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Calcium Ions to Crosslink Supramolecular Nanofibers to Tune the Elasticity of Hydrogels over Orders of Magnitude

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Abstract

Learning from the stabilization of protein structures, we combine aromatic-aromatic and electrostatic interactions to tailor the elasticity of supramolecular hydrogels. Specifically, calcium ions allow interfiber crosslinks among the supramolecular nanofibers of small peptides that consist of multiple carboxylic acid and aromatic groups. A small change of the concentration of calcium increases the elasticity of hydrogels over several orders of magnitude. This simple approach not only demonstrates a biomimetic design of materials, but also confirms that the combination of multiple aromatic-aromatic interactions and multiple calcium salt bridges is a feasible way to control the mechanical properties of soft nanomaterials.

Keywords

Self-Assembly; Supramolecular; Hydrogels; Nanofibers; Crosslink; Calcium

Introduction

This article reports the use of calcium ions (Ca^{2+}) to crosslink the nanofibers of self-assembled small peptides to tune the elasticity of supramolecular hydrogels up to five orders of magnitude. Containing large amount of water and being soft, hydrogels bear considerable physical resemblance to human tissues, which has resulted in intensive exploration of hydrogels as biomaterials for applications ranging from tissue engineering to drug delivery.¹⁻³ Particularly, the demonstration of supramolecular nanofibers of peptides as the bioactive matrices of hydrogels for regenerative medicine^{4, 5} has illustrated the promising potentials of the hydrogels formed due to non-covalent interactions.⁶⁻⁸ The successful application of hydrogels not only requires certain biological functionalities to be incorporated in the hydrogels, but also demands proper elasticity of the hydrogels because the fate of the cells is sensitive to tissue level elasticity.⁹ For example, mesenchymal stem cells (MSCs) commit to the lineage specified by matrix elasticity after several weeks in culture,¹⁰ which implies that, in addition to biofunctionalities, the modulation of physical effects (e.g., elasticity) of the hydrogels is a necessary aspect for mimicking the *in vivo* microenvironment of cells.

In fact, besides the conventional method that varies the density of crosslinks for adjusting stiffness of hydrogels,¹¹⁻¹³ several elegant approaches have provided alternatives for

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Supporting Information **Available**. Materials and methods, synthesis, rheology, and control experiment are available free of charge via the Internet at <http://pubs.acs.org> at <http://pubs.acs.org>.

controlling the elasticity of the hydrogels. For example, Gong and coworkers have used double-network (DN) structures to form polymeric hydrogels that exhibit extremely high mechanical strength and exceptionally low frictions^{14, 15} or form highly extensible self-assembling anisotropic structures.^{16, 17} Aida et al. have demonstrated the mix of clay microparticles and a dendritic molecular binder to produce a high-water-content moldable hydrogels that bear several useful characteristics including self-healing and catalysis.¹⁸ Okumura et al. have chemically cross-linked polyrotaxane that consists of α -cyclodextrins and polyethylene glycol (PEG) and have obtained a new kind, topologically interlocked hydrogels that undergo large volume change upon the absorption of water.¹⁹ Stupp and coworkers have pioneered to use calcium ions to crosslink self-assembled nanofibers of peptide amphiphile molecules, and have successfully tailored the mechanical properties of the resulted hydrogels based largely on intrafiber crosslinking²⁰ or simultaneous increase of the concentrations of the peptides and calcium cations.²¹

