Organogels Based on Self-Assembly of Diphenylalanine Peptide and Their Application To Immobilize Quantum Dots

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We report that a single dipeptide (L-Phe-L-Phe, FF), which is probably one of the smallest peptide gelators, can self-assemble into long nanofibrils in organic solvents and entangle further to form gels. The obtained FF gels are responsive to temperature, and the FF sol–gel process is thermoreversible. The formation of such gels may be driven by the hydrogen bond of peptide main chains and the $\pi-\pi$ interactions between aromatic residues of the peptide. Lipophilic nanocrystals can be encapsulated into the gel through gelating the organic solution of corresponding nanocrystals using the FF gelator at room temperature. Quantum dots (QDs) are encapsulated into the FF gel by adopting the above method. The resulting gels with the incorporated QDs still remain photoluminescent (PL). It is an effective method to protect QDs from oxidation and improve the stability of the QDs. This strategy is generally suited for encapsulation of lipophilic nanocrystals.

Introduction

Molecular self-assembly is becoming a popular tool to construct different types of micro- and nanostructured materials.[1–4] Low-molecular-weight organogelators are known as distinct soft materials and can self-assemble into various types of fibrils, strands, and tapes in organic solvents via weak intermolecular interactions.[5–9] Discovery and development of low-molecular-weight gelators, including some synthetic peptides, have potential applications in the creation of new materials for nano- and biotechnology.[10–16] Assemblies of inorganic nanocrystals into two- or three-dimensional (2D/3D) architectures are of fundamental interest because of their unique optical and electronic properties.[17,18] Many efforts have been exerted in organizing water-soluble nanocrystals into an ordered assembly because the templates used require water as a solvent.[19–22] An immobilization or accumulation of inorganic nanocrystals around the cross-linking nanofibrils made of gelators can create a 3D network scaffold. It has been reported that lipophilic nanocrystals can be assembled into a gel under spatial control using a functional amphiphilic gelator.[23,24]

The diphenylalanine peptide is a suitable bottom−up building block. Previously, Reches and Gazit reported that Alzheimer's β-amyloid diphenylalanine core recognition motif could self-assemble into discrete and extraordinary stiff nanotubes in an aqueous solution, which could serve as a casting mold for producing silver nanowires.[25] A cationic dipeptide derived from diphenylalanine was self-assembled into nanotubes at physiological pH, and the resulting nanotubes were spontaneously transformed into vesicle-like structures by diluting the nanotube dispersion.[26] A 9-fluorenylmethoxycarbonyl (Fmoc)-modified dipeptide related to diphenylalanine had been presented as a hydrogelator.[27,28] Here, we report that this single dipeptide molecule (L-Phe-

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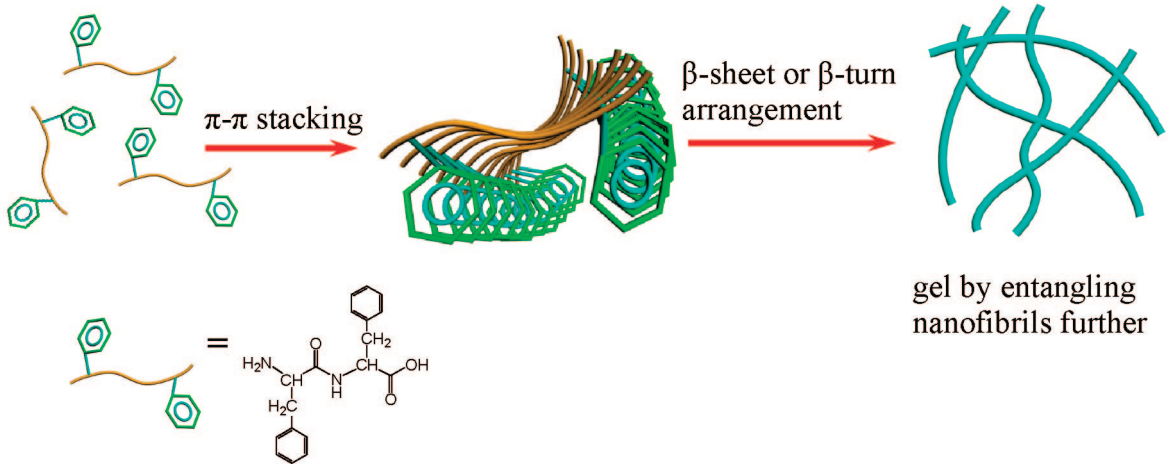

