-wiring it to produce electricity carries the promise of inexpensive and environmentally friendly solar power. We here report that dry PS-I stabilized by surfactant peptides functioned as both the light-harvester and charge separator in solar cells self-assembled on nanostructured semiconductors. Contrary to previous attempts at biophotovoltaics requiring elaborate surface chemistries, thin film deposition, and illumination concentrated into narrow wavelength ranges the devices described here are straightforward and inexpensive to fabricate and perform well under standard sunlight yielding open circuit photovoltage of 0.5 V, fill factor of 71%, electrical power density of 81 µW/cm$^2$ and photocurrent density of 362 µA/cm$^2$, over four orders of magnitude higher than any photosystem-based biophotovoltaic to date.

PS-I precisely orchestrates 96 chlorophyll molecules with electron donors and acceptors$^1$ (Fig 1 a) achieving efficient coherent energy transfer$^2$ and near-unity charge separation quantum yield at ambient temperatures$^3,4$. This is a feat unmatched by any man-made photovoltaic device and has led to PS-I being studied as a candidate for many nanobioelectronic applications$^5-8$, as well as being the original inspiration behind the dye-sensitized solar cell (DSC)$^9$. So far, research on PS-I biophotovoltaics has focused on proof-of-principle devices, studying immobilized PS-I complexes and isolated reaction centers (RC) in self-assembled monolayers (SAMs) on flat electrodes$^5-8$.

Two main obstacles hinder biophotovoltaics from being a more widely studied technology, constantly improved by many independent researchers. Firstly, while extracting PS-I from a variety of abundant sources is easy, drying this extract on electrodes results in rapid loss of function due to denaturation. Secondly, the electrical power output of biophotovoltaics to date has been so low$^5-8$, that they were of little practical interest and the characterization necessary to improve their performance required cumbersome, expensive to iterate-optimize methods. For instance, in order to obtain measureable photocurrents it was necessary to make up for the low absorption cross sections of the nearly transparent active SAMs. In prior studies this was addressed by using either laser light with power equivalent to 100 times that of standard air-mass 1.5 (AM1.5) sunlight$^7$, or incoherent monochromatic light$^6$ in both cases precisely tuned to the pigment absorption maxima—an unrealistic emulation of real-world conditions requiring elaboration instrumentation.

Results
We have removed these two obstacles by designing a PS-I biophotovoltaic whose IV characteristics can be easily studied under regular sunlight and its design and fabrication are amenable to low-cost, iterative optimization. To avoid denaturation, we treated PS-I with designer peptide surfactants$^1$. To improve photovoltaic performance we increased the light absorption cross-section without changing the footprint by departing from the traditional flat
We used nanocrystalline TiO2 and ZnO nanowires to provide a lyte and platinized glass as is common for conventional DSCs. Conducting substrates and the circuits were completed by liquid electrolytes, PS-I molecules were air-dried on nanostructured semiconducting electrodes (TiO2 nanocrystals and ZnO nanowires). Finally, we showed how high affinity peptide motifs bioengineered to promote selective adsorption to specific substrates can enhance photovoltaic performance. These materials, geometries and design resulted in simple, robust biophotovoltaic devices of unprecedented performance.

Photochemically active, trimeric PS-I was isolated and characterized from the thylakoids of the thermophilic cyanobacteria Thermosynechococcus elongatus as described in detail in Fig. 2 of Iwuchukwu et al. This PS-I was stabilized for several months in solution and in dry form by designer peptide surfactants (Fig. 2). To build devices, PS-I molecules were air-dried on nanostructured semiconducting substrates and the circuits were completed by liquid electrolyte and platinized glass as is common for conventional DSCs (Fig. 1b). We used nanocrystalline TiO2 and ZnO nanowires to provide a large effective surface area (Aeff) for PS-I adsorption and light harvesting (Fig. 3) and without any further optimization, these devices achieved electrical power outputs Pout of up to 81 µW/cm² and area-normalized short-circuit current densities Isc,Norm of up to 362 µA/cm² (Fig. 4). These parameters are to be compared to the over 10,000 times lower Isc,Norm (~30 nA/cm²) reported previously with monochromatic illumination tuned to the ~800 nm absorption peak of a monolayer of PS-I on a thin film of gold (actual power efficiency not reported) and to the up to 120 µA/cm² Isc,Norm observed when isolated RCs were chemically bound to a series of evaporated metallic and semiconducting thin films and illuminated with 10 W/cm² laser light (one-hundred times the power density of AM1.5 sunlight) all concentrated into the 808 nm absorption peak of RCs. While there have been various estimated and theoretical maximum efficiencies (e.g. an upper possible limit of 20% efficiency for RC-biophotovoltaics), there have been no reports of conversion efficiency η for PS-I (where η = Pout / Pinc the total power of the incident light and Pout the total resulting electrical power). Our photocurrent measurements were carried out under AM1.5 standard simulated sunlight with precisely controlled active surface areas (0.159 cm²) and continuously-calibrated, spectral-mismatch corrected sunlamps, as is the standard in the conventional photovoltaic industry. Under these conditions, that closely emulate outdoor sunlight, the total external efficiency of conversion of incident sunlight to useable electricity was η ~ 0.08%. This must not be confused with the sometimes very high quantum or internal efficiencies routinely reported for organic optoelectronics.

