Nanostructures

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# Oligomeric Hydrogels Self-Assembled from Reduction-Controlled Condensation**

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***Abstract:*** *Polymer hydrogels and small-molecule-based (SMB) supramolecular hydrogels have been widely explored. But oligomeric hydrogels have remained a challenge because synthetic difficulties of the oligomers and control of their amphiphilicities. Reported herein is the rational design of two precursors Cys(SEt)-Lys-CBT (**1**) and (Cys-Lys-CBT)$_2$ (**2**) (CBT = 2-cyano-6-aminobenzothiazole) and the use of a biocompatible condensation to prepare oligomeric hydrogels. Glutathione reduction of **1** or **2** yields the same gelator Cys-Lys-CBT (**3**) which condenses with each other to yield amphiphilic cyclic oligomers. The oligomers instantly self-assemble into nanofibers and form oligomeric hydrogels with similar mechanic properties. Chemical analyses indicated that the major condensation product in both two hydrogels is a cyclic dimer. Considering its biocompatibility, optimal mechanical strength, and biodegradability, we believe that our oligomeric hydrogel might be useful for long-term drug delivery in the future.*

Hydrogels have received significant attention because of their broad biomedical applications such as enzyme immobilization and screening, drug delivery, and tissue engineering.[1] Currently hydrogels can be roughly divided into two types: polymer hydrogels and small-molecule-based (SMB) supramolecular hydrogels. Polymer hydrogels, composed of polymers with covalent or noncovalent bonds, have shown prevailing advantages over SMB hydrogels in materials fabrication, electricity storage, and long-term drug delivery given their excellent mechanic properties.[2] But the main issues existing for polymer hydrogels are their biocompatibility and biodegradability, which are respectively introduced by the toxic reactants used for their syntheses and the polymeric covalent bonds formed.[3] SMB hydrogels, formed by small-molecule self-assembly through weak interactions (e.g., hydrogen bond, π–π interactions, electrostatic interaction, etc.) are much more biocompatible and degradable than polymer hydrogels.[4] But their weak mechanical strength limits them as scaffolds for tissue engineering or long-term drug delivery.[1c,5] Oligomers, referred to as one type of molecular complex, each of which is composed of a few monomer units, are usually basic composition units of a macromolecular or supramolecular complex formed by noncovalent interactions between the oligomers. To date, many examples have shown the importance of oligomers in biochemistry and their wide applications in material chemistry.[6] Theoretically, oligomer hydrogels should have stronger mechanical strength than SMB hydrogels while at the same time they should be more compatible and degradable than polymer hydrogels. Thus, oligomeric hydrogels should have unique advantages for tissue engineering and drug delivery when compared to polymer hydrogels or SMB hydrogels. But given the synthetic difficulties of the oligomers and difficulty in adjusting their amphiphilicities to form hydrogels, oligomeric hydrogels[7] are reported less often than either polymer hydrogels or SMB hydrogels. In 2010, Rao and co-workers reported a biocompatible click condensation reaction between the 1,2-aminothiol group of cysteine and the cyano group of 2-cyano-6-aminobenzothiazole (CBT), and it could be controlled by pH, reduction, or protease.[8] This click condensation reaction has a second-order reaction rate of $9.19\,M^{-1}s^{-1}$ and was used to efficiently synthesize cyclic oligomers.[8,9]

Inspired by these reports, we rationally designed two precursors, **1** and **2** (Figure 1), and employed this condensation reaction to synthesize amphiphilic cyclic oligomers for the self-assembly of oligomeric hydrogels (i.e., Gel I from **1** and Gel II from **2**). Upon glutathione (GSH) reduction, the disulfide bond of either **1** or **2** is cleaved to afford the active gelator **3** whose 1,2-aminothiol group on the cysteine motif is exposed and instantly condenses with the cyano group on the CBT motif at pH 6 to yield amphiphilic cyclic oligomers (red parts indicate the hydrophobic structures and blue parts indicate the hydrophilic structures). The amphiphilic cyclic oligomers quickly self-assemble into nanofibrous networks through π–π interactions and thereafter form oligomeric hydrogels.