Figure 1. Proposed self-assembly mechanism of the dipeptide. Aromatic groups of FF peptides stack through $\pi-\pi$ interactions, and the resulting molecular stacks further assemble to form nanofibrils. Such nanofibrils may entangle to form the peptide organogel.

L-Phe, FF; see Figure 1 for its structure), which is probably one of the smallest peptide gelators, can self-assemble into long nanofibrils in organic solvents and entangle further to form gels. Such gels can be readily used to encapsulate quantum dots (QDs) and gold nanoparticles through gelating the organic solution of nanocrystals. The obtained gels with the incorporated QDs display an obvious photoluminescence (PL). Encapsulation using the FF gelator provides an effective method to protect QDs from oxidation and can improve the stability of the QDs. These organic–inorganic complexes may find applications as optical and electronic materials and devices.

Experimental Section

Materials. The dipeptide (L-Phe-L-Phe), 1,1,1,3,3,3-hexafluoro-2-propanol (HFP), and chloroform were purchased from Sigma-Aldrich. Dimethyldioctadecyl ammonium bromide was obtained from Acros. CdSeS nanocrystals (QD523, green; QD556, yellow; QD576, orange; and QD622, red) were purchased from Tianjin Ura quantum dots Corp. in China. Other solvents were obtained from Beijing chemical reagent Corp. and used as received.

Preparation of Gels and Encapsulation of Nanocrystals. A typical protocol is as follows: To fabricate the FF gel, a 20 μL 0.4 M FF/HFP solution was diluted to a final concentration of 8 mM in chloroform or 16 mM in toluene. A 200 μL 1 mg mL^{-1} QD or Au nanoparticle solution was dispersed in toluene and adjusted to 1 mL. The above nanocrystals solution was added to an 80 μL 0.4 M FF/HFP solution. The gel with the entrapped nanocrystals was formed rapidly at room temperature.

Microscopy. Gel samples were carefully picked up and applied to conductive polymer or stainless-steel stubs and allowed to dry followed by sputtering a thin layer of gold/Pt (3:2). The images were taken using a S-4300 (HITACHI, Japan) scanning electron microscope. A small amount of gel sample was pipetted on mica and allowed to dry. The atomic force microscopy (AFM) images were recorded with a Nanoscope IIIa (Digital Instruments, Veeco Metrology Group) in tapping mode in air. A small piece of gel with the encapsulated nanocrystals was carefully placed onto a carbon-on-Formvar TEM grid and allowed to dry. The images were obtained using a Philips CM200-FEG (120 kV). A piece of gel was placed on a microscope glass coverslip and imaged using an Olympus IX-70 fluorescence microscope equipped with a CCD camera.

Spectroscopy. Circular dichroism (CD) spectra between 190 and 300 nm were recorded in a JASCO 815 spectrometer at room temperature. Data were collected with a gel sample on a quartz chip at a scan speed of 25 nm min^{-1}, with a 0.5 nm step size. Each spectrum was the average of four measurements. FTIR spectra were measured using a TENSOR 27 FTIR spectrometer (BRUKER) with the gel sample on a CaF_2 plate. A Hitachi Model F-4500 spectrofluorometer was used to measure the fluorescence of gels, FF solution in DMF, fluorescent CdSeS nanocrystals, and gels of the encapsulated CdSeS nanocrystals. The fluorescence spectra of gel samples were obtained on a quartz chip. Others were measured in a 1.0 cm quartz cuvette at a scanning speed of 125 nm min^{-1}. Pure gel and FF solution of DMF were excited at 259 nm, and the emission spectra were collected from 269 to 500 nm. Fluorescent CdSeS nanocrystals and gels of the encapsulated CdSeS nanocrystals were excited at 365 nm, and the emission spectra were collected from 450 to 700 nm. XRD data of gel samples were collected at 3−60° using a Rigaku D/max-2500 instrument (Cu/Kα-1).

