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Manipulating assembly of cationic dipeptides using sulfonic azobenzenes†

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Three sulfonic azobenzenes with tiny differences in the terminal were used to manipulate the assembly of cationic dipeptides (CDP); assemblies with diverse morphologies including urchin-like, flower-like and plate-like structures were formed.

The fabrication of functional nanomaterials by the "bottom-up" strategy needs to rationally design and accurately control nanostructures at the molecular level.[1] To date, different versatile small-molecule-based nanostructures with the same chemical structures have been obtained by external stimuli, such as light,[2] enzyme,[3] pH,[4] redox[5] and introducing other small molecules.[6] However, it remains a great challenge to efficiently control the assembly of biomolecules into desired nanostructures by regulating the interaction between them.[7]

Recently, many oligopeptides with several amino acid residues have been developed as building blocks for various functional nanomaterials in the fields of tissue engineering,[8] drug delivery,[9] and vaccines.[10] Among them, a diphenylalanine peptide (FF) and its derivatives have attracted wide attention and nanostructures with diverse morphologies such as flower-like and plate-like structures have been assembled.[11] Controlled assembly of such peptides has also been performed to construct well-defined supramolecular nanostructures.[12] For example, biomolecular necklaces were fabricated by controlled co-assembly of FF and its *tert*-butyl dicarbonate (Boc) protected analogue.[13] In our previous reports, interestingly, a cationic dipeptide (CDP, H–Phe–Phe–NH_2·HCl), a cationic derivative of FF, could assemble into nanotubes and vesicles at physiological pH and a reversible transformation between nanotubes and vesicles could take place at controlled concentrations.[14] Furthermore, controlling the assembly of CDP using phosphotungstic acid (PTA) through electrostatic interaction led to the formation of porous nanospheres.[15]

Herein, we manipulated the assembly of CDP using three azobenzenes with analogous structures (Fig. 1). Tiny differences among the azobenzene molecules lead to formation of nanostructures with entirely different morphologies. Azobenzene molecules were chosen as the manipulating units because they are analogs of Congo red which is important species in the therapy of Alzheimer's disease both for detecting amyloid aggregation and amyloid inhibitors.[16] This context may help to shed light on the diagnosis and therapy of Alzheimer's disease and apply in the fabrication of novel peptide-based functional nanomaterials.

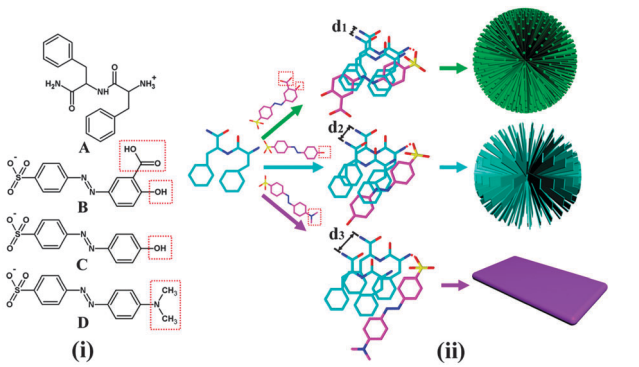

Fig. 1 (i) Chemical structures of CDP (A) and azobenzenes CPABS (B), HPABS (C) and MO (D); (ii) schematic representation of the proposed model for the formation of the structures by co-assembly of CDP and azobenzenes (*d* means the molecular distance between CDP molecules, and the order is $d_1 < d_2 < d_3$).

The molecular structures of CDP and azobenzene molecules are illustrated in Fig. 1(i). Fig. 1(i)A shows structure of CDP while Fig. 1(i)B–D show structures of three water-soluble azobenzenes including 4-[(3-carboxyl-4-hydroxy)phenylazo]benzenesulfonic acid (CPABS), 4-[(4-hydroxy)phenylazo]benzenesulfonic acid (HPABS) and methyl orange (MO), respectively. All the azobenzenes are sulfonic azobenzenes possessing analogous structures and only containing tiny differences in the terminal.

Because CDP is water-insoluble, 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) was added to help dissolve CDP. Upon adding aqueous solutions of azobenzenes to HFIP solution of CDP (in a 1 : 1 mole ratio) at room temperature, yellow precipitates formed rapidly, indicating generation of co-assembled nanostructures. The as-prepared precipitates were then collected and characterized by SEM. The SEM results reveal that urchin-like, flower-like

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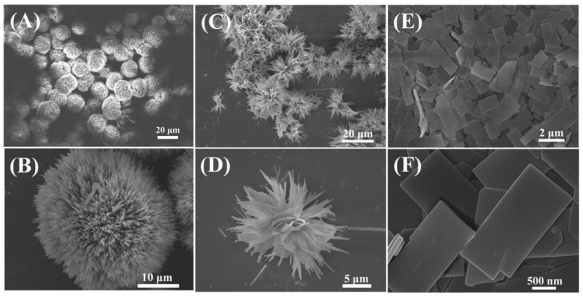

