Communication

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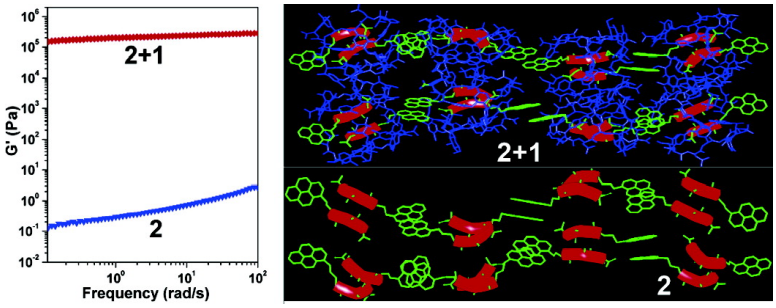

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Molecular Recognition Remolds the Self-Assembly of Hydrogelators and Increases the Elasticity of the Hydrogel by 10^6-Fold

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This communication describes an effective strategy based on molecular recognition to enhance the mechanical properties of hydrogels formed by self-assembling small molecules in water.[1] Three-dimensional (3D) matrixes, which mimic the extracellular matrix (ECM) in living systems, present new opportunities in cell biology and tissue engineering.[2] The successful use of Matrigel (a hydrogel made of water and the substances extracted from the ECM of particular types of cells[3]) for 3D cell culture has stimulated interest in syntheszing materials for specific biological applications, such as hydrogels based on chemically modified natural polymers or synthetic polymers as the media for drug delivery and tissue engineering.[4] As a class of tailored 3D matrixes, hydrogels of small molecules self-assembling into proteinlike nanofibers[5,6] have served as scaffolds for the growth of neurons or to promote cell adhesion.[6,7] To use such small-molecule hydrogels in a wider scope of applications, however, their mechanical properties must be improved.

Although in situ polymerization allows one to enhance the stability of small-molecule gels,[8] such a covalent cross-linking approach usually requires additional chemical synthesis, alters the properties of the hydrogelators, and may result in the loss of biocompatibility and biodegradability, which are readily possible in small-molecule hydrogels. Therefore, we opt for molecular recognition to enhance the elasticity of the small-molecule hydrogels based on our previous work on low-molecular-weight hydrogelators.[9] In this work, we show that the addition of the receptor into the mechanically weak hydrogels of a derivative of the ligand leads to up to a $\sim 10^6$-fold increase of the storage modulus of the hydrogel. Our study on the microstructure and the molecular structure of the hydrogels, before and after the addition of the receptor, indicates that molecular recognition not only rectifies the defects in the self-assembly of the hydrogels that consist only of water and the ligands but also remolds the self-assembled one-dimensional linear superstructure to a highly cross-linked two-dimensional sheet. Compared to directly using covalent bonds, the cross-links generated by molecular recognition conserve the noncovalent interactions between molecules in the supramolecular hydrogels, allow the biocompatibility and biodegradability of the hydrogel to be upheld easily, and may deliver additional biofunctions that associate inherently with the ligands or receptors that act as the cross-linkers.

We choose vancomycin (Van) as the receptor (**1**) and a D-Ala-D-Ala derivative as the ligand (**2**) because of the well-established molecular recognition event (Figure 1A) between **1** and **2** in aqueous solution.[10,11] **2** gels water at the minimum gelation concentration of ~30 mM and pH = 9.5. In contrast, the mixture of **1** and **2** (mol ratio = 1:1) forms a hydrogel at the minimum gel concentration of 5 mM and pH = 9.5. We used dynamic oscillatory