Results and Discussion

Fabrication of Organogels. Such a gelator is eventually insoluble in a general organic solvent; thus, FF dipeptide is first dissolved in a minimum amount of 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) and then the liquid is added to the above FF solution to be gelled. The gelation process can be observed exclusively in chloroform or aromatic solvents, such as toluene or xylene, while in other solvents, such as methanol, acetone, cyclohexane, dichloromethane, and *N*,*N*-dimethylformamide (DMF), FF dipeptide fails to form gels regardless of the FF concentration. A stable semitransparent gel was rapidly obtained when a 20 μL 0.4 M FF/HFP solution was diluted by chloroform to a final concentration of 8 mM (Figure 2a). Although the gel contains less than 0.2 wt % peptide, it can quickly form a 3D network. We find that the obtained FF gels are responsive to temperature and that the transition of FF gel to sol occurs around 67 °C. As the system is cooled from 67 °C to room temperature, the FF solution (Figure 2b) becomes a gel within 10 min

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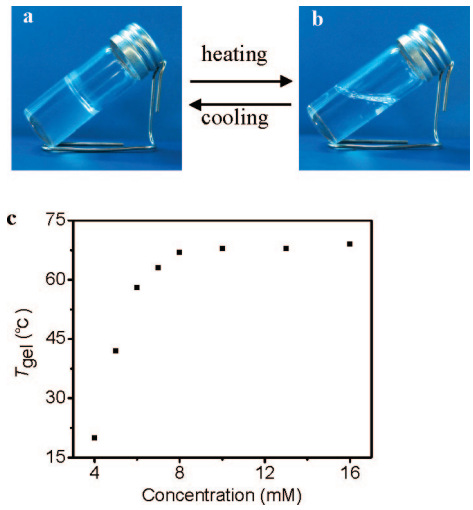

Figure 2. Thermoreversible gelation of a 8 mM FF solution in chloroform. (a) FF gel in chloroform at 25 °C. (b) FF sol in chloroform after heating to 67 °C. (c) Effect of the molecular concentration on the gel−sol transition temperature of the gel in chloroform.

(Figure 2a). This shows that the FF sol–gel process is thermoreversible. We take the gel−sol phase transition temperature (T_{gel}) to monitor the effect of the molar concentration on the FF gelation in chloroform (Figure 2c). It is found that the T_{gel} value initially increases with the molar concentration and then remains constant as the FF molecular concentration reaches ca. 8 mM. It indicates that, at this critical concentration point, the gelation of the solvent approaches saturation.

To gain better insight into the molecular organization of the FF gel, scanning electron microscopy (SEM) and atomic force microscopy (AFM) were used to observe the dried samples of gel-phase materials in both chloroform and toluene. The SEM image shows that the FF gel in chloroform consists of a fibrous network with long fibrils (Figure 3a). The magnified SEM image shows that the fibrils form a network with branches and entangle each other with diameters ranging from 10 to 50 nm (inset in Figure 3a). The AFM observation shows the same order of the fibrils with an average height of 20 nm (Figure 3b). In comparison to the gel in chloroform, the FF gel in toluene has a slightly different morphology (Figure 3c). Both fibrils and ribbon structures with a width of about 2 μm and a length of tens of micrometers were observed by SEM (inset in Figure 3c). The AFM image also shows the coexistence of fibrils and ribbons (Figure 3d).