Encouraged by the above progresses, we choose to use calcium ions to crosslink the self-assembled nanofibers of peptides that favor aromatic-aromatic interactions for several reasons. (i) Aromatic-aromatic interactions stabilize the structure of protein.²² (ii) Calcium-binding proteins (e.g., parvalbumin) contain extensive aromatic-aromatic interactions in their hydrophobic core rich in neighboring aromatic residues.²³ (iii) It is feasible and effective to employ aromatic-aromatic interaction for promoting molecular self-assembly in water and forming hydrogels.²⁴⁻³³ These results imply that the cooperativity between aromatic-aromatic interactions and calcium ion bridges, as a useful combination, is advantageous for increasing the mechanical strength of the nanofibers in the hydrogels. Thus, we design three small molecule hydrogelators (**1**, **2**, and **3**) consisting of multiple aromatic groups for enhancing aromatic-aromatic interactions and multiple carboxylate groups for establishing calcium bridges via the coordination of COO^- groups to Ca^{2+} ions. At the concentrations as low as 0.8 wt% (or around 10 mM) and at slightly acid condition, these hydrogelators self-assemble in water to form nanofibers and result in hydrogels. At near neutral pH, the hydrogelators and water form viscous solutions, which, upon the addition of calcium ions, turn to hydrogels. Interestingly, it also appear that worm-like micelles form in the solution of **2** (Shown in the Figure S3).³⁴ The storage moduli of the hydrogels increase drastically (i.e., orders of magnitude) with relatively small increase of concentration of calcium ions. In addition, the crosslinking density of the nanofibers in the hydrogel also increases with the concentration of calcium ions, indicating that the calcium ions participate both intrafiber and interfiber coordination (Scheme 1). To reach the storage moduli over 1.0×10^5 Pa, gels usually have gelators in quiet high concentration.¹⁸ The hydrogels reported in this work can reach such high moduli (up to 1.6×10^5 Pa) at a quite low concentration of the hydrogelators (0.8 wt %), and recover rapidly after mechanical deformation. Since there are increasingly clear and affirmative evidences suggesting the dependence of cellular behaviors on the mechanical properties of matrix or scaffolds,^{9, 35, 36} the understanding and modulation of the mechanical properties of supramolecular hydrogels, illustrated in this work, offer both insights and an alternative approach to develop soft nanomaterials for bioengineering and biomedical applications.

Results and Discussion

Figure 1A shows the molecular structures of the three hydrogelators investigated in this work. All these molecules share a common motif of N'F, where N' is a naphthyl acetic acid and F is a phenylalanine. As shown in Figure 1B, the naphthyl and phenyl rings from N'F likely provide significant intermolecular aromatic-aromatic interaction to promote the self-assembly of the hydrogelators in water, because, in the case of N'FF,³⁷ the intermolecular distance (3.5 Å) of naphthyl and phenyl rings of N'F segment is shorter than intramolecular ones (3.7 Å). Despite it is unknown whether the obtained crystal structure is the exactly

molecular packing in the nanofibers, the intermolecular interactions in the single crystal provide useful insight into possible molecular arrangement.³⁷ Unlike **1**, hydrogelators **2** and **3** have one cysteine, which is able to form disulfide bond upon oxidation. However, **3** precipitated after oxidation by H₂O₂, which differs from the behavior of peptidic amphiphiles.³⁸ While **2** contains of one aspartic acid, **1** or **3** has two aspartic acids (D), which are able to bind calcium ions. When such coordination occurs at intermolecular mode (as the calcium bridge), it serves as the molecular base for the formation of reversible crosslinks between the hydrogelators as well as the nanofibers formed by the hydrogelators (Scheme 1). Unlike **1**, compounds **2** and **3** have an additional phenylalanine that certainly increases the hydrophobicity of the hydrogelators. Using solid-phase for peptide synthesis and reverse-phase high-performance liquid chromatography (HPLC) for purification, we obtain **1**, **2**, and **3** in the total yield of 63%, 50%, and 40 %, respectively.