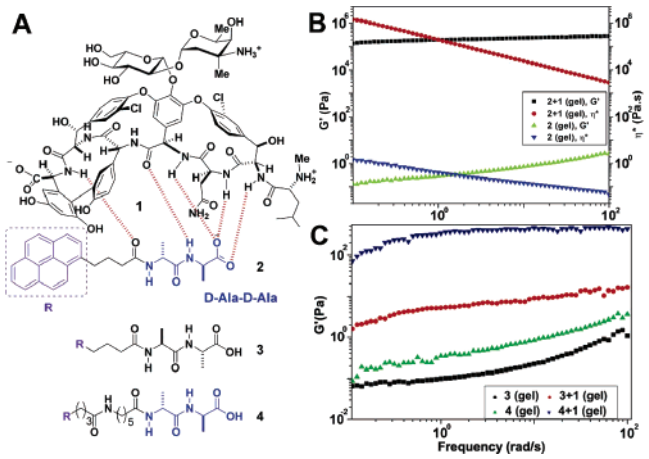

Figure 1. (A) Molecular structures of the receptor, vancomycin (**1**), and the derivatives of the ligands (**2**−**4**). (B) Linear viscoelastic frequency sweep responses of the hydrogels of **2** and **2**+**1** at strain of 1% and 0.1%, respectively. (C) Linear viscoelastic frequency sweep responses of the hydrogels of **3**, **4**, **3**+**1**, and **4**+**1** at 1% strain. The concentrations of **1**, **2**, **3**, and **4** are all 30 mM.

measurements[12] to evaluate the viscoelastic behavior of these two hydrogels at the same concentration (30 mM). To ensure that the hydrogels are reversible upon applying a shear force, all the frequency sweep measurements followed the determination of the linear viscoelastic regime by a strain sweep. As shown in the linear viscoelastic frequency sweep response of the hydrogels (Figure 1B), the storage modulus (G') of the hydrogel of **2** is 0.12 Pa at 0.1 rad/s. The frequency dependence versus complex viscosity ($\eta^* \propto (\text{frequency})^{n-1}$, $n = 0.47 \pm 0.006$) and a nonlinear frequency response started at 100 rad/s indicate that **2** can form only a liquidlike hydrogel. At the concentration of 30 mM, G' of the hydrogel of **2**+**1** is 1.6×10^5 Pa at 0.1 rad/s, and its frequency dependence versus complex viscosity ($\eta^* \propto (\text{frequency})^{n-1}$, $n = 0.15 \pm 0.006$) indicates the solidlike and highly elastic features of the hydrogel. Increasing the mol ratio of **1** (compared to **2**) from zero to one increases G' of the hydrogel of **2**+**1**, following a power law ($G' \propto [\mathbf{1}]^n$, $n = 5.93 \pm 0.31$), suggesting that **1** acts as a cross-linker.[12]

We used **3** (the enantiomer of **2**) and **4** (a derivative of **2**) to verify the function of **1**. As shown in Figure 1C, compared to the G' of the hydrogel of **3**, the G' of the hydrogel of **3**+**1** increases only 10-fold over that of the hydrogel of **3**, which implies that there is a slight increase of the density of the solution (from **3** to **3**+**1**) and that the weak interaction[13] between **1** and **3** contribute little to the increase in the G'. Therefore, we estimate that molecular recognition between **1** and **2** provides at least a 10^5-fold increase of the storage modulus of the hydrogel of **2**+**1**. The G' of the hydrogel of **4**+**1** increases only 420-fold over that of the hydrogel of **4**. This moderate increase in the G' upon **1** binding to **4** mainly

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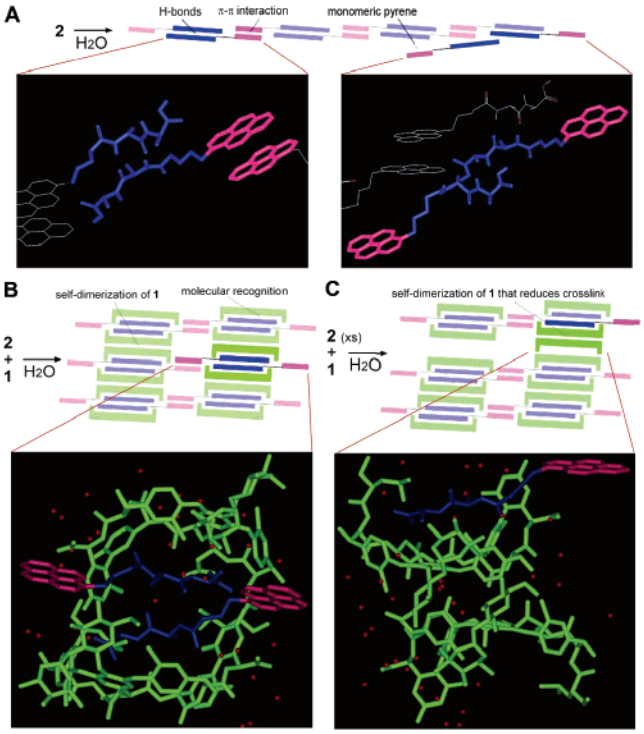