Fig. 2 SEM images of microstructures through co-assembly of CDP and CPABS (A, B), HPABS (C, D) and MO (E, F).

and plate-like structures were generated through assembly of CDP manipulated by CPABS, HPABS and MO, respectively. In detail, as shown in Fig. 2A, when CPABS was used to manipulate assembly of CDP, a large number of microspheres with a diameter of *ca.* 25 μm emerged. The enlarged image (Fig. 2B) reveals that the microspheres which were well formed and uniform in size have urchin-like morphology containing plenty of nanorods on their surface. Then, CDP assembly manipulated by HPABS led to formation of flower-like structures with the average diameter of *ca.* 15 μm (Fig. 2C and D). The flower-like structures contain plate-like substructures on the surface. Furthermore, Fig. 2E and F show the nanostructures formed by assembly of CDP manipulated by MO. Plate-like structures which possess rectangular shape were formed with *ca.* 2 μm in length and 1 μm in width. From the above SEM results it can be revealed that nanostructures with different morphologies were generated although the three kinds of manipulating azobenzene molecules share similar molecular structures. Therefore, it is reasonable to suppose that the distinct morphologies of assembled nanostructures are intrinsically related to different molecular packing types in these systems.

To understand the interactions between CDP and azobenzenes, Fourier transform infrared (FTIR) spectroscopy was performed to investigate these assembly structures at the molecular level. Fig. 3A–C show the FTIR spectra of azobenzenes and relevant co-assembled structures. The FTIR spectrum of CPABS (Fig. 3A (below)) shows asymmetric and symmetric O═S═O stretching vibrations of the sulfonic group at 1171 cm^{-1} and 1036 cm^{-1}.[17] After being assembled into urchin-like microspheres, these bands shift to 1180 cm^{-1} and 1033 cm^{-1}, respectively, indicating the electrostatic interaction between CDP and CPABS. The same sorts of shifts are also observed in flower-like structure (Fig. 3B) and plate-like structure (Fig. 3C), revealing the existence of electrostatic interaction between CDP and these azobenzene molecules in the corresponding structures. A new peak appearing at 1555 cm^{-1} in Fig. 3A (above) belongs to the aromatic ring skeleton vibrations.[18] The appearance of this new peak may be due to the stacking of aromatic rings. Similarly, the new peak appearing at 1558 cm^{-1} in Fig. 3B (above) is also ascribed to the same reason. However, there is no new peak in the similar region in Fig. 3C (above). The reason for this may be that the molecular arrangement in plate-like structures was looser than those in the other two kinds of assembly structures. Furthermore, as shown in Fig. S1 (ESI†), the peaks of amido bonds in CDP molecules also shift after being modulated by azobenzenes, from 1664 cm^{-1} to 1671 cm^{-1}, 1676 cm^{-1} and 1672 cm^{-1} for CPABS, HPABS and MO respectively. It also indicates that the introduction of azobenzenes changed the molecular arrangements of CDP.

To demonstrate the molecular packing in these three assembly systems, X-ray diffraction (XRD) analysis is performed for the as-prepared microstructures. Fig. 4 shows the XRD patterns of urchin-like structures, flower-like structures, plate-like structures and CDP powder, respectively. The inset in Fig. 4 shows the corresponding pattern with the 2θ values from 3° to 35°. The XRD pattern at wide angles (2θ = 3–35°) reveals that all of the three assembly structures and the CDP powder possess strong and numerous diffraction peaks, indicating extremely ordered molecular packing in these co-assembly systems. However, compared to original patterns of the CDP powder, the XRD patterns of co-assembled structures changed greatly after being manipulated by azobenzenes. To clarify the relationship of XRD patterns and the morphology of the assembly structures, we investigated the XRD patterns at a small angle area (3–10°). According to the Bragg equation, the molecular layer spacing of urchin-like, flower-like, plate-like structures and the CDP powder can be calculated from the first peak to be 1.55 nm, 1.68 nm, 1.80 nm and 1.37 nm, respectively. One can see that the molecular layer spacing increased after co-assembly in the order $D_{CPABS} < D_{HPABS} < D_{MO}$. It indicated the insertion of azobenzene molecules

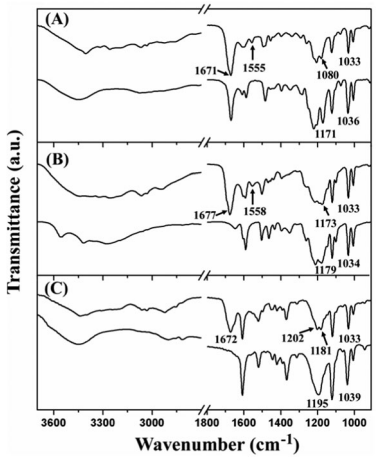

Fig. 3 FTIR spectra of co-assembled structures (above) and azobenzene powder (below): (A) urchin-like structures and CPABS; (B) flower-like structures and HPABS; (C) plate-like structures and MO.