**PS-I Biophotovoltaic Solar Cell.** In photosynthetic organisms, PS-I catalyses light-driven electron transfer from reduced plastocyanin located in the lumen, to ferredoxin in the stroma providing a path across the membrane consisting of a chain of cofactors (Fig. 1a). Light absorption results in excitation of the primary electron donor (P700), transfer to primary and secondary electron acceptors and finally crossing of the membrane. In our biophotovoltaic solar cell, the role of plastocyanin was played by the Co(II)/Co(III) ion-containing electrolyte Z813, and ferredoxin was replaced by either nanocrystalline TiO2 (Fig. 1b left) or ZnO-nanowires (Fig. 1b right) to provide large-surface area electron acceptors.

When using TiO2, we chose the pore size of the nanocrystalline film to be double the diameter of our PS-I particles to ensure a high probability of physisorption. When using ZnO nanowires, we substituted (by a self-assembly exchange reaction) the naturally-occurring electron acceptor PsAE subunit with one that contained an amino acid sequence with high affinity for ZnO: RSNTRMTARQHRSANHKSTQRARS.
(PsaE-ZnO) thus promoting adhesion and minimizing the distance that electrons must travel to the anode (Fig. 2 a).

**Stabilization of Native and Bioengineered PS-I.** Stabilization of dry PS-I extract on glass and on the transparent conductor Indium-Tin-Oxide (ITO) for at least three weeks has been described elsewhere. Here, we mixed PS-I at 0.4 mg/mL in a 1:1 ratio with 0.1% (w/v) of the 2.4 nm long cationic peptide surfactant Ac-AAAAAAK-NH₂ (A₆K) consisting of six alanines and a lysine at the amidated C-terminus and observed enhanced stability (Fig. 2 b). The bioengineered, self-assembled PS-I containing PsaE-ZnO exhibited low-temperature fluorescence peaks identical to unmanipulated PS-I extract undergoing identical treatment (Fig. 2 c), indicating that subunit substitution did not adversely affect structure and we expect the photochemical-activity enhancing effect of A₆K to be similar in both cases.