After obtaining the design compounds, we test the conditions for them to form hydrogels. Table 1 shows the typical conditions of hydrogelation and the optical images of the hydrogels or the solutions of **1**, **2**, and **3**. Each compound dissolves in water at basic pH (e.g., pH 9). After adding HCl (0.1 M) to adjust the solution to slightly acidic or simply adding CaCl₂ (0.1 M) at near the neutral pH (and the pH was measured by pH paper), these molecules self-assemble in water to form nanofibers and result in hydrogels at the concentration as low as 0.8 wt%. Being different from that Adams et al. have observed the formation of less homogeneous gels when they used HCl to adjust pH,³⁹ the TEM images the Gels-HCl (Figure 2) indicate that the gels are relatively homogenous in this case. While **2** forms the hydrogel at pH 4, **1** or **3** forms the hydrogel at pH 6. If hydrophobicity was the major factor for hydrogelation, **1** and **3** would unlikely form the hydrogels at the pH higher than that for the hydrogelation of **2**. Thus, this result indicates that the number of carboxylic acid groups on the hydrogelators dictates the pH for hydrogelation and that carboxylic acids participate intermolecular hydrogen bonding for the self-assembly. Since carboxylates bind to calcium ions, we dissolve **1**, **2**, or **3** in the buffers (0.1 M Tris-HCl, pH 7.5) before adding the solution of CaCl₂ (0.1 M). As expected, the addition of CaCl₂ to the solution of **1**, **2**, or **3** results the formation of a transparent hydrogel (from now on, we refer the hydrogel formed by adding HCl as Gel-**1** (or **2** or **3**)-HCl and the hydrogel formed by adding CaCl₂ as Gel-**1** (or **2** or **3**)-CaCl₂). Two additional control experiments also validate that the coordination between Ca²⁺ and carboxylates is responsible for the hydrogelation of these hydrogelators at pH 7.5. First, the addition of NaCl ([NaCl] / [COOH] = 1.0 or 5.0) in the solution of **1**, **2**, or **3** at pH 7.5 fails to afford a hydrogel, suggesting that the increase of ionic strength unlikely causes the hydrogelation. Second, the addition of certain amount of EDTA ([EDTA]/[CaCl₂] = 4.0) into the Gel **1**-CaCl₂ affords a clear solution overnight (shown in the Figure S10), indicating that the coordination between Ca²⁺ and the carboxylates on the hydrogelators is indispensable for the hydrogelation.

To evaluate the difference of the microstructures of the hydrogels formed by adjusting pH or by adding CaCl₂, we use transmission electron microscope (TEM) to examine the nanofibers that serve as the matrices of the hydrogels. Figure 2 shows the TEM images of matrices of Gels-CaCl₂ and Gels-HCl. The molecules of **1**, **2**, or **3** all self-assemble in water to form nanofibers that physically crosslink to form a fibrous network as the matrix of the hydrogel, but the diameters and the densities of their nanofibers differ significantly, depending on the molecules as well as the presence of calcium ions. For example, the TEM image of Gel **1**-CaCl₂ shows the helical nanofibers with diameter of 8 nm to intertwine to form double helix-like structures (See Figure S6), probably due to calcium ion bridges between nanofibers of **1**. The TEM image of Gel **1**-HCl reveals the fibril with minimum diameter of 9 nm. While the nanofibers of Gel **2**-HCl twist partially and have widths about 9 nm, the nanofibers of Gel **2**-CaCl₂ have diameters 11 nm and exhibit little twisting. While the TEM image of Gel **3**-HCl exhibits quite uniform nanofibers with average diameter of 12 nm, the

TEM image of Gel **3**-CaCl₂ shows the widths of the nanofibers about 13 nm. Although the diameters of the nanofibers of the Gels-CaCl₂ close to those of corresponding Gels-HCl, the network densities of Gels-CaCl₂ are much higher than those in Gels-HCl, which likely contributes to the high storage moduli of Gels-CaCl₂. These results clearly support that the combination between aromatic-aromatic interaction and electrostatic interaction allows the formation of highly crosslinked networks, which also agree with the rheological results shown in the following section.

To understand the effect of the calcium bridges on the mechanical properties of the hydrogels, we compare storage moduli of Gels-CaCl₂ and Gels-HCl. As shown in the Figure 3A, Gels-CaCl₂ exhibit stiffness orders of magnitude higher than these of corresponding Gels-HCl. Notably, the storage moduli of Gel **2**-CaCl₂ (4.3×10^4 Pa) and Gel **3**-CaCl₂ (3.0×10^4 Pa) are two orders of magnitude over those of Gel **2**-HCl (3.5×10^2 Pa) and Gel **3**-HCl (2.1×10^2 Pa), respectively, which is consistent with that the densities of the nanofibers in Gels-CaCl₂ are higher than these of Gels-HCl. Interestingly, the storage modulus of Gel **1**-HCl is two orders of magnitude higher than those of Gel **2**-HCl and Gel **3**-HCl, indicating that the additional phenylalanine and cysteine residues on **2** and **3** likely contribute to the low elasticity of Gels-**2** and **3**. The storage moduli of Gel **2**-HCl and Gel **3**-HCl are comparable, suggesting that the extra aspartic acid on **3** barely enhance the elasticity of the hydrogels.