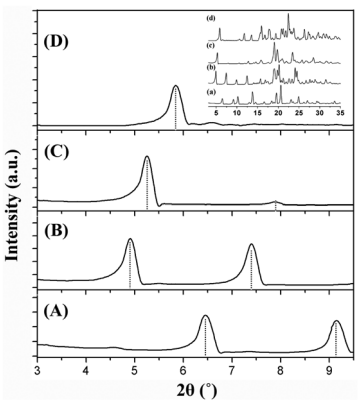

Fig. 4 XRD patterns of the CDP powder (A, a) and co-assembled structures of CDP and MO (B, b), HPABS (C, c), CPABS (D, d); the inset image shows the wide angle XRD patterns with 2θ from 3° to 35°.

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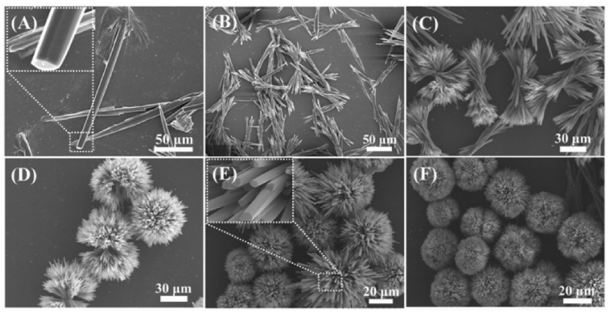

Fig. 5 SEM images of co-assembly at different ratios (CDP to CPABS): (A) 0.05; (B) 0.10; (C) 0.26; (D) 0.41; (E) 0.52; (F) 0.70.

into the arrangement of CDP molecules with different degrees.[19] Moreover, the relative intensity of the peaks (Ri) is also changed. For the CDP powder in Fig. 4A, the Ri value is about 1.16. After co-assembly with MO, although the peaks both shifted to smaller angle, the ratio of their intensity is 1.12 which almost remains the same with the CDP powder. When HPABS was used, the intensity of the second peak decreases rapidly and the Ri value rises to 10.2. Nevertheless, when CPABS was used, the second peak disappeared, thus the Ri value rises up to infinity. It reveals that when the three kinds of azobenzene molecules with tiny differences inserted into the CDP molecular arrangement, they showed different co-assembly behaviors, which could induce different nanostructures.

We could also regulate the assembly process by increasing the amount of one of the building blocks while keeping another one fixed. For example, we added CPABS to a dispersed solution of CDP nanotubes and observed morphology changes from nanotubes to network structures and finally urchin-like microspheres (Fig. S2, ESI†). The whole morphology change process proves that the addition of CPABS would destroy the intrinsic interactions of CDP in nanotubes and generate new interactions between CDP and CPABS to form CDP-CPABS co-assembly. Then we fixed the amount of CPABS and increased the ratio of CDP and CPABS gradually. Morphology changes from one-dimensional (1D) to three-dimensional (3D) ordered structures were observed when the molar ratio (CDP to CPABS) was increased from 0.05 to 0.70 (Fig. 5). Enlarged SEM images in Fig. 5A and E indicate that the initial formed microrods and those microrods on the surface of urchin-like microspheres both possess polygonal shape. This result not only reveals extremely ordered molecular arrangement in these structures, but also sheds light on the morphology evolution from 1D to 3D structures. Furthermore, in the case of CPABS, confocal laser scanning microscopy (CLSM) and photoluminescence (PL) were employed to investigate optical properties. Red emission was observed when the urchin-like microspheres were irradiated by laser light. The fluorescence spectrum of the microspheres with the peak located at 590 nm is shown in Fig. S3 (ESI†). However, fluorescence spectra of pure CPABS molecules did not show any peak at the same region, neither did CDP.[15] The new optical characteristics of co-assembly indicates that there may exist π–π interaction between CDP and CPABS molecules in the assembly structures, which is in accordance with the FTIR result (Fig. 3A).

In conclusion, the assembly of CDP could be manipulated into urchin-like, flower-like and plate-like nanostructures by three azobenzenes with tiny differences. The introduction of azobenzenes would destroy interactions between CDP molecules and generated new non-covalent interactions between CDP and azobenzenes. Tiny differences among azobenzenes which inserted into CDP molecular arrangement caused different molecular packing, which was the key factor for the formation of these structures. Taking CPABS as an example, assembly of CDP could also be regulated by changing the amount of CDP or azobenzene with the other one fixed. We hope this approach could help to fabricate novel peptide-based functional nanomaterials by co-assembly through adjusting molecular structures.

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