Organic & Biomolecular Chemistry

Cite this: *Org. Biomol. Chem.*, 2011, **9**, 2149

www.rsc.org/obc

PAPER

Supramolecular hydrogels based on short peptides linked with conformational switch†

Yucheng Huang,[a] Zhenjun Qiu,[a] Yanmei Xu,[a] Junfeng Shi,[b] Hongkun Lin[a] and Yan Zhang*[a]

Received 20th November 2010, Accepted 14th December 2010
DOI: 10.1039/c0ob01057j

Short peptides appropriately linked with an azobenzene conformational switch were found to be motif and pH dependant supramolecular hydrogelators. The hydrogelation properties of the short peptides linked with the conformational switch were studied in detail with respect to dependence on amino acid residue, pH and salt effect. The presence of amino acids with aromatic side chains such as Phe and Tyr was found to be favorable for the short peptides to gel water at an appropriate pH range. Cationic amino acid residues such as Arg and Lys in the short peptides were found to be unfavorable for hydrogelation. pH and salt effect were also found to be important factors for the hydrogelation properties of the short peptides. A series of short peptides with bioactive sequences were linked with the conformational switch and their hydrogelation properties were investigated. Photoresponsive supramolecular hydrogels were realized based on the *E-/Z-* transition of the conformational switch upon light irradiation. Proper combination of amino acid residues in the short peptides resulted in smart supramolecular hydrogels with responses to multiple stimuli.

Introduction

Peptide-based hydrogels are important biocompatible functional materials with diverse applications.[1] With increasing research interest on stimuli-responsive soft materials in recent years,[2] peptide-based supramolecular hydrogels have been designed as "smart" materials with response to various stimuli.[3] The sol–gel or gel–sol phase transition of a hydrogel induced by external stimuli such as light, pH, enzyme and chemicals presents potential applications in drug delivery, controlled release, cell encapsulation *etc.*[4,5] Among all the stimuli-responsive soft materials, photo-responsive hydrogels are of particular interest in materials science and engineering since their property and function are subject to spatio-temporally resolved manipulation through light irradiation.[6] Compared with a large family of photo-responsive polymer hydrogels, only a few examples of photo-responsive small molecular hydrogels have been reported up to now.[7–9]

Incorporation of fumaric amide as a conformational switch has proved to be an effective strategy to construct photo-responsive supramolecular hydrogels with potential applications.[7] Another conventional conformational switch, azobenzene, has been used in the construction of photo-responsive polymer hydrogels,[10] organogels[11] and host–guest type supramolecular hydrogels.[12] Although azobenzene-linked sugar molecules have been found to gel water at low concentrations[13] and an azobenzene substituted short peptide showed reversible dispersion and reorganization of fibrous self-assembling,[14] azobenzene based small molecular gelators which can gel pure water and show photo-induced phase transition have rarely been reported.[9]

It is therefore interesting for us to explore the scope of photo-responsive hydrogels based on azobenzene substituted short peptides. Through detailed study of the hydrogelation properties of short peptides appropriately linked to the conformational switch azobenzene, we demonstrate herein the dependence of the hydrogelation properties of the short peptides on amino acid residues, pH and salt effect. Photo-response of the hydrogels with these substituted short peptides is also demonstrated, indicating that the appropriate combination of azobenzene moiety and short peptide sequence could be an efficient way to construct various photo-responsive supramolecular hydrogel systems with different functions and applications.

Results and discussion

Molecular design and synthesis

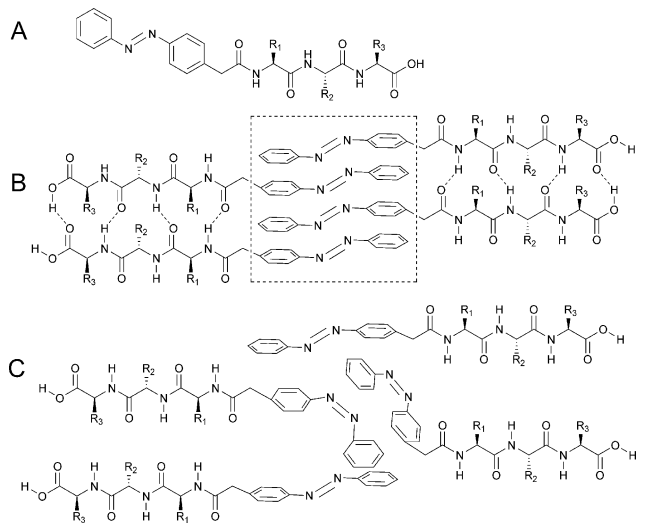
Unlike the previously reported hydrogelators with a conformational switch in between the hydrophobic and hydrophilic components,[7] the conformational switch of the peptide

[a]*School of Chemistry and Chemical Engineering, Key Lab of Analytical Chemistry for Life Science, Ministry of Education of China, Nanjing University, Nanjing, 210093, China. E-mail: njuzy@nju.edu.cn; Fax: (86)-25-83685976; Tel: (86)-25-83593072*

[b]*Department of Chemistry, Brandeis University, 415 South Street, Waltham, Massachusetts, 02454, USA*

† Electronic supplementary information (ESI) available: Molecular modelling on the self-assembly of azo-D-Lys-Phe-Ala; UV spectra of the hydrogels before and after UV-irradiation; SEM of the cryo-dried samples of hydrogels formed by azo-Lys-D-Phe-D-Ala and azo-Gln-Phe-Ala before and after UV-irradiation; characterization of the azo-peptides. See DOI: 10.1039/c0ob01057j

hydrogelators developed by us was at the N-terminal of the short peptides. A representative structure of the small molecular hydrogelators with conformational switch is shown in Scheme 1A. Instead of using (*E*)-4-(phenyldiazenyl)benzoic acid to couple with the N-terminal amino group,[14] we used (*E*)-2-(4-(phenyldiazenyl)phenyl) acetic acid as the N-terminal substitution group of the short peptides. This subtle structural difference resulted in great improvement on the ability of the substituted short peptides to dissolve in water without any organic solvents and form hydrogels.



