Biomimetic Self-Organization

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Mimicking Primitive Photobacteria: Sustainable Hydrogen Evolution Based on Peptide–Porphyrin Co-Assemblies with a Self-Mineralized Reaction Center

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***Abstract:** Molecular evolution, with self-organization of simple molecules towards complex functional systems, provides a new strategy for biomimetic architectonics and perspectives for understanding the complex processes of life. However, there remain many challenges to fabrication of systems comprising different types of units, which interact with one another to perform desired functions. Challenges arise from a lack of stability, dynamic properties, and functionalities that reconcile with a given environment. A co-assembling fiber system composed of simple peptide and porphyrin is presented. This material is considered a prebiotic assembly of molecules that can be rather stable and flexibly self-functionalized with the assistance of visible light in a "prebiotic soup"; acidic (pH 2), hot (70°C), and mineral-containing (Na^+, Ti^{4+}, Pt^{2+}, and so forth) water. The co-assembled peptide–porphyrin fiber, with self-mineralized reaction centers, may serve as a primitive photobacteria-like cellular model to achieve light harvesting, energy transfer, and ultimately sustainable hydrogen evolution.*

Life-inspired systems with dynamic architectonics and functions are interesting in nanotechnology and materials science.[1] They can direct the flow of mass, energy, or information in a manner analogous to living matter with the help of the harmonized actions of molecular systems.[2] Photosystems are masterpieces of nature for solar energy conversion by an ensemble organized through proteins, pigments, and various cofactors.[3] The pigment–protein complexes in green plants are rather complicated, and are very difficult to replicate in artificial photosynthetic devices. By contrast, photobacteria, representative of primitive photosynthesis, provides an elegant natural inspiration to mimic the simpler architectural principles.[4] For example, in green sulfur bacteria chlorosomes self-assembled mainly by pigments, capture light and then transfer the energy to reaction centers mediated by the Fenna–Matthews–Olson (FMO) protein complex.[5] Therefore, mimicking photobacteria, may not only provide a blueprint for solar energy utilization, but also help us to understand the complex evolution processes of photosynthesis. Biomimetic photosystems derived from synthetic or inorganic–biological hybrid strategies have been developed with great success.[6] However, designing and fabricating dynamic molecular assemblies possessing the characteristics of photobacteria still remains a big challenge.

Molecular evolution with self-organization of photoactive molecules is generally regarded as an important process in prebiotic photosystem evolution.[7] Porphyrins have been proposed as possible precursors of photosynthetic pigment systems.[8] Peptides are of particular interest in prebiotic evolution for easy availability and enabling various functions, such as self-replication, localization of RNA, and catalysis of multiple reactions.[9] Simple peptides can also tune self-assembly of functional components, such as porphyrins, into complex structures with enhanced functions.[10] A plausible prebiotic environment is required for photosystem evolution. Hydrothermal systems, such as hot, acidic, mineral pools, generated from rainwater-fed systems around volcanoes enriched in sulfide and minerals, provide a plausible scenario for the habitat and nature of early life;[11] *Sulfolobus* requires 70–75°C and pH 2–3 for growth.[12]

Herein, we develop a scenario for molecular evolution towards mimicking primitive hydrogen-producing photobacteria by self-organization of porphyrin and peptide in a "prebiotic soup" (acidic (pH 2), hot (70°C), and mineral-containing (Na^+, Ti^{4+}, Pt^{2+}, and so forth) water under visible light illumination), which is a plausible prebiotic environment similar to that of volcanic hydrothermal vents (Scheme 1). The peptide (L-Lys-L-Lys, KK) and porphyrin ((tetrakis(4-sulfonatophenyl)porphine, TPPS) molecules in the "prebiotic soup" (Supporting Information, Figure S1), can spontaneously self-assemble into hierarchical fiber bundles with an organization similar to that of pigments in chlorosomes. The fibers have positively charged peptide envelopes that can facilitate the mineralization of TiO_2 nanoparticles (NPs). Pt NPs can also mineralize on the fibers in situ after photoreduction of potassium tetrachloroplatinate(II). TiO_2/Pt can be regarded as a primitive reaction center model, which uses the energy from light harvesting porphyrin assemblies to achieve charge separation and catalyze proton reduction for generation of hydrogen. Thus, the peptide modulated porphyrin/TiO_2/Pt fibers resemble the architectonics of green