To characterize the recovery property of the hydrogels, we subject Gels-HCl and Gels-CaCl₂ to large amplitude oscillation (100% strain for 10 min) until they breakdown, then measure the values of storage moduli (G') in the linear regime as a function of time immediately after the removal of the external shear force. As shown in Figure 3B and 3C, these gels recover about 60% of their original mechanical stiffness almost instantly (within 10 s). Within 10 minutes, Gels **1**-HCl, **2**-HCl, and **3**-HCl recover 81, 99, and 96% of their original elasticity, respectively. Under similar condition, Gels **1**-CaCl₂, **2**-CaCl₂, and **3**-CaCl₂ recover 93, 100, and 87% of their original moduli, respectively. Therefore, the recovery abilities of Gels-CaCl₂ are comparable to those of Gels-HCl. This result differs from the case of calcium ions crosslinked nanofibers of peptide amphiphile, in which the addition of CaCl₂ impairs the recovery ability of the hydrogel (only recovered 35% of original elasticity).²¹ Therefore, the extensive aromatic-aromatic interactions offered by hydrogelators (**1**, **2**, and **3**) likely contribute to such a rheological distinction. Interestingly, Gel **2**-CaCl₂ recovers more than 100% of its original elasticity after the removal of the external force and under shearing for over 10 min. this rapid recovery is similar to the case of injectable solid hydrogels,⁴⁰ in which the fibrillar networks likely fracture into large particles/planes then easily reform after shear. In all measurements, the phase of the elastic burst completes in several seconds, indicating a rapid recovery of the nanofibers formed via non-covalent interactions of the small molecules. These results not only agree that rapid recovery is common feature of supramolecular hydrogels,⁴¹⁻⁴⁴ but also support that the calcium-induced hydrogels are useful for applications requiring rapid recovery, such as injectable matrices for encapsulation of drugs or cells.^{45, 46}

To further establish the mode of the interaction between calcium ions and the hydrogelators, we investigate the storage moduli (G_0 , the maximum of storage modulus obtained from the linear regime of strain sweep⁴⁷ (at strain = 1.0% and frequency = 1 Hz)) as a function of [CaCl₂]/[COOH] for the Gels-CaCl₂. As shown in Figure 4, the storage moduli of the hydrogels change significantly after the addition of calcium ions. The most notable feature is that the storage moduli of the hydrogels almost exhibit a “switch” response to the increase of the amount of the calcium ions. After the initial addition of calcium at [CaCl₂]/[COOH] of 0.13, 0.1, and 0.02, the storage moduli of aqueous solutions of **1**, **2**, and **3** are 1.3 Pa, 5.5 Pa, and 1.4 Pa, respectively. When the values of [CaCl₂]/[COOH] increase to 0.77, 0.77, and

0.56, the storage moduli of Gels **1**-CaCl₂, **2**-CaCl₂, and **3**-CaCl₂ increase nonlinearly to 1.6×10^5 Pa, 4.3×10^4 Pa, and 3.0×10^4 Pa, respectively. For example, **1** only form a semi gel at pH 7.5 when the [CaCl₂]/[COOH] is 0.50, then the storage modulus of Gel **1**-CaCl₂ increase from 1.4×10^3 Pa to 2.3×10^4 Pa when its [CaCl₂]/[COOH] grows from 0.5 to 0.56. With higher molar ratio between calcium and carboxylate group, the storage modulus of Gel **1**-CaCl₂ rapidly climbs to 1.6×10^5 Pa after [CaCl₂]/[COOH] increases to 0.77. The hydrogels of **2** or **3** exhibits behavior similar to that of **1** after the addition of the calcium ions. The storage modulus of Gel **2**-CaCl₂ quickly increases from 5.9×10^2 to 4.3×10^4 Pa when the values of [CaCl₂]/[COOH] changes from 0.25 to 0.77. Similarly, the storage modulus of Gel **3**-CaCl₂ jumps from 1.4×10^2 Pa to 3.0×10^4 when values of [CaCl₂]/[COOH] increase from 0.25 to 0.56. Although the subtle structural differences of **1**, **2**, and **3** likely affect the exact values of [CaCl₂]/[COOH] for the drastic increase of the elasticity, the sudden exponential increase of elasticity indicates that there is a threshold concentration of the calcium ions, at which the critical fraction of the nanofibers crosslink to form the networks of the hydrogels.⁴⁸ It's noted that pyromellitimide organogels show the exponential increase of the viscosity with the time, which associated with the mechanism of aggregation mode.⁴⁹ Thus, our results might fundamentally differ with the case pyromellitimide organogels. Second, the crosslink also can occur in the carboxyl group of individual fibers,⁵⁰ which increases the storage modulus of resulted hydrogel upon the addition of calcium ion. This result clearly differs from the increase of the mechanical properties of the hydrogels caused by the increase of the concentration of the hydrogelators, also distincts from the rigid hydrogel formed by intermolecular folding of synthetic peptide,⁵¹ but it resembles to a phenomenon only occurring in the case of actin⁵² that the increase of the stiffness of a hydrogel via solely changing the amount of calcium ion.