Scheme 1 A) Representative chemical structure of the short peptides with azobenzene as conformational switch; B) balanced intermolecular interactions between hydrogen bonding and π–π stacking C) disturbed balance of intermolecular interaction due to partial *E*–*Z* isomerisation of azobenzene.

The rationale for the molecular design was to use the conformationally switchable azobenzene as the main contributor to hydrophobic interaction in the self-assembly system through effective intermolecular π–π stacking of the phenyl rings on the azobenzene moiety. Meanwhile, the peptide bone would provide hydrogen bonding sites for intermolecular hydrophilic interactions (Scheme 1B). When a delicate balance between the π–π stacking interaction and hydrogen bonding is achieved, the self-assembled system can form a three dimensional fibrous network and gel water. Conformational switching of the azobenzene moiety from *E*- to *Z*- would change the relative position of the phenyl rings and make the π–π stacking less effective (Scheme 1C), which may disassemble the fibrous network and induce phase change of the hydrogel. Therefore it would be possible to construct photo-responsive supramolecular hydrogels with the short peptides linked with azobenzene at the N-terminal.

The advantage of putting azobenzene at the N-terminal of the peptides lies mainly on the convenience of synthesizing peptides with diverse sequences to test their hydrogelation properties. The azobenzene moiety can be easily attached using (*E*)-2-(4-(phenyldiazenyl)phenyl)acetic acid to couple with the free N-terminal of the short peptides on resin during solid phase peptide synthesis. It is therefore convenient to screen various kinds of photo-responsive functional hydrogelators with easy chemistry.

Hydrogelation properties of azobenzene substituted dipeptides

Different types of amino acid residues have been used in the combination of dipeptides to get comprehensive information on the dependence of hydrogelation ability on the motifs in the azobenzene substituted short peptides. Representative amino acid residues in the dipeptides include Phe and Tyr which have aromatic side chains, Arg and Lys which have cationic side chains, Glu which contains an anionic side chain, Ser and Gln which have hydrophilic side chains and Ala which contains an aliphatic side chain. The combination of the amino acids resulted in the azo-dipeptides shown in Table 1. Most of these azo-dipeptides listed in Table 1 could be water soluble under appropriate pH range without the aid of any organic solvent. For azo-dipeptides which could not form hydrogels, adjustment of pH either resulted in precipitation or clear solution. Only for azo-dipeptide hydrogelators could the intermediate gel state be achieved through careful adjustment of pH. Typical conditions for different azo-dipeptides to gel water are also listed in Table 1.

From Table 1 it can be seen that the hydrogelation properties of azobenzene substituted dipeptides are highly motif-dependant. The amino acid side chain should play a pivotal role in the hydrogelation process. Azo-dipeptides with aromatic amino acid residues such as Phe and Tyr seemed to be prone to forming hydrogels. The combination of Phe with various types of amino acids including anionic Glu, hydrophilic Ser, aliphatic Ala and aromatic Phe or Tyr could all result in hydrogelators. The

Table 1 Hydrogelation conditions for azobenzene substituted dipeptides

azo-dipeptide	pH	C (mg mL^{-1})[a]	azo-dipeptide	pH	C (mg mL^{-1})[a]
Azo-Phe-Glu	2.1	8.5	Azo-Gln-Tyr	4.8	1.9
Azo-Glu-Phe	2.5	5.0	Azo-Gln-Gln	—[b]	—
Azo-Phe-Ser	2.0	4.3	Azo-Gln-Ala	2.5	6.0
Azo-Ser-Phe	5.7	2.0	Azo-Glu-Ala	2.4	4.2
Azo-Phe-Ala	7.8	3.2	Azo-Arg-Ala	—	—
Azo-Ala-Phe	9.5	3.5	Azo-Arg-Phe	—	—
Azo-Phe-Phe	9.7	2.8	Azo-Arg-Gln	—	—
Azo-Phe-Tyr	8.6	5.8	Azo-Ser-Ala	—	—
Azo-Tyr-Ala	3.0	5.0	Azo-Lys-Ala	—	—
Azo-Ala-Tyr	5.1	2.5	Azo-Arg-Lys	—	—
Azo-Tyr-Tyr	4.8	0.8	Azo-Glu-Lys	—	—
Azo-Glu-Tyr	4.8	1.9	Azo-Tyr-Lys	2.2	7.8

[a] Lowest concentration of hydrogelation at the corresponding pH. [b] Unable to form hydrogel under tested conditions.

additional hydrophobic interaction induced by the aromatic side chain was shown to be favorable for the self-assembling of the azo-dipeptides. On the other hand, azo-dipeptides with cationic amino acid residues such as Arg and Lys seemed to be either soluble or totally insoluble in water and therefore showed poor hydrogelation ability.

It is also noteworthy that the distance between the Phe or Tyr residue and the N-terminal azobenzene showed influence on the pH and concentration required for the hydrogelation of corresponding azo-dipeptides. Comparison of the gelation condition for azo-Phe-Glu to azo-Glu-Phe, azo-Phe-Ser to azo-Ser-Phe and azo-Tyr-Ala to azo-Ala-Tyr clearly shows consistent change including higher pH and lower concentration required for hydrogelation. This indicated that hydrophobic interactions induced by the aromatic side chain of Phe or Tyr should be more effective when Phe or Tyr was away from the hydrophobic head of the substituted dipeptides and the enhanced hydrophobic interaction could lead to higher pH being required for hydrogelation. Therefore it should be possible to adjust the hydrogelation conditions for known azo-dipeptides through insertion of aromatic amino acid residues at appropriate positions, which has been confirmed by the results shown in the following part.

