Published in final edited form as:

Nat Chem. 2010 November ; 2(11): 929–936. doi:10.1038/nchem.822.

Photoelectrochemical complexes for solar energy conversion that chemically and autonomously regenerate

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Abstract

Naturally occurring photosynthetic systems use elaborate pathways of self-repair to limit the impact of photo-damage. Herein, we demonstrate a complex that mimics this process consisting of two recombinant proteins, phospholipids and a carbon nanotube. The components self-assemble into a configuration in which an array of lipid bilayers aggregate on the surface of the carbon nanotube, creating a platform for the attachment of light-converting proteins. The system can disassemble upon the addition of a surfactant and reassemble on its removal over an indefinite number of cycles. The assembly is thermodynamically meta-stable and can only transition reversibly if the rate of surfactant removal exceeds about 10^{-5} sec^{-1} . Only in the assembled state

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Author contributions

M.H.H., J.H.C., A.A.B. and M.S.S. designed the research. M.H.H., J.H.C., A.A.B., R.A.G. and D.A.H. synthesized the complexes. M.H.H. performed the photoelectrochemical experiments. J.H.C. purified the complexes and performed the spectroscopic experiments with A.C.C. A.A.B. performed kinetic modeling of the complex formation. E.S.J. performed the modeling of the DMPC configuration on the SWNT. A.M. and C.A.W. supplied the photosynthetic reaction centers. Y.V.G. and S.G.S. supplied the membrane scaffold proteins. T.H.B., A.S.Z. and K.J.V. performed AFM measurements. E.K.H. performed SANS measurements. M.S.S. originated the concept for the paper. M.H.H., J.H.C., A.A.B. and M.S.S. co-wrote the manuscript with input from S.G.S. and C.A.W.

We develop the first synthetic photoelectrochemical complex capable of mimicking the self-repair cycle in plants. The reversible self-assembly of this meta-stable complex is driven via chemical signaling alone. We demonstrate a regeneration cycle to increase photo-conversion efficiencies by over 300% over 168 hours and extend lifetime indefinitely.

do the complexes exhibit photoelectrochemical activity. We demonstrate a regeneration cycle that uses surfactant to switch between assembled and disassembled states, resulting in increased photo-conversion efficiency of more than 300% over 168 hours and an indefinite extension of the system's lifetime.

The self-repair process in plants, algae, and photosynthetic bacteria uses molecular recognition and meta-stable thermodynamic states to make protein complexes that can be continually repaired by partial disassembly and reassembly with new components, initiated by chemical signals alone. For example, the repair of photo-damaged D1 protein in photosystem II (PS II) is initiated by both acceptor side- and donor side-induced photoinactivation of the protein, resulting in peptide bond scissions that alter protein conformation and drive the dissociation of damaged complex from the large PS II assemblies embedded within membrane stacks inside the chloroplast of a plant cell¹. The separated complex diffuses laterally, out from within the stacks of membranes toward the outer membrane regions, where it disassembles into peripheral light-harvesting complex II (LHC II) and a PS II core complex². The damaged D1 component of the PS II core is then fully degraded and the depleted complex equilibrates with newly biosynthesized D1 protein, resulting in the self-assembly of a tightly-bound, repaired complex. The repaired complex returns within the membrane stacks where it re-docks with the extended light-harvesting systems inside the membranes, thus completing the repair cycle¹. Central to this self-repair is molecular recognition of the components and thermodynamic meta-stability, allowing the system to reversibly transition between kinetically trapped and disassembled states. In this work, we extend these concepts to develop the first synthetic photoelectrochemical complex capable of mimicking key elements of this self-repair cycle.

