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Self-Assembly

Photo-induced Reversible Structural Transition of Cationic Diphenylalanine Peptide Self-Assembly

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Stimuli-responsive supramolecular assemblies have generated much research interest because of their applications in drug carriers, sensors, protein probes, and memory storage.[1] A variety of external stimuli, including temperature, pH, enzymes, oxidizing/reducing agents, and light, have been used in these systems.[2] Recently, light-triggered assembling has attracted considerable attention because it works reversibly, rapidly, and remotely without generating any undesired substances.[3] Thus various photo-responsive groups have been employed as photoswitching units to manipulate the assembly of structures and their relevant functions in numerous supramolecular assembly systems.[4] Among them, azobenzene and its derivatives have been proven to be particularly advantageous as photoswitches because of their *trans–cis* conformational change, which can be triggered by UV-vis light irradiation. They have, therefore, been widely used in various supramolecular assemblies for optical control of these systems, including controlling dramatic structure changes and molecular property variations.

Diphenylalanine (FF) peptide, the core recognition motif of Alzheimer's β-amyloid polypeptide, is an excellent biomolecular building block to fabricate bio-functional nanomaterials. A variety of defined supramolecular structures, such as, discrete nanotubes, nanowires, macroporous honeycomb scaffolds, and peony-flower-like hierarchical nanostructures, can be formed through self-assembly of diphenylalanine and its derivatives.[5] Recently, researchers are not just interested in assembling such peptides into diverse structures, the controlled assembly of these peptides has attracted even more attention. Li et al. reported a structural transition from organogels to flower-like microcrystals by introducing ethanol as a co-solvent to FF organogels formed in toluene.[6] Reches and co-workers fabricated biomolecular necklaces by controlling the molecular mole ratio of the two units in the co-assembly of FF and its tert-butyl dicarbonate protected analogue.[7] Very recently, Ulijn and co-workers prepared a supramolecular self-assembly system using FF-based derivatives, which displays coupled light switching, biocatalytic condensation/hydrolysis, and gelation.[8] However, it is still a challenge to control the reversible self-assembly of the dipeptide for biological applications.[9]

In this report, we designed a photoswitchable sulfonic-azobenzene 4-[(4-ethoxy)phenylazo]benzenesulfonic acid (EPABS), aiming to optically manipulate the self-assembly of a cationic diphenylalanine peptide (CDP, H-Phe-Phe-NH_2·HCl). The photo-induced *trans–cis* conformational change of EPABS significantly influenced the CDP assembly and a reversible structural transition between a branched microstructure and a vesicle-like nanostructure was observed. Sulfonic-azobenzene is the analog of Congo red, which is an important medical molecule for the detection and therapy of Alzheimer's disease.[10] Thus we hope that this approach could contribute to shed light on the light-based diagnosis and therapy of Alzheimer's disease and fabrication of novel smart biomaterials.

Figure 1(i) illustrates the molecular structures of EPABS (*trans*- and *cis*-form) and CDP. Before utilization EPABS was dissolved in water to investigate its photoswitching property. As expected, the EPABS molecules could be photoisomerized under UV-vis irradiation. As shown in Figure 1b, upon irradiation by UV light (λ = 365 nm) the absorption band at around 352 nm decreased significantly, whereas the band at around 432 nm increased slightly. These absorption bands located around 352 and 432 nm are related to the π–π* and n–π* transitions, respectively.[11] The variation in the absorption bands under UV irradiation indicates the photoisomerization of EPABS from the *trans*-state to the *cis*-state. After UV irradiation the above solution was irradiated by visible light and, interestingly, the π–π* absorption band increased again whereas the n–π* absorption band decreased slightly after irradiation, revealing that EPABS underwent a *cis–trans* photoisomerization changing back to its *trans* state.

The supramolecular assemblies formed based on CDP and EPABS were prepared by adding an aqueous solution of EPABS to the 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) solution of CDP with a mole ratio of 1:1 at room temperature. Upon mixing, a clear yellow solution was initially obtained; then some suspended solids appeared gradually and the clear solution turned completely turbid in several minutes. The formation of the suspended solids indicates the generation of the supramolecular assemblies. After aging for two hours, the suspended solids were separated from the suspension and characterized by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) (**Figure 2**). Both the SEM and TEM results indicate that branched structures, about 60 μm in size, were generated via co-assembly