Interestingly, being treated by 0.1 N CaCl₂, the solution of **1** at pH 7.5 turns into suspension from a clear solution, the suspension becomes a clear gel after aging several minutes, which apparently helps the calcium ion disperse evenly in the dynamic self-assembly process.⁵³ This behavior only associates with hydrogelator **1**, since the solution of **2** or **3**, when being treated by calcium, directly changes to a transparent hydrogel. In order to investigate the phenomenon, we use the rheometer to monitor the storage moduli and loss moduli as a function of time for the aqueous solution or hydrogels of **1** at pH 7.5 at different concentration of Ca²⁺. As shown in the Figure 5, the storage modulus hardly changes even more than 50 min at the [CaCl₂]/[COOH] of 0.25, indicating there is insufficient supramolecular interactions for hydrogelation at this condition. While the value of [CaCl₂]/[COOH] increases to 0.50, the elasticity increase to 1.5×10^2 Pa within 10 min, which further confirm the coordination between Ca²⁺ and carboxylates is critical for the hydrogelation. The storage modulus increase sharply to 1.2×10^3 Pa within 4 min after the addition of calcium at the [CaCl₂]/[COOH] of 0.67, suggesting that the crosslinking rate increase at the higher calcium concentration.

The increase of the elasticity Gels-**1**, **2**, and **3** after adding calcium also suggests the change of the morphologies of the hydrogels after the calcium ions reach the threshold concentration. To investigate the effect of calcium ion on microstructure of the hydrogels, we use TEM to examine the nanofibers in the case of Gel **1**-CaCl₂ at different concentrations of CaCl₂ (Figure 6). Obviously, the increase of values of [CaCl₂]/[COOH] results in different morphology, that is, the densities, diameters, lengths of nanofibers for Gel **1**-CaCl₂ vary significantly upon the addition of CaCl₂, which agrees with the mechanical properties (Figure 4A). The nanofibers in Figure 6A are short and distribute loosely, which explains that **1** fail to form a strong hydrogel at low [CaCl₂]/[COOH] (0.5). The nanofibrils in the Figure 6B with minimum diameter of 7 nm appear quite randomly but with a slightly higher density, agreeing with that **1** self-assembles to form a stable hydrogel at [CaCl₂]/[COOH] = 0.56. With the increase of [CaCl₂]/[COOH] to 0.59, 0.63, and 0.67,

the nanofibers in the Gels **1**-CaCl₂ exhibit an average widths of 9 nm, 9 nm, and 11 nm, respectively (Figure 6C, 6D, and 6E). The densities of nanofibers from Figure 6B to Figure 6D increase gradually, however, there is subtle difference between Figure 6D and Figure 6E, which is consistent with results in rheological measurement. These results confirm that the higher densities and bigger sizes of the nanofibers lead to the stronger matrices in the hydrogels and higher values of elasticity. Interestingly, the TEM image in the Figure 6F ([CaCl₂]/[COOH] = 0.77) reveals the helical nanofibers with diameter 8 nm intertwine to form a helix-like structures, likely due to the calcium ion bridges between nanofibers of **1**. These results also support that calcium ions participate interfiber crosslink of the supramolecular nanofibers. Based on these results, we speculate that the mechanism of the calcium caused crosslinking. As shown in scheme 1, the hydrogelator **1** only forms soluble aggregates at pH 7.5 before the addition of calcium ions. The initial addition of calcium mostly contributes to intrafiber intermolecular coordination, which gradually stabilize the nanofibers. After reaching the threshold concentration, the addition of calcium contributes more to the interfiber intermolecular coordination, thus promoting the crosslinking of the fibers and increase of storage moduli.

