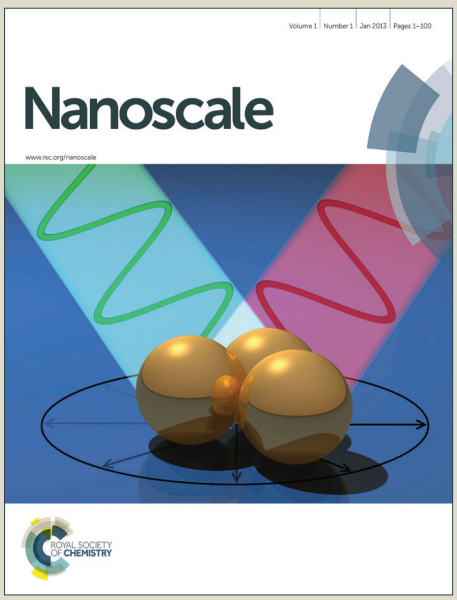
Nanoscale

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/nanoscale

Nanoscale

RSCPublishing

COMMUNICATION

Mechanics of single peptide hydrogelator fibrils†

Cite this: DOI: 10.1039/x0xx00000x

Ying Li*[#a], Yang Sun[#b], Meng Qin[b], Yi Cao*[b] and Wei Wang*[b]

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

The rigidity of peptide fibers is essential to their chemical and biological functions, despite that it remains largely unexplored. Here, we present the first direct measurement of the mechanics of individual fibers in peptide hydrogels by AFM imaging and statistical analysis and find that the intermolecular interactions play a considerable role.

Protein fibrils are important structural constituents of living organisms. [1] Inside the cell, actin fibrils and microtubules are the major components of cytoskeleton. [2] Outside the cell, collagen fibers are abundant in the extracellular matrices. [3] Nature has designed many fibril-forming proteins that could give rise to fibrils of distinct mechanical properties essential for the structure and activity of living systems. [4] On the opposite, functional proteins can also form amyloid-like fibrils under pathological conditions, which may lead to many severe diseases, such as Parkinson's and Alzheimer's. [5, 6] Nanomechanical properties of those amyloid fibrils are important to their toxicity: Both the conversion of the amyloid proteins from the soluble form to their fibrillar state and their transmissibility are directly related to the mechanical properties of amyloid fibrils. [7-11]

Inspired by the protein fibrils found in nature, many *de novo* designed short peptides that are capable of forming fibrous structures have been designed for biological applications. [12-26] These peptides have attracted significant attention because of their great designability in structure and chemical functionality as well as broad biomedical applications. [27-37] Despite that great efforts have been devoted to the development of novel peptide fibers, our knowledge to the intrinsic mechanical properties of these fibers is still limited. [38] Herein, we extended the studies of the mechanical properties of protein fibrils to the *de novo* designed peptide hydrogelator fibers. It was found that many *de novo* designed peptide fibers could form hydrogels under certain conditions. Mechanical properties of the hydrogels have been recognized as an important factor that dictates their biological applications. [39-41] Especially for cell culture, the mechanical properties of the hydrogels could directly control cell growth, spreading and differentiation. [42, 43] However, it is unclear whether the mechanical properties of individual fibrils are also related to their function. On the more fundamental level, it is also intriguing whether the mechanical properties of the peptide fibers affect their hydrogel forming capability. In order to answer these questions, the mechanical properties of individual *de novo* designed peptide hydrogelator fibrils should be measured.