Except for the dependence of hydrogenation properties on amino acid residues, we also investigated pH effect and salt effect on the hydrogelation properties of given azo-dipeptides. For the azo-dipeptides with Phe residues, hydrogelation happened at specific pH as listed in Table 1. Under pH lower than the listed pH for hydrogelation, the dipeptides precipitated out and the precipitate would not dissolve even upon heating. However, the azo-dipeptides with Tyr showed hydrogelation ability within broad pH range. Fig. 1A shows the pH effect at the lowest concentration required for hydrogelation for three dipeptides containing Tyr. The lowest concentration for hydrogelation of these three dipeptides could be less than 1% at pH around 4. Solubility of the dipeptides increased with increase in pH; therefore the concentration needed for gelation showed consistent increase with pH. Salt effect was also studied on these three azo-dipeptide hydrogelators. As shown in Fig. 1B, for azo-Ala-Tyr and azo-Tyr-Tyr, using buffer containing higher concentration of Na_2HPO_4/NaH_2PO_4 decreased the concentration required for hydrogelation. It indicated that electrostatic interactions presented in the gel matrix could enhance the intermolecular interaction between the short peptides. However, for the dipeptide azo-Glu-Tyr which contains an anionic Glu, salt effect was almost negligible compared with the other two.

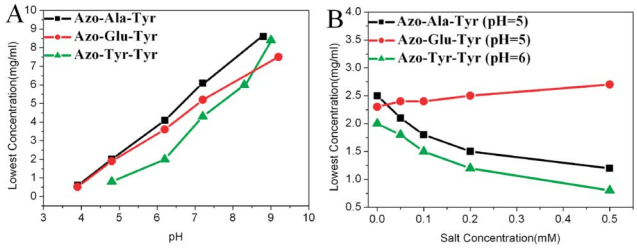


Fig. 1 Lowest concentration of hydrogelation for azo-dipeptides under different pH (A) and in solutions containing different concentration of salt (B).

Hydrogelation properties of azobenzene substituted tripeptides

Azobenzene substituted tripeptides with Phe or Tyr between two other amino acid residues were prepared and their hydrogelation properties were studied. As shown in Table 2, insertion of Phe in between the two amino acids of the azo-dipeptides showed a regulatory role in hydrogelation property as rationalized above. Azo-Gln-Phe-Ala could gel water at higher pH and lower concentration compared to azo-Gln-Ala. After insertion of Phe in azo-Arg-Ala, azo-Ser-Ala and azo-Lys-Ala which could not form gel as dipeptides, the resulting tripeptides could gel water under acidic conditions (entry 5, 6 and 9). Insertion of Tyr instead of Phe showed a similar effect on the regulation of gelation properties. The tripeptides with cationic Arg or Lys residues (entry 5 and 9-12) could gel water under quite acidic conditions.

It is noteworthy that the configuration of amino acid residues in the tripeptides also had a slight effect on the hydrogelation properties of these tripeptides. The tripeptides with amino acids in uniform configurations such as azo-Lys-Phe-Ala and azo-D-Lys-D-Phe-D-Ala could form gel at lower concentration than azo-Lys-D-Phe-D-Ala and azo-D-Lys-Phe-Ala which have both D- and L- amino acids in the tripeptide chains. Additionally, lower phase transition temperatures for gels formed by azo-Lys-D-Phe-D-Ala or azo-D-Lys-Phe-Ala than gels by azo-Lys-Phe-Ala or azo-D-Lys-D-Phe-D-Ala indicated weaker intermolecular strength.

Table 2 Hydrogelation conditions for azobenzene substituted tripeptides and time needed for initial photo-responses

Entry	Azo-tripeptide	pH	C (mg mL^{-1})[a]	T (°C)[b]	t_{init} (min)[c]
1	Azo-Gln-Phe-Ala	7.8	3.7	65	3
2	Azo-Glu-Phe-Ala	4.7	4.2	85	—[d]
3	Azo-Leu-Phe-Ala	8.6	4.7	71	15
4	Azo-Gly-Phe-Ala	7.5	7.2	48	5
5	Azo-Arg-Phe-Ala	1.6	2.8	47	8
6	Azo-Ser-Phe-Ala	3.7	3.2	54	—[d]
7	Azo-Gln-Tyr-Ala	3.1	2.5	81	—
8	Azo-Glu-Tyr-Ala	3.0	3.0	69	—
9	Azo-Lys-Phe-Ala	1.6	3.1	59	30
10	Azo-Lys-D-Phe-D-Ala	1.2	6.8	42	7
11	Azo-D-Lys-D-Phe-D-Ala	1.6	3.0	60	15
12	Azo-D-Lys-Phe-Ala	1.4	6.8	39	4

[a] Lowest concentration to gel water at specific pH; [b] Temperature for gel to solution phase transition; [c] Time needed for initial collapse of the gel upon UV irradiation; [d] No obvious change upon UV irradiation.

CD spectra of the hydrogels are shown in Fig. 2. The sharp peak around 194 nm and the trough around 214 nm indicate that the hydrogelators were mainly assembled in β-sheet-like superstuctures in the gel state. The signal around 330 nm is due to the π–π* transition of the *E*-azobenzene group.[15] CD spectra of the hydrogels formed by azo-D-Lys-D-Phe-D-Ala and azo-Lys-Phe-Ala showed symmetric signals as shown in Fig. 2B. Molecular modelling on the self-assembly of azo-D-Lys-Phe-Ala (ESI†) using Materials Studio as suggested before[16] showed consistent results on the morphology and microstructure of the fibrous network.

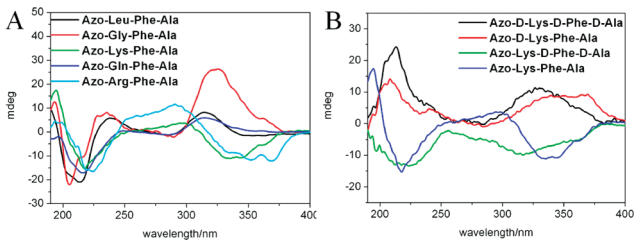


Fig. 2 A) CD spectra of hydrogels formed by azo-X-Phe-Ala; B) CD spectra of hydrogels formed by azo-D-Lys-D-Phe-D-Ala, azo-D-Lys-Phe-Ala, azo-Lys-D-Phe-D-Ala and azo-Lys-Phe-Ala.