We began the study with the syntheses of the precursors **1** and **2** (see Schemes S1 and S2 in the Supporting Information). After obtaining **1** and **2**, we tested their gelation abilities. In brief, 10.0 mg of **1** or 8.7 mg of **2** were dissolved in 300 μL water (71.4 mM for **1**, or 35.7 mM for **2**), thus resulting

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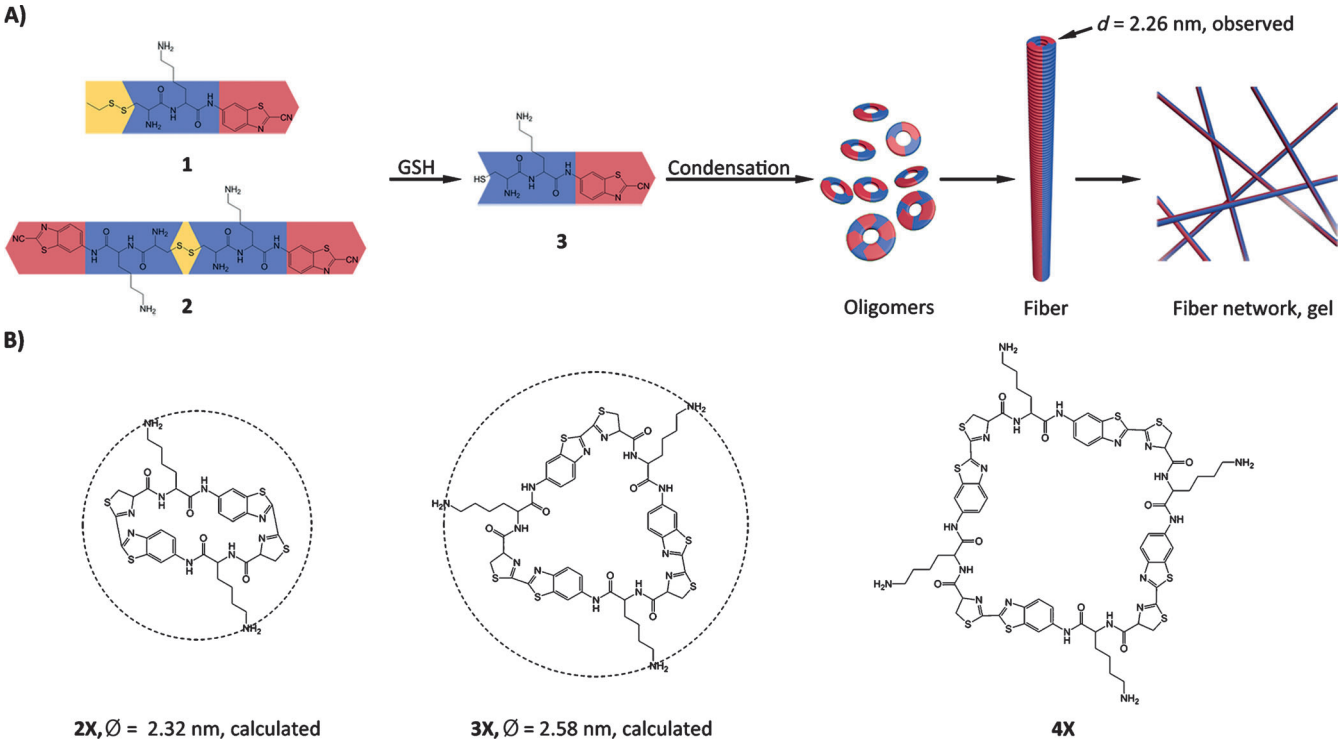


***Figure 1.*** A) GSH-controlled condensation reactions to yield cyclic amphiphilic oligomers which self-assemble into nanofibrous networks to form oligomeric hydrogels. Blue sections indicate the hydrophilic structures and red sections indicate the hydrophobic structures. B) Chemical structures of dimer, trimer, and tetramer for self-assembly.