Results and discussion

To develop such a complex, we examined the use of phospholipid-based light-harvesting nanostructures. Phospholipids have been used previously to disperse single- and multi-walled carbon nanotubes³, and the dialysis of phospholipids such as 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) in the presence of an amphipathic apolipoprotein (membrane scaffold protein; MSP) creates a lipid bilayer nanodisc (ND) approximately 10 nm in diameter and 5 nm high, as shown previously⁴. We find that such discs assemble onto a single-walled carbon nanotube (SWNT) such that the diameter is parallel to a nanotube, creating a platform for attaching membrane proteins (Fig. 1). One protein of interest for photoelectrochemical conversion is the photosynthetic reaction center (RC) isolated from the purple bacterium, *Rhodobacter sphaeroides*⁵. This bacterial reaction center is a protein complex composed of 4 bacteriochlorophylls (Bchls), 2 bacteriopheophytins (Bphes), and primary and secondary ubiquinones (Q_A and Q_B). Upon photoabsorption, the complex acts as a photoconverter, shuttling the formed exciton to the Bchl dimer (called the primary donor, P) where charge is separated, with the hole remaining (P⁺) and the electron transferred to the Q_B site on the other side of the reaction center via electron transfer reaction⁶. The incorporation of the reaction center into the nanodisc places the hole injection site (P⁺) directly facing the carbon nanotube, which may then act as a molecular hole conducting wire. We find that this ordered assembly of lipids, membrane scaffold protein,

nanodisc, and SWNT forms spontaneously when a sodium cholate-suspended mixture of each of the components is dialyzed to remove the surfactant. Control experiments (Supplementary Fig. 7) confirm that all components are necessary to form the structure. The complexes are broken apart upon the re-introduction of 2 wt% sodium cholate in a cycle that can be repeated indefinitely with no irreversible degradation of the photoelectrochemical properties of the assembled state, as described below.

We confirmed the parallel arrangement of the nanodiscs along the nanotube surface using atomic force microscopy (AFM) and small-angle neutron scattering (SANS). Typical AFM image shown in Fig. 2a reveals either free nanodisc stacks or nanodisc assembled along the nanotube axis. The height profile (Fig. 2b) along the nanotube, which indicates a height of 8 ± 0.4 nm, is consistent with a bilayer stack on either side of the nanotube. The specific orientation of the discs is confirmed by SANS (Supplementary Information section 1). Figures 2c and 2d show the scattering intensity versus reciprocal lattice vector for nanodisc (red, Fig. 2c) and ND-SWNT (red, Fig. 2d) described by the best fit model of an isotropic suspension of monodisperse 8 nm diameter by 4 nm high discs (blue, Fig. 2c) and a series of parallel discs in linear arrangement (black, Fig. 2d), respectively. The parallel arrangement leads to a maximum in scattering at $q = 2\pi/(2R)$, where R is the radius. In contrast, the blue curve in Fig. 2d compares the same data to that of an isotropic dispersion, highlighting the difference. These experimental results confirm the disc dimensions of 8 nm diameter with a 4 nm height and the particular parallel stacking arrangement along the nanotube. This arrangement allows for membrane proteins to orientate orthogonal to the nanotube surface. Specifically, this arrangement conveniently projects the hole injection site of the RC in close proximity to the nanotube surface, as depicted in Fig. 1.

We used density gradient centrifugation to specifically isolate the complexes from background components and further verify their structure⁷. The mixtures were added to a 5 mL stop-layer of 60 v/v% iodixanol, and subsequently, 5 density gradient layers of 50, 40, 30, 20, and 10 v/v% iodixanol/water solutions (1 mL each) were serially added in a centrifuge tube. Assembled ND-SWNT, RC-ND, and free lipids (i.e. no MSP) were also examined as controls. After centrifugation at 30,000 rpm for 7 hours, each sample in the tube was fractionated in twenty 250 μ L increments along the density gradient into a 96-wellplate. A fluorescence wellplate-reader tracked the fluorescent emission of a lipid-soluble Laurdan dye⁸, which indicates the presence of hydrophobic phases (Fig. 3a). Free lipid is significantly less dense than the hydrophobic nanodisc phases, all of which demonstrate a narrow peak near 1052 kg/m^3 assigned as the lipid bilayer nanodisc. A homebuilt near infrared (NIR) plate-reader tracked the fluorescence of the reaction centers themselves at 866 nm (Fig. 3b) and the fluorescence of the (9,1) SWNT (Fig. 3c). When reaction centers are included in the mixture, they reconstitute into the nanodisc phase, as seen by the peak in reaction center photoluminescence (PL) centered at 1050 kg/m^3 in the absence of SWNT (Fig. 3b). In contrast, when the reaction centers are added to the ND-SWNT complex, this peak shifts to 1077 kg/m^3 , precisely where the SWNT show a maximum for the RC-ND-SWNT and the empty ND-SWNT systems in the gradient. Both these photoluminescences show overlapping peaks at 1077 kg/m^3 (Fig. 3c) where the reaction center concentration is a maximum. This analysis unambiguously confirms that the reaction center is reconstituted in the nanodisc attached along the SWNT. Within the resolution of our experiment, the