Photoresponse of the hydrogels

Since the azobenzene moiety was supposed to contribute to the self-assembly of the azo-peptides through intermolecular π–π stacking, the photo-induced *E*- to *Z*- isomerization should have influence on the self-assembling system through disturbance of the intermolecular hydrophobic interaction. Therefore we expect that photo-response could be realized in hydrogels based on azo-peptides. Our results showed that the hydrogels formed by azo-tripeptides did have reversible photo-response. Take the gel formed by azo-Gln-Phe-Ala for example: photo-response and the related *E*- to *Z*- transformation are shown in Fig. 3A. Upon UV-irradiation, the gel formed by *E*-azo-Gln-Phe-Ala began to collapse within 3 minutes. A complete conversion of the hydrogel into a homogeneous non-viscous solution took less than 20 min during which a heat effect was avoided. The resulting solution

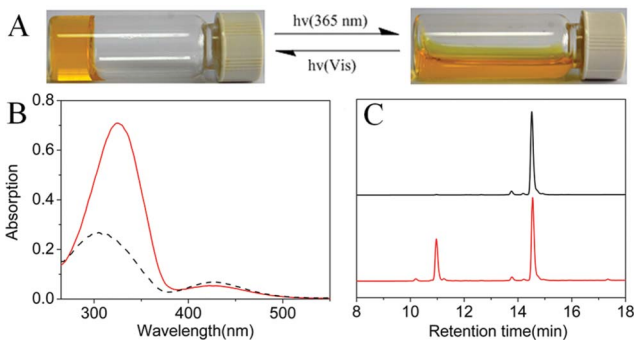


Fig. 3 A) Optical images of photo-induced phase change of the hydrogels formed by azo-Gln-Phe-Ala and the corresponding structure change; B) UV spectrum of the azo-Gln-Phe-Ala hydrogel before (red solid line) and after (black dash line) UV irradiation; C) HPLC monitoring of the components of the hydrogel formed by azo-Gln-Phe-Ala before (upper) and after (lower) photo-induced phase change.

could turn back into gel upon ambient visible light irradiation for an average time span of two days.

UV and HPLC monitoring on the components of the azo-Gln-Phe-Ala gel before and after photo-induced phase transition showed an obvious increase in the ratio of *Z*- to *E*- isomers during the process. As shown in Fig. 3B, for the sample corresponding to the original hydrogel before photo-irradiation, the characteristic absorption of *E*-azobenzene around 330 nm predominated. After photo-irradiation and phase change, there was distinct decrease in the absorption around 330 nm along with an increased absorption around 430 nm which is characteristic of the *Z*-azobenzene. HPLC analysis on the components showed that the *E*-azo-Gln-Phe-Ala, which formed the hydrogel matrix, partially turned into *Z*-azo-Gln-Phe-Ala after the photo-induced phase transition of the hydrogel. (Fig. 3C) It therefore indicates that *E*- to *Z*-photoisomerization of the azobenzene moiety happened in the gel matrix upon UV irradiation. It also suggested that partial isomerization of the azobenzene moiety was able to disturb the delicate balance in the self-assembling system and induce phase transition of the small molecular hydrogel.

Hydrogels formed by several other tripeptides such as azo-Gly-Phe-Ala and azo-Lys-D-Phe-D-Ala also showed fast response upon UV irradiation. Changes in the viscoelastic properties of the hydrogel formed by azo-Lys-D-Phe-D-Ala before and after UV irradiation has also been confirmed by oscillatory rheology as shown in Fig. 4. The dynamic strain sweep test (**A**) showed that the storage modulus of the hydrogel before UV irradiation was 70 times higher than the one after UV irradiation. At the same time, the dynamic frequency sweep test (**B**) showed more than 100 times decrease on the storage modulus of the hydrogel after UV irradiation.

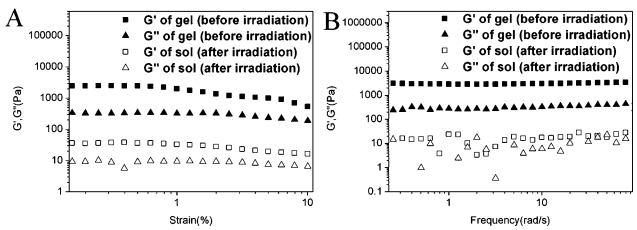


Fig. 4 The strain sweep test (A) and the frequency sweep test (B) of the gel formed by azo-Lys-D-Phe-D-Ala before and after UV irradiation.

It is noteworthy that the photo-response of the hydrogels formed by azo-tripeptides was not uniform. Sensitivity of the hydrogels to UV-irradiation was shown in Table 2 as the total irradiation time needed for the gel began to collapse (t_{init}). The azo-Gly-Phe-Ala gel and azo-Lys-D-Phe-D-Ala gel showed similar sensitivity (5 min and 7 min respectively). The gel formed by azo-Lys-Phe-Ala showed partial phase-transition after 30 min irradiation and no photo-response was observed on the gel formed by azo-Glu-Phe-Ala even after 5 h UV-irradiation. The reason for such differences in photo-response could be rationalized by the photo-induced *E*- to *Z*- isomerization process in different gels. UV absorption spectra of the gel before and after UV irradiation (ESI†) revealed different *E*- to *Z*- isomerization processes for different azo-tripeptide gels. It indicated that the photo-isomerization of the azobenzene moiety was partially or completely restricted in some gel matrices. Such restriction on the photoisomerization of the azobenzene moiety

has also been found in other azobenzene related systems[17] and it might explain why there were only a few examples of photo-responsive supramolecular hydrogels based on azobene-integrated small molecules up to now.

