Cation Tuning toward the Inference of the Gelation Behavior of Supramolecular Gels

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We serendipitously discovered that the tripeptide Asp–Phe–Phe trifluoroacetic acid salt (hereafter abbreviated as \(\beta\)-AspFF) formed a reversible thermotropic gel in chloroform solution (at temperatures higher than the boiling point of chloroform), and a stable gel in toluene solution (at equal to or lower than the room temperature). Experimental results indicate that doping metal ions into \(\beta\)-AspFF toluene gels can trigger morphological variations in the gel skeleton, thereby increasing gel volume and inducing the collapse of organogels. Investigation on the cation-tuned gelation behavior of \(\beta\)-AspFF can be used to elucidate heating-induced gel collapse (of normal gel) or reverse thermotropic gelation as well as select carbamide and acetamide as activators of \(\beta\)-AspFF gels in chloroform solution at room temperature.

Advances in the use of supramolecular gelators for fabrication of polymer-like physical gels have inspired scholars to develop functional soft materials1–5. Well-designed low-molecular-weight gelators (LMWGs, \(<500\) amu) contain necessary covalently linked moieties, which promote the growth of noncovalently associated building blocks through self-assembly6–9. Although LMWGs have been the research focus in supramolecular science, prediction of candidate gelator compounds remains unsuccessful because of the complex noncovalent interactions among individual gelators10,11.

The heat-set, jelly-like networks of LMWGs have attracted intensive attention as novel thermoresponsive materials. The reversible self-assembly of the first thermotropic LMWG is an enthalpically driven process, in contrast to the entropy-driven heat-set gelation of polymers and proteins12. Insufficient information on the structure–activity relationship of known thermotropic gels limits the development of new gelators.

“Molecular tunability” is universally used to fabricate objective three-dimensional (3D) structures through simple self-assembly because of the “weak force” maintained nature of supramolecular gels13–16. Cations or anions are used as a chemical stimulus to effectively elucidate the self-assembly pathway during gelation. Incorporating metals in supramolecular gels (metallogels) has also been extensively studied17,18. Furthermore, the binding effect of cations on supramolecular gels has begun to attract widespread attention19–22.

In this paper, we introduce a facile example of using cations as a chemical stimulus to investigate the structure–activity relationship of a thermotropic gel. The gelator \(\beta\)-AspFF is a tripeptide that contains diphenylalanine (FF) as the assembly core and aspartic acid moiety as the metal binding unit (Figure S1).

Results

Characterization of \(\beta\)-AspFF organogel. The gelator formed gels in toluene and chloroform solutions. Interestingly, gels formed in the chloroform and toluene solutions are thermotropic and normal gels, respectively. The cryo-dried scaffolds of these gels exhibit well-organized 3D networks of nanofibrils (Fig. 1, detailed experiment in SI). Scanning electron microscopy (SEM) results showed that the \(\beta\)-AspFF toluene gel consists of fibrous networks with entangled long fibrils, with diameters ranging from 50 nm to 100 nm (Figure S2). This finding was confirmed by transmission electron microscopy (TEM) observations, in which fibrils possess an average diameter of less than 100 nm (Fig. 1a). Natural dried organogel in toluene generated umbrella-like (upside-down) porous residues at the bottom of the vessel; this gel still presents a 3D fibril network (Fig. 1c).

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Cryo-dried gel formed in the chloroform solution exhibits morphological structure, similar to that of gels formed in the toluene solution, but manifests reverse thermodynamic behavior. Considering its limited information and distinct gelation pathway, thermotropic gel formation using LMWG must be investigated. However, imaging tools, such as SEM, TEM, and atomic force microscopy (AFM), cannot provide in situ structural information about gelation. This limitation is particularly irremediable for studying the structure–activity relationship of thermotropic gels.