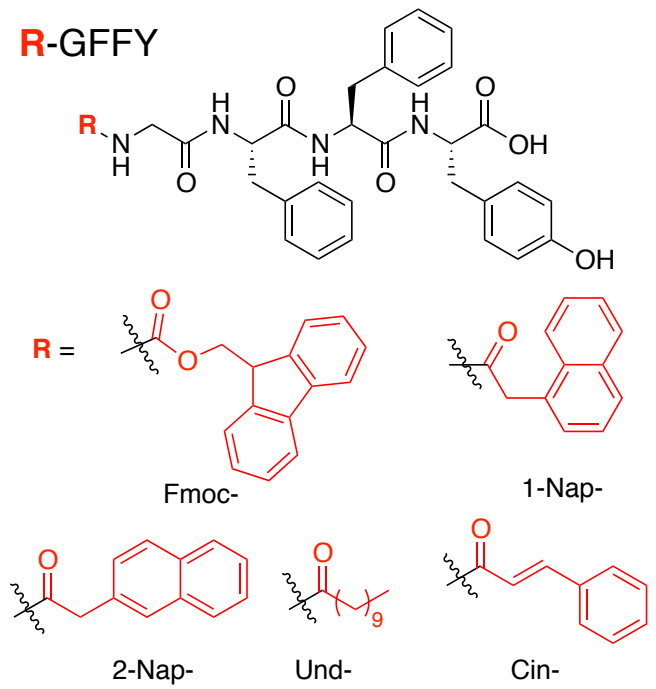


Fig. 1 Chemical structures of designed peptide hydrogelators

We focused on a class of peptide fibrils containing the diphenylalanine (FF) motif, which has been recognized as the minimum core recognition motif of the β-amyloid polypeptide that forms tubular structures *in vivo*. [22] Inspired by such nature, many FF containing short peptides with different N-terminal capping groups were found to be able to form hydrogels, despite that the sizes and structures of the resulting fibrils are distinct. [39, 44-50] In this paper, we studied five peptide gelators with the same peptide sequence of GFFY (glycine-phenylalanine-phenylalanine-tyrosine) but different N-terminal capping groups as Fmoc-, 1-Nap-, 2-Nap-, Und- and Cin- (Figure 1). As the formation of hydrogels is mainly kinetically controlled, we used a method that allows hydrogels being formed in a reproducible fashion by dispersing the DMSO solution of the peptide into PBS buffer (pH ~ 7.4). The formation of hydrogels was confirmed by both the tube inverting method and the rheology measurement (Figure S5 and Figure S6).