**Nanocrystalline TiO₂ and ZnO Nanowires as Large Surface Area Photoanodes.** $I_{sc}$ is directly proportional to electrical power output and is controlled by light absorption. To increase the useful light incidence angle and optical cross-section of our biophotovoltaics, we used two types of rough, large surface area semiconductors as photoanodes. This made our devices able to absorb light from nearly a $2\pi$ solid angle and provided an increased effective area for PS-I. **Figure 2** | (a) To promote attachment and orientation of the entire PS-I complex to ZnO nanowires, we fused the ZnO-binding peptide tag RSNTRMTARQHRSANHKSTQRARS (expressed in E.coli) to the N-terminus of the PsaE subunit. Upon exchanging native PsaE in favor of PsaE-ZnO and self-assembly, the modified PS-I preferentially binds to ZnO nanowires by the electron acceptor side, minimizing distance between electron acceptor and electrode and maximizing electron transfer. (b) The marked increase in the rate of methyl viologen (MV)-mediated oxygen reduction by PS-I in the presence of the designer surfactant peptide A₆K, indicates that A₆K maintains the ability of PS-I to catalyze photochemical charge separation and MV-mediated O₂ uptake relative to the control treatment with either the non-ionic detergent Triton X-100 (middle) or DDM present in the isolation buffer (left). Increased O₂ uptake activity cannot be due to free chlorophyll-mediated O₂ consumption (via chlorophyll) since treating cyanobacterial PS-I with strong detergents (1% SDS) leads to only minimal loss of chlorophyll from PS-I. All activity tests are normalized per mol of PS-I. (c) Low-temperature fluorescence of PS-I self-assembled in the presence of excess PsaE-ZnO (red) peaks at the same two wavelengths as unaltered PS-I extract (blue) indicating that bulk chlorophyll organization is preserved and the stabilizing interaction with A₆K is likely similar in both cases.
SAM adsorption: $A_{\text{eff}} \sim 200$ times that of the flat footprint for TiO$_2$ nanocrystals (Fig 3 a) and $\sim 30$ times with ZnO nanowires (Fig 3 b). In addition to providing an inexpensive alternative to TiO$_2$, the charge carrier mobility of ZnO nanowires is one-hundred times faster than TiO$_2$ and large-scale, ambient temperature solution-growth of ZnO nanowires is simple, requiring fewer steps, less energy and is easily adaptable to flexible conducting substrates. However, ZnO DSC photoanodes have so far always underperformed when compared with identically sensitized TiO$_2$. This is due to their lower roughness factor $\rho$, poor dye loading, and the shunting of the photocurrent by the corrosion of ZnO by common DSC dyes and electrolytes. The IV behavior of our biophotovoltaics indicated that tagging PS-I with an amino acid sequence that binds to ZnO promoted orientation and/or binding to ZnO nanowires with $\eta = 0.03\%$, for PsaE-ZnO, while $\eta = 0.00\%$ for the histidine-tagged control (Fig. 4 d and e respectively). The $I_{\text{sc}}$ achieved with ZnO (Fig. 4 d) is roughly a factor of ten lower than that with TiO$_2$, consistent with the ratio of the two $A_{\text{eff}}$ ($\rho_{\text{ZnO}} \sim 30$, $\rho_{\text{TiO}_2} \sim 200$) suggesting that $A_{\text{eff}}$ is the primary control of $I_{\text{sc}}$.

**Photocurrent Measurements Under Realistically Simulated Solar Illumination.** The shapes of the IV curves (Fig. 4) obtained upon exposing our devices to AM1.5 sunlight for both PS-I complexes on TiO$_2$ and bioengineered PS-I on ZnO are similar to conventional DSCs. After accounting for the UV-excited photocurrent (Fig. 4b) the following four types of control devices, containing all buffer components and electrolyte, did not yield any additional photovoltaic action: i) devices made with denatured PS-I (boiled for 10 min), ii) blanks without PS-I or A$_6$K , iii) blanks without PS-I but with A$_6$K , iv) devices with PS-I but no A$_6$K. This leaves stabilized, non-denatured PS-I as the only possible agent behind 80% of the maximum measured photocurrent (the remaining 20% being due to UV excitation of TiO$_2$ and ZnO). We emphasize that these photocurrents cannot be attributed to sensitization by leached chlorophyll, because unless precisely organized by the PS-I scaffold, chlorophyll alone does not act as a photovoltaic sensitizer, the interaction between the chlorophyll ester units with the TiO$_2$ surface is weak and chlorophyll does not adsorb on TiO$_2$ or ZnO.