**Nano-assemblies of β-AspFF in different solvents.** The nano-assemblies of β-AspFF in solvents were first characterized (Fig. 2). At room temperature, β-AspFF self-assembled into nanoribbon structures in

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**Figure 1.** TEM images of cryo-dried scaffolds of β-AspFF nanofibrils at a concentration of 2 mg·mL⁻¹ in different organogels (a) toluene organogel and (b) chloroform organogel; (c) SEM images of toluene gel after solvent evaporation; insert picture is a digital photo; and d: thermo-reversible gelation of β-AspFF gel at a concentration of 5 mg·mL⁻¹ in chloroform.

**Figure 2.** Morphologies of cryo-dried scaffolds of β-AspFF aggregates assembled in different solvents (at a concentration of 10 mg·mL⁻¹ in DMSO, methanol, ethanol and THF; at a concentration of 2 mg·mL⁻¹ in acetonitrile and deionized H₂O).
The SEM images of the cryo-dried sample suggested that Al $^3+$ bond acceptor (HBA) ability, and surface tension $^2$. lalanine peptide formed nanotubes / nanowires in hexafluoroisopropanol (HFIP)–H$_2$O but generated intertwined the 3D arrangements of gels doped with Cu$^{2+}$. We assumed that the -AspFF gel skeleton could not bound to Al$^3+$.

Table 1. Property parameters of pure organic solvents. $^a$The phase behavior is organogels.

methanol, nanospheres in DMSO, nanofibers in ethanol, nanosheet assemblies in acetonitrile, and microtape structures in deionized H$_2$O. The SEM results indicated that -AspFF nanoaggregates were formed in water, and THF exhibits highly similar structures to those of nanofibril interactions in the toluene gel. Hence, formation of fibrillar structures may be a necessary condition but insufficient for gel formation.

The physical and chemical properties of solvents play important roles in gelation. In this study, we summarized several main solvent parameters, such as polarity parameter, hydrogen bond donor (HBD) ability, hydrogen bond acceptor (HBA) ability, and surface tension $^3$.

Table 1 presents that -AspFF prefers to be gelled in solvents with low HBD and HBA values$^2$. This finding confirmed that -AspFF tends to self-assemble to well-organized aggregates through inter-molecular $\pi$–$\pi$ and hydrogen bond interactions. By contrast, the intermolecular hydrogen bond interaction between gelator and solvent molecules weakened in solvents with low HBD and HBA abilities. Thus, the inter-molecular hydrogen bond interaction in -AspFF organogels is not the main driving force to trap and immobilize large volumes of solvent molecules.

The nature of the initial solvent (stock solution) also plays a key role in the gelation pathway$^{25,26}$. The diphenylalanine peptide formed nanotubes / nanowires in hexafluoroisopropanol (HFIP)–H$_2$O but generated intertwined nanofibrils in pure H$_2$O. The experimental results suggest that the interaction between the initial solvent and the main solvent primarily affects -AspFF gel. For instance, -AspFF forms intertwined nanofibrinous networks when first dissolved in HFIP. Followed by dilution with toluene or chloroform to a final concentration of 3 mg mL$^{-1}$; these networks could bind solvent molecules to the gel. We replaced HFIP with DMSO as the initial stock solvent and failed to obtain organogels in toluene and chloroform solutions. Only dispersion nanosticks were observed in these systems, as shown in Figure S3 and Table S1.

The fabricated organogel is sensitive to organic acids and bases. Adding one drop (10 μL) of triethylamine (TEA) into a prepared -AspFF gel can trigger sol transition. The TEM observations suggested that nanofibrils in the -AspFF gel are partly converted into nanospheres after TEA addition (Figure S4a). One drop of trifluoroacetic acid (TFA) can transform the gel into a clarified solution after several minutes. The TEM images revealed that the clarified solution contains irregularly shaped nanoparticles (Figure S4b). Significant pH changes can trigger 3D morphological rearrangements of the gelator, leading to failure to maintain network structure and finally inducing gel collapse. Hence, the nanofibril structure is necessary for efficient grasping of solvent molecules by the gelator to form a gel.