reaction center does not appear to alter the density of the ND-SWNT complex significantly, but the results confirm that the RC-ND-SWNT complex is stable and can be isolated free from constituent components.

A closer examination of the photoluminescence from the SWNT before and after nanodisc formation reveals that near armchair ($n, n-1$) species exhibit a large red-shift (49 to 60 nm) of the emission wavelength (Fig. 4a). Before assembly, the SWNT fluorescence peak maxima are consistent for samples suspended in 2 wt% sodium cholate. Upon removal of the sodium cholate by dialysis for 30 min and subsequent formation of the RC-ND-SWNT structure described above, only the near armchair species in the mixture, (6,5), (7,6), and (8,7), demonstrate this large photoluminescence red-shift (Fig. 4b). The density separation analysis gives no indication that significant changes to the macromolecular structure occur for only these species. Because the chiral angle is so prominently recognized in spite of the wide variation in diameter and length among these three SWNT species, we hypothesize that the lipid bilayer in the nanodisc adsorbs such that the hydrocarbon chain of DMPC partially registers in a very specific manner with the graphene lattice, independent of the curvature of the SWNT sidewall. We note that the flexible hydrophobic chains are spaced such that they can align parallel to the unit vectors along the carbon atoms of the graphene lattice. Registration of hydrocarbon chains on SWNT has been observed previously⁴ for various surfactant molecules, dissolved in water, that are similar in size and properties to DMPC. However, in this work the photoluminescence shift is only observed when the DMPC molecules are bound with the membrane scaffold protein and the entire nanodisc structure is formed. Since the DMPC head group is hydrophilic, we expect that only the two tails contribute significantly to the DMPC adsorption to SWNT. Therefore, molecular dynamics was used to energetically relax the DMPC molecule relative to the graphene using Hyperchem software, and the AMBER force field⁹ was used to calculate the van der Waals energetic contribution for alignment to the graphene lattice of one of the two DMPC tails (13 carbon hydrophobic chain) (Supplementary Information section 2). The van der Waals energy of the aligned chain is 1 kcal/mol more thermodynamically favorable than that of the random chain orientation with graphene. This result is in agreement with our energy minimizing simulations that indicate that the tails of DMPC have a tendency to align with the graphene lattice, regardless of the initial DMPC conformation and orientation (Fig. 4d). This favorable alignment is consistent with a registry to the graphene lattice that is specific to the species of nanotube.

One useful aspect of the SWNT photoluminescence shift is that it is only observed when the nanodisc phase forms at the nanotube sidewall, and molecules that denature or disrupt the phase decrease the photoluminescence red-shift of the near armchair species back to values commensurate with other SWNT species (Fig. 4b). Nanotube suspension added to other preparations such as membrane scaffold protein or lipid alone via dialysis do not cause the photoluminescence shift (Supplementary Fig. 7), illustrating that it is a useful optical probe of the assembly/disassembly processes.