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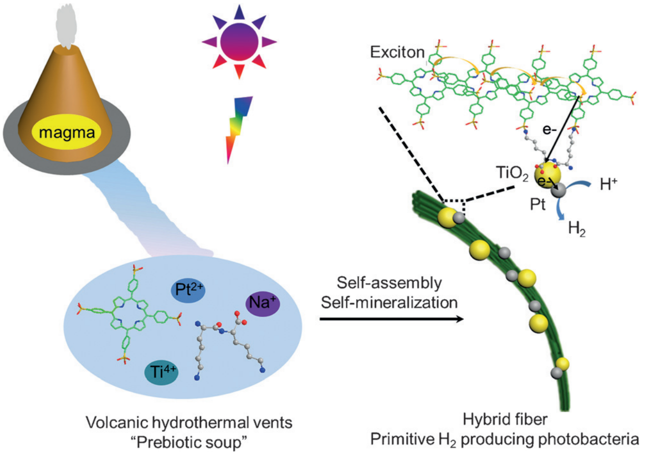

Scheme 1. Illustration of the molecular evolution of porphyrins and peptides. A model of a primitive photosystem in a volcanic hydrothermal "prebiotic soup".

sulfur bacteria. Such a system is based on the assumption that a primitive photosystem, or at least a plausible individual functional unit, may gradually evolve from the prebiotic mix into assemblies of molecules. Our study gives a strategy for fabrication of biomimetic photosystems based on molecular self-organization in combination with self-mineralization. Additionally, this process shows the characteristics of prebiotic molecular evolution, such as spontaneous and dynamical self-organization of simple molecules into complex systems in a plausible prebiotic environment.[13] Thus, in a sense this system provides an experimental case for prebiotic evolution of photosynthesis.

In the designed prebiotic soup, fibrous structures can spontaneously form (Figure 1a; Supporting Information, Figure S2). To clarify the process of molecular evolution with self-organization, each step was investigated in detail. TPPS molecules can form J-aggregates by slipped face-to-face stacking in acidic solution (pH 2). Hydrophilic KK can direct the J-aggregated structures into orientationally organized fiber bundles by multi-scale synergy of interactions, including electrostatically enhanced hydrogen bonds, hydrophilic anchoring, and surface tension.[14] The fiber bundles show a dynamic assembly behavior, which is sensitive to ionic strength (for example, NaCl concentration; Supporting Information, Figure S3). The width and length of the fibers obviously increases after addition of NaCl (Supporting Information, Figure S4). The intense exciton split circular dichroism (CD) signal located at 494 nm, corresponding to the chirality of aggregated TPPS, increases with NaCl concentration and reaches a maximum at 0.2 M NaCl (Supporting Information, Figure S5), which is in agreement with the size of the assembled fiber. NaCl can enhance the chirality of TPPS, probably because of the enrichment of counter ions around the TPPS molecule, thus reducing electrostatic repulsion between the TPPS molecules, and facilitating the growth of the assembled fiber. Such complex patterns with dynamic behavior indicate that a large ensemble of molecules is in dynamic equilibrium, which are easily adjusted to be responsive to the surrounding conditions.[15] Interestingly, the assembled fiber can also form and be stable even at high temperature, at 70 °C for example (Supporting Information, Figure S6). The resistance of the assembled fiber to high temperatures possibly results from multiple intermolecular interactions, which tend to be much stronger than the corresponding monovalent interactions, a phenomenon that is often necessary to regulate physiological processes.[16]

Self-mineralization occurs following the self-assembly of fiber bundles in the designed prebiotic soup. Mineralized TiO_2 on the surface of the fibers appears (Figure 1c,e; Supporting Information, Figure S7) after the titanium(IV) bis(ammonium lactato)dihydroxide (TiBALDH) aqueous solution is mixed with KK/TPPS fibers and incubated at 70 °C for 12 h. The high-resolution TEM (HRTEM) analysis reveals that the single crystals have the orientation of the anatase (101) plane (Figure 1b). The fiber may be associated with the TiO_2 NP mineralization (Supporting Information, Figure S8). We suggest that anionic TiBALDH molecules initially bind to the amino groups of KK through electrostatic interaction. Subsequently, the mineralization reaction follows the acid–base catalysis of the peptide molecules, leading to the hydrolysis of the TiBALDH complexes, followed by condensation of trihydroxyl species, and ultimately resulting in the synthesis of TiO_2.[17] The peptide (KK) has positive charges, which may increase the amount of TiO_2 formed.[18] After illumination, Pt NPs are generated in situ on the surface of TPPS/KK/TiO_2 fibers (Figure 1c,f), probably by a photochemical reduction route.[10a,b] The HRTEM image shows, that most Pt NPs have an average diameter of about 3 nm and are oriented along the (111) axis (Figure 1b).