Doping of metal ions into the gel system. To investigate the relationship between the structural features and gelation ability of -AspFF, we added metal ions into the gel system under acidic conditions (to avoid coordination effect). Our experimental results confirmed that -AspFF formed gels in acidic toluene solution in the presence of several metal ions, such as Co$^{2+}$, Ni$^{2+}$, Cu$^{2+}$, Zn$^{2+}$, and Mg$^{2+}$ (Fig. 3).

The SEM images confirmed that the -AspFF gelator doped with Co$^{2+}$ assembled into nanofibers and minimally differed from pure -AspFF gel. Ni(Ac)$_2$-doped organogel are mainly composed of well-organized fibrils, and the final volume of the gels slightly increased compared with that of Co(Ac)$_2$-doped organogel. Gels doped with Cu$^{2+}$, Zn$^{2+}$, and Mg$^{2+}$ possess larger volumes but poorer appearance compared with Co$^{2+}$-doped gel. The SEM observations suggested that gels doped with Cu$^{2+}$ and Zn$^{2+}$ are supported by 3D networks of shorter but more rigid nano-bags, whereas Mg$^{2+}$-doped gel is composed of bundled nanosticks. Evidently, the 3D arrangements of gels doped with Cu$^{2+}$, Zn$^{2+}$, and Mg$^{2+}$ are considerably less compact than that of Co$^{2+}$-doped gel. The cryo-dried skeleton of gels doped with Cu$^{2+}$ and Zn$^{2+}$ share highly similar structures to those of pure -AspFF aggregates in methanol and ethanol solutions.

In contrast to divalent metal ions, Al$^{3+}$-doped -AspFF toluene system cannot form a gel; moreover, addition of a small amount of AlCl$_3$ into the well-maintained pure -AspFF toluene gel could lead to gel collapse. The SEM images of the cryo-dried sample suggested that Al$^{3+}$-doped -AspFF formed micro-sized spherical particles. After sonication-induced gelation, permeate dispersion M(Ac)$_2$-doped organogel exhibited a clearer and cleaner appearance than MCl$_2$-doped system. Metal ions (M = Co$^{2+}$, Ni$^{2+}$, Cu$^{2+}$, Zn$^{2+}$) feature similar ionic radius. [CH$_3$COO]$^-$ possesses the smallest ionic radius among the ions that were distributed in the gel channel, and Co(Ac)$_2$, Ni(Ac)$_2$, Cu(Ac)$_2$, and Zn(Ac)$_2$ present similar transparency. Meanwhile, Cl$^-$ shows large ionic radius, which could block the pore and appear turbid. However, the size of Al$^{3+}$ is smaller than those of the other ions. We assumed that the -AspFF gel skeleton could not bound to Al$^{3+}$; thus, free ions wander to break the gel.

| Solvent  | Polarity index | HBD ability | HBA ability | Surface tension | Assemblies   |
|---------|----------------|-------------|-------------|-----------------|--------------|
| Toluene | 2.40           | 0.00        | 0.11        | 28.53           | Nanofiber$^a$|
| Chloroform | 4.10          | 0.20        | 0.10        | 27.16           | Nanofiber$^a$|
| THF     | 4.2            | 20.0        | 8.0         | 26.4            | Nanofiber    |
| EA      | 4.3            | 17.1        | 9.3         | 26.29           | Nanofiber    |
| Ethanol | 4.3            | 0.86        | 0.75        | 22.27           | Nanofiber    |
| Acetonitrile | 6.2          | 0.19        | 0.4         | 19.1            | Nanosheet    |
| Methanol | 6.6           | 0.98        | 0.66        | 22.55           | Nanofiber    |
| DMSO    | 7.99           | 29.8        | 19.3        | 43.6            | Nanosheet    |
| Water   | 10.20          | 1.17        | 0.47        | 72.80           | Microtapes   |
skeleton into nanospheres, resulting in gel collapse (the ionic radii of Co$^{2+}$, Ni$^{2+}$, Cu$^{2+}$, Zn$^{2+}$, Mg$^{2+}$, Al$^{3+}$, and Cl$^{-}$ are 75, 70, 73, 75, 72, 53, and 181 pm, respectively).