To further prove that aromatic-aromatic interactions play an essential role for the molecular self-assembly of small molecules in water, we replaced the naphthyl group and phenylalanine with a cyclohexyl group to attach to the GLDD at the N-terminal to obtain cyclohexyl-GLDD (**4**), which fails to form a hydrogel under the same condition used for the formation of Gel **1**-HCl or Gel **1**-CaCl₂ (Figure S9). This result confirms that aromatic-aromatic interactions contribute significantly for the self-assembly of the hydrogelators. In addition, fluorescence spectra show red shifts in the gel states of **1** (Figure S5), suggesting aromatic-aromatic interactions.

Conclusion

In conclusion, by taking the lesson from the stabilization of protein structure,²² we combine aromatic and electrostatic interactions to tailor the elasticity of supramolecular hydrogels up to five orders magnitudes (i.e, from 1.3 Pa to 1.6×10^5 Pa in the case of **1**). Specifically, calcium ions offer interfiber crosslinks to the supramolecular nanofibers of small peptides that consist of multiple carboxylic acid and aromatic groups. This simple approach provides a feasible way to control the mechanical properties of soft nanomaterials for bioengineering and biomedical applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Estroff LA, Hamilton AD. Chem Rev. 2004; 104:1201. [PubMed: 15008620]
2. Lee KY, Mooney DJ. Chem Rev. 2001; 101:1869. [PubMed: 11710233]
3. Langer R, Peppas NA. AIChE J. 2003; 49:2990.
4. Slaughter BV, Khurshid SS, Fisher OZ, Khademhosseini A, Peppas NA. Adv Mater. 2009; 21:3307. [PubMed: 20882499]
5. Shin H, Jo S, Mikos AG. Biomaterials. 2003; 24:4353. [PubMed: 12922148]