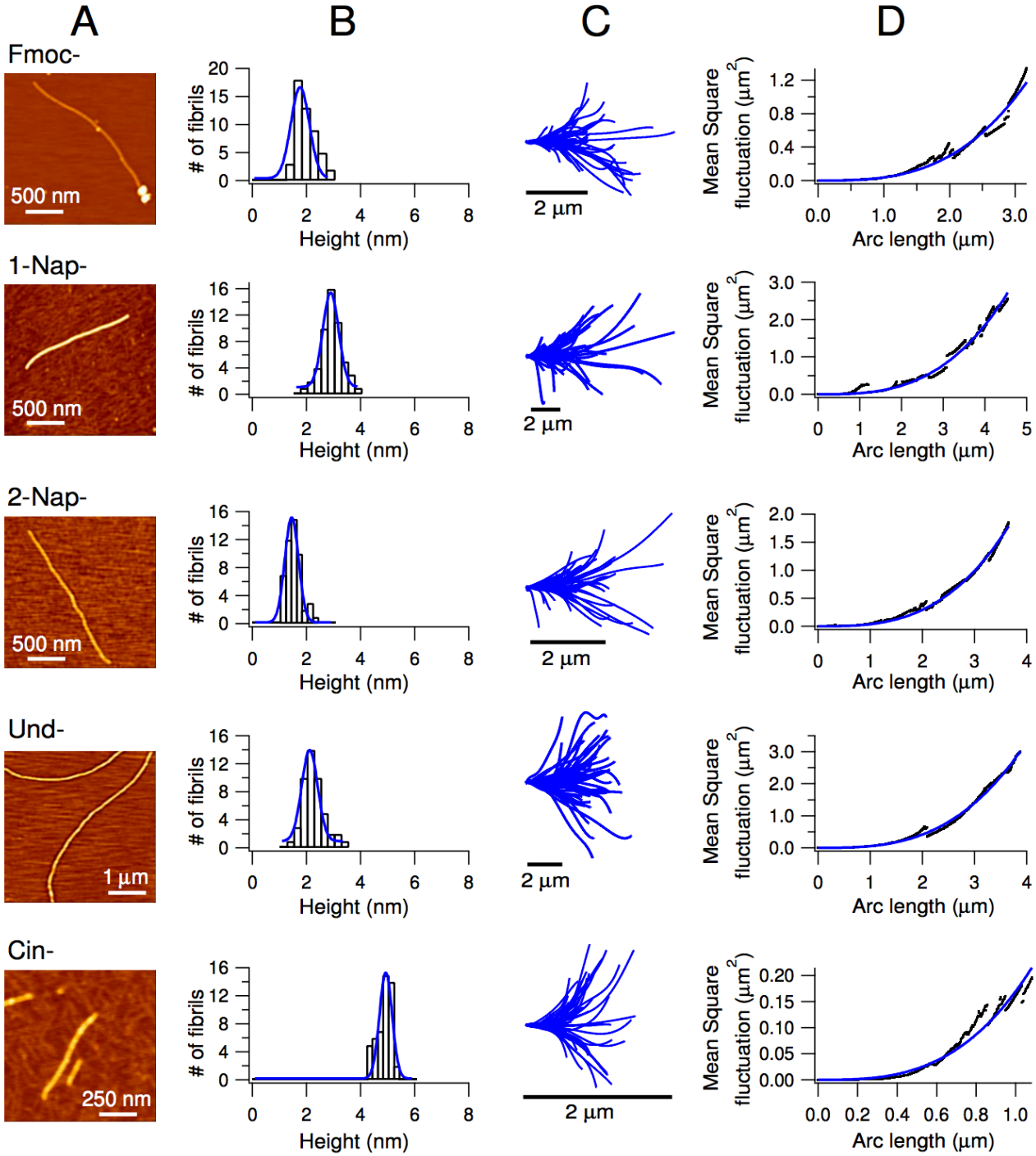


Fig. 2 AFM images of single peptide hydrogelator fibrils and statistical analysis to obtain their mechanical properties. (A) Representative AFM height images of five peptide hydrogelator fibrils. (B) Height distribution of the fibrils. (C) Contour trajectories of fibrils aligned to the first tangent. (D) Mean square fluctuation of fibrils at different arc lengths.

We then used the atomic force microscopy (AFM) based imaging method to capture the mechanical properties of the individual hydrogel fibrils. [11, 51, 52] To avoid the entanglement of the fibrils in AFM images, hydrogel samples (5 mg mL^{-1}) were diluted 10 times with deionized water, deposited on the mica surface and nitrogen gas-dried for the measurement. The dilution and drying process did not change the shape and width of the fibrils, as confirmed by the Cryo-EM images of the hydrogels (Figure S7). Representative fibrils of these peptides are shown in Figure 2A. We imaged ~ 50 individual fibrils of each sample. The heights (h) were summarized in Figure 2B. The 2-Nap- fibrils show the lowest height of ~1.5 nm and the Cin- fibrils showed the highest height of ~4.9 nm. The cross-sectional moments of area (I) for the fibrils could be calculated from the heights of the sample by using a tubular model, in which $I = (\pi h^4)/4$. [53, 54] The tubular model may overestimate I, as the self-assembled may adopt helical ribbon or hollow tube structures. [52] The diverse cross-sectional moments of area suggest that the underlying intermolecular interactions stabilizing these hydrogelator fibrils are distinct and the N-terminal capping groups play an important role in the self-assembly process.

Next, we estimated the bending rigidity (C_B) of the fibrils by measuring their shape fluctuations. The fluctuation of fibrils led to the decay of tangent correlation along the fibril. The faster the decay, the softer the fibril is, as described by theories of semi-flexible polymers. [54] We therefore analyzed the tangent angles of these fibrils by aligning the fibrils of each peptide along the first tangent (Figure 2C). Then, the mean square fluctuation ($\langle u^2 \rangle$) at different arc length (L) was calculated and shown in Figure 2D, based on the statistical model, $\langle u^2 \rangle = k_B T L^3/48 C_B$, where k_B is the Boltzmann constant and T is temperature. [53, 54] Fitting this model to the experimental data yielded the bending rigidity for all five types of hydrogelator fibrils. Interestingly, although these fibrils are of distinct cross-sectional moment of area, their bending rigidities are similar and in the range of $4.8 \times 10^{-28} \sim 3.0 \times 10^{-27}$ N m^2. The Young's moduli (Y) for the fibrils can also be calculated ($Y = C_B/I$) and are quite diverse, ranging from 1.7×10^7 Pa for Cin-GFFY to 1.1×10^{10} Pa for 2-Nap-GFFY.

In order to understand whether such mechanical properties are suitable for biological applications, we then set to compare them with those for other biomacromolecular or synthetic fibrils. As summarized in Figure 3, the bending rigidity of the hydrogelator fibrils (filled squares) is more than one order of magnitude lower than that of amyloid fibrils (open squares) but higher than that of protofibrils (open circles). This indicates that they may not have any tendencies to form amyloid-like toxic species *in vivo*. Comparing with native biological fibrils (open triangles), the bending rigidity of hydrogelator fibrils is lower than many cytoskeleton fibrils, such as actin and tubulin, and is similar to that of individual collagen fibrils of extracellular matrix. [10] This explains why cytoskeleton exists as a viscous fluid instead of a gel even in the presence of higher protein concentrations. Formation of peptide hydrogelator fibrils inside cells could lead to cell death. [55-57] It is possible that low