Doping of urea into the β-AspFF chloroform solution. We selected urea to increase the enthalpy of β-AspFF chloroform system (thermotropic gel)$^{27,28}$. Our experimental tests revealed that urea is an effective reagent used to decrease the gelation transition temperature of β-AspFF in chloroform. Fluorescence spectrum was also used to trace the in situ structural changes of β-AspFF during heat- and urea-induced gelation. Figure S5 shows that, at room temperature, fluorescence signal increased with increasing β-AspFF concentration within low ranges (<1 mg·mL$^{-1}$). The strongest fluorescence signal appeared at 1 mg·mL$^{-1}$, and further increase in the concentration of β-AspFF caused the aggregation-induced fluorescent quenching phenomenon. Below the gelation temperature (<323 K), fluorescence signal minimally changed with increasing temperature (Fig. 4a). An abrupt fluorescence peak appeared at 367 nm when heated at 323 K; continuous heating of the system (323 K to 333 K) led to enhanced fluorescence strength. The gel transferred to a clear sol at 353 K, whereas the corresponding fluorescence peaks at 367 and 470 nm significantly decreased. This finding indicated that peaks at 367 and 470 nm are due to gelation-induced fluorescence enhancement.

We used a sample with β-AspFF to determine changes in fluorescence signals in urea-induced β-AspFF gelation. Characteristic peaks at 367 and 470 nm emerged with increasing urea concentration. A remarkable parallel
result during heating above 323 K confirmed that addition of urea to the β-AspFF chloroform system may induce structural transformations similar to those of heating at 323 K.

The cation-induced gelation behavior is highly similar to our observations when heating chloroform gel. The key advantage of using cation-induced gelation to mimic heat-induced gel collapse or reverse heat-set gelation is that well-controlled intermediate structures can be obtained in heating-related gelation by carefully tuning the concentration of cations.

Theoretically, increase in temperature of a system will accelerate molecular motion for all species, referring to a process of entropy increase. However, gel formation is a phenomenon that requires entropy reduction. As such, increased temperature could induce gel collapse rather than sol-to-gel transition, unless the energy reduction of structural changes caused by heating can compensate the entropy production effect.

Discussion
The lack of well-developed experimental techniques to monitor the intermediate structure of gelators in a heating process considerably limits studies on the structure–activity relationship of thermotropic LMWG gelator. To date, most known thermotropic gels are polymer gels, and LMWG gel are stable at temperatures equal to or lower than the room temperatures. In this study, we introduced a simple strategy of using metal ions to progressively tune the morphological structure of the nano-assemblies of peptidic LMWG and consequently adjust the gelation behavior of the thermotropic LMWG gelator. Our experimental results confirmed the importance of special morphological nanostructures in gel stability. This unique gelation property renders β-AspFF as a potent template for studies on the distinctive gelation pathway of thermotropic gelators, particularly LMWG gelators.

Methods
Procedure for gel preparation. The β-AspFF tripeptide was first dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP). Subsequently, 50 μL of 0.1 M β-AspFF / HFIP solution was diluted to a final concentration of 5 mM in toluene or chloroform solution. Sonication for 5 minutes could obtain gels, whereas chloroform gels required longer time and were found at more than 323 K.

Nano-assemblies in different solvents. β-AspFF 100 mg·mL−1 HFIP stock-solutions were prepared and diluted to 10 mg·mL−1 by adding dissolvent or 2 mg·mL−1 solvent with poor solubility. The samples were incubated for an hour and then dropped to silicon wafer. After drying, electron microscopy analyses were performed.