**Discussion**

Using inexpensive raw materials and simple processes, we have achieved record biophotovoltaic performances. We isolated PS-I from thermophilic cyanobacteria, but the structural similarity between this and higher plant PS-I suggests that many other
Figure 4 | Photocurrent measurements of PS-I biophotovoltaic devices under AM1.5 simulated insolation at 298 K. Illuminated surface 0.159 cm² (a) 40 µl of PS-I (0.2 mg/mL) stabilized by 1:1 0.1%w/v designer surfactant peptide A₆K (resulting in a total of 8 µg of protein) dried on a 3.8 µm thick layer of 60 nm-pore TiO₂ produces an IV curve typical of a DSC. Fill factor (ff) ranged from 64% to 71% (b) Eliminating ultraviolet (UV) wavelengths below 350 nm resulted in a ~20% reduction in the normalized short circuit current (JₘNorm) and a ~10% reduction in the open circuit voltage (Voc) indicating that 80% of the total electrical power generated is due to PS-I (the rest due to UV photovoltaic response of TiO₂). These photocurrents cannot be attributed to sensitization of TiO₂ by leached chlorophyll derivatives rapper. A blank control containing A₆K generated no power when exposed to UV-less sunlight of any intensity, neither did controls built with PS-I denatured by boiling for 10 minutes, nor devices built with PS-I not treated with A₆K (data not shown). Total incident-light to electrical external power conversion efficiency η was 0.08% with UV, 0.07% without. (c) Linearity test of PS-I photocurrent at intensities from 0.01x to 1.0x AM1.5 shows behavior typical of a DSC. (d) IV of PS-I self-assembled in the presence of an overabundance of PsaE-ZnO electron-accepting subunit yields a total power conversion efficiency, η=0.03%. (e) Control: IV of PS-I self-assembled with an overabundance of non-ZnO specific histidine-tag containing PsaE subunit yields lower Voc, JₘNorm and η=0.00% as expected, suggesting that the PsaE-ZnO tag either enhanced binding of PS-I to the ZnO nanowires or favored the optimal orientation, or both. Z813 Co(II)/Co(III) electrolyte and platinized glass were used to complete all devices.
abundant sources of highly pigmented thylakoids can also be used including the normally discarded leafy parts of common agricultural crops or timber. PS-I is indeed a promising raw material for ultra-low-cost biophotovoltaics as postulated by LaVan et al., but significant optimization challenges must be met. The devices characterized here indicate merely the lowest limit of PS-I biophotovoltaic performance possibilities. Since we did not perform any optimization, significant efficiency gains can be expected from increased loading, better oriented and more tightly coupled PS-I to photoanode, customization of stabilizing agents and better matching of bio-friendly electrolytes with photoanode/phothocathode substrates. The short-term stability and photoactivity of various treatments of PS-I is summarized in Fig. 2 and discussed in detail elsewhere11,12 and is encouraging. Clearly, tracking biophotovoltaic performance over the long-term is an important future step but was beyond the scope of the present study11. While we used centrifugation, equally pure PS-I can be isolated from plant or bacterial extracts by porous membrane bioseparation, an inexpensive method that can be scaled up to industrial levels by simple yet highly efficient affinity binding with protein-specific epitope tags19. The design and methodology principles described here are suitable to biophotovoltaics that can be characterized under normal sunlight. We hope these results encourage optimization efforts to deliver biosolar power that is truly “green”.

Methods

Cloning and expression of ZnO-binding subunits. While we here show data on PsAE only (Fig. 2c), plasmids coding for PsAE (Uniprot accession number Q62007) and also PsD (Uniprot accession number P34982) from Cyanobacterium Mastigocladus laminosus were expressed and studied. Both self-assemble near the final electron acceptor and ejection site of the PS-I complex and are ideally placed to appropriately attach and orient the molecule to a photoanode. The peptide tag RSNTRMTARQHRSANHKSTQRARS10, was fused to the N-terminal of the protein-specific epitope tags19. The design and methodology principles described here are suitable to biophotovoltaics that can be characterized under normal sunlight. We hope these results encourage optimization efforts to deliver biosolar power that is truly “green”.

Subunit exchange. To exchange the native PsAE (or PsD) in PS-I to the recombinant PsAE-ZnO (or PsD-ZnO), the recombinant proteins were incubated with PS-I in a 50:1 molar ratio for 2 hours at 4 °C. Free PsAE (or PsD) was removed by centrifuging the sample over an YM-100 filter (Millipore) leaving unbound PsAE (or PsD) in the flow-through (Fig. 2a). These exchange reaction protocols are expected to yield exchange efficiency of >65%. Since we used a dual isolation system using both IMAC and sucrose density exchange, we know with certainty that our efficiency is higher than 65%.

Stability test of immobilized PS-I via low temperature fluorescence spectroscopy. Low-temperature fluorescence spectroscopy was used to ascertain stability of the PS-I complex when immobilized on ZnO nanowires. Each 1 cm × 1 cm ZnO nanowire chip covered in PS-I (drops standardized to contain a total of 2.9 μg of protein) was placed on a custom made rod holder and cooled under liquid nitrogen (−196.15 °C) in a cryostat with glass windows at right angles. Fluorescence was excited optically using a 408 nm laser incident at a ~45° angle to the normal to the chip surface. Steady-state emission spectra were recorded using a CCD spectrometer (slit width 20 μm) with an optical fiber input oriented +45° to the normal thus detecting from a right angle to the excitation beam. The fluorescence spectrum of each sample was qualitatively normalized and the peaks were found to be identical to those of native PS-I (Fig. 2c), as expected if no structural changes resulted from the exchange reaction or immobilization on the ZnO nanowires.

