ChemComm

COMMUNICATION

View Article Online
View Journal | View Issue



Cite this: *Chem. Commun.*, 2017, 53, 12450

Received 11th October 2017,
Accepted 26th October 2017

DOI: 10.1039/c7cc07859e

rsc.li/chemcomm

# Hierarchical supramolecular hydrogels: self-assembly by peptides and photo-controlled release *via* host–guest interaction†

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**A hierarchical supramolecular hydrogel was self-assembled from a Fmoc-RGDS tetrapeptide and showed photo-controlled release directed by host–guest interaction. Multiple payloads, including vesicles, were successively released from a single peptide hydrogel.**

Hydrogels are versatile biomedical materials owing to their porous structure with high-water content, which have inherent biocompatibility and similarity to human tissue.[1–3] Supramolecular hydrogels formed either by macromolecules[4–7] or small molecules[8–11] have gained increasing interest in recent years. Several research groups have focused on polymeric supramolecular hydrogels based on host–guest interaction and cyclodextrins (CDs) are especially useful as excellent host molecules in aqueous media.[4,5,12–16] Compared to traditional CD-decorated polymers, cyclodextrin vesicles (CDVs) offer another possibility to act as multivalent non-covalent cross-linkers.[17] We have reported a polymeric supramolecular hydrogel containing CDVs as 3D junctions. Self-thinning and self-healing properties of the gel were observed and as a result, an injectable hydrogel was obtained.[18] Furthermore, the non-covalent interaction enables the response of these soft materials to various stimuli, for example, light,[12–14] redox,[15,16] metal-ion[19] and temperature,[20] based on responsive guest molecules.

Self-assembly of low molecular weight gelators (LMWGs), on the other hand, has also attracted considerable attention in the past decade.[21] Various LMWGs, such as peptides,[22–25] carbohydrates[26] and synthetic organic compounds,[11] have been reported. Aromatic peptides are especially attractive due to their facile synthesis and strong π–π interactions in aqueous solution.[23,25] Different sequences of amino acids, used as hydrophilic head group, were optimized for various applications.[21,27,28] The Fmoc-protecting group is frequently applied as hydrophobic part since it is widely used in solid-phase peptide synthesis (SPPS).[21,22,29] Similar to polymeric hydrogels, the non-covalent interaction of peptide hydrogels also permits the development of multi-stimuli hydrogels. Several stimuli, including chemical stimulus,[30,31] enzyme[32,33] and light,[34] were reported. Among them, light as trigger is of particular interest since it allows to target a specific area of the gel with high resolution in space and time and also, light is a non-invasive stimulus. Photo-switchable moieties are notably fascinating because they support the establishment of light-responsive peptide hydrogels. LMWGs comprising azobenzenes[35–37] and stilbenes[26,38,39] are two important variations of this approach. Azobenzene is unique since it can be readily photo-isomerized as well as form photo-responsive inclusion complex with β-CD *via* host–guest interaction in water.[40] It was also reported that azobenzene containing dipeptides show a photo-induced change of self-assembly in presence of α-CD.[41] However, to the best of our knowledge, a light-responsive LMWG based on host–guest interaction is so far unprecedented.

Herein, we introduce a novel hierarchical supramolecular hydrogel by combination of self-assembly of an amphiphilic peptide and host–guest interaction as additional cross-linking motif to stabilize the gel. Recently, a new type of water soluble photoswitch, arylazopyrazole (AAP), has been developed in our group.[42] Its nearly quantitative photo-isomerization combined with the selective affinity of *trans*-AAP to β-cyclodextrin facilitate better light reversible host–guest systems compared to typical azobenzene.[43–45] By introduction of AAP as light-responsive guest and CDV as cross-linkers, photo-controlled release of multiple payloads from a single peptide hydrogel is achieved.