6. Capito RM, Azevedo HS, Velichko YS, Mata A, Stupp SI. *Science*. 2008; 319:1812. [PubMed: 18369143]
7. Gao Y, Yang ZM, Kuang Y, Ma ML, Li JY, Zhao F, Xu B. *Biopolymers*. 2010; 94:19. [PubMed: 20091873]
8. Shi JF, Gao YA, Yang ZM, Xu B. *Beilstein J Org Chem*. 2011; 7:167. [PubMed: 21448260]
9. Discher DE, Janmey P, Wang YL. *Science*. 2005; 310:1139. [PubMed: 16293750]
10. Engler AJ, Sen S, Sweeney HL, Discher DE. *Cell*. 2006; 126:677. [PubMed: 16923388]
11. Kuo CK, Ma PX. *Biomaterials*. 2001; 22:511. [PubMed: 11219714]
12. Stokke BT, Draget KI, Smidsrod O, Yuguchi Y, Urakawa H, Kajiwar K. *Macromolecules*. 2000; 33:1853.
13. Ohtsuka A, Watanabe T. *Carbohydr Polym*. 1996; 30:135.
14. Gong JP, Katsuyama Y, Kurokawa T, Osada Y. *Adv Mater*. 2003; 15:1155.
15. Gong JP. *Soft Matter*. 2006; 2:544.
16. Yang W, Furukawa H, Gong JP. *Adv Mater*. 2008; 20:4499.
17. Haque MA, Kamita G, Kurokawa T, Tsujii K, Gong JP. *Adv Mater*. 2010; 22:5110. [PubMed: 20839252]
18. Wang Q, Mynar JL, Yoshida M, Lee E, Lee M, Okuro K, Kinbara K, Aida T. *Nature*. 2010; 463:339. [PubMed: 20090750]
19. Okumura Y, Ito K. *Adv Mater*. 2001; 13:485.
20. Stendahl JC, Rao MS, Guler MO, Stupp SI. *Adv Funct Mater*. 2006; 16:499.
21. Greenfield MA, Hoffman JR, de la Cruz MO, Stupp SI. *Langmuir*. 2010; 26:3641. [PubMed: 19817454]
22. Burley SK, Petsko GA. *Science*. 1985; 229:23. [PubMed: 3892686]
23. Nickolds CE, Bradshaw RA, Kretsing Rh, Coffee CJ. *Proc Natl Acad Sci U S A*. 1972; 69:581. [PubMed: 4501574]
24. Yang ZM, Liang GL, Ma ML, Gao Y, Xu B. *J Mater Chem*. 2007; 17:850.
25. Ma ML, Kuang Y, Gao Y, Zhang Y, Gao P, Xu B. *J Am Chem Soc*. 2010; 132:2719. [PubMed: 20131781]
26. Smith AM, Williams RJ, Tang C, Coppo P, Collins RF, Turner ML, Saiani A, Ulijn RV. *Adv Mater*. 2008; 20:37.
27. Gazit E. *Chem Soc Rev*. 2007; 36:1263. [PubMed: 17619686]
28. Shao H, Parquette JR. *Chem Commun*. 2010; 46:4285.
29. Adams DJ, Topham PD. *Soft Matter*. 2010; 6:3707.
30. Castelletto V, Cheng G, Greenland BW, Hamley IW, Harris PJF. *Langmuir*. 2011; 27:2980.
31. Ryan DM, Doran TM, Anderson SB, Nilsson BL. *Langmuir*. 2011; 27:4029. [PubMed: 21401045]
32. Cheng G, Castelletto V, Moulton CM, Newby GE, Hamley IW. *Langmuir*. 2010; 26:4990. [PubMed: 20073495]
33. Gao J, Wang HM, Wang L, Wang JY, Kong DL, Yang ZM. *J Am Chem Soc*. 2009; 131:11286. [PubMed: 19630424]
34. Lin Z. *Langmuir*. 1996; 12:1729.
35. Pelham RJ, Wang YL. *Mole Biol Cell*. 1996; 7:2429.
36. Kong HJ, Liu JD, Riddle K, Matsumoto T, Leach K, Mooney DJ. *Nat Mater*. 2005; 4:460. [PubMed: 15895097]
37. Zhang Y, Kuang Y, Gao YA, Xu B. *Langmuir*. 2011; 27:529. [PubMed: 20608718]
38. Hartgerink JD, Beniash E, Stupp SI. *Science*. 2001; 294:1684. [PubMed: 11721046]
39. Adams DJ, Butler MF, Frith WJ, Kirkland M, Mullen L, Sanderson P. *Soft Matter*. 2009; 5:1856.
40. Yan C, Altunbas A, Yucel T, Nagarkar RP, Schneider JP, Pochan DJ. *Soft Matter*. 2010; 6:5143. [PubMed: 21566690]
41. Pochan DJ, Schneider JP, Kretsinger J, Ozbas B, Rajagopal K, Haines L. *J Am Chem Soc*. 2003; 125:11802. [PubMed: 14505386]

42. Nowak AP, Breedveld V, Pakstis L, Ozbas B, Pine DJ, Pochan D, Deming TJ. *Nature*. 2002; 417:424. [PubMed: 12024209]
43. Yan CQ, Pochan DJ. *Chem Soc Rev*. 2010; 39:3528. [PubMed: 20422104]
44. Ozbas B, Kretsinger J, Rajagopal K, Schneider JP, Pochan DJ. *Macromolecules*. 2004; 37:7331.
45. Beniash E, Hartgerink JD, Storrie H, Stendahl JC, Stupp SI. *Acta Biomater*. 2005; 1:387. [PubMed: 16701820]
46. Silva GA, Czeisler C, Niece KL, Beniash E, Harrington DA, Kessler JA, Stupp SI. *Science*. 2004; 303:1352. [PubMed: 14739465]
47. Song F, Zhang LM, Li NN, Shi JF. *Biomacromolecules*. 2009; 10:959. [PubMed: 19275215]
48. Qiao Y, Lin YY, Wang YJ, Li ZB, Huang JB. *Langmuir*. 2011; 27:1718. [PubMed: 21218845]
49. Tong KWK, Dehn S, Webb JEA, Nakamura K, Braet F, Thordarson P. *Langmuir*. 2009; 25:8586. [PubMed: 20050045]
50. Ozbas B, Rajagopal K, Haines-Butterick L, Schneider JP, Pochan DJ. *J Phys Chem B*. 2007; 111:13901. [PubMed: 18044866]
51. Ozbas B, Rajagopal K, Schneider JP, Pochan DJ. *Phys Rev Lett*. 2004; 93
52. Gardel ML, Shin JH, MacKintosh FC, Mahadevan L, Matsudaira P, Weitz DA. *Science*. 2004; 304:1301. [PubMed: 15166374]
53. Lehn JM. *Proc Natl Acad Sci U S A*. 2002; 99:4763. [PubMed: 11929970]