Remarkably, the complex can be chemically disassembled by adding 2 wt% sodium cholate into the initial separated components and subsequently reassembled upon its dialysis from the system. Figure 4c shows the process of repeated assembly/disassembly cycles as

monitored using the emission wavelength of the (6,5) nanotube. A dialysis flow chamber allowed for spectroscopic monitoring of a solution of 7 nM RC-ND-SWNT complex as a continuously supplied buffer was switched between surfactant-free and 2 wt% surfactant buffer. As the buffer is switched to surfactant-free media, the sodium cholate is dialyzed from the sample, resulting in the assembly and subsequent photoluminescence red-shift of the (6,5) nanotube as described above. Switching the buffer back to sodium cholate buffer causes the surfactant to diffuse back into the dialysis cell, disassembling the mixture into its starting components and blue-shifting the emission. The process can be repeatedly cycled in this way with no loss in fidelity for at least 5 cycles over 15 hours. Photoelectrochemical activity is also preserved even after repeated assembly/disassembly in this manner, as shown below.

The reversible assembly/disassembly capabilities of such a specifically orientated complex have no analog in any other synthetic photoelectrochemical structure, and we note that this is the first synthetic photoelectrochemical structure to mimic the dynamic equilibrium that forms the basis of natural self-repair. As discussed, this evolutionarily conserved self-repair process present in photosynthetic bacteria, algae, and higher plants results in the replacement of damaged D1 proteins with a newly synthesized protein. In this self-repair process, the new protein is reassembled into a functional PS II using the same intermolecular forces that drive the self-assembly process demonstrated in the synthetic system in this work¹.

Both the natural complexes in living systems and the engineered complex introduced in this work can be considered meta-stable thermodynamic phases that are kinetically trapped into their functional state. To understand the dynamics behind the formation and disassembly process, we developed a kinetic model with 15 mechanistic steps (Supplementary Information section 4), as illustrated in Fig. 5. The surfactant removal rate controls the formation of ND-SWNT. There is a threshold rate of approximately $1.0 \times 10^{-5} \text{ sec}^{-1}$ below which the system irreversibly forms pure lipid, protein, and SWNT particulate phases. The system can only cycle reversibly between the meta-stable ND-SWNT and disassembled components by transitioning at a rate above this threshold. The magnitude of this limiting rate is fixed by the differences in kinetic timescales between the rapid ND-SWNT assembly process and the thermodynamically-favored, but kinetically slower, homogeneous phases.

Surprisingly, once formed, this dynamically-assembled, purified RC-ND-SWNT complex has a photoelectrochemical activity that is present only in the assembled state (Fig. 6b). We monitored the photoresponse of the system using a double mediator scheme containing ferrocyanide/ferricyanide (70 μM) and ubiquinone/ubiquinol (70 μM) redox couples in a photoelectrochemical cell with a transparent bottom mounted on an inverted microscope (Fig. 6a). A SWNT film cast on a glass substrate was utilized as the transparent electrode^{10,11} as it was found to produce a root mean square (rms) noise current of 1 nA, reduced by a factor of 50 over typical ITO electrodes (Supplementary Fig. 8). A 700 nM RC-ND-SWNT solution in standard Tris buffer produces, under open-circuit conditions, a current that saturates at 20 nA and upon 20 mW laser illumination at 785 nm, as shown in Fig. 6b. This current of 20 nA translates into an external quantum efficiency of $8.0 \times 10^{-5}\%$

for a solution containing 8.4×10^{12} RC-ND-SWNT complexes (Supplementary Information section 6). When the light is turned off, the current returns to the baseline.