Fmoc-RGDS was chosen as building block for the peptide backbone due to its good water solubility and high biocompatibility. It has been reported that Fmoc-RGDS can form hydrogel and serve as cell adhesion motif.[27] The π–π interaction between hydrophobic Fmoc groups induces the self-assembly of the amphiphilic peptide in water. As the supramolecular polymer grows beyond a certain length, the entanglement between the self-assembled fibers reinforces the gel formation (Fig. 1a). Synthesis of Fmoc-RGDS was carried out by SPPS. To introduce light responsive unit into

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† Electronic supplementary information (ESI) available: Experimental procedures and supporting data. See DOI: 10.1039/c7cc07859e

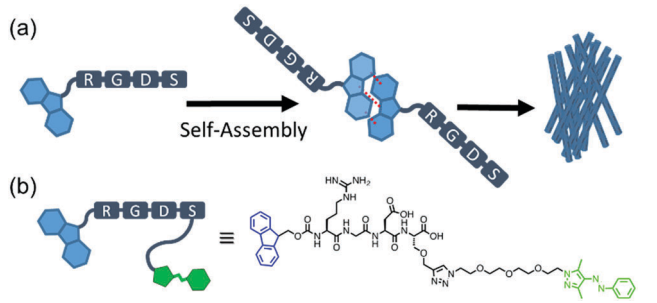


Fig. 1 (a) Schematic representation of the self-assembly of Fmoc-RGDS in water and (b) structure of AAP-functionalized peptide Fmoc-RGDS-AAP obtained *via* CuAAC and SPPS.

peptide backbone, Fmoc-L-Ser-TEG-AAP was synthesized *via* copper-catalyzed azide–alkyne cycloaddition (CuAAC) and used as first amino acid in SPPS. Fmoc-RGDS-AAP was obtained after stepwise elongation. Details of the synthesis and analysis of the peptide are provided as ESI.†

To investigate the photophysical properties of Fmoc-RGDS-AAP, UV/vis spectroscopy was used. AAP is a light-responsive motif and undergoes isomerization upon UV (365 nm) and visible (520 nm) irradiation. Indeed, Fmoc-RGDS-AAP displayed a similar spectrum as our first report on AAP,[42] *i.e.* a $\pi \rightarrow \pi^*$ band at 334 nm and an $n \rightarrow \pi^*$ band around 430 nm. Under UV irradiation (365 nm), the $\pi \rightarrow \pi^*$ absorbance is diminished whereas the $n \rightarrow \pi^*$ band is increased. This observation reveals that the *trans*- to *cis*-isomerization is complete after 15 min irradiation. After irradiation with visible light (520 nm) for 15 min, the original spectrum is obtained, indicating near complete *cis*- to *trans*-isomerization. The slightly higher absorbance at 334 nm compared to the original spectrum may be due to the presence of *cis*-isomer as trace amount in the peptide as prepared. Upon UV and vis irradiation, the absorbance of the Fmoc group between 250–300 nm remains almost constant (Fig. 2a). The photo-induced switching of Fmoc-RGDS-AAP is fully reversible for at least three cycles (Fig. 2b).

Another appealing feature of AAP is that it provides not only superior photoswitching behavior but moreover *trans*-AAP has strong affinity to β-CD, which broadens the application in cyclodextrin-based supramolecular systems. Host–guest interaction between Fmoc-RGDS-AAP and β-CD was investigated by ITC (Fig. S1, ESI†). A 1 : 1 stoichiometry and a binding constant $K_a = 16.9 \times 10^2\ M^{-1}$ was determined, which is comparable to our previous report.[42]

The secondary structure of Fmoc-RGDS was studied by circular dichroism. In accordance with the literature, the self-assembled Fmoc-RGDS showed a disordered structure and a low signal at around 200 nm in the circular dichroism spectrum.[27] To further understand the thermal stability of the self-assembled peptide structure, spectra at different temperature were recorded. No change was observed in the spectra, suggesting that the self-assembled peptide is stable up to 45 °C (Fig. S2a, ESI†). Moreover, insertion of Fmoc-RGDS-AAP into Fmoc-RGDS has little influence on the self-assembled structure. By comparison of circular dichroism spectra for 0.01% Fmoc-RGDS and 0.01% Fmoc-RGDS containing 0.001% Fmoc-RGDS-AAP, a very similar shape and intensity of the curves was observed (Fig. S2b, ESI†). These findings show that insertion of Fmoc-RGDS-AAP did not affect the peptide fibril assembly. However, the slightly diminished peak at 200 nm suggested that there might be a secondary π–π interaction between neighboring AAP groups, which is further explained by microscopic images (see below).