Figure 1.

(A) The molecular structures of the hydrogelators investigated in this work. (B) The crystal structure of N'FF shows the intermolecular and intramolecular aromatic-aromatic interactions with the distance of ring at 3.5 and 3.7 Å, respectively.³⁷

Figure 2. Transmission electron micrographs (TEM) of self-assembled nanofibers in the hydrogels formed by adjusting the pH or adding CaCl₂. (See conditions of the hydrogelation in Table 1).

Figure 3.

(A) The rheological properties for the hydrogels formed by adjusting the amount of HCl and CaCl_2 . Recovery of storage moduli of the hydrogels formed by adjusting (B) pH or (C) $[\text{CaCl}_2]$ after being exposed to 100% strain for 10 min, then recovery at a certain strain. (The applied strains in the recovery experiments for Gels-1, 2, 3-HCl and Gels-1, 2, 3- CaCl_2 are 0.2%, 0.25%, 1.0%, 0.2%, 0.5%, and 0.5%, respectively. See conditions of the hydrogelation in Table 1).

Figure 4.

The storage modulus (G') versus $[\text{CaCl}_2]/[\text{COOH}]$ of the hydrogels of (A) **1**; (B) **2**; and (C) **3**. The optimal values of $[\text{CaCl}_2]/[\text{COOH}]$ for the hydrogels of **1**, **2**, and **3** are 0.77, 0.77, and 0.56, respectively. The data was fitted sigmoidal line, the samples were kept overnight at room temperature before measurement.

Figure 5.

The storage modulus (G' , filled) and loss modulus (G'' , blank) versus the time for the aqueous solution or hydrogels of **1** (0.8 wt %) prepared at different $[\text{CaCl}_2]/[\text{COOH}]$ ($\blacktriangle \triangle$) for 0.25, $\blacksquare \square$ for 0.50, $\bullet \circ$ for 0.67) and pH 7.5. (Strain = 1.0%, frequency = 1Hz)

Figure 6.
The TEM images of the solution or hydrogels of **1** with [CaCl₂]/[COOH] at (A) 0.50, (B) 0.56, (C) 0.59, (D) 0.63, (E) 0.67, and (F) 0.77, respectively.

Scheme 1.

Illustration of the hydrogelation process induced by calcium ions.

Conditions of hydrogelation and the optical images of the hydrogels and the solutions of **1**, **2**, and **3**.

Table 1

Conc (wt %/mM)	Gel-CaCl ₂ ^a		[CaCl ₂]/[COOH]	Gel-HCl		pH	Sol-NaCl ^b	
	Gel			Gel			Sol	[Na Cl]/[COOH]
0.8/10.9			0.77			6		1.0
0.8/9.21			0.77			4		1.0

Conc (wt %/mM)	Gel-CaCl ₂ ^a		[CaCl ₂]/[COOH]		Gel-HCl		pH		Sol-NaCl ^b		[NaCl]/[COOH]	
	Gel				Gel				Sol			
0.8/8.13			0.56				6				1.0	

^a-CaCl₂ formed by adjusting the amount of CaCl₂ in the Tris-HCl (pH = 7.5) buffer or 3-NaCl prepared at pH 7.5, and [NaCl]/[COOH] = 1.0.