Doping of metal ions into the β-AspFF toluene gel system. Briefly, 10 mg·mL−1 metal ions were dissolved in 50 μL of HFIP and then doped into 5 mM β-AspFF toluene gel. The dispersion was permeated for several hours to obtain various metal ion gels, except Al3+. 4

Doping of urea into the β-AspFF chloroform solution. A certain concentration of the urea solution was dispersed in chloroform, and the final volume was adjusted to 950 μL. The urea solution was then added to 50 μL of 40 mg·mL−1 β-AspFF/HFIP solution.

References
1. Luo, Z. & Zhang, S. Designer nanomaterials using chiral self-assembling peptide systems and their emerging benefit for society. Chem. Soc. Rev. 41, 4736–4754 (2012).
2. Raeburn, J., Zamith Cardoso, A. & Adams, D. J. The importance of the self-assembly process to control mechanical properties of low molecular weight hydrogels. Chem. Soc. Rev. 42, 5143–5156 (2013).
3. Zhang, S. Fabrication of novel biomaterials through molecular self-assembly. Nat. Biotechol. 21, 1171–1178 (2003).
4. Aggeli, A. et al. Responsive gels formed by the spontaneous self-assembly of peptides into polymeric β-Sheet Tapes. Nature 386, 239–262 (1997).
5. Gazit, E. Self-assembled peptide nanostructures: the design of molecular building blocks and their technological utilization. Chem. Soc. Rev. 36, 1263–1269 (2007).
6. Zheng, W., Gao, J., Song, L., Chen, C., Guan, D., Wang, Z., Li, Z., Kong, D. & Yang, Z. Surface-induced hydrogelation inhibits platelet aggregation. J. Am. Chem. Soc. 135, 266–271 (2013).
7. Yang, Z., Liang, G., Guo, Z., Guo, Z. & Xu, B. Intracellular hydrogelation of small molecules inhibits bacterial growth. Angew. Chem. Int. Ed. 46, 8216–8219 (2007).
8. Roy, S. & Banerjee, A. Amino acid based smart hydrogel: formation, characterization and fluorescence properties of silver nanoclusters within the hydrogel matrix. Soft Matter. 7, 5300–5308 (2011).
9. Tan, L., Liu, Y., Ha, W., Ding, L., Peng, S., Zhang, S. & Li, B. Stimuli-induced gel-sol transition of multi-sensitive supramolecular β-cyclodextrin grafted Alginate:Ferrocene modified pluronic hydrogel. Soft Matter. 8, 5746–5749 (2012).
10. Mahler, A., Reches, M., Rechter, M., Cohen, S. & Gazit, E. Rigid, Self-assembled hydrogel composed of a modified aromatic dipeptide. Adv. Mater. 18, 1365–1370 (2006).
11. Du, X., Zhou, J., Shi, J. & Xu, B. Supramolecular hydrogelators and hydrogels: from soft matter to molecular biomaterials. Chem. Rev. 115, 131165–13307 (2015).
12. Kuroiwa, K., Shibata, T., Takada, A., Nemoto, N. & Kimizuka, N. Heat-set gel-like networks of lipophilic Co(II) triazole complexes in organic media and their thermochromic structural transitions. J. Am. Chem. Soc. 126, 2016–2021 (2004).
13. Fleming, S. & Ulijn, R. V. Design of nanostructures based on aromatic peptide amphiphiles. Chem. Soc. Rev. 43, 8150–8177 (2014).
14. Irwansyah, I., Li, Y., Shi, W., Qi, D., Leow, W., Tang, M., Li, S. & Chen, X. Gram-positive antimicrobial activity of amino acid-based hydrogels. Adv. Mater. 27, 648–654 (2015).
15. Yan, X. H., Cui, Y., He, Q., Wang, K. W. & Li, J. R. Organogels based on self-assembly of diphenylalanine peptide and their application to immobilize quantum dots. Chem. Mater. 20, 1522–1526 (2008).
16. Sun, Z., Li, Z., He, Y., Shen, R., Deng, L., Yang, M. & Zhang, Y. Ferrocenyl phenylalnine: A new strategy toward supramolecular hydrogels with multistimuli responsive properties. J. Am. Chem. Soc. 135, 13379–13386 (2013).
