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Smart hydrogels from laterally-grafted peptide assembly†

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Small peptides carrying laterally-grafted azobenzene units self-assemble into photo-responsive hydrogels which are applied as a smart matrix for controlling the dye molecules release. We demonstrate that a delicate balance among peptides interactions plays a pivotal role in the photo-responsive gel–sol transition.

Supramolecular hydrogels with a stimuli responsive functionality have been of immense interest to the field of nano-scale materials.[1,2] This interest stems from the fact that the responsive gels allow the development of smart biomaterials, such as controlled drug delivery, sensors, and tissue engineering.[3,4] Smart hydrogels obtained from natural biomolecules are appealing since they are more likely to be biodegradable, biocompatible, and bioresorbable.[5,6] Rational peptide design and engineering are thus emerging as particularly promising routes to such materials due to the relative easiness in their synthesis.[7–9] An essential requirement is that the peptides should have a propensity to form 1D self-assembly nanofibers, which can entangle to form 3D networks with high water content.[10] To advance the utility of smart hydrogels, a lot of effort has been focused on triggering the self-assembly and gelation behavior of small peptides in various stimuli-responsive manners, such as pH,[11] temperature,[12] ionic strength,[13] oxidation–reduction,[14] enzymatic manipulation,[15] and light.[16] Among them, light, especially the photoisomerization of azobenzene, is an interesting choice as it can be manipulated spatially and temporally in a non-contact fashion,[17,18] and the photochromic reaction is repeatable at will without apparent fatigue.[19]

Small peptides with β-sheet structures are commonly favourable for the formation of 1D nanofibers due to the directional hydrogen bonding within the peptide backbones.[20] Although a few β-sheet peptides with azobenzene units have been synthesized to create smart hydrogels, the photo-responsive gel–sol transitions are complicated.[21,22] In other words, UV-light irradiation is not enough to destroy the peptide 3D networks, and the gel to sol transition process needs additional assistance reagents, such as sodium hydroxide[21] or enzyme.[22] The main reason is that the intermolecular interactions among β-sheet peptides are too strong to be broken by the conformational transition of the azobenzene units. This character led us to consider that weakening the interactions by optimizing the molecular design should be a rational strategy for creating very simple photo-responsive hydrogels. With this in mind, we designed two small peptides (**1** and **3**) containing light sensitive azobenzene units in a lateral way and terminal lysine groups (Fig. 1). It is expected that the electrostatic repulsions between protonated lysine groups reduce the secondary interactions of peptides and result in simple photo-responsive gels. Peptide **2** with glutamic acid at one terminal was synthesized for elucidating the importance of the electrostatic repulsions. Herein, we present the photo-responsive self-assembly and the gelation behavior of the synthesized peptides. We also demonstrated that the smart hydrogel can be used further as a vehicle to control the dye molecules release.

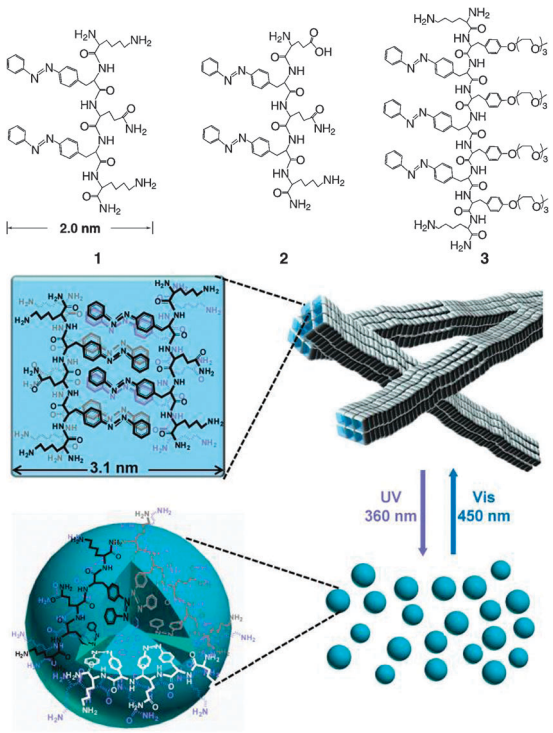

Fig. 1 Molecular structures of the synthesized peptides and the schematic presentation of the photo-responsive self-assembly structures of peptide **1**.