Scanning electronic microscopy (SEM) was exploited to investigate the surface morphology of the peptide self-assembly. In this experiment, samples of 2.5% Fmoc-RGDS and 2.5% Fmoc-RGDS with 0.25% Fmoc-RGDS-AAP were prepared (see ESI† for sample preparation methods) and multiple areas per sample were scanned. Fibril structures were found in both cases and entanglement of fibers was also observed, which is consistent with reported literature.[27] In presence of Fmoc-RGDS-AAP, SEM showed more cross-linking fibers (Fig. 3) compared to only Fmoc-RGDS (Fig. S3, ESI†). A reasonable explanation for this observation might be the formation of additional π–π interactions between AAPs protruding from the peptide fibrils.

Next, a light responsive hierarchical supramolecular hydrogel was developed by combination of Fmoc-RGDS, Fmoc-RGDS-AAP and CDV. The molecular structure, size and preparation of CDV are provided in the ESI† (Fig. S6). The average size of CDV obtained by DLS is *ca.* 140 nm. Hydrogels were prepared by dissolving several milligrams of peptides in $ddH_2O$, followed by gently heating at 45 °C for 5 min. After gelation overnight, all gels passed the "reverse vial" test. Various compositions were tested and versatile light-responsive hydrogels were achieved in presence of 2.5% Fmoc-RGDS (38 mM), 0.25% Fmoc-RGDS-AAP (2.3 mM) and 50 μM CDV (Fig. 4). The hydrogels were formed not only by entanglement of self-assembled supramolecular

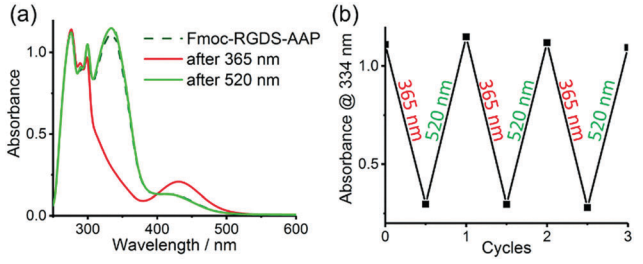


Fig. 2 (a) UV/vis spectra of *trans*- and *cis*-isomers of Fmoc-RGDS-AAP and (b) three cycles of fully reversible photo-isomerization of Fmoc-RGDS-AAP.

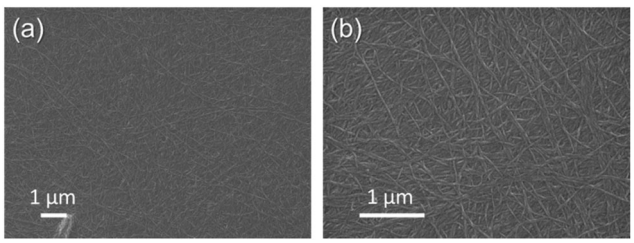


Fig. 3 SEM images of 2.5 wt% Fmoc-RGDS in presence of 0.25 wt% Fmoc-RGDS-AAP at (a) 10k× and (b) 25k× magnification.

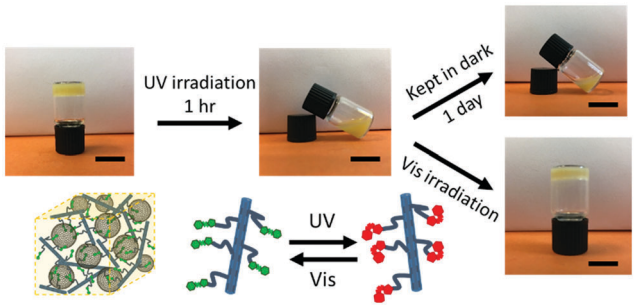


Fig. 4 Reversible photoswitching of a supramolecular hydrogel composed of 2.5 wt% Fmoc-RGDS, 0.25 wt% Fmoc-RGDS-AAP and 50 μM of CDV (scale bar: 1 cm).