In this reaction scheme, ferrocyanide either donates an electron directly to the P^+ site of the reaction center or to the SWNT, which shuttles it to the photo-reduced P^+ site on the reaction center ($P^+ + e^- \rightarrow P$). The nanodisc assembly places this P site in close proximity to the nanotube. After electron transfer, ferricyanide travels to the working electrode where it is reduced ($Fe(CN)_6^{3-} + e^- \rightarrow Fe(CN)_6^{4-}$). On the opposite side of the reaction center, ubiquinone reduces to ubiquinol by accepting two electrons from the Q_A site in sequential turnovers of the reaction center, shuttling the electrons to the anode¹²⁻¹⁴. Some interaction between the redox couples may also take place under these conditions¹⁴. The SWNT acts primarily as a scaffold on which to collect approximately 100 reaction centers in a single linear complex. The evidence shows that the photoresponse is substantially enhanced with this dual mediator system in the presence of SWNT compared with the case of the ubiquinone mediator alone. However, the existence of a photoresponse in the presence of ubiquinone alone indicates that a process whereby direct electron transfer to the P^+ site of the reaction center is likely, however a detailed mechanism is under investigation (Supplementary Figs. 11 and 12 and Supplementary Table 1).

As shown in Fig. 6c, the photocurrent increases linearly with increasing the RCND-SWNT complex concentration. This implies that the photoresponsive output is limited by the number of photoactive complexes per volume. Embedding 10 mM of these complexes into a 1 μ m-thick thin-film device would result in absorbance comparable to those exhibited by typical CdTe films with similar thicknesses (Supplementary Information section 7)¹⁵. The requirements for such films are that the diffusion time of regeneration components must be shorter than the cycle time. Given that the largest diffusing component is the reaction center, we calculate a minimum required diffusion time of 27 sec for a 1 μ m-thick film (Supplementary Information section 7), which is significantly shorter than the 2-hour cycle time allotted for reassembly of the complex. This motivates the exploration of thin-film geometries that utilize reaction center-embedded matrices (Supplementary Fig. 14). Work that is on-going in our laboratory is exploring both re-concentrated colloidal cells and matrix materials that still allow for regeneration^{16,17}.

The complex enables the construction of a photoelectrochemical cell where a regeneration cycle can be prompted using a chemical signal, sodium cholate addition or removal, alone. Figure 7a outlines the cell with two re-circulating membrane dialyzers: one 1000 kDa and the other 12–14 kDa for disassembly and reassembly, respectively. All components except the nanotube scaffold (damaged reaction centers, lipids, and membrane scaffold proteins) can permeate the former when sodium cholate addition signals disassembly (Supplementary Fig. 13). The sodium cholate is then removed via the latter dialyzer, and the remaining lipids and proteins, supplemented from outside of the loop, re-form the complexes. Without the regeneration cycle, the photocurrent falls off rapidly to 50% after 5 hours, and to 20% after 32 hours (Fig. 7b). While some photoelectrochemical cells exhibit stability over 1000 hours¹⁸⁻²⁰, many in the literature demonstrate deactivation rate constants comparable to our un-regenerated cell, as illustrated by recent measurements on quasi-solid-state dye-sensitized solar cells (DSSC) showing deactivation to zero photocurrent after 60 hours²¹.

We find that immediately following each regeneration cycle, which is initiated every 32 hours, the photocurrent is restored to the previous maximum followed by a similar deactivation curve. Repeated regeneration appears to extend the lifetime of the device for over 168 hours (Fig 7b), and increases the photo-conversion efficiency by more than 300%. The increase is limited by the frequency of regeneration steps, which we arbitrarily set at 8.7×10^{-6} Hz, and the length of the regeneration cycle (8 hours). More efficient dialyzers and mass transfer, such as those encountered in a microfluidic platform, would shorten both times. In theory, the device could regenerate just as easily from biological components derived from waste biomass²²⁻²⁴, or by coupling directly to conventional biosynthesis in a manner similar to natural chloroplast operation²⁵⁻²⁷.

In conclusion, we have demonstrated the first synthetic photoelectrochemical complex capable of chemically triggered disassembly and specifically orientated reassembly based solely upon intermolecular forces and thermodynamic equilibrium. This reversible assembly and disassembly process enables the synthesis of a photoelectrochemical cell that can autonomously regenerate using only a chemical signal (surfactant addition and removal). To date, only natural photosynthetic systems have shown the ability to disintegrate complex light harvesting machinery that precisely reassemble after repair. By more closely mimicking such dynamic systems, we may be able to design more robust, fault-tolerant solar energy conversion schemes that approach the process that took nature over 100 million years of evolution to develop.