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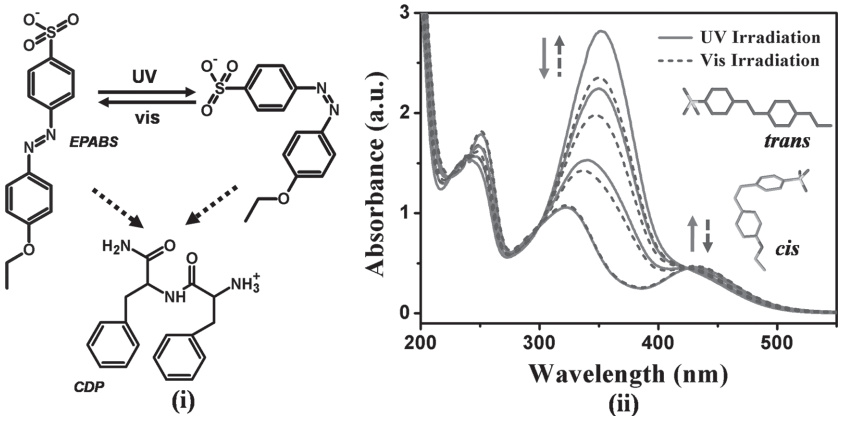

Figure 1. i) Molecular structure of EPABS and CDP; ii) UV–vis spectrum of an aqueous solution of EPABS after UV or visible light irradiation.

of CDP and EPABS. The detailed SEM images shown in Figure 2B–E reveal that the branched nanostructures are built by elongated nanoplates and helical nanobelts that are interconnected through a center core so as to construct the final co-assembled structure. The elongated nanoplates (Figure 2D) with a size of about 30 μm in length, 2 μm in width, and 1 μm in thickness, majorly serve as the building blocks to form the branched structure. The helical nanobelts, which are also found in the TEM images (Figure 2F), are about 30 μm in length. However, they are thinner than the nanoplates, only hundreds of nanometers in thickness (Figure 2E). Various reports have demonstrated the formation of nanoplates and hierarchical structures composed of nanoplates during the self-assembly of diphenylalanine peptides.[5h,6,12] However, the formation of helical structures in the assembly of diphenylalanine peptides has not been reported thus far. It has been demonstrated that the *trans*-azobenzene unit contains a planar molecular configuration that favors cooperative packing in a helical form because of the strong interactions and steric hindrance of the adjacent molecules.[13] Thus, we propose that the helical structures formed here are the result of the existence of EPABS in the supramolecular assemblies.

Fourier-transform infrared spectroscopy (FTIR) was performed to further clarify the formation of the as-prepared branched structure at molecular level. **Figure 3** shows the FTIR spectra of CDP, EPABS, and the branched structures. In Figure 3A the bands located at 1201 cm^{-1} and 1039 cm^{-1} can be ascribed to the asymmetric and symmetric O=S=O stretching vibrations of the sulfonic group.[14] After co-assembly with CDP to form branched structures, these two bands were shifted to 1183 cm^{-1} and 1033 cm^{-1}, respectively, suggesting strong electrostatic interactions between CDP and EPABS. Moreover, a new band located at 1547 cm^{-1} appeared in the branched structure. This new band may be ascribed to stacking of the aromatic rings, which was also the case in our previous work.[15] Furthermore, after co-assembly with CDP, the band located at 1664 cm^{-1}, which belongs to the amido group of the CDP molecules, also shifted compared to that of pure CDP to 1644 cm^{-1}, indicating a strong molecular interaction between the two kinds of building blocks.

Because of the special *trans–cis* isomerization of EPABS under irradiation with UV light, it is reasonable to expect that the co-assembly system formed by CDP and EPABS may also possess this photoswitching property. To verify this speculation, the suspension was transferred to a quartz tube and irradiated under UV light at 365 nm. Interestingly, upon irradiation, the suspended solids disappeared gradually, and the suspension became completely clear after being irradiated for an hour (**Figure 4**A). After that, the clear solution obtained above was irradiated by visible light, and an increased opalescence, which was visible by the naked eye, occurred. After irradiation for about fifteen minutes, the whole system changed into a completely turbid suspension again. Figure S1 in the Supporting Information shows the SEM images of the assembled structures in the above suspension with a different scale. It can be seen that the branched structure regenerated after being irradiated by visible light again, which indicates that EPABS returned to its *trans*-form. To prove the key role of light in the structural transition process, we put the sample (in its clear state, i.e., after UV irradiation) in the dark; and the sample remained almost completely clear even after several hours (Figure S2, Supporting Information). Moreover, we found that the reversible transition can be repeated for many times without significant attenuation (Figure 4B). UV–vis spectroscopy was performed to monitor the state transition of the co-assembly system. As shown in **Figure 5**A, the UV-vis spectrum of the co-assembly system after UV irradiation was in accordance with that of the pure EPABS under the same conditions. This confirms that EPABS is in the *cis*-state in the supramolecular assemblies after UV irradiation, which indicates that the photo-induced *trans–cis* isomerization of