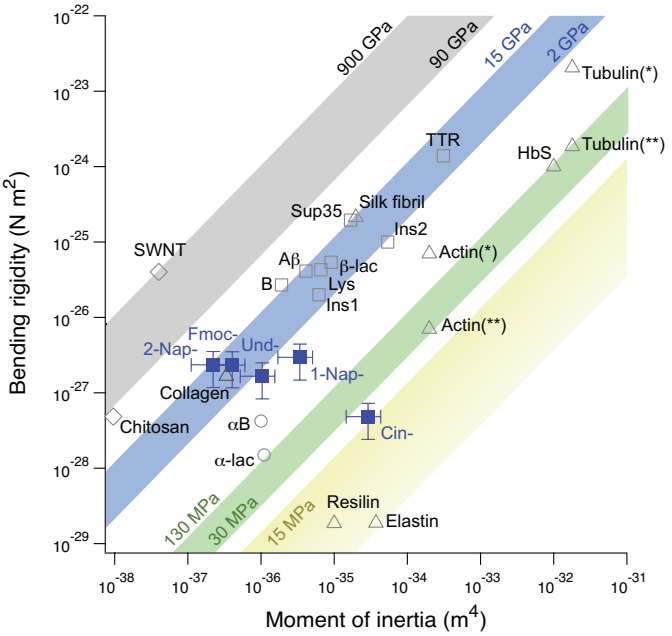


Fig. 3 Comparison of the mechanical properties of peptide hydrogelator fibrils with naturally occurring protein fibrils and other macromolecule or carbon materials. The shield areas with different color scheme have been adapted from Knowles *et al.* for comparison [51]. The gray band represents materials made of covalent or metal bonding interactions. The light blue band represents non-covalent fibrils with ordered hydrogen bonding network. The light green band represents non-covalent fibrils without ordered hydrogen bonding network, and the light yellow band corresponds to materials of pure entropic elasticity. Most peptide hydrogelator fibrils show ordered structures with hydrogen bonding network. The calculated moment of inertia of Cin- fibril may be a bit overestimated due to the presence of hollow structures.

bending rigidity is beneficial for the formation of inter-fibrillar entanglements and therefore facilitates the construction of hydrogel matrix. Such unique mechanical feature may suggest that they are suitable to serve as artificial extracellular matrix for cell culture and regenerative medicine. Although more systematic studies are required, this study paves the way for the understanding of mechanics of individual hydrogelator fibers on the cell-substrate interactions and the subsequent cellular responses. Moreover, there is no direct correlation between the mechanics of individual fibrils and that of the hydrogels (Figure 4). Given that receptors (e.g. focal adhesion proteins) on cell surfaces can only sense the local mechanical properties of extracellular matrix, it will be interesting to study the effects of the mechanics of hydrogel fibrils on the cellular response.

It is also important to understand the molecular origin of such different mechanics of these fibrils. We characterized the structures of these fibrils using XRD (Figure 5A) and Infrared spectroscopy (Figure 5B). Surprisingly, although these fibrils show distinct mechanical properties, they all adopt similar β-sheet structures. As shown in the XRD data, all major peaks are at the same positions, indicating that the molecular packing of these peptide fibrils is similar. Moreover, the relative IR absorbance for amide I and amide II regions of all peptides is also similar, featuring an anti-parallel *β*-sheet structure. This suggests that the N-terminal capping groups do not significantly affect the arrangement of the hydrogen bonds in the GFFY

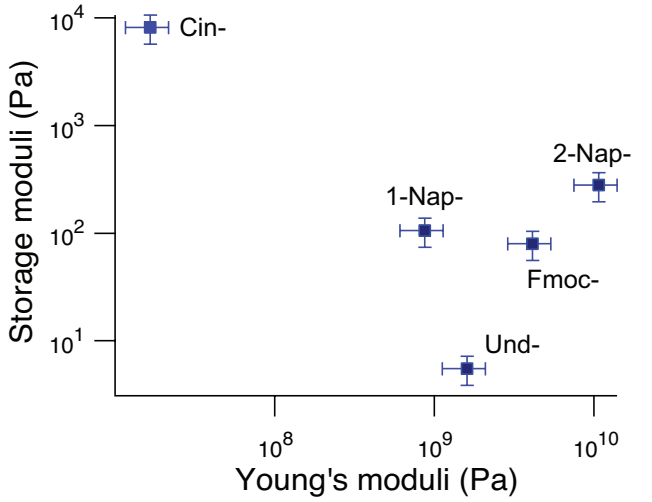


Fig. 4 Relationship between Young's moduli of the hydrogelator fibrils and the storage moduli of the hydrogels. Peptide concentration: 5 mg mL^{-1}.

region. Therefore, the diverse mechanical properties of the hydrogelator fibrils may mainly stem from the intermolecular interactions between the N-terminal capping groups. This finding is in good agreement with previous studies on amyloid fibrils, in which it was found that the mechanical properties of amyloid fibrils are mainly determined by the strength of intermolecular interactions. [51] However, understanding the molecular determinant of the mechanical stability of fibrils will require more accurate structural characterization and molecular dynamics simulations. [58, 59]