Intermolecular Interaction Mechanism. To further understand the mechanism governing the self-assembly of FF gels, Fourier transform infrared spectra (FTIR) (Figure S1 in the Supporting Information) and circular dichroism (CD) (Figure 4a) were used to characterize the gels in chloroform and in toluene, respectively. The FTIR spectrum of the gel in chloroform showed significant β-turn character based on the position of the amide I band at 1650 and 1686 cm^{-1}.[29] The peak at 1650 cm^{-1} is assigned to aperiodic secondary structures involving type I, II, VIa, and VIII β turns. The

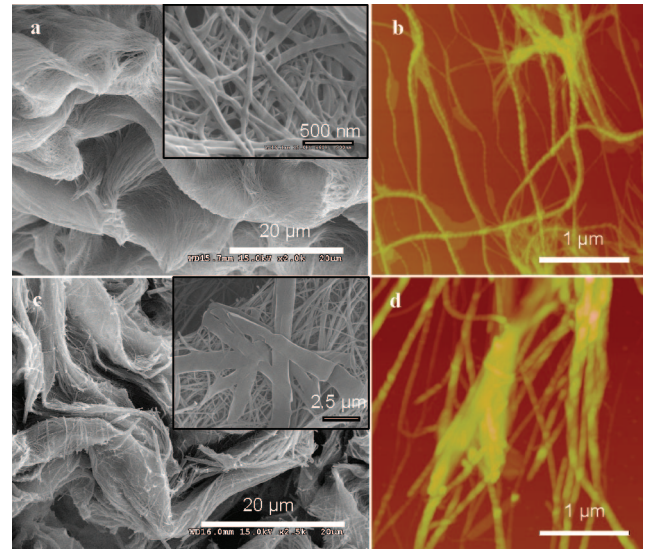

Figure 3. Microscopic analysis of the gels self-assembled by the FF dipeptide. (a) SEM image of the dried FF gel in chloroform (inset, the magnified image of nanofibrils). (b) AFM image of FF gel in chloroform (3×3 μm^2, z scale = 100 nm). (c) SEM image of the dried FF gel in toluene (inset, the magnified image of nanofibrils). (d) AFM image of FF gel in toluene (3×3 μm^2, z scale = 100 nm).

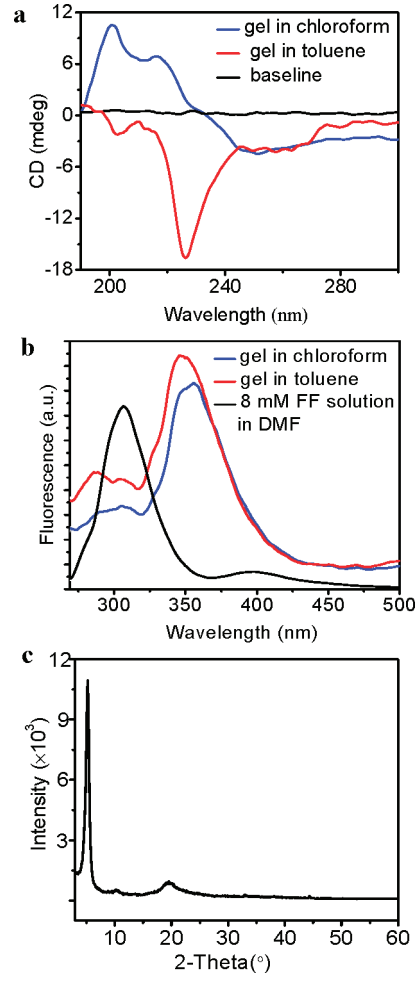

Figure 4. Intermolecular interaction between the dipeptides in the gel. (a) CD spectra of dried FF gel. (b) Florescence emission ($\lambda_{excitation}$ = 259 nm) of dried FF gel. (c) X-ray diffractogram of the dried gel in toluene.

peak at 1686 cm^{-1} can be assigned as a marker band for the β-turn conformation adopted by the molecule.[30–33] An