A multi-response hydrogel

It is rational to integrate specific peptide sequences with photo-sensitive functional groups to develop hydrogels with response not only to light but also to other external stimuli such as ligand–receptor interaction. We have demonstrated that the short peptide D-Ala-D-Ala integrated with a light-sensitive spiropyran moiety could form hydrogel with dual response to light and to ligand–receptor interaction.[9] Compared with spiropyran-substituted peptides, the azobenzene substituted peptides had similar photo-sensitivity and better thermal stability. Therefore it was possible to produce a hydrogel with multi-response to light, heat and ligand–receptor interaction based on azobenzene substituted tripeptides. Since D-Phe-D-Ala has been reported to be a synthetic ligand for vancomycin,[18] it was integrated with azobenzene in the tripeptide azo-Lys-D-Phe-D-Ala to test whether the hydrogel could respond not only to light but also to its receptor, vancomycin.

Response of the hydrogel to ligand–receptor interaction between D-Phe-D-Ala and vancomycin was tested. When one equivalent of vancomycin hydrochloride was added to the surface of the azo-Lys-D-Phe-D-Ala gel, the yellow gel changed gradually into a homogeneous solution. A control experiment on the azo-Lys-Phe-Ala gel showed no phase change upon addition of vancomycin, which indicated the response of azo-Lys-D-Phe-D-Ala gel to vancomycin was due to the specific ligand–receptor interaction between D-Phe-D-Ala and vancomycin. In addition to the response to ligand–receptor interaction, the azo-Lys-D-Phe-D-Ala gel could also respond to heat and UV-irradiation. Upon heating to 42 °C, the gel could turn into a yellow solution, which upon cooling could turn back into gel with the aid of ultrasonification. Gel-to-solution and the reverse solution-to-gel transition could also be controlled through UV or visible light irradiation. Therefore the azobenzene substituted tripeptide azo-Lys-D-Phe-D-Ala could form hydrogel with multiple responses to light, heat and ligand–receptor interaction (Fig. 5). This indicates that it is possible to construct smart supramolecular hydrogels with multiple responses not only to light but also to other external stimulus through appropriate combination of an azobenzene moiety with specific peptide sequences with recognition sites.

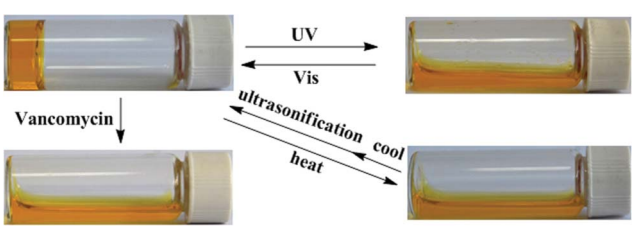
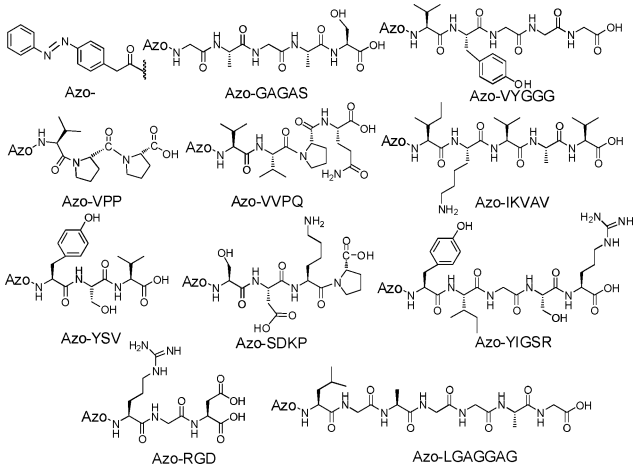


Fig. 5 Multiple responses of the hydrogel formed by azo-Lys-D-Phe-D-Ala to light, heat and addition of vancomycin.

Azobenzene-linked bioactive short peptides

To further extend potential applications of photo-responsive peptidic hydrogels based on azobenzene substituted short peptides, preliminary studies on the hydrogelation properties of various bioactive short peptides linked to an azobenzene moiety at the N-terminal have also been carried out. The structures of the bioactive short peptides linked with azobenzene is shown in Scheme 2. The bioactive tripeptide sequences we have tested include RGD, VPP and YSV. RGD is well-known as ligand to integrin $\alpha_v\beta_3$.[19] VPP is a casein-derived tripeptide which can modulate monocyte adhesion to the vascular endothelium.[20] The tyroservaltide tripeptide YSV has been proven to have inhibitory effects on human hepatocarcinoma.[21] Hydrogelation ability of azo-RGD, azo-VPP and azo-YSV was tested. The results showed that azo-RGD and azo-VPP could not form gels while azo-YSV could gel water at acidic pH. The presence of Pro or Arg residue in the peptides might have prevented the azo-tripeptides from self-assembling.



Scheme 2 Chemical structure of azobenzene-linked bioactive short peptides.

The two azo-tetrapeptides azo-VVPQ and azo-SDKP with Pro residue also could not form hydrogel. Five pentapeptide sequences were linked with azobenzene and three of these five azo-pentapeptides showed gelation ability. Among the pentapeptides which have been proved to be hydrogelators when attached to Fmoc or Pyrene,[22] GAGAS and VYGGG with N-terminal linked to azobenzene could form hydrogel under acidic conditions. The analogues of laminin active fragment YIGSR,[23] when linked with azobenzene, showed very poor solubility and could not form hydrogel, while the other laminin active fragment IKVAV[24] derived azo-IKVAV could form gel at the concentration around 4.9 mg ml^{-1} at pH around 4.5. Azobenzene substituted heptapeptide azo-LGAGGAG was also found to be a hydrogelator under acidic conditions. Conditions for the azobenzene substituted bio-active short peptides to form hydrogel are listed in Table 3.