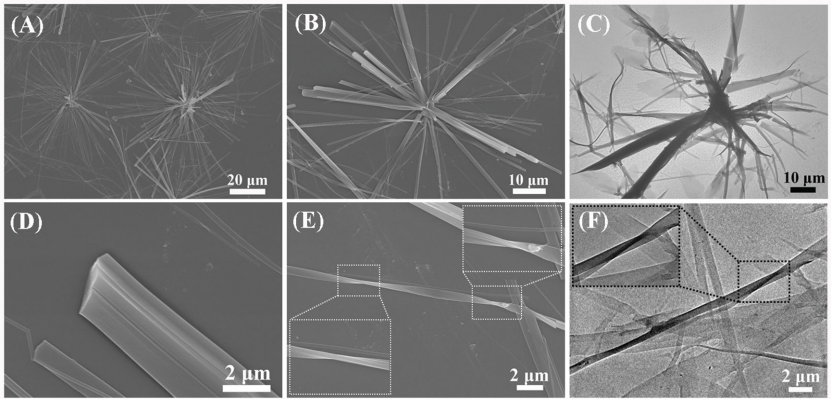

Figure 2. A,B,D,E) SEM images and C,F) TEM images of microstructures formed by co-assembly of CDP and EPABS under visible light.

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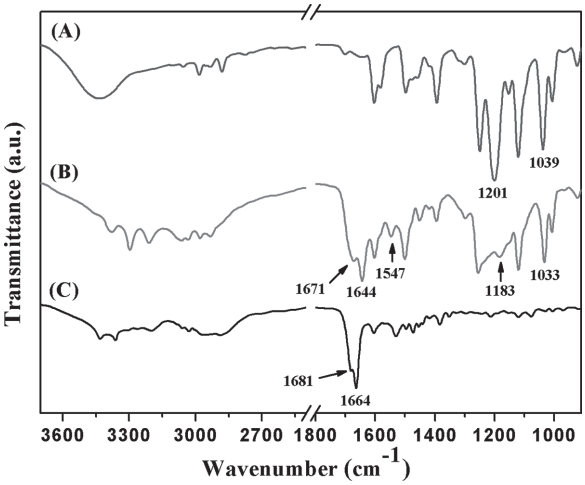

Figure 3. A–C) FTIR spectrum of EPABS (A), the co-assembly structure (B), and CDP (C).

EPABS was the driving force for the state transition of the co-assembly system.

In order to clarify the structural transition of the supramolecular assembly system, TEM (transmission electron microscopy) was performed to investigate the clear solution obtained under UV light irradiation. As shown in the TEM image (Figure 5B), vesicle-like structures were obtained after UV irradiation. These vesicles, ranging in size from 100 nm to 300 nm, as confirmed by dynamic light scattering (DLS) measurements (Figure S3, Supporting Information), coincide well with the vesicle-like structures formed by the self-assembly of CDP in dilute aqueous solutions.[16] Furthermore,

