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Artificial Photosynthesis

Self-Assembled Light-Harvesting Peptide Nanotubes for Mimicking Natural Photosynthesis**

*Jae Hong Kim, Minah Lee, Joon Seok Lee, and Chan Beum Park**

Solar energy has recently attracted a great deal of interest as a sustainable and environmentally acceptable energy source.[1] In nature, green plants store solar energy in chemical fuels through photosynthesis. Photoinduced electron transfer during the light reactions regenerates reducing power in the form of nicotinamide cofactors, NAD(P)H, which is then used for the synthesis of carbohydrates in the Calvin cycle (Figure 1 a).[1a] Light-harvesting by natural photosynthesis occurs by means of two large protein complexes called photosystem I and II, which are composed of light-harvesting antenna (i.e., chlorophyll a and b) and catalytic metal clusters embedded within proteins.[1b] The photosynthetic units are constructed through self-assembly, and their sophisticated structure leads to an efficient transfer of photoinduced electrons during photosynthesis.[1c] Despite the various efforts made thus far to mimic natural photosynthesis using photosensitizers, such as organic dyes[2a] or inorganic nanocrystals[2b,c] an integrated photocatalytic platform[2d,e] for photochemical synthesis in the visible light range is critically needed and should thus be developed.

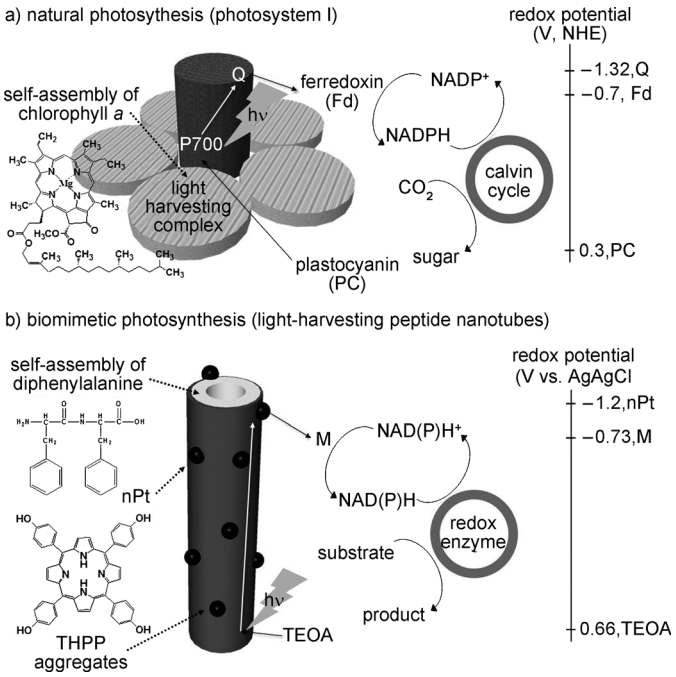

Figure 1. Schematic illustration of a) structure, biocatalytic reaction, and redox potential of natural photosynthesis by photosystem I and b) biomimetic photosynthesis by light-harvesting peptide nanotubes. White and black arrows indicate photoinduced electron excitation and electron transfer, respectively.

On the other hand, the self-assembly of bio-organic molecules into nanostructures is an attractive route to fabricate functional materials.[3] For example, diphenylalanine (Phe–Phe, FF), an aromatic dipeptide consisting of two covalently linked phenylalanine units, can form various nanostructures such as nanotubes, nanowires, and nanospheres under different processing conditions.[3f,g] FF-based nanostructures can readily self-assemble in a simple way and possess the functional flexibility and molecular recognition capability suitable for a wide range of applications, such as biosensors, imaging, guest encapsulation, and nanofabrication.[3a] For example, we recently demonstrated that peptide nanotubes can act as a host matrix for photosensitizers and lanthanide ions.[4] The incorporation of lanthanide complexes into peptide nanotubes enabled a high synergistic effect on the enhancement of photoluminescence through a cascade energy-transfer mechanism.

Herein, we report on the development of light-harvesting peptide nanotubes that integrate photosynthetic units, thus mimicking natural photosynthesis. As shown in Figure 1 b, the light-harvesting peptide nanotubes were synthesized by the self-assembly of FF and porphyrin. Porphyrins are macrocyclic compounds that include the chlorophyll molecules found in light-harvesting photosystems of green plants.[5] We further incorporated platinum nanoparticles (nPt) on the surface of the FF/porphyrin nanotubes by means of self-metallization. Similar to quinone and ferredoxin, which act as an electron separator and a mediator in natural photosynthetic systems, respectively, nPt was introduced here in order to efficiently separate and transfer the exited electrons from porphyrin to an electron mediator (i.e., $M = [Cp^*Rh(bpy)H_2O]^{2+}$, $Cp^* = C_5Me_5$, bpy = 2,2′-bipyridine). M can facilitate the selective and efficient regeneration of nicotinamide cofactors.[6] We found that light-harvesting peptide nanotubes synthesized in this way are able to harvest solar energy, thereby regenerating NAD(P)H for the production of fine chemicals by means of redox enzymes in a manner similar to natural photosynthesis, in which photochemically regen-