polymers but additionally by host–guest interaction between AAP and CDV. After irradiation with UV-light, the self-supporting hydrogel was weakened due to loss of host–guest interaction, resulting from isomerization of *trans*-AAP to *cis*-AAP. Since *cis*-AAP does not bind to β-CD, the non-covalent interaction between supramolecular fiber and CDV is strongly reduced. As a result, a very soft, non-self-supporting gel was obtained. Reversible stiffening of the hydrogel was demonstrated by storing the very soft gel either in dark or under visible light. After one day, the sample kept in dark remained as very soft gel while the sample that was exposed to light recovered as a self-supporting hydrogel. The stiffening of the hydrogel is due to reconstruction of host–guest interaction between *trans*-AAP and CDV. Since the half-life time of isomerization from *cis*- to *trans*-AAP is approximately four days,[42] the sample kept in dark cannot recover as a hydrogel within one day. This observation supports our claim that the light responsive hydrogel is stabilized *via* host–guest interaction.

Rheological measurements revealed the visco-elastic properties of the supramolecular hydrogels. Two different concentrations of Fmoc-RGDS were compared in oscillatory rheological measurements. In Fig. S4a (ESI†), 5% Fmoc-RGDS showed approximately two orders of magnitude higher storage modulus $G'$ than 2.5% Fmoc-RGDS. These findings suggested that stiffer gels can be obtained with a growing number of entanglements of the peptide fibers. Similar observations were made if the concentration of CDV was increased (Fig. 5a). Hydrogels containing 2.5 wt% Fmoc-RGDS, 0.25 wt% Fmoc-RGDS-AAP and increasing concentration of CDV were compared. The highest storage ($G'$) and loss modulus ($G''$) was found in hydrogels containing of 200 μM CDV, whereas the lowest was observed for hydrogels containing only 25 μM CDV. This observation shows that a higher concentration of CDV leads to a higher degree of cross-linking so that a more rigid supramolecular material is obtained. Since the hydrogel is based on supramolecular polymers, the response to shear is interesting to examine (Fig. S4b, ESI†). Initially, a lower shear rate ($\dot{\gamma} = 0.5\ s^{-1}$) was applied and constant viscosity for all hydrogels was observed. Then, a high shear rate ($\dot{\gamma} = 500\ s^{-1}$) resulted a decline in viscosity, indicating disruption of the supramolecular structure under shear. Finally, a rapid recovery of viscosity was received after lowering shear rate back to 0.5 $s^{-1}$. Thus, all hydrogels showed shear-thinning behaviour (thixotropy). Furthermore, the change of viscoelastic properties under irradiation were studied. A frequency sweep measurement of samples comprising 2.5 wt% Fmoc-RGDS, 0.25 wt% Fmoc-RGDS-AAP and 50 μM CDV was recorded. In this case, rheology of the hydrogel showed a large decrease of both storage ($G'$) and loss modulus ($G''$) after UV irradiation. Upon visible light irradiation, although the plateau modulus ($G'$) did not recover quite as high as before, over one order increase was achieved (Fig. 5b). These rheology measurements clearly show that the hierarchical peptide gel is stabilized by host–guest interaction of cyclodextrin in the CDV and AAP on the peptide and that alternating photo-isomerization of *trans*-AAP to *cis*-AAP induces a reversible softening and stiffening of the gel.

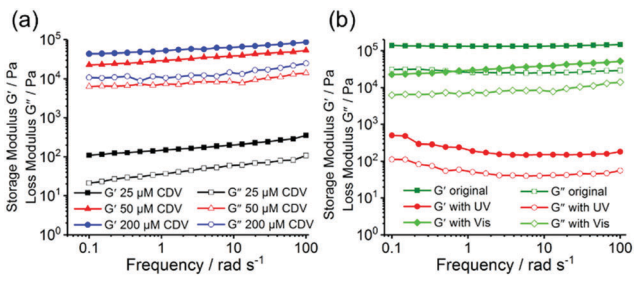


Fig. 5 (a) Frequency sweep of oscillatory rheological measurements performed at 0.5% strain for increasing concentration of CDV. (b) Photoswitching experiment performed in presence of 2.5 wt% Fmoc-RGDS, 0.25 wt% Fmoc-RGDS-AAP and 50 μM CDV.