in clear solutions (Figure 2 A,C; vials on left). After the addition of 4 equivalents of GSH to the above solutions and adjustment of the pH values to 6 with 10 M NaOH, the disulfide bonds of both **1** and **2** were reduced and exposed the 1,2-aminothiol groups, which condensed with the cyano groups on the CBT motifs to yield macrocyclized amphiphilic oligomers (e.g., cyclic dimers (**2X**), trimers (**3X**), and tetramers (**4X**); Figure 1 B) and the opaque Gel I (3.33 wt %) and Gel II (2.90 wt %) were afforded within two seconds (Figure 2 A,C, vial on right). Interestingly, when the concentration of **2** was decreased to 1.00 wt % (12.3 mM), it took a longer time (about two minutes) for **2** to afford Gel II, thus confirming that the condensation reaction is concentration-dependent.[8] Inverted tube tests indicated that the minimum gelation concentrations (MGCs) for Gel I and Gel II are (1.15 ± 0.05) wt % (24.6 ± 1.1 mM) and 0.95 ± 0.05 wt % (11.7 ± 0.6 mM), respectively (see Figure S11).

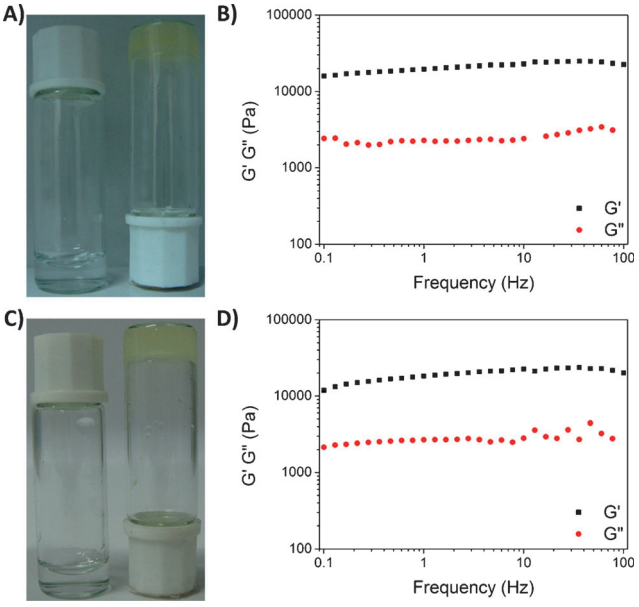


***Figure 2.*** A) Optical image of a solution of **1** at 3.33 wt % (left) and Gel I (right). B) Dynamic frequency of storage modulus (G′) and the loss modulus (G″) of Gel I at the strain of 0.1 %. Conditions: [**1**] = 71.4 mM (3.33 wt %), [**1**]/[GSH] = 1:4, pH 6, 25 °C. C) Optical image of a solution of **2** at 2.90 wt % (left) and Gel II (right). D) G′ and G″ values of Gel II at the strain of 0.1 %. Conditions: [**2**] = 35.7 mM (2.90 wt %), [**2**]/[GSH] = 1:4, pH 6, 25 °C.

To evaluate the viscoelastic properties of the gels, we firstly used dynamic strain sweep to determine the proper conditions for the dynamic frequency sweep of 3.33 wt % Gel I (71.4 mM) and 2.90 wt % Gel II (35.7 mM). As shown in Figure S5, the values of the storage modulus (G′) and the loss modulus (G″) of Gel I and Gel II exhibit a weak dependence from 0.01 % to 1.00 % strain (with G′ dominating G″), thus indicating that the samples are hydrogels. After setting the strain amplitude at 0.10 % (within the linear response regime of strain amplitude), we used dynamic frequency sweep to study Gel I and Gel II. As shown in Figure 2 B,D, the G′ and G″ values of these two gels slightly increase with the increase of frequency from 0.1 to 100 Hz. The values of G′ are about 8 to 10 times larger than those of G″ within the 0.1 to 100 Hz range, thus suggesting that both gels are fairly tolerant to

external shear force. Besides, the similarity of G′ and G″ values of 3.33 wt% Gel I to those of 2.90 wt% Gel II, it was also consistent that the concentration of the gelators (i.e., **3**) are the same in these two gels. Furthermore, the G′ values are about 20,000 Pa and the G″ values are about 2,000 Pa for both Gel I and Gel II, and they are higher than those of most SMB hydrogels but lower than those of some polymer hydrogels reported (see Tables S2 and S3), thus suggesting that our oligomeric hydrogels have optimal stiffness. However, both of the two gels were broken down under the strain larger than 5%, thus indicating weak elasticity of both gels (see Figure S5). Further study of the Gel II at lower concentration (i.e., 1.00 wt%, 12.3 mM) exhibited a weaker strength but stronger elasticity, thus suggesting that the viscoelastic properties of the gel are affected by its gelator concentration (see Figure S6).