Methods

Self-assembly via membrane dialysis

Nanodisc synthesis procedures are described elsewhere^{4,8}. Briefly, DMPC (Avanti Polar Lipids) and Laurdan (6-dodecanoyl-2-dimethylaminonaphthalene, Molecular Probes) in chloroform were dried with high-purity N₂ and in a vacuum chamber before suspension in aqueous solution with 0.1 M sodium cholate. Membrane scaffold proteins were produced using a BioFlo 410 fermenter according to the protocols described previously⁴. Membrane scaffold proteins are a class of amphiphatic proteins based on the apolipoprotein A-I sequence without a globular N-terminal domain that is present in the native proteins. The proteins were isolated using a Ni-affinity resin and the quality verified by electro-spray mass spectrometry and SDS-PAGE electrophoresis. Membrane scaffold proteins were added to the solution at a molar ratio of DMPC:membrane scaffold protein:Laurdan = 100:1:1. As-prepared HiPco or CoMoCAT nanotubes were obtained from Rice University and Southwest Nanotechnologies Inc., respectively. Reaction centers were isolated from *Rhodobacter sphaeroides*, and suspended in 0.1% LDAO and 0.1 M tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) at pH 8.0^{28,29}. SWNT, initially dispersed in D.I. water with 2 wt% sodium cholate, and reaction centers were added to yield final concentrations of 4–20 mgL⁻¹ and 3–7 mM, respectively. The mixture solutions are dialyzed against the Tris buffer using 12–14 kDa pore membranes (Spectrum laboratories). The buffer was replaced every 8 hours, and self-assembled nanomaterials are collected after 24 hours.

Atomic force microscopy and small-angle neutron scattering

The self-assembled complexes were visualized with an AFM (Veeco Metrology) in contact mode. The complexes were placed on a mica surface in a fluid cell with imaging buffer (10 mM Tris (pH 8.0), 0.15 M NaCl and 10 mM MgCl₂). SANS experiments of the complexes were carried out on the 30 m NG7 beamline at the National Institute of Standards and Technology (NIST) Center for Neutron Research (NCNR) (Supplementary Information section 1). All solutions were dialyzed against D₂O prior to measurements to improve scattering contrast against the pure hydrogenated components.

Material purification via ultracentrifugation

The dialyzed materials contain a mixture of self-assembled components, which are separated based upon their density differences using an ultracentrifuge (Optima L-100 XP, Beckman Coulter), following a procedure developed by Arnold *et al.*⁷ Specifically, each dialyzed sample was added onto a 5 mL stop-layer (60% iodixanol, Optiprep, Sigma) in a centrifuge tube, followed by serial addition of 50, 40, 30, 20, and 10% gradient layers (1 mL each). After centrifugation at 30,000 rpm for 7 hours, a fraction recovery system (Beckman Coulter) was used to extract 250 μ L aliquots from the centrifuge tube into each well of a 96-wellplate with a programmed translational stage. The densities of fractionated portions were determined by measuring the mass of 100 μ L water from each well after ultracentrifugation and fractionation under the same conditions as other samples.

Spectroscopic characterization

Optical density was determined using a UV-vis-NIR spectrophotometer (UC-3101PC, Shimadzu). A plate-reader (Varioskan Flash, Thermo Scientific) measured optical absorption and Laurdan fluorescence from fractionated samples. The samples were also characterized with resonance Raman (Kaiser Optical Systems) and photoluminescence (PI Action) spectroscopy using a 785 nm laser diode (Ocean Optics) for excitation. The fluorescence of (9,1) nanotubes at ~925 nm is used to detect SWNT presence, since it demonstrates a strong photoluminescence response. Steady-state photoluminescence excitation (PLE) spectra were obtained with a home-built scanning spectrofluorometer equipped with a Xe lamp and a cryogenically-cooled Ge detector. 10- and 4-nm increments were employed in the excitation and emission monochromators.