To investigate this supramolecular hydrogel as photo-controlled release system, two fluorescent dyes with different molecular weight (FITC-isomer I, $M_W$ = 389 g $mol^{-1}$ and FITC-Dex4000, $M_W$ = 4000 g $mol^{-1}$) were encapsulated inside the hydrogel. 200 μL of hydrogel samples were prepared one day before (see ESI† for preparation details) at the bottom of cuvettes. And additional 800 μL of $ddH_2O$ was added carefully, minimizing the disruption of hydrogel interface. Release of fluorescent dye was monitored by fluorescence spectroscopy with and without UV irradiation (Fig. 6). The percentage of release was calculated by dividing the fluorescence intensity of fully dissolved hydrogels obtained by heating and gently stirring. Significant releases of both dyes were detected under UV irradiation for 4 h. FITC-Dex4000 showed a slower and less extensive release, which means that it was trapped longer and tighter inside the gels due to its larger size than FITC-Isomer I. (Fig. 6a, red and blue lines). Control experiments were also performed by monitoring diffusion of fluorescent from hydrogel to aqueous solution in the dark. No significant release was observed within 4 h, but depending on the molecular weight of dyes, 20% and 55% of release were observed after 20 h (Fig. S5, ESI†).

Another appealing feature of this responsive soft material is the photo-controlled release of CDV from the supramolecular peptide hydrogel. In order to examine CDV release from hydrogels, hydrophobic NBD-cholesterol was embedded in the membrane of CDV. Otherwise, the same experimental setup was employed as described above (Fig. 6b). Compared to the release of FITC-I and FITC-Dex4000, the release of CDV is considerably slower. A significant release of CDV is only observed after 2 h of irradiation (Fig. 6a, black line). This result is consistent with our expectation that the rate of release from

 

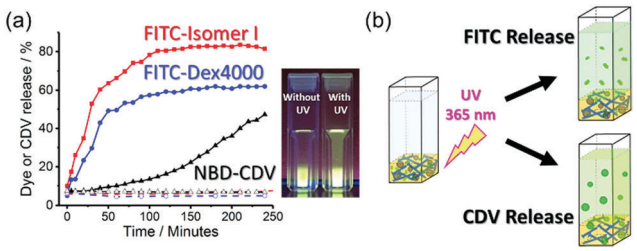


Fig. 6 (a) Overlay of the release of FITC-I, FITC-Dex4000 and CDV under UV irradiation (red: FITC-Isomer I; blue: FITC-Dex4000; black: NBD-cholesterol CDV) and the corresponding control experiments (no irradiation, hollow symbols). (b) Schematic presentation of fluorescent dyes and CDV release under UV-irradiation.

the hydrogel is size dependent. Moreover, CDV is not only physically entrapped in the hydrogel but in addition non-covalently bound as cross-linker between the peptide fibrils. These data clearly show that the supramolecular peptide hydrogel can serve as a reservoir for stepwise photo-release of as many as three different payloads depending on their molecular weight and their interaction with the peptide matrix.

In conclusion, we demonstrated the first example of photo-responsive hydrogels by combination of self-assembly of amphiphilic peptides and introduction of host–guest interaction in peptide hydrogels. Responsive peptide hydrogels were obtained in optimal composition of Fmoc-RGDS, Fmoc-RGDS-AAP and CDV. These hydrogels were further developed as light-triggered release platform not only for small and large molecules but also for vesicles as payload. These findings suggest that this supramolecular hydrogel has potential for the development of photo-controlled drug or protein release materials.

We are grateful for the financial support from EC H2020 – Marie Skłodowska-Curie Actions – Innovative Training Network, Multi-App (project number: 642793). We thank PD Dr Cramer-Kellers and Mrs Pröbsting for advice on rheology. Markus Börner is acknowledged for SEM measurements and Luca Cochet and Benedikt Nowak are acknowledged for peptide synthesis.

## Conflicts of interest

There are no conflicts to declare.

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