Participation of the luciferin moiety in the gelation was investigated by measuring the fluorescence spectra of the oligomers formed by either **1** or **2** at sequentially diluted concentrations in water. Gel I and Gel II above were dispersed in 0.01M phosphate buffer (pH 6) with their gelator concentration ranging from 1.0 μM to 1,000 μM. The dispersions were excited at $\lambda = 325$ nm. The results indicated that the fluorescence emission maxima of Gel I and Gel II increased from $\lambda = 421$ to 436 nm and $\lambda = 425$ to 440 nm, respectively, with an increase in their concentration (see Figure S7). Plots of fluorescence emission maximum versus concentration revealed two regimes, thus indicating critical micelle concentrations (CMCs) of 14.0 μM for **3** in Gel I and 15.1 μM for **3** in Gel II (see Figure S7). In principle, condensation of **1** or **2** under GSH-reduction yielded oligomeric mixtures (i.e., dimer, trimer, tetramer, and higher-order oligomers) to form Gel I and Gel II, respectively. HPLC and matrix-assisted laser desorption/mass spectroscopic (MALDI/MS) analyses were employed to analyze the chemical compositions of the gels. As shown in Figure 3A, in 3.33 wt% Gel I, most of the precursor **1** was converted into its dimer (**2X**; 90.7%), while a very tiny proportion of **1** was converted into higher-order oligomers (9.3%). The MALDI/MS spectrum of the reaction mixture in Gel I clearly showed the presence of the dimer (**2X**), trimer (**3X**), tetramer (**4X**), etc. (Figure 3B). Interestingly, as shown in the HPLC traces in Figure 3C, while the dimer (**2X**) was the main product in 2.90 wt% Gel II, accounting for 64.8% of total oligomers,

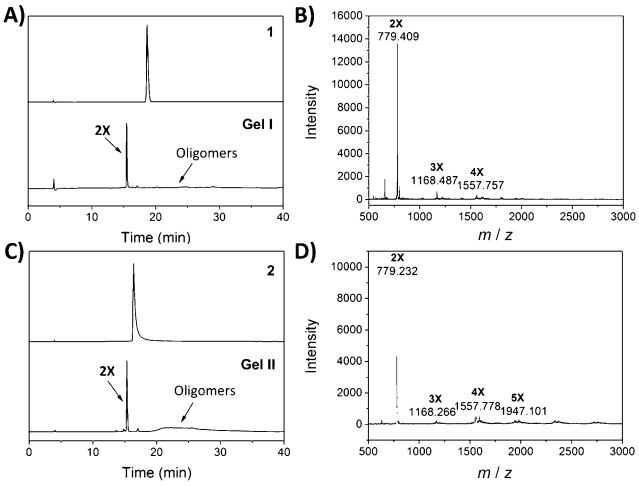


***Figure 3.*** A) HPLC traces of **1** (top) and 3.33 wt% Gel I (71.4 mM, bottom). B) MALDI/MS spectrum of 3.33 wt% Gel I (71.4 mM). C) HPLC traces of **2** (top) and 2.90 wt% Gel II (35.7 mM, bottom). D) MALDI/MS spectrum of 2.90 wt% Gel II (35.7 mM). **2X**, **3X**, **4X**, and **5X** are abbreviations for dimer, trimer, tetramer, and pentamer, respectively.

a broad peak of higher-order oligomers accounts for 35.2% of the total oligomeric products. This data suggests that **2** tends to yield more higher-order oligomers than **1** does, and it probably results from the fact that one molecule of **2** contains two gelator molecules **3** linked by a disulfide bond while one molecule of **1** only contains one gelator molecule **3** protected by a disulfide bond with a SEt group. Thus, it is easier to yield higher-order oligomers of **2** by intermolecular condensation than it is with **1**.