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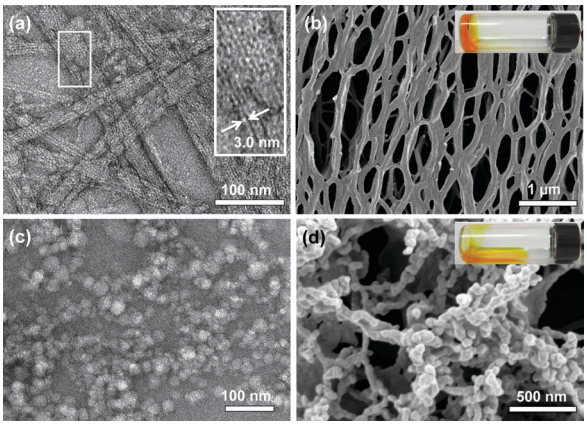

Fig. 2 Photo-responsive self-assembly structures of peptide **1**: (a) TEM image of **1** in 40 μM aqueous solution; (b) SEM image of **1** gel (inset, optical image of **1** gel); (c) TEM image of **1** solution and (d) SEM image of **1** gel sample (inset, optical image of **1** sol) after exposing to 360 nm UV light for 15 min.

A transmission electron microscopy (TEM) image revealed that peptide **1** self-assembled into long nanofibers with a uniform diameter of 3 nm (Fig. 2a and inset) in diluted aqueous solution. With increasing concentration, the nanofibers of peptide **1** entangled with each other to form 3D networks (Fig. 2b), corresponding to stable hydrogel formation (Fig. 2b, inset). The critical gel concentration (cgc) of **1** is 5.1 wt% based on the inverting tube method. The high cgc value is probably due to the presence of electrostatic repulsions between the protonated lysine groups ($pK_a \sim 10.5$) in aqueous solution, which depresses the aggregation to some extent.[23]

Circular dichroism (CD) spectra of peptide **1** (Fig. 3a) displayed a negative Cotton effect at 220 nm, indicative of typical β-sheet conformation. Fourier transform infrared (FT-IR) spectra of the gels (Fig. 3b) showed well-defined amide I bands centered at 1629 cm^{-1} and 1679 cm^{-1}, which suggests that the nanofibers of peptide **1** consist of antiparallel β-strands.[24] The X-ray diffraction of the peptide **1** xerogels (Fig. S3, ESI†) indicated layered structures with a distance (*d*) of 3.03 nm, which is consistent with the TEM observation. The molecular length (*l*) between the hydrophilic amine at the ε-position of lysine and the azobenzene units was estimated to be 2.0 nm. Considering the obtained layer spacing located at $l < d < 2l$, we propose that the nanofibers consist of a bilayer structure with intercalated azobenzene units, as shown in Fig. 1.

Notably, the gels of **1** are responsive to UV irradiation through a conformational transition of azobenzene moieties from *trans* to *cis* states (see UV spectra, Fig. S4 (ESI†)). This

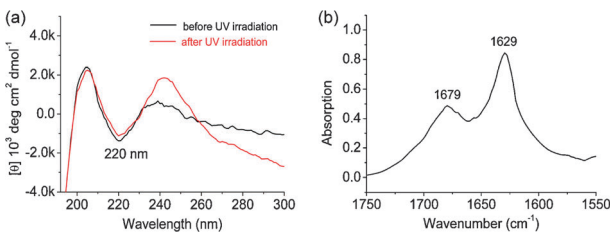

Fig. 3 (a) Circular dichroism (CD) spectra of peptide **1** aqueous solution before (black line) and after (red line) UV irradiation; (b) FT-IR spectrum of peptide **1** gel.

conformational change enforces long nanofibers to break up into discrete spherical aggregates (Fig. 2c and d). Consequently, the disassociation of the nanofibers caused by photoisomerization of azobenzene units drove the gels to transform into a fluid solution (Fig. 2d, inset). After aging the solution of spherical structures for two hours under daylight, long nanofibers and self-supporting hydrogels were observed again (Fig. S5, ESI†), indicating a reversible sol–gel transition with light. Interestingly, circular dichroism (CD) spectra showed that the β-sheet structure of peptide **1** remained almost unchanged after UV irradiation (Fig. 3a), indicating that the photoisomerization of azobenzene did not cause a significant variation in the backbone of **1**. It is clear that the stacking of azobenzene groups has an important influence on the self-assembly structures even though the backbone structure of peptide **1** remains constant. Actually, the hydrophobic azobenzene moieties have a strong tendency to associate with each other to minimize their contact with water. The planar geometry of *trans*-azobenzene side groups provides an energetic contribution as well as the directional hydrogen bonding for the formation of crossed β-sheet structures, which further stack into extended 1D structure. When exposing the solution of nanofibers to UV irradiation, the nonplanar *cis* azobenzene units caused an increased bulky effect, which does not facilitate the peptides alignment into 1D arrangement. This result is similar to the self-assembly of aromatic dipeptides reported by Reches and Gazit.[25] Combining with the electrostatic repulsions between protonated lysine groups, these factors resulted in random β-sheet structures, which were not competent for 1D growth. Some molecular simulations suggested that the random β-sheets had a compromise propensity to adopt a spherical interfacial curvature.[26,27] We also studied the photo-responsive gelation behavior of **1** after treatment with salt and basic reagents. In those cases, the electrostatic repulsions between protonated lysine groups would be suppressed significantly. The cgc value of peptide **1** is down to 3.4 wt% and 3.1 wt% for ammonia water (pH ~ 10.5) and salt solution (10 mM NaCl), respectively. However, the resulting gels did not transform into fluid solution even after exposing to UV light for 30 min (Fig. S6, ESI†), implying that the enhanced peptides interactions are strong enough to inhibit the disassociation of the nanofibers.