[*] J. H. Kim, M. Lee, J. S. Lee, Prof. C. B. Park
Department of Materials Science and Engineering, Korea Advanced Institute of Science and Technology
335 Science Road, Daejeon 305-701 (Republic of Korea)
E-mail: parkcb@kaist.ac.kr

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erated NAD(P)H is consumed by enzymatic reduction reactions in the Calvin cycle.

From the range of porphyrins, we selected tetra(*p*-hydroxyphenyl) porphyrin (THPP) as a model light-harvesting molecule, as it is hydrophobic enough to interact with FF, while possessing similar optical and electrochemical properties to those of chlorophyll *a*. THPP was simply incorporated into FF nanotubes by means of a self-assembly process. Briefly, FF and THPP were dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) and then diluted in a phosphate buffer (100 mM, pH 6.0). After the treatment, we observed the formation of a green precipitate, the color of which significantly differed from pure FF nanotubes (white) and THPP (pink; Figure S1 and S2). The results indicate that FF nanotubes serve as a template for the self-assembly of THPP monomers. According to our SEM analysis, the green precipitates consisted of nanotubes with a "rough" surface, unlike pure FF nanotubes (Figure S3 A and S4); this roughness is attributed to the incorporation of a THPP layer on the outer surface of the nanotubes during the self-assembly of FF. The powder X-ray diffraction pattern of FF/porphyrin nanotubes in Figure S3B displays the typical six-fold symmetry of pure FF nanotubes,[7] which indicates that the crystal structure of FF nanotubes was not affected by the incorporation of THPP. We further investigated the spectroscopic properties of FF/THPP nanotubes. The absorption spectra of free THPP monomers exhibited a strong Soret band, centered at 419 nm, and four weak Q bands at 516, 554, 592, and 651 nm, respectively (Figure 2 a). The Soret band of THPP became broader and red-shifted from 419 to 444 nm when incorporated into FF nanotubes. Furthermore, a new peak was observed for the FF/THPP nanotubes at 693 nm. The red-shift and the new peak indicate that the inner core of THPP has been protonated to become H_2THPP^{2+}.[8] In general, an acidic solution leads to the protonation of the inner core of the porphyrin. In case of our experiment, the protonated THPP was formed by hexafluoro-2-propanol (HFIP), which is a convenient protonation medium.[9] The Soret band of THPP red-shifted from 419 nm to 426 nm when dissolved in HFIP (Figure S5 and S6), indicating that THPP was partially protonated by HFIP. According to literature,[10] FF nanotubes are negatively charged in the phosphate buffer (pH 6.0) and can interact with protonated THPP monomers. In addition to partial electrostatic interactions, FF nanotubes may bind with the hydroxyl groups of THPP through hydrogen bonding since FF nanotubes have carboxylic acid groups (Figure S7). According to literature,[4d] peptide-based nanotubes can interact with porphyrins by hydrogen bonding.

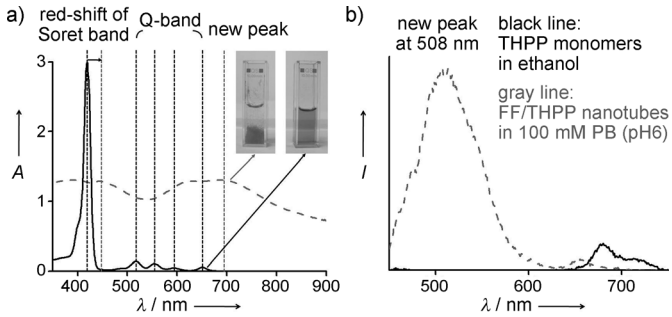

Figure 2. Optical properties of light-harvesting peptide nanotubes. a) Absorption and b) emission spectra of THPP monomers (black line) and FF/THPP nanotubes (gray line). The excitation wavelength was 420 nm. Inset in (a) contains photographs of FF/THPP nanotubes (left) and THPP suspensions (right) in quartz-glass cuvettes.