To investigate the morphologies of Gel I and Gel II, we performed cryo transmission electron microscopy (cryo-TEM) observations. The microscopic structure of 3.33 wt% Gel I under cryo-TEM exhibited short fibers with an average width of $(2.26 \pm 0.14)$ nm and an average length of $(40.83 \pm 3.56)$ nm (Figure 4B). A cryo-TEM image of 1.00 wt% Gel II showed not only short fibers, as those observed in Gel I, with an average width of $(2.26 \pm 0.16)$ nm and a longer length of $(83.45 \pm 7.10)$ nm (see Figure S9), but also longer and regular fibers whose lengths are more than several hundreds of a nanometer (Figure 4C). The difference of the fiber length between these two gels might be caused by the difference in

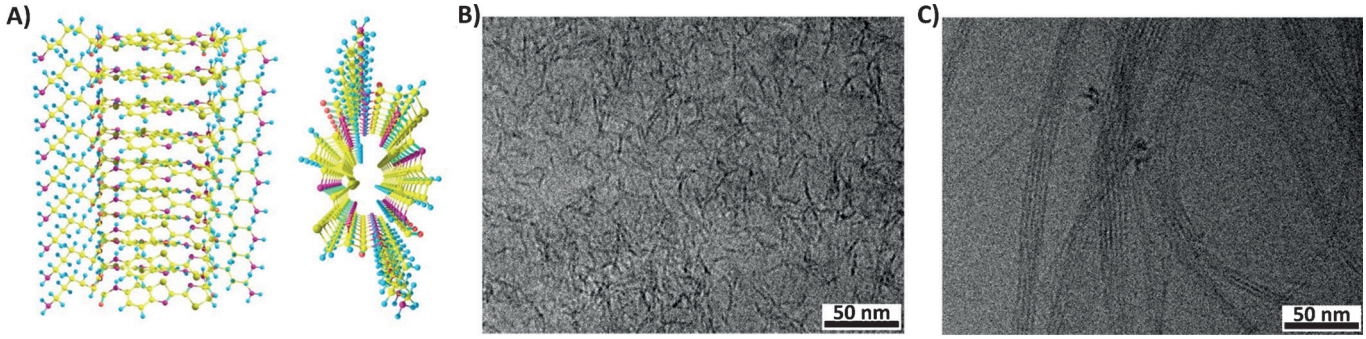


***Figure 4.*** A) The proposed molecular arrangement of dimer, side (left) and top (right) view. B) Cryo-TEM image of Gel I (3.33 wt%). C) Cryo-TEM image of long fibers in Gel II (1.00 wt%).

  

the duration of their gelation processes. While 3.33 wt % Gel I forms instantly (within two seconds), 1.00 wt % Gel II was afforded in two minutes, and we can therefore deduce that longer gelation times offer more time for the oligomers to interact with each other (π–π stacking), thus leading to longer fibers. Assuming that oligomers just interact with oligomers of the same order to form regular fibers (e.g., dimers only π–π stack with dimers) and according to the amphiphilic structure of the oligomers, we proposed possible molecular arrangements for the nanofibers in both gels obtained during the gelation process in water.[10] As shown in Figure 4 A, the dimers are arranged layer by layer, and the luciferin structures are packed face to face to offer strong π–π stacking with a layer distance of 0.33 nm. From this molecular arrangement model, the nanofiber of dimers has a calculated diameter of 2.32 nm, and is in good agreement with the cryo-TEM measurement (i.e., 2.26 nm). Similarly, fibers formed by trimers have a calculated diameter of 2.58 nm and a layer distance of 0.37 nm (see Figure S10). This result indicated that only a small proportion of the nanofibers in the cryo-TEM images might possibly result from the self-assembly of trimers, or even higher-order oligomers. The HPLC traces in Figure 3 in combination with the molecular arrangement above led us to conclude that the main composition of the oligomeric hydrogels is that of dimers.

In summary, we have rationally designed two precursors, **1** and **2**, which instantly and efficiently yield amphiphilic oligomers and self-assemble into oligomeric hydrogels (i.e., Gel I from **1** and Gel II from **2**) upon GSH-controlled condensation. Since **1** and **2** yield the same gelator **3**, Gel I and Gel II have similar mechanic properties (i.e., higher concentration of precursor leads to stronger strength but weaker elasticity). Chemical analyses indicated that the major condensation product in both hydrogels is the cyclic dimer and Gel II has more oligomers of higher-order than does Gel I. Since **1** releases toxic ethanethiol upon GSH-reduction, Gel II which was formed from biocompatible GSH-controlled condensation of **2** might find broad applications in tissue engineering and long-term drug delivery in the future.

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