Application of the photo-responsive hydrogel to controlled release

As a preliminary study on the bio-applications of the photo-responsive supramolecular hydrogel, we demonstrate herein the controlled release of vitamin B12 from the gel matrix realized by

Table 3 Hydrogelation properties of azobenzene-linked bioactive short peptides

azo-peptide	pH	C (mg mL^{-1})
azo-GAGAS	3.9	4.9
azo-VYGGG	2.8	4.4
azo-VPP	—	—
azo-VVPQ	—	—
azo-IKVAV	4.5	4.9
azo-YSV	1.8	5.6
azo-SDKP	—	—
azo-YIGSR	—	—
azo-RGD	—	—
azo-LGAGGAG	1.6	6.7

photo-irradiation. As shown in Fig. 6A, vitamin B12 could be trapped in the hydrogel without disturbing the gelation ability of azo-Gln-Phe-Ala. Without photo-irradiation, the release of vitamin B12 could only be realized by concentration-gradient motivated diffusion from the gel to the upper layer water and the complete release process took more than two days. Photo-irradiation on the mixture greatly accelerated the release process.

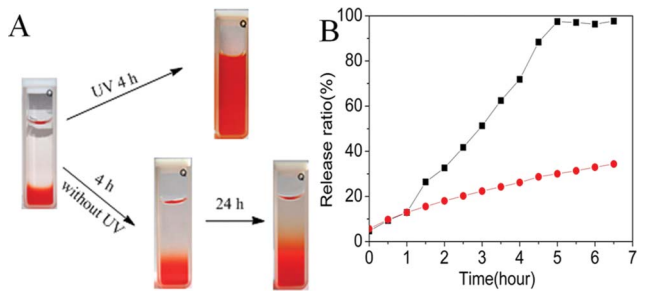


Fig. 6 A) Optical images of the controlled release of vitamin B12 through UV irradiation (upper image) compared with spontaneous diffusion (lower images); B) Quantitative comparison of the release ratio of vitamin B12 with (black square) and without (red dot) UV irradiation.

The release ratio of vitamin B12 from the gel matrix to the upper layer water could be quantified by measuring the UV absorption of the solution at a wavelength corresponding to the absorption of vitamin B12. UV absorption of the two parallel samples which had the same behavior within the first 1 h without photo-irradiation was measured in due time courses. Once the sample was exposed to UV irradiation, a steep increase on the release ratio as shown in Fig. 6B (black square) was observed. After 4 h irradiation, a photo-controlled release ratio of 97% was achieved compared with the diffusion-controlled release around 30%.

Conclusions

We have synthesized several series of azobenzene substituted short peptides and studied their motif and pH dependant hydrogelation. Aromatic amino acids including Phe and Tyr have been found to promote hydrogelation of azo-dipeptides while cationic amino acids such as Arg were unfavorable for the corresponding azo-short peptides to form hydrogels. Azo-dipeptides containing Tyr could form hydrogels within a wide range of pH and the lowest concentration for hydrogelation increases with increase in pH. Salt effect has also been observed in hydrogels formed by azo-Ala-Tyr and azo-Tyr-Tyr. Photo-response of the hydrogels formed by azo-short peptides was realized through photo-induced *E*- to *Z*-isomerization of the azobenzene moiety followed by the disassembling of the previously balanced system induced by the conformational change. Hydrogels with different strength intermolecular interactions in the gel matrix showed different sensitivity to light irradiation. The hydrogel based on azo-Lys-D-Phe-D-Ala showed multiple responses to heat, light and ligand–receptor interaction between D-Phe-D-Ala and vancomycin. Moreover, azobenzene substituted bio-active short peptides also showed hydrogelation ability. The azo-Gln-Phe-Ala gel has been demonstrated as promising photo-responsive soft material for controlled release of drug molecules. Comprehensive study on the gelation ability and photo-response of azobenzene substituted short peptides may lead to more photo-responsive supramolecular systems with diverse applications.

Experimental

Synthesis of the azobenzene substituted peptides

(4-Phenylazophenyl) acetic acid was synthesized from nitrobenzene and (4-aminophenyl)acetic acid ethyl ester according to the reported method.[25] The azobenzene substituted short peptides were then synthesized by solid phase peptide synthesis from 2-chlorotrityl chloride resin and corresponding Fmoc-protected amino acids. After (4-phenylazophenyl) acetic acid was coupled to the N-terminal of the peptides on resin, the resulted azobenzene substituted peptides were cleaved form the resin using TFA. After removal of TFA by rotary evaporation, the crude oily product was further dispersed in diethyl ether followed by centrifugation to be transformed into an amorphous powder. Purity of the substituted peptide products was proved to be higher than 95% by HPLC. Most of the azo-peptides have been characterized by ^{1}HNMR or MS (ESI†).

Preparation of Hydrogels

A homogeneous solution of the azobenzene substituted short peptide was prepared in a glass vial *via* pH adjustment and heating. Upon cooling to room temperature, hydrogel formed instantaneously for azo-Gln-Phe-Ala, azo-Lys-Phe-Ala, azo-Leu-Phe-Ala, azo-D-Lys-D-Phe-D-Ala and azo-Arg-Phe-Ala. For azo-Lys-D-Phe-D-Ala and azo-D-Lys-Phe-Ala, it was necessary to use ultrasound sonification to facilitate the hydrogelation. Conversion of the azo-Gly-Phe-Ala solution to a stable gel needs not only ultrasound sonification but also more than 40 h standing at room temperature. The hydrogels of azo-Ser-Phe-Ala, azo-Gln-Tyr-Ala, azo-Glu-Tyr-Ala and azo-Glu-Phe-Ala could be prepared by acidifying the basic solution to the pH shown in Table 2.