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additional contribution at 1618 cm^{-1} indicates that the peptide region also has a β-sheet structure. The peak at 1620 cm^{-1} from the gel in toluene reveals that the dipeptide has a predominant β-sheet conformation.[10] The observed CD spectrum of the gel in chloroform yields a feature similar to the CD signature of the β-turn peptide containing aromatic residues.[29,34] A strong positive band near 200 nm corresponds to the β-turn $\pi-\pi^*$ transition, and a second positive band at 218 nm is indicative of a $n-\pi^*$ transition. In contrast to the CD signature of the gel in chloroform, the Cotton effect at 226 nm ($n-\pi^*$ transition) of the gel in toluene can be interpreted as a signature for the dominant β-sheet arrangements of FF molecules.[35,36] FTIR and CD results altogether show that the observed fibril or ribbon structures in gels exhibit different molecular arrangements of dipeptide in chloroform and toluene. It may thus lead to different types of self-assembly of the dipeptide as the solvent is different.

Fluorescence spectroscopy was used to measure the emission spectra of the gels in chloroform, toluene, and the solution of FF in DMF of Figure 4b, respectively. In the solution of FF, the phenyl groups have an emission peak at 306 nm, which shifts to 357 nm for the gel in chloroform and 347 nm for the gel in toluene. The obvious red-shifts suggest that the phenyl groups overlap efficiently between FF molecules in a parallel mode similar to the major organization of $\pi-\pi$ interactions in proteins[37] and self-assembly of amyloid fibrils.[38] In comparison to the gel in chloroform, the small blue-shift of the corresponding peak of the $\pi-\pi$ interaction for the gel in toluene is more likely because toluene joins the aromatic stacking interaction. Additionally, FF gels in both chloroform and toluene can display obvious fluorescence when they are excited ($\lambda_{excitation}$ = 330−385 nm) by a high-pressure mercury lamp (100 W) under a fluorescence microscope (parts a and b of Figure S3 in the Supporting Information). As expected, the solution of FF gives no fluorescence when it is excited at the same condition. This result further demonstrates the conjugating $\pi-\pi$ interactions between FF aromatic residues. An X-ray diffraction (XRD) pattern of the dried gel in toluene was shown in Figure 4c. In comparison to the X-ray diffractogram of FF dipeptide nanotube,[39] FF molecular arrangement in the gel phase is somehow different by its XRD diffraction pattern of a sharp peak at 17 Å (2θ = 5.2°). The result indicates that lamellar thickness of β-sheet monolayers in the gel phase is about 1.7 nm.

From above, we suggest that the $\pi-\pi$ interactions between the phenyl groups provide the required driving force in forming extended supramolecular structures. The interaction via hydrogen bonds also assists the gelation process. The single sharp peak at 357 or 347 nm in the emission spectra of the gels suggests that there should only be one mode of overlapping for the aromatic moieties of FF. As illustrated in Figure 1, we suppose that aromatic groups in the FF peptides first connect through $\pi-\pi$ interactions, and the resulting stacking units further assemble into nanofibrils via hydrogen bond and $\pi-\pi$ interactions to form gels.

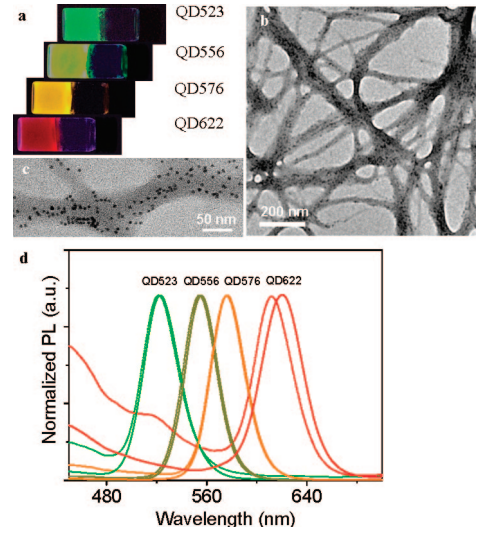

Figure 5. Encapsulation of the CdSeS nanocrystals in gel. (a) PL photograph of four different encapulated QDs gels. (b) TEM image of the encapsulated QD523 nanocrystals in the fibril network. (c) Magnified TEM image of the QD523 nanocrystals immobilized to the fibril. (d) Emission spectra ($\lambda_{excitation}$ = 365 nm) of the free QDs in toluene (solid line) and the encapsulated QDs in gel (dash dot).