Figure 2. Illustrations of supramolecular arrangements. (A) Plausible self-assembly of **2** results in both monomeric and dimeric pyrene groups. (B) When **2**:**1** ≈ 1:1, molecular recognition between **2** and **1** and self-dimerization of **1** promote cross-links. (C) When **2**:**1** > 1:1, self-dimerization of **1** reduces cross-links.

results from the long and flexible carbon chain between pyrene and D-Ala-D-Ala, which actually increases the distance between the cross-linking points. In addition, the excess amount of **1** decreases G' rapidly (e.g., $G' = 371$ Pa at 0.1 rad/s when **1** is 1.6 equiv of **2**),[14] suggesting that the excess amount of the molecules of **1** reduces the cross-link by self-dimerization.[11] These results further prove that **1** indeed acts as a cross-linker via two interactions: (i) the molecular recognition between **1** and **2** and (ii) the dimerization of **1** (Figure 2B).

A spectroscopic analysis offers a plausible molecular organization in gels of **2** and **2**+**1**. The emission spectra of the gel of **2** show maxima at 399 and 417 nm and a broad band centered at 476 nm.[14] The former two peaks indicate the presence of monomeric pyrene moieties, and the last emission suggests the dimerization of the pyrene groups (due to $\pi-\pi$ stacking) in the hydrogel. The circular dichroism (CD) of the hydrogel of **2** exhibits two troughs at 202 ($\pi\pi^*$ transition) and 228 ($n\pi^*$ transition), indicating that the two alanine residues self-assemble into a helical arrangement, which induces the similar superhelical organization of the pyrene group to give the CD signals from 255 to 389 nm.[14] Adding about one equivalent of **1** into the gel of **2** changes both the emission spectra and the CD of the resulting hydrogel. The emissions originating from the monomeric pyrene (i.e., maxima at 399 and 417 nm) almost disappear while the maximum at 476 nm increases substantially, indicating that the interaction between **1** and **2** promotes the $\pi-\pi$ stacking of the pyrene groups of **2** in the hydrogel. The CD signals in the 190−240 nm region decrease significantly, which is consistent with the formation of hydrogen bonds between **1** and **2**.[11]

The transformation of the self-assembled molecular structures, governed by the molecular recognition between **1** and **2**, causes the changes in the microstructure of the hydrogels, as confirmed by SEM and TEM.[14] The networks of the nanofibers (∼30 nm wide) and the their bundles in the hydrogel of **2** convert into interconnected sheetlike two-dimensional structures as the result of the extensive cross-links in the **2**+**1** hydrogel.

On the basis of the above measurements, we suggest the following possible supramolecular structures in the hydrogels of **2** and **2**+**1**. As illustrated in Figure 2A, the hydrogen bonds and the $\pi-\pi$ interactions lead to the formation of supramolecular polymer chains of **2**, which aggregate to form nanofibers, whose networks serve as the matrixes of the hydrogels. In the **2**+**1** hydrogel, the binding of **1** to **2** cooperatively enhances the self-association of **1**[11] (plus the supramolecular polymer chains of **2**), which resulted in two-dimensional self-assembled polymers as the matrixes of the hydrogel (Figure 2B and C). In conclusion, this work demonstrates the use of molecular recognition for regulating the self-assembly of small-molecule hydrogelators to control the elasticity of the resulting hydrogels. On the basis of our results, we suggest that an effective cross-linker for supramolecular hydrogels should meet the following requirements: (1) high affinity to the hydrogelator and (2) high tendency of self-association. This previously unexplored supramolecular approach should offer a versatile alternative to design and to create sophisticated hydrogels as desirable biomaterials for tissue engineering, drug delivery, and biomimetics.

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Supporting Information Available: Synthesis of **2**, **3**, and **4**, frequency sweep responses of G'', the dependence of the G' of the hydrogel of **2**+**1** vs [**1**], emission and CD spectra, and SEM and TEM images. This material is available free of charge via the Internet at http://pub.acs.org.

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