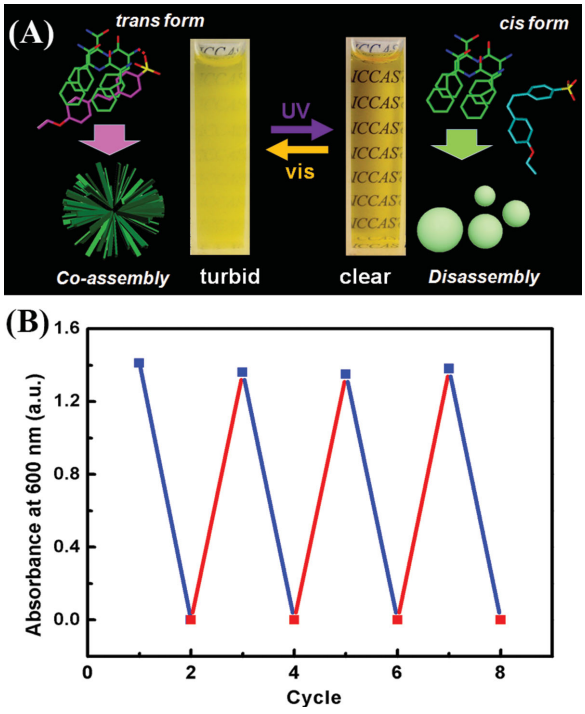

Figure 4. A) The illustration of the photo-responsive property of the supramolecular assembly. B) The turbidity of the supramolecular assembly sample (absorbance at 600 nm) by alternate irradiation at UV light (blue solid square) and vis light (red solid square), respectively.

by reducing the molecular ratio of EPABS and CDP to 0.5:1, both branched structures and vesicle-like structures were formed (Figure S4, Supporting Information). So it is reasonable to assume that the vesicle-like structures were formed by assembly of the CDP molecules. Previous reports have demonstrated that azobenzene molecules in the *cis*-form are more hydrophilic and show a higher steric hindrance than molecules in the *trans*-form.[17] Huang et al. have investigated supramolecular assemblies based on cetyltrimethylammonium bromide (CTAB) and sodium (4-phenylazo-phenoxy)-acetate (AzoNa) through electrostatic interactions, and they have found that the *cis*-AzoNa escapes from the supramolecular assemblies after UV irradiation because of its stronger hydrophilic property and higher steric hindrance.[18] A similar phenomenon has also been reported by Gröhn and co-workers in a supramolecular assembly system based on a dendrimeric macro-ion and diazo dyes.[19] Moreover, to clarify the importance of electrostatic interactions, *t*-butyloxycarbonyl (Boc)-FF (*N*-Boc-Phe-Phe-COOH), an anionic diphenylalanine peptide, was also co-assembled with EPABS. However, both the illumination experiments (Figure S5, Supporting Information) and the SEM images (Figure S6, Supporting Information) indicate that the assembly system does not show any photoswitching property because of the absence of electrostatic interactions. Thus it can be deduced that the EPABS molecules escape from the co-assembly structures after being irradiated by UV light, leading to the formation of vesicle-like structures from the self-assembly of the CDP molecules.

Combing the results described above, we propose a possible mechanism of the assembly and transformation process, which is illustrated in Figure 4A. Firstly, before UV illumination, *trans*-EPABS is inserted into the CDP molecular arrangement driven by electrostatic interactions and π–π interactions.[15a] According to the FTIR results (Figure 3B), the aromatic rings of EPABS overlap with the CDP aromatic rings, which results in the formation of branched structures. After being irradiated by UV light, *trans*-EPABS in the branched structure is gradually transformed into *cis*-EPABS. The higher hydrophilic property and steric hindrance of *cis*-EPABS may lead to *cis*-EPABS escaping from the branched structures, indicating a disassembly of the co-assembled structure. Free CDP molecules can then perform a self-assembly procedure to form vesicle-like structures. When this system was then exposed to visible light, EPABS would return to its *trans*-form again, leading to the regeneration of the co-assembled branched structures.

In conclusion, we have successfully manipulated the self-assembly of CDP molecules using EPABS. Branched structures composed of elongated nanoplates and helical nanobelts were obtained by the co-assembly of CDP and EPABS under visible light. After UV irradiation, a *trans*–*cis* photoisomerization of EPABS results in the disassembly of the structure, leading to a structural transition from branched structures to vesicle-like structures. The photo-induced structural transition is reversible and can be repeated many times without significant attenuation. This work may help to understand and design smart assemblies to reversibly control the action of biosystems.

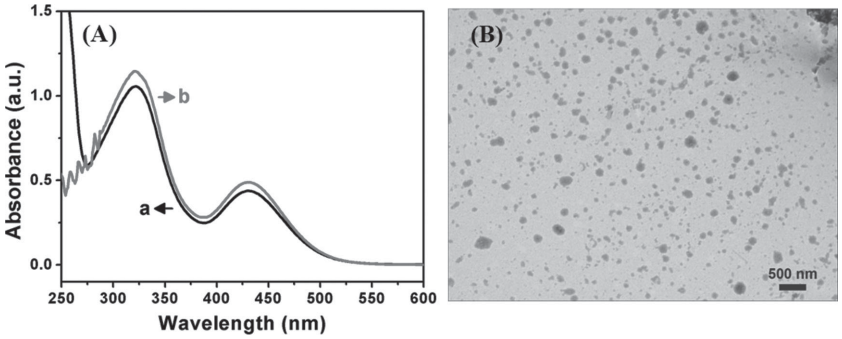

Figure 5. A) UV–vis spectra of pure EPABS (a) and the assembly system (b) under UV light; B) TEM images of nanostructures formed by supramolecular assembly under UV light.