17. Qin, L., Duan, P., Xie, F., Zhang, L. & Liu, M. A metal ion triggered shrinkable supramolecular hydrogel and controlled release by an amphiphilic peptide dendron. Chem. Commun. 49, 10823–10825 (2013).
18. Jin, Q., Zhang, L., Zhu, X., Duan, P. & Liu, M. Amphiphilic Schiff base organogels: metal-ion-mediated chiral twist and chiral recognition. Chem. Eur. J. 18, 4916–4922 (2012).
19. Sun, Z., Lv, F., Cao, L., Liu, L., Zhang, Y. & Lu, Z. Multistimuli-responsive, moldable supramolecular hydrogels cross-linked by ultrafast complexation of metal ions and biopolymers. Angew. Chem. Int. Ed. 54, 944–9748 (2015).
20. Diaz Diaz, D., Kuhbeck, D. & Koopmans, R. J. Stimuli-responsive gels as reaction vessels and reusable catalysts. Chem. Soc. Rev. 40, 427–448 (2011).
21. Piepenbrock, M.-O. M., Lloyd, G. O., Clarke, N. & Steed, J. W. Metal- anion-binding supramolecular gels. Chem. Rev. 110, 1960–2004 (2010).
22. Zhang, Z., He, T., Yuan, M., Shen, R., Deng, L., Yi, L., Sun, Z. & Zhang, Y. In situ synthesis of Ag/amino acid biopolymer hydrogels as mouldable wound dressing. Chem. Commun. 51, 15862–15865 (2015).
23. Malavolta, L., Oliveira, E., Cilli, E. M. & Nakaike, C. R. Solvation of polymers as model for solvent effect investigation: proposition of a novel polarity scale. Tetrahedron 58, 4383–4394 (2002).
24. Huang, R., Qi, W., Su, R., Zhao, J. & He, Z. Solvent and surface controlled self-assembly of diphenylalanine peptide: from microtubes to nanoﬁbers. Soft Matter. 7, 6418–6421 (2011).
25. Su, Y., Yan, X., Wang, A., Fei, J., He, Q. & Li, J. A Peony-flower-like hierarchical mesocrystal formed by diphenylalanine. J. Mater. Chem. 20, 6734–6740 (2010).
26. Du, M., Zhu, P., Yan, X., Su, Y., Song, W. & Li, J. Honeycomb Self-assembled peptide scaffolds by the breath ﬁgure method. Chem. Eur. J. 17, 4238–4245 (2011).
27. Abu-Handisyah, M. & Shehabuddin, A. Transfer enthalpies and entropies of amino acids from water to urea solutions. J. Chem. Eng. Data. 27, 74–76 (1982).
28. Kreshock, G. C. & Scheraga, H. A. The temperature dependence of the enthalpy of formation of the amide hydrogen bond; the urea model1. J. Phys. Chem. 69, 1704–1706 (1965).

Acknowledgements

This work was supported by the National Nature Science Foundation of China (21261022, 21473257, 21162027, and 91127024), the Graduate Research and Innovation Program of Xinjiang (XJGRI2014024), and the Tian Shan scholar program of Xinjiang University. The authors thank the Special Program for Applied Research on Super Computation of the NSFC-Guangdong Joint Fund (the second phase) and the National High Technology Research and Development Program of China (2015AA020502).

Author Contributions

P.X., Y.Z. and J.W. designed the experiment. P.X. performed, analyzed, interpreted data, performed characterization, and contributed to the writing of the manuscript. Y.Z. analyzed, interpreted data, and contributed to the editing of the manuscript. H.W., X.W., T.H., R.S., F.Y. and J.W. contributed to the analysis of the experimental data.

Additional Information

Supplementary information accompanies this paper at http://www.nature.com/srep

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Xue, P. et al. Cation Tuning toward the Inference of the Gelation Behavior of Supramolecular Gels. Sci. Rep. 6, 25390; doi: 10.1038/srep25390 (2016).