Photoelectrochemical measurement

Photoelectrochemical properties were probed with a potentiostat (Princeton Applied Research, 273A), a coiled Pt wire auxiliary counter electrode and an Ag/AgCl reference electrode (BASi). A transparent SWNT film cast on glass substrate was used as a working electrode (Supplementary Information section 5)^{10,11}. The photoactive solution was contained within a PDMS (Sylgard 184, Dow Corning) mold with a cylindrical hole, which was clamped onto the glass substrate. This setup was mounted on the inverted microscope and illuminated with a 785 nm laser diode with an irradiance of 20 mW (Fig. 6a). To detect the photoelectrochemical response, 70 μ M ferrocyanide (K₄Fe(CN)₆) and 70 μ M ubiquinone-2 (C₁₉H₂₆O₄) were used as redox mediators in Tris buffer solution. The photoresponse of our system is measured under open-circuit conditions by turning on and

off the light source. To examine the lifetime using regeneration, we devised a photoelectrochemical system consisting of a cell connected to two dialyzers with membranes with 12–14 kDa and 1000 kDa pores for assembly and disassembly, respectively. While the mixture solution flows through the 12–14 kDa pore membrane, the complex self-assembles upon surfactant removal. After measuring photoelectrochemical activity for 32 hours, the complex is dialyzed for 6 hours against the surfactant buffer using a 1000 kDa pore membrane to disassemble the complex and subsequently remove used reaction centers, membrane scaffold proteins, and lipids while retaining the SWNT. The complex is reassembled upon surfactant removal for 2 hours by adding new components, including reaction centers. This assembly-disassembly process is repeated throughout lifetime measurements.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was financially supported by a grant from ENI Petroleum Co. Inc. Eni S.p.A. under the Eni-MIT Alliance Solar Frontiers Program, seed funding from the MIT Energy Initiative (MITEI) and the U.S. Department of Energy (grant number ER46488). M.H.H. is grateful for support from the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (KRF-2007-357-D00133). J.H.C. acknowledges financial support from Purdue University. Membrane scaffold proteins were produced under support from NIH GM33775. Correspondence and requests for materials should be addressed to M.S.S.

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Figure 1. Schematic of self-assembled photoelectrochemical complexes

Molecular model of self-assembly process of carbon nanotubes with photosynthetic reaction centers upon surfactant (sodium cholate) removal. Membrane dialysis induces spontaneous self-assembly of DMPC and membrane scaffold proteins to form nanodiscs, which reconstitute reaction centers while suspending nanotubes in aqueous solution. The resulting, highly ordered complex is shown in the right-hand panel. Addition of sodium cholate completely decomposes the complexes into individual components that are in the initial condition (left-hand panel).

Figure 2. Structural characterizations of self-assembled photoelectrochemical complexes
a, AFM image showing a nanodisc and a NDSWNT. **b**, The height profile indicates that these nanostructures are ~8 nm in height. **c,d**, SANS measurements are shown in red for nanodisc (**c**) and ND-SWNT (**d**). The blue curve in **c** denotes a model fit to the form factor of an isotropic suspension of monodisperse discs, whereas the blue curve in **d** generates an unsatisfactory fit assuming isotropic disc form factor. The model fit in black (**d**) is in good agreement with the experimental data, assuming the factor for sequentially adsorbed discs, $q = 2\pi/(2R)$. These results confirm that nanodiscs have a diameter of approximately 8 nm with a 4 nm height, and nanodiscs are stacked along the carbon nanotubes.

Figure 3. Purification of self-assembled photoelectrochemical complexes

Fluorescence intensity distributions of Laurdan dye (a), reaction center (b), and SWNT (c) of the self-assembled complexes as a function of density after ultracentrifugation at 30,000 rpm for 7 hours. After ultracentrifugation, each sample in a centrifuge tube is fractionated in every 250 μL into a 96-wellplate. The RC-ND and ND-SWNT samples are also examined as controls. Laurdan is used to identify the nanodisc distribution. Note that free lipids (i.e., no membrane scaffold protein) are located only in the layer of density below 1050 kg/m^3 . The highlighted portions are collected for structural and photoelectrochemical characterizations.