We further analyzed FF/THPP nanotubes using spectrofluorometry and Raman spectroscopy. When we compared the fluorescence spectrum of FF/THPP nanotubes with that of free THPP monomers, a new fluorescence peak at 508 nm appeared after the incorporation of THPP into FF nanotubes (Figure 2 b). This result is attributed to exciton coupling between S_2 states of porphyrin molecules, similar to electronic interactions in an extended aggregate of porphyrin. According to literature,[11] the S_2 fluorescence of porphyrin aggregates formed by the Langmuir-Blodgett method was enhanced by the triplet–triplet annihilation between porphyrin molecules. In addition, we found a significant change in the Raman spectrum of FF/THPP nanotubes when compared to that of free THPP monomers (Figure S8). According to literature,[12] protonated THPP monomers can form J-aggregates through hydrogen bonding between four N–H bonds at the inner core of THPP and the peripheral hydroxyl group of adjacent THPP. Taken together, THPP monomers were self-assembled into J-aggregates by means of electrostatic attractions between THPP and FF and hydrogen bonding between THPP, resulting in a stronger exciton coupling between THPP monomers. The exciton coupling of THPP J-aggregates self-assembled with FF is similar to the role of the natural light-harvesting complex that contains chlorophyll aggregates. Chlorophyll aggregates harvest solar energy through a strong transition-dipole moment along the head-to-tail arrangement of the chromophores through J-aggregation.[5]

To examine whether photoinduced electrons from FF/THPP nanotubes can be transferred to the electron mediator (i.e., M), we measured the photocurrent of FF/THPP nanotubes deposited on an indium tin oxide (ITO) glass in phosphate buffer (100 mM, pH 6.0) containing 15 w/v% triethanolamine (TEOA) as an electron donor. According to our results, the anodic photoinduced response of FF/THPP nanotubes was observed only when visible light ($\lambda > 400$ nm) was used for irradiation (Figure 3 a), indicating that the electrons excited by visible light were transferred from TEOA to the ITO glass through the FF/THPP nanotubes. This phenomenon suggests that THPP can act as a photoactive molecule by generating photocurrent through a favorable energetic relationship with TEOA. We further analyzed the redox potential of FF/THPP nanotubes and M through the use of cyclic voltammetry to investigate a detailed mechanism of the photoinduced electron transfer. The reduction potential at the cathodic peak current of the FF/THPP nanotubes and M was observed at −1.2 and −0.7 V (vs. Ag/AgCl), respectively (Figure 3 b, black solid and dotted lines). The energetic relationship between FF/THPP nanotubes and M is similar to that found in natural light harvesting; for example, chlorophyll *a* dimer (−1.32 V vs. NHE) and iron-sulfur clusters F_A/F_B (−0.45 V) work as a photosensitizer and a

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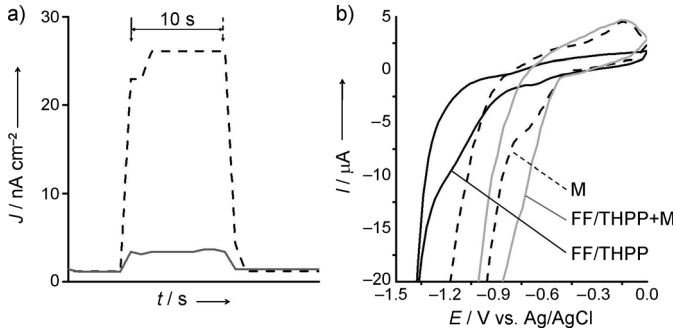

Figure 3. a) Photocurrent response of FF/THPP nanotubes on an ITO electrode (black dotted line) and ITO electrode alone (gray solid line). Solid and dotted black arrows indicate light-on and -off, respectively. b) Cyclic voltammogram of M alone (black dotted line), and FF/THPP nanotubes with M (gray solid line) and without M (black solid line).

component of the electron-transport system, respectively, in natural photosynthetic organisms.[13] We also observed that the cathodic current of M at its reduction potential exhibited a strong increase in the presence of the FF/THPP nanotubes (Figure 3b, gray solid line), indicating that the excited electrons from the FF/THPP nanotubes were utilized to reduce M. We further observed fluorescence decrease of FF/THPP nanotubes with the addition of M, which suggests that the excited electrons are transferred from FF/THPP nanotubes to M (Figure S10). Taken together, FF/THPP nanotubes can transfer photoinduced electrons from the electron donor (TEOA) to the electron mediator (M) such as the reaction center in photosystem I (see Figure 1).

In natural photosynthesis, quinone works as an electron separator to increase the efficiency of photoinduced electron transfer. To mimic the role of quinone, many researchers have attempted to enhance charge separation efficiencies in chromophore/metal nanohybrids and the photocatalytic activity of metal/semiconductor nanoassemblies.[14] In our work, we have deposited platinum nanoparticles (nPt) onto the light-harvesting peptide nanotubes by a self-metallization process. For the formation of nPt on the FF/THPP nanotubes, a suspension containing the nanotubes, ascorbic acid (reducing agent), and K_2PtCl_4 (metal precursor) was exposed to visible light ($\lambda > 400$ nm) for 30 min. We analyzed the resulting FF/THPP/nPt using transmission electron microscopy (TEM) and linear sweep voltammetry (LSV), after washing and centrifugation steps. Figure S11 shows the TEM image of the FF/THPP nanotubes coated with nPt clusters of approximately 30 nm in size. TEM image of the FF/THPP nanotubes without nPt (Figure S11) and energy-dispersive x-ray spectroscopy (EDS) analysis (Figure S11, inset) confirm that the clusters observed on the FF/THPP nanotubes consist of nPt. We further studied the redox properties of nPt-decorated FF/THPP nanotubes using LSV analysis (Figure S12). Surprisingly, the cathodic current of nPt-decorated FF/THPP nanotubes increased at a rate approximately 1000 times greater than that of the FF/THPP nanotubes, thus suggesting that nPt efficiently separated electrons from excitons in the FF/THPP nanotubes.

We applied FF/THPP/nPt to visible-light driven regeneration of NADH to mimic the light reaction of photosynthesis. Following a 1-hour regeneration reaction starting from 1 mM NAD^+, we obtained 0.178 mM of regenerated NADH with FF/THPP/nPt, while approximately 0.009 mM, 0.01 mM, and 0.035 mM of NADH were regenerated with THPP monomers, THPP monomers with nPt, and FF/THPP nanotubes, respectively (Figure 4a, inset). The turnover frequency of nPt-decorated FF/THPP nanotubes was estimated to be approx-

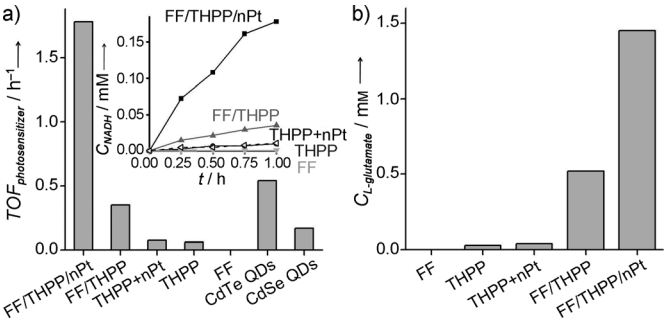

Figure 4. a) Turnover frequency of FF nanotubes only, THPP monomers, THPP/nPt, FF/THPP nanotubes, and FF/THPP/nPt nanotubes in comparison with other inorganic photosensitizers. Inset in (a) shows the temporal change of NADH concentration in the presence of FF nanotubes, THPP monomers, THPP/nPt, FF/THPP nanotubes, and FF/THPP/nPt nanotubes, respectively. b) Photosynthesis of L-glutamate by glutamate dehydrogenase (GDH) with FF nanotubes, THPP monomers, THPP/nPt, FF/THPP nanotubes, and FF/THPP/nPt nanotubes.

imately 1.78 h^{-1} (Figure 4a), which is much higher than those of inorganic nanomaterials, such as CdTe (0.540 h^{-1}) and CdSe (0.158 h^{-1}) quantum dots, *p*-doped TiO_2 (0.003 h^{-1}), and $W_2Fe_4Ta_2O_{17}$ (0.002 h^{-1}).[2b] To test the recycled usability of FF/THPP/nPt, we separated them from the reaction medium after NADH regeneration for 1 hour, and reused them. As shown in Figure S13, the recycling efficiency slightly decreased after the third cycle, hence indicating a good recycling capability for light-harvesting peptide nanotubes. We further coupled the visible-light-driven NADH regeneration to a redox enzymatic reaction using glutamate dehydrogenase as a model enzyme, which can convert α-ketoglutarate to L-glutamate only in the presence of NADH (Figure 4b). We found that the conversion yield (1.45 mM) of L-glutamate with FF/THPP/nPt was 2.7 and 48.3 times higher than those of the FF/THPP nanotubes and the THPP monomers only, respectively. FF/nPt alone exhibited a negligible photocatalytic effect on NADH regeneration (Figure S14). Thus, our results demonstrate that FF/THPP/nPt nanotubes accelerate NADH regeneration and redox enzymatic synthesis under visible light.

In summary, we synthesized light-harvesting peptide nanotubes incorporated with THPP and nPt. The J-aggregation of THPP occurred during the self-assembly of the FF nanotubes by electrostatic attraction and hydrogen bonding. nPt was synthesized on the FF/THPP nanotubes through a self-metallization process under visible light. nPt-coated FF/

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THPP hybrid materials were suitable for mimicking photosynthesis because of their structure and electrochemical properties similar to photosystem I. Our results show that the integrated photocatalytic system is effective for visible-light-driven NADH regeneration coupled with redox enzymatic synthesis of L-glutamate.

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