Analysis of pH and salt effects on the gelation properties

pH effect. Azo-dipeptides were mixed with a small amount of water and the mixtures were adjusted to the desired pH with 1 M HCl or NaOH, then heated to about 80–90 °C to get a clear solution. For each, a yellow hydrogel was formed upon cooling to room temperature. The lowest concentration for hydrogelation at a specific pH was obtained by successive dilution of the hydrogel using aqueous solution with the same pH for hydrogelation to the point when the hydrogel could not form any more.

Salt effect. Buffer solutions at different pH (5 and 6) and different concentrations (0.05 M, 0.1 M, 0.2 M and 0.5 M) were prepared by mixing Na_2HPO_4 and NaH_2PO_4. Azo-dipeptides were mixed with a small quality of buffer and heated to about 80–90 °C to get a clear solution. Yellow hydrogels formed upon cooling to room temperature. The lowest concentration for hydrogelation at specific pH was obtained by successive dilution of the hydrogel using buffer solution with the same concentration of salt to the point when the hydrogel could not form any more.

Photo-response tests on the hydrogels

The light source for UV irradiation was a high pressure mercury lamp (500 W) with a filter (300–400 nm, cut visible light off). A control experiment was performed in parallel using the same hydrogel in a tin foil covered glass vial to exclude the possibility of response induced by any external stimulus other than light irradiation. Photo-response of the hydrogels was confirmed only when obvious gel-to-solution phase change was observed in the sample under irradiation but not in the control sample.

Rheology test on the hydrogel before and after UV irradiation

All rheological measurements were performed using an ARES-G2 Rheometer with a cone and a plate (25 mm diameter plate and 0.0999 rad cone angle); the gap opening between the cone and the plate was set to be 0.0282 mm. The dynamic strain sweep test was carried out at 6.282 rad s^{-1}, and the dynamic frequency sweep test was investigated at critical strain, which was determined from the storage-strain profile.

Acknowledgements

We would like to acknowledge financial support from the National Science Foundation of China (20702024, 20972067), National Basic Research Program of China (No. 2011CB935800) and Fok Ying Tung Education Foundation (114012).

Notes and references

1 R. V. Ulijn and A. M. Smith, *Chem. Soc. Rev.*, 2008, **37**, 664–675; L. Ruan, H. Zhang, H. Luo, J. Liu, F. Tang, Y.-K. Shi and X. Zhao, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 5105–5110; A. M. Smith, R. J. Williams, C. Tang, P. Coppo, R. F. Collins, M. L. Turner, A. Saiani and R. V. Ulijn, *Adv. Mater.*, 2008, **20**, 37–41.

2 P. Dastidar, *Chem. Soc. Rev.*, 2008, **37**, 2699–2715; A. R. Hirst, B. Escuder, J. F. Miravet and D. K. Smith, *Angew. Chem., Int. Ed.*, 2008, **47**, 8002–8018; Z. Yang, G. Liang and B. Xu, *Acc. Chem. Res.*, 2008, **41**, 315–326; Y. Yang, T. Chen, J.-F. Xiang, H.-J. Yan, C.-F. Chen and L.-J. Wan, *Chem.–Eur. J.*, 2008, **14**, 5742–5746; J.-W. Liu, Y. Yang, C.-F. Chen and J.-T. Ma, *Langmuir*, 2010, **26**, 9040–9044.

3 F. Peng, G. Li, X. Liu, S. Wu and Z. Tong, *J. Am. Chem. Soc.*, 2008, **130**, 16166–16167; T. Z. Grove, C. O. Osuji, J. D. Forster, E. R. Dufresne and L. Regan, *J. Am. Chem. Soc.*, 2010, **132**, 14024–14026; S. W. Liao, T.-B. Yu and Z. Guan, *J. Am. Chem. Soc.*, 2009, **131**, 17638–17646.

4 S.-L. Zhou, S. Matsumoto, H.-D. Tian, H. Yamane, A. Ojida, S. Kiyonaka and I. Hamachi, *Chem.–Eur. J.*, 2005, **11**, 1130–1136; Y. Zhang, H. Gu, Z. Yang and B. Xu, *J. Am. Chem. Soc.*, 2003, **125**, 13680–13681; Y. Zhang, Z. Yang, F. Yuan, H. Gu, P. Gao and B. Xu, *J. Am. Chem. Soc.*, 2004, **126**, 15028–15029; J. Gao, H. Wang, L. Wang, J. Wang, D. Kong and Z. Yang, *J. Am. Chem. Soc.*, 2009, **131**, 11286–11287; Z. Yang, P.-L. Ho, G. Liang, K. H. Chow, Q. Wang, Y. Cao, Z. Guo and B. Xu, *J. Am. Chem. Soc.*, 2007, **129**, 266–267; A. K. Das, R. Collins and R. V. Ulijn, *Small*, 2008, **4**, 279–287; T. O. McDonald, H. Qu, B. R. Saunders and R. V. Ulijn, *Soft Matter*, 2009, **5**, 1728–1734.

5 M. Ikeda, S. Ueno, S. Matsumoto, Y. Shimizu, H. Komatsu, K. Kusumoto and I. Hamachi, *Chem.–Eur. J.*, 2008, **14**, 10808–10815; S. Wang, D. Nagrath, P. C. Chen, F. Berthiaume and M. L. Yarmush, *Tissue Eng. A*, 2008, **14**, 227–236; F. Zhao, M. L. Ma and B. Xu, *Chem. Soc. Rev.*, 2009, **38**, 883–891; Y. Hu, H. Wang, J. Wang, S. Wang, W. Liao, Y. Yang, Y. Zhang, D. Kong and Z. Yang, *Org. Biomol. Chem.*, 2010, **8**, 3267–3271.

6 A. M. Kloxin, A. M. Kasko, C. N. Salinas and K. S. Anseth, *Science*, 2009, **324**, 59–63; S. Yagai, T. Karatsu and A. Kitamura, *Chem.–Eur. J.*, 2005, **11**, 4054–4063; C. Gong, K.-L. Wong and M. H. W. Lam, *Chem. Mater.*, 2008, **20**, 1353–1358; M. A. Kostiainen, D. K. Smith and O. Ikkala, *Angew. Chem., Int. Ed.*, 2007, **46**, 7600–7604.