To gain further insight into the strength of β-sheets of peptide **1**, we have prepared the analogous one bearing a glutamic acid at the one end of the molecules (Fig. 1, peptide **2**). This molecular design would lead to enhanced β-sheet strength through increased electrostatic interactions between glutamic acid and lysine units of the adjacent molecules (Fig. S7, ESI†). Indeed, peptide **2** self-assembles into rigid nanofibers (Fig. 4a), which further form 3D networks and stable gels (Fig. 4b and inset) at lower concentrations (~2.6 wt%). However, the gels do not show responsive characteristics after transformation of the azobenzene units into *cis*-conformation by UV irradiation (Fig. S8, ESI†). The TEM (Fig. 4c), SEM (Fig. 4d), and optical images (Fig. 4d, inset) show that the fibrillar structures, 3D network structures, and the gel matrix remain unchanged, respectively, even after UV irradiation for long time (above 30 min).

The above results strongly demonstrate that construction of photo-responsive hydrogels requires a delicate balance among peptide interactions. In our system, the introduction

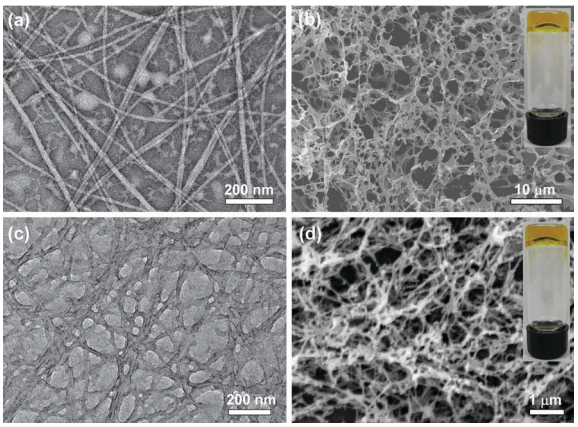

Fig. 4 Self-assembly structures of peptide **2**: (a) TEM image of **2** in 40 μM aqueous solution; (b) SEM image of **2** gel (inset, optical image of **2** gel); (c) TEM image of **2** solution and (d) SEM image of **2** gel sample (inset, optical image of **2** gel) after exposing to 360 nm UV light for 30 min.

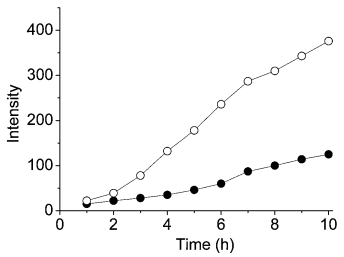

Fig. 5 Time course of the rhodamine B release, with (cycles) and without (black dot) UV irradiation. (The ordinate axis corresponds to the emission intensity (at 581 nm) of RhB in distilled water.)

of electrostatic repulsions into β-sheet peptides is a simple strategy to design smart hydrogels. To further support this proposal, we have synthesized another peptide with more azobenzene units (**3**). Peptide **3** formed stable gels above the concentration of 4.03 wt%, which showed responsive characteristics after transformation of the azobenzene units into *cis*-conformation by UV irradiation (Fig. S9, ESI†).

With the photo-responsive characteristics, the gels are considered as a smart material to control the dye release. Rhodamine B (RhB) was firstly mixed with the peptide **1** gel. We then investigated the release of RhB to distilled water with and without UV irradiation by detecting the emission intensity of RhB at 581 nm. As shown in Fig. 5, the emission intensity within 10 hours is near 125 in the absence of UV irradiation. In contrast, when applying the UV irradiation, a more rapid release of RhB is observed in the composite hydrogels together with the moderate decrease in the gel volume, because of the gel-to-sol phase transition, causing the gradual release of RhB molecules embedded in the gel matrix.

In conclusion, we designed and synthesized small peptides with laterally-grafted azobenzene units. Peptides **1** and **3** with lysine groups at both terminals formed photo-responsive supramolecular hydrogels, due to the presence of electrostatic repulsions between protonated lysine groups. When enhancing the β-sheet strength through the introduction of ammonia water, salts, or electrostatic interaction, the resulting hydrogels did not show responsive characteristics any more even after UV irradiation for longer time. The results here suggest that construction of photo-responsive hydrogels requires appropriate secondary interactions of peptides. The photo-responsive characteristics of such peptide gels allowed us to further tune the release of dye molecules with UV light. Although the present smart gels still need to be improved in the future for applying under physiological conditions, we believe that this report will provide an important insight into rational molecular design for photo-responsive peptide hydrogels.

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