Figure 4. Optical signatures of assembled RC-ND-SWNT complex

a, Photoluminescence excitation contour of carbon nanotubes before (top) and after (bottom) dialysis. A few SWNT species demonstrate large fluorescence red-shifts upon sodium cholate removal, which is used as indicative of nanodisc formation along the nanotube axis. **b,** SWNT fluorescence wavelength shifts upon nanodisc formation on SWNT, identified from photoluminescence excitation spectra. Near armchair species (n, n-1) demonstrate large red-shifts of 49–60 nm, whereas the spectral changes in other species are moderate. **c,** A plot of (6,5) photoluminescence maxima in a spectral window of 985–1015 nm as a function of time during serial self-assembly and decomposition. **d,** The initial structure of DMPC, with the axis oriented at 25° relative to the graphene lattice vector, based on simulation is shown in the upper panel. The lower panel shows that the energy minimized structure based on simulation has both DMPC tails aligned to the carbon atoms of the graphene lattice. Alignment of each tail contributes 1 kcal/mol of favorable van der Waals attraction compared to the randomly oriented tail.

Figure 5. Kinetic model illustrating ND-SWNT concentration throughout dialysis

Sodium cholate concentration refers to the total amount of free and micellar sodium cholate aggregates normalized with respect to the maximum concentration achieved at each dialysis rate. ND-SWNT complexes form below the critical micelle (CMC) and critical bilayer (CBC) concentrations, and concentration increases as the sample is dialyzed to low sodium cholate concentrations at high rates, with a minimum threshold rate of $1.0 \times 10^{-5} \text{ sec}^{-1}$.

Figure 6. Photoelectrochemical activity of assembled RC-ND-SWNT complex in a photoelectrochemical cell

a, Schematic of the photoelectrochemical measurement apparatus that includes a potentiostat in a three-electrode configuration. A transparent SWNT film cast on glass substrate was used as a working electrode, while an Ag/AgCl and a coiled Pt wire were the counter and reference electrodes, respectively. A 785 nm laser diode illuminates the solution at 20 mW through an inverted microscope. **b**, Photoresponse of 700 nM RC-ND-SWNT complex solution using 70 μM ferrocyanide and 70 μM ubiquinone as a double mediator in the photoelectrochemical cell. The photoelectrochemical activity disappears when the complex is broken apart by introducing sodium cholate, and is resurrected by reassembling the complex upon surfactant removal. **c**, Dependence of photocurrent on RC-ND-SWNT complex concentration ranging from 700 nM to 33.7 μM in the photoelectrochemical cell. The photocurrent increases linearly with complex concentration.

Figure 7. Photoelectrochemical activity of a RC-ND-SWNT complex that autonomously regenerates

a, Schematic of the photoelectrochemical system that consists of a photoelectrochemical cell incorporated to two re-circulating membrane dialyzers. Dialyzers 1 and 2 are used for assembly and disassembly of the complex with 12–14 and 1000 kDa pore membranes. The photo-damaged reaction centers along with membrane scaffold proteins and lipids are removed during dialysis for disassembly of the complex and replaced with new components while SWNT are retained. The disappearance of reaction center photoluminescence peaks after disassembly confirms the complete removal of photo-damaged reaction centers prior to reassembly (Supplementary Fig. 13). **b**, Temporal photoresponse of the RC-ND-SWNT with and without regeneration. Without regeneration (black curve), the photocurrent decreases sharply, falling to 50% after 5 hours, 10% after 80 hours. Deactivation is comparable to DSSC data published in the literature (green curve)²¹. Operating the regeneration cycle every 32 hours for 8 hours in duration restores the photocurrent to the previous maximum and extends the device lifetime indefinitely. Over 168 hours, the efficiency is increased more than 300%.