The hybrid fiber can capture light, which is the first step in primitive photosynthesis. The hybrid fibers have maximal absorption around 426, 492, and 712 nm (Figure 2a), which is similar to that of aggregated bacteriochlorophylls in green sulfur

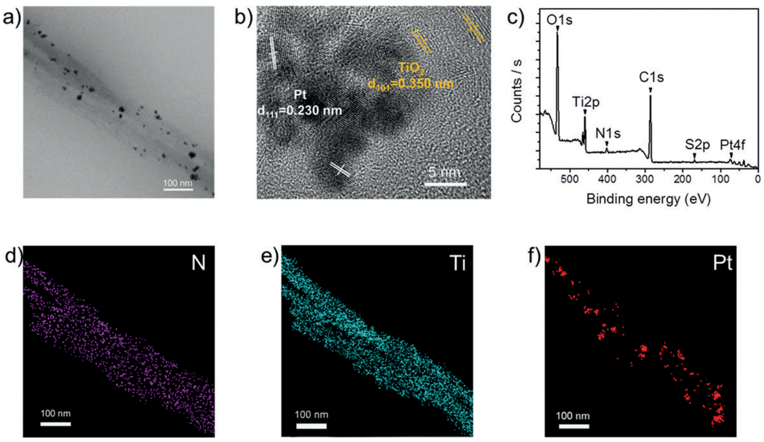

Figure 1. a) TEM image of the hybrid fibers showing mineralized nanoparticles. b) HRTEM image of the mineralized TiO_2 and Pt nanoparticles on the hybrid fibers. c) XPS of the hybrid fibers, indicating that the valence state of Ti and Pt is +4 and 0, respectively (Supporting Information, Figure S9). d–f) Elemental mapping of the hybrid fibers showing the N, Ti, and Pt components, respectively.

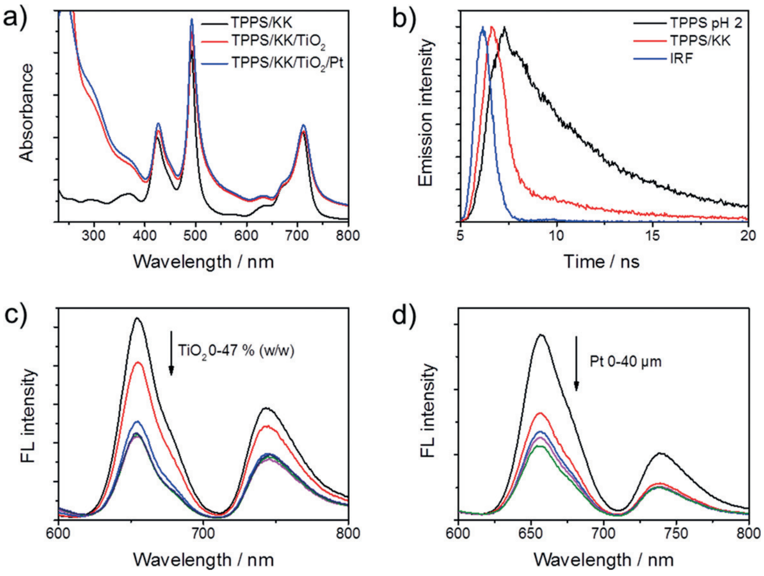

Figure 2. a) UV/Vis absorption spectra of aqueous TPPS/KK, TPPS/KK/TiO_2, and TPPS/KK/TiO_2/Pt fibers. b) Time-resolved fluorescence spectra of aqueous J-aggregate TPPS, TPPS/KK, and TPPS/KK/TiO_2 fibers ($\lambda_{ex} = 450$ nm, $\lambda_{em} = 740$ nm). Inset: instrument response function (IRF). c) Fluorescence spectra of aqueous TPPS/KK after mineralization of TiO_2 ($\lambda_{ex} = 450$ nm). d) Fluorescence spectra of aqueous TPPS/KK/TiO_2 after photoreduction of Pt ($\lambda_{ex} = 450$ nm).