Experimental Section

Materials: The cationic dipeptide (CDP, H-Phe-Phe-NH_2·HCl) was purchased from Bachem (Budendorf, Switzerland). Boc-FF (*N*-Boc-Phe-Phe-COOH) was purchased from Alfa Aesar. 1,1,3,3,6,6-hexafluoro-2-propanol (HFIP) was obtained from Sigma-Aldrich. EPABS was synthesized according to the literature.[20]

Synthesis of 4-[(4-Hydroxy)phenylazo]benzenesulfonic Acid (HPABS): To synthesize HPABS, *p*-aminobenzene sulfonic acid (3.5 g, 20 mmol) was dissolved in a mixture of 3 mL of concentrated HCl and a small amount of water, which was cooled to 0 °C using an ice bath. Then, another 3 mL of concentrated HCl was added under vigorous stirring. After that, a $NaNO_2$ solution (1.5 g $NaNO_2$ dissolved in 10 mL water) was slowly added dropwise to the mixture at 0 °C. Under strong stirring a phenol solution (1.9 g (20 mmol) of phenol dissolved in 50 mL of K_2CO_3 aqueous solution) was slowly added dropwise to the above mixture at 0 °C. After reacting for 2 h, the pH value of the reaction liquid was adjusted to 2.0 using 0.1 M HCl solution. The yellow precipitate was filtrated out and dried under vacuum. The crude product was purified by recrystallization from water/ethanol (yield: 76%). 1H-NMR (400 MHz, [D6]-DMSO, 25 °C, 7.85 (d, 2H; Ar-H), 7.8 (s, 4H; Ar-H), 6.95 (d, 2H; Ar-H)).

Synthesis of 4-[(4-Ethoxyl)phenylazo]benzenesulfonic Acid (EPABS): For the synthesis of EPABS, HPABS [1.0 g (3.4542 mmol)] was first dissolved in 20 mL of anhydrous dimethylformamide (DMF). Then 1.1 g (3 eq.) of anhydrous sodium carbonate, 0.3553 g (1 eq.) of sodium iodide, and 1.4 ml (4 eq.) of 1-bromoethane were added under stirring. After reacting for 5 d at 40 °C, the reaction liquid was rotovaporated to remove any solvent and excess iodomethane. Then a small amount of water was added to the remaining mixture, which was then heated, and cooled down again. The final precipitate was recuperated by filtration, rinsed successively with water (small amount), acetone, and chloroform, then dried in vacuum at 70 °C for 2 d, yielding an orange powder (0.86 g, yield 79%). 1H-NMR (400 MHz, [D6]-DMSO, 25 °C, 7.92 (d, 2H, Ar-H), 7.8 (q, 4H, Ar-H), 7.13 (d, 2H, Ar-H), 4.06 (t, 2H), 1.45 (t, 3H).

Preparation and Characterization of Co-assembled Structures of CDP and EPABS: In a typical procedure, an aqueous solution of 1.6 mM azobenzene (1 mL) was added to a 10 μL of 160 mM CDP/HFIP solution at room temperature. This immediately resulted in the formation of a yellow precipitate. After aging for a while, the yellow suspended solids were collected for further characterization.

Characterization: The SEM images of the samples were taken using an S-4800 (HITACHI, Japan) scanning electron microscope. The obtained suspended solids were carefully picked up and transferred onto a silicon substrate for SEM measurements. Then the sample was dried under vacuum and coated by a thin layer of platinum. The TEM images of the samples were carried out using a Philips CM200-FEG transmission electron microscope. 1H-NMR spectra were recorded on a Bruker AV400 (400 MHz) spectrometer. The FTIR spectra were recorded on a Bruker TENSOR-27 spectrophotometer. The samples were dried under vacuum and then pressed into KBr pellet for FTIR spectroscopy. A Hitachi U-3010 spectrophotometer was used to record the UV–vis spectra of the various samples. The samples were irradiated with a Xe light using a filter with a wavelength of 365 nm. Visible-light irradiation was achieved by holding the samples under sun light for fifteen minutes.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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