Encapsulation of Nanocrystals. Inorganic nanocrystal composite gels are of potential interest for applications in electronic and optical devices.[23,24] In this context, it is intriguing to study the basic properties of the nanocrystal gel system prepared from the FF dipeptide. Four different luminescent QDs were dispersed into toluene, and the FF gelator was used to gelate the resulting solution of QDs. As shown in Figure 5a, gels of the encapsulated QDs with different emission colors are achieved under UV irradiation, indicating that QDs are firmly entrapped in the gel network and retain the original luminescence colors. It is noted that gels of the encapsulated QDs are stable, and no precipitation occurs after 2 weeks. The TEM image shows a cross-linking 3D stucture of fibrous aggregates of QDs in the gel with the encapsulated QD523 nanocrystals (Figure 5b). In comparison to the TEM image of fibrils in the absence of QDs (Figure S2a in the Supporting Information), these fibrous aggregates comprise individual QDs, as shown in Figure 5c. This result reveals that QD nanocrystals are attached to the FF fibrils. A similar phenomenon can be observed from the TEM images of other gels to encapsulate QDs (parts b−d of Figure S3 in the Supporting Information). Furthermore, a few free QDs can also be observed in the fibrous network. The normalized PL emission spectra of the QDs and the encapsulated QDs in gel are compared in Figure 5d. The maxima of the emission spectra after encapsulating the QDs in the gels are blue-shifted (approximate 2 nm in QD523, QD556, and QD576 and 10 nm in QD622) com-

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pared to those of the free QDs in toluene (Figure S4 in the Supporting Information). This behavior had been observed for the QDs coated by a silica shell.[40] Blue shifts in the emission spectra of the encapsulated QDs in gels likely occur as a result of which QDs are linked to the dipeptide fibrils. These results indicate that some nanocrystals are attached to the fibrils likely because of the ligand interaction between the dipeptide molecule and nanocrystal, while others are dispersed in the interspace of the fibril network.

To validate the general application of the method to encapsulate nanocrystals in gel, 3–5 nm octanethiol-stabilized gold nanoparticles were prepared according to the procedure of Brust et al.[41] The toluene solution of Au nanoparticles was added to the FF/HFP solution. The brown-colored gel was formed rapidly at room temperature (Figure S5a in the Supporting Information). TEM images show that the fibrils of the attached Au nanoparticles are clearly observed (parts b and c of Figure S5 in the Supporting Information). To investigate suitability for encapsulating the more size nanoparticle, Au particles with the size of 20 nm were synthesized in aqueous solution[42] and transferred into the organic phase using phase transferring agent (dimethyldioctadecyl ammonium bromide). TEM image reveals that the majority of Au particles are well-dispersed in the gel network (Figure S6a in the Supporting Information) and a fraction of them are attached to the FF fibrils (Figure S6b in the Supporting Information). Taken together, the dipeptide gelator is a good candidate to fabricate 3D organic–inorganic scaffolds.

Conclusions

In summary, we have demonstrated the formation of a self-assembled organogel by a small molecular building block, diphenylalanine peptide. Fabrication of this dipeptide gel is supposed to be driven by the hydrogen bond of the peptide main chains and the $\pi-\pi$ interactions between aromatic residues of the peptide. Such an organogelator can be used to gelate the lipophilic nanocrystals in organic solvent at room temperature. The dipeptide gels can be readily manufactured and also be decorated chemically. Thus, gel materials with different optical, electronic, and magnetic properties can possibly be achieved via gelating the corresponding functional nanocrystals.

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Supporting Information Available: IR spectra, fluorescence images, magnified PL emission spectra, additional TEM images, and encapsulation of Au nanoparticles in gel. This material is available free of charge via the Internet at http://pub.acs.org.

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