7 S. Matsumoto, S. Yamaguchi, A. Wada, T. Matsui, M. Ikeda and I. Hamachi, *Chem. Commun.*, 2008, 1545–1547; H. Komatsu, S. Matsumoto, S. Tamaru, K. Kaneko, M. Ikeda and I. Hamachi, *J. Am. Chem. Soc.*, 2009, **131**, 5580–5585; S. Matsumoto, S. Yamaguchi, S. Ueno, H. Komatsu, M. Ikeda, K. Ishizuka, Y. Iko, K. V. Tabata, H. Aoki, S. Ito, H. Noji and I. Hamachi, *Chem.–Eur. J.*, 2008, **14**, 3977–3986; Y. Sako and Y. Takaguchi, *Org. Biomol. Chem.*, 2008, **6**, 3843–3847.

8 L. A. Haines, K. Rajagopal, B. Ozbas, D. A. Salick, D. J. Pochan and J. P. Schneider, *J. Am. Chem. Soc.*, 2005, **127**, 17025–17029; T. Muraoka, C.-Y. Koh, H. Cui and S. I. Stupp, *Angew. Chem., Int. Ed.*, 2009, **48**, 5946–5949.

9 Z. Qiu, H. Yu, J. Li, Y. Wang and Y. Zhang, *Chem. Commun.*, 2009, 3342–3344; X. Li, Y. Gao, Y. Kuang and B. Xu, *Chem. Commun.*, 2010, **46**, 5364–5366.

10 Y. Inoue, P. Kuad, Y. Okumura, Y. Takashima, H. Yamaguchi and A. Harada, *J. Am. Chem. Soc.*, 2007, **129**, 6396–6397; Y. Ji, G.-C. Kuang, X.-R. Jia, E.-Q. Chen, B.-B. Wang, W.-S. Li, Y. Wei and J. Lei, *Chem. Commun.*, 2007, 4233–4235; T. Sakai, H. Murayama, S. Nagano, Y. Takeoka, M. Kidowaki, K. Ito and T. Seki, *Adv. Mater.*, 2007, **19**, 2023–2025; S. Kume, K. Kuroiwa and N. Kimizuka, *Chem. Commun.*, 2006, 2442–2444.

11 C. Wang, Q. Chen, F. Sun, D. Zhang, G. Zhang, Y. Huang, R. Zhao and D. Zhu, *J. Am. Chem. Soc.*, 2010, **132**, 3092–3096; K. Murata, M. Aoki, T. Suzuki, T. Harada, H. Kawabata, T. Komori, F. Ohseto, K. Ueda and S. Shinkai, *J. Am. Chem. Soc.*, 1994, **116**, 6664–6676; M. George and R. G. Weiss, *Acc. Chem. Res.*, 2006, **39**, 489–497; K. Murata, M. Aoki, T. Nishi, A. Ikeda and S. Shinkai, *J. Chem. Soc., Chem. Commun.*, 1991, 1715–1718; T. Ishi-i and S. Shinkai, *Top. Curr. Chem.*, 2005, **258**, 119–160.

12 Y.-L. Zhao and J. F. Stoddart, *Langmuir*, 2009, **25**, 8442–8446; S. Tamesue, Y. Takashima, H. Yamaguchi, S. Shinkai and A. Harada, *Angew. Chem., Int. Ed.*, 2010, **49**, 7461–7464.

13 H. Kobayashi, A. Friggeri, K. Koumoto, M. Amaike, S. Shinkai and D. N. Reinhoudt, *Org. Lett.*, 2002, **4**, 1423–1426.

14 Y. Matsuzawa and N. Tamaoki, *J. Phys. Chem. B*, 2010, **114**, 1586–1590.

15 M. Susudo, Y. Ishikawa, K. Itoh and S. Tazuke, *Macromolecules*, 1991, **24**, 3993–3998.

16 Y.-T. Chan, C. N. Moorefield and G. R. Newkome, *Chem. Commun.*, 2009, 6928–6930.

17 K. Uchida, S. Yamaguchi, H. Yamada, M. Akazawa, T. Katayama, Y. Ishibashi and H. Miyasaka, *Chem. Commun.*, 2009, 4420–4422.

18 S. Boonyarattanakalin, J. Hu, S. A. Dykstra-Rummel, A. August and B. R. Peterson, *J. Am. Chem. Soc.*, 2007, **129**, 268–269.

19 J.-P. Xiong, T. Stehle, R. Zhang, A. Joachimiak, M. Frech, S. L. Goodman and M. A. Arnaout, *Science*, 2002, **296**, 151–155.

20 K. Aihara, H. Ishii and M. Yoshida, *J. Atheroscler. Thromb.*, 2009, **16**, 594–603.

21 J. Jia, R. Lu, S. Qiu, H. Li, X. Che, P. Zhao, M. Jin, H. Yang, G. Lin and Z. Yao, *Cancer Biol. Ther.*, 2005, **4**, 993–997.

22 M. Ma, Y. Kuang, Y. Gao, Y. Zhang, P. Gao and B. Xu, *J. Am. Chem. Soc.*, 2010, **132**, 2719–2728.

23 E. Witkowska, A. Oriowska, J. Izdebski, J. Salwa, J. Wietrzyk and A. Opolski, *J. Pept. Sci.*, 2004, **10**, 285–290.

24 R. S. Bresalier, B. Schwartz, Y. S. Kim, Q.-Y. Duh, H. K. Kleinman and P. M. Sullam, *Cancer Res.*, 1995, **55**, 2476–2480.

25 G. H. Coleman, C. M. McCloskey and F. A. Stuart, *Org. Synth.*, 1955, **Coll. 3**, 668; N. Iyi, K. Kurashima and T. Fujita, *Chem. Mater.*, 2002, **14**, 583–589.