Fabrication of sealed solar cells, current-voltage (IV) and control measurements. Devices (Fig 1) were made by allowing a 40 μL drop of PS-I solution to dry at room temperature on two different nanostructured semiconducting electrodes: a TiO2 of 60 nm average pore size, 3.8 μm film thickness, roughness factor: pTiO2 ~200 (i.e. surface area increases by ~50% per μm of film thickness) fabricated as described previously21 (Fig 3a) and a mat of 3 μm tall, ZnO nanowires (grown on ZnO nanoparticle-seededITO glass as described elsewhere22) with pZnO ~30 (Fig 3b). Cells were sealed with 40 μm thick heat-treated Syrlin gaskets and Z813 Co(II)/Co(III) electrolyte composed of:

0.5 M Co(OH)3(bis(trifuoromethanesulfonfyl)imide)2
0.05M Co(OH)2(bis(trifuoromethanesulfonfyl)imide)
0.2 M LiClO4 dissolved in a 60%Ethylene Carbonate and 40% acetonitrile (v/v) solvent, which was introduced by capillary action. Platinum-coated, fluorine-doped tin-oxide (FTO) glass was used as the counter electrode. In all cases, photoanodes of 0.5 mm diameter and IV curves for all scans were used and IV curves were taken at semiconductor area. All measurements were performed using a thermally-corrected-calibrated solar simulator with neutral density filters as described in detail by Ito et al.60 devices total were tested, the results reported in Fig 4 belong to individual devices exhibiting typical behavior, not averages over many devices. The error bars in all IV curves are included in the plots but are smaller than the size of the data point markers. Control electrolyte made with TiO2 and electrolyte but containing no PS-I and exposed to full sunlight (including UV) gave Pmax 28 μW/cm2, with 277 μA/cm2, 257 mV Voc with a fill factor of 0.39. It was impossible to obtain IPCE curves of control unsensitized devices due to very low currents, as expected (see supplemental materials).

PS-I Purification. Our PS-I was identical to that used in Iwuchukwu et al.11. Briefly, PS-I was extracted from the thylakoid membranes of the thermophylic cyanobacteria T. elongatus. Bacteria were grown in 2 L airlift fermenters (Bethesda Research Labs, Bethesda MD) to late log phase at 56°C. Bacterial growth was followed by incubation with 0.25% (w/v) lysosome for 2 hours at 37°C under gentle agitation. Cells were lysed with the French press; unlysed cells were removed at 3,000 g for 5 min and membranes were collected at 20,000 RPM. The membranes were washed and solubilized as in Fromme and Witt6 with the exception that in the final wash 3 M NaBr was used. Then the supernatant was loaded on a 10-30% linear sucrose gradient (20 mM MES pH 7.0, 10 mM MgCl2, 10 mM CaCl2 and 0.3% w/v n-dodecyl-b-D-maltopyranoside (DDM), for 16 hours at 24,000 RPM. The lower green band was collected (see Fig. 2 of Iwuchukwu et al.11), pooled and stored at −20°C. Purity was confirmed by Tris-tricine SDS-PAGE gel electrophoresis. The chlorophyll content of PS-I was measured as described previously11.


**Acknowledgements**

We thank the Intel Corporation for their unrestricted gift partially seed-funding this work. LK gratefully acknowledges her fellowship by the Knut and Alice Wallenberg foundation. SZ gratefully acknowledges the John Simon Guggenheim Foundation for his Guggenheim Fellowship to pursue this research. We are grateful to Sloan Kulper for creating panels c, d & e of Figure 3. We are indebted to S. M. Zakeerrudin, Jun-Ho Yum, Peter Chen and Jiang Liu for their assistance. BB and SZ were partially supported from an NSF NIRT award. BB was partially supported from a gift from the Gibson Family Foundation.

**Author contributions**

AM, BB and SZ wrote the main manuscript text. AM, KM, LK, DY, MV, MN, BB performed the experiments. AM, KM, LK, MV, BB prepared figures 1–4. All authors reviewed the manuscript.

**Additional information**

Supplementary Information accompanies this paper at http://www.nature.com/scientificreports

**Competing financial interests:** The authors declare no competing financial interests

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**How to cite this article:** Mershin, A. *et al.* Self-assembled photosystem-I biophotovoltaics on nanostructured TiO2 and ZnO. *Sci. Rep.* **2**, 234; DOI:10.1038/srep00234 (2012).