bacteria.[19] The coupling of TPPS in the fiber through self-assembly affords the capture of a wider range of photons through the extended spectral absorption (Supporting Information, Figure S10). Strong absorption below a wavelength of 340 nm is associated with the optical band gap of TiO_2. No apparent peaks of Pt NPs were observed, possibly because of the low Pt loading. Compared with bare TPPS/KK fiber, mineralization of TiO_2 and Pt NPs does not influence the visible light absorbance of the hybrid fiber (Figure 2 a) because the aggregated structure of TPPS has negligible change, in agreement with the CD analysis (Supporting Information, Figure S11). The time-resolved emission kinetics show that the radiative S1 exciton lifetime of the assembled fiber is shorter than that of TPPS J-aggregates induced by acid (0.71 ns vs. 4.44 ns; Figure 2 b). The decrease of the radiative lifetime is probably a result of the increased exciton coherence length of the J-aggregates.[20] The exciton produced in these assemblies can spread over multiple unit molecules, facilitating energy transfer, and representing a high potential-energy state that can be utilized to drive charge transfer.

A reaction center is indispensable in photosynthesis because of its capacity to funnel light energy. Herein, the mineralized TiO_2 NPs on the fibers make up the reaction center, allowing photoactivated charge separation.[21] The in situ mineralized TiO_2 can quench the emission of the assembled fiber by 66 % (quenching is concentration dependent; Figure 2 c; Supporting Information, Figure S12). This may be because of electron injection from the porphyrin to TiO_2 NPs.[22] Generally, strong electronic coupling is required to overcome thermodynamic and kinetic barriers in interfacial exciton dissociation.[20] In our case, TiO_2 is chelated with the carboxyl group of KK, as indicated by IR analysis (Supporting Information, Figure S13). As TiO_2 NPs do not directly bind to TPPS, the back electron transfer may be weaker than in strong coupling systems (for example, binding of TiO_2 and porphyrin by direct linkage).[23] Additionally, J-aggregates can enhance the photoinduced charge separation at the porphyrin/TiO_2 interface as a result of the energy funnel effect and large spatial charge separation.[20] The hybrid fibers emerge with preferable spectral coverage and exciton dynamics, which are suitable for light harvesting and energy transfer.

The emission of the hybrid fiber corresponding to the singlet-excited state of TPPS is further quenched by in situ generated Pt NPs (Figure 2 d). The emission intensity is reduced by 60 % if the concentration of Pt atoms is increased from 0 to 40 μM. This suggests, that there is transfer of excited electrons between the TiO_2 and Pt NPs, confirmed by the transient photocurrent experiment (Supporting Information, Figure S14). Such a reaction center, formed with local mineralization of inorganic mineral ions by light, may be of critical importance for the corresponding bioreactions, such as H_2 evolution. Almost all bioreactions (also H_2 synthesis) involve coupling of redox reactions to transfer of more than one electron to achieve photocatalysis. Therefore, the reaction center also functions as an electron storage unit. In an ideal system, the reaction center should be positioned at the point where it can receive a maximum of electrons or photons; that is, it should function as a funnel. In terms of self-organization this should occur by synthesizing the reaction center with the aid of light. It would then also be positioned where most of the light passes. In some sense, fiber bundles with long-range order mimic such an architectonic scaffold, in which the reaction center can effectively work for photocatalytic synthesis.

After illumination by visible light, the primitive photosynthetic reaction for H_2-evolution occurs on the hybrid fiber in the prebiotic soup (Figure 3 a). H_2 was not effectively produced when TiO_2 (Pt) was omitted or inefficiently coupled to the system (Figure 3 b; Supporting Information, Figure S15). The evident enhancement of H_2 production after mineralization of TiO_2 may be attributed to the efficient mediation of electron transfer from photoexcited TPPS to Pt NPs. Pt is also necessary to complete the electron-transfer pathway for photocatalytic H_2 evolution. A close neighbor of TiO_2 and Pt components (Figure 1 b), achieved by the photochemical deposition method, is believed to favor the vectorial transfer of photogenerated electrons from TiO_2 to Pt NPs, thus enhancing charge separation and photocatalytic efficiency. After 6 h of irradiation, the hybrid fiber did not exhibit any significant change in its absorption spectrum (Supporting Information, Figure S16), indicating its high structural and optical stability during photocatalytic H_2 production.

Interestingly, the H_2 evolution activity of the hybrid fiber turns out to be dramatically enhanced when increasing the NaCl concentration of the prebiotic soup. The hybrid fiber at 0.2 M NaCl has a turnover number (TON) of 365 after 6 h, up

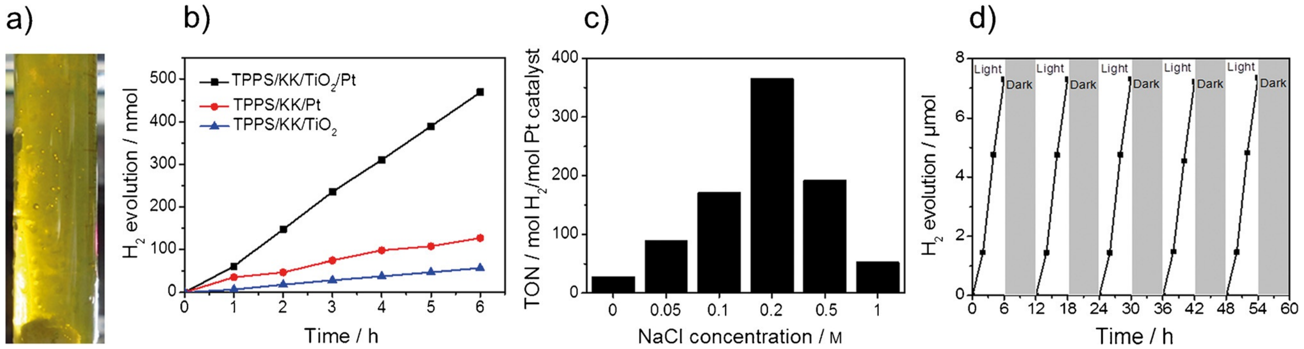

Figure 3. a) Photograph of H_2 bubbles produced from the hybrid fibers in a test tube during illumination. b) Time dependence of the H_2 production of TPPS/KK/Pt, TPPS/KK/TiO_2, and TPPS/KK/TiO_2/Pt fibers in the absence of NaCl. c) Effect of NaCl on the hydrogen production of the hybrid fibers. d) Long-term photocatalytic performance of the hybrid fibers in NaCl (0.2 M).

to 13-times compared to that without NaCl, with a TON of 27 (Figure 3c). NaCl can increase the intermolecular electronic coupling between the stacked TPPS molecules (Supporting Information, Figure S5), which leads to enhancement of the delocalization of the exciton along a certain number of electronically coupled monomers. NaCl can also enhance the coupling of nanotubular subunits in TPPS/KK fibers (Supporting Information, Figure S4), which facilitates delocalization of excitons between nanotubular subunits.[24] Both result in increased exciton coherence and energy transfer to the reaction center. NaCl may improve the conductivity of the reaction system, facilitating H_2 production-related electron transfer. Additionally, the hybrid fiber shows sustainable H_2 production in response to light illumination (Figure 3d). The behaviors for NaCl-enhanced H_2 production and rhythmic change according to light suggest a case of adaptability to the prebiotic environment in prebiotic evolution.

In summary, in the designed acidic, hot, and mineral prebiotic soup, peptides and porphyrins can spontaneously self-organize into ordered hybrid fibers by molecular self-assembly and self-mineralization with the assistance of visible light. The different components in the hybrid fibers are effectively coupled, to collaboratively realize light harvesting, charge separation, and photocatalytic hydrogen evolution. Primitive photosynthesis may work, as the pigment cyclically photooxidizes donors, such as ferrous ions, sulfhydryl groups, or organic molecules, emitting hydrogen.[25] We envisage that the inorganic component may be important in prebiotic metabolism and reaction.[26] Therefore, the hybrid fibers, with a structural and working principle similar to the photosynthetic architecture in photobacteria, can be regarded as a primitive hydrogen-producing photobacterial model. Our findings support a simple but robust and effective strategy to construct biomimetic photosystems based on molecular self-organization and self-mineralization. Simultaneously, this system is reconciled with prebiotic evolution of photosystems as a result of the plausible prebiotic environment and molecular evolution, thus providing a novel system for studying the construction of primitive membrane-free protocells by organization of key structural and metabolic building blocks. Taken together, these findings may offer a plausible concept and an effective pathway toward establishment of primitive photosynthetic models; thereby expanding the approaches for biomimetic energy conversion and deepening the insight into prebiotic organization, energy conversion, molecular and life evolution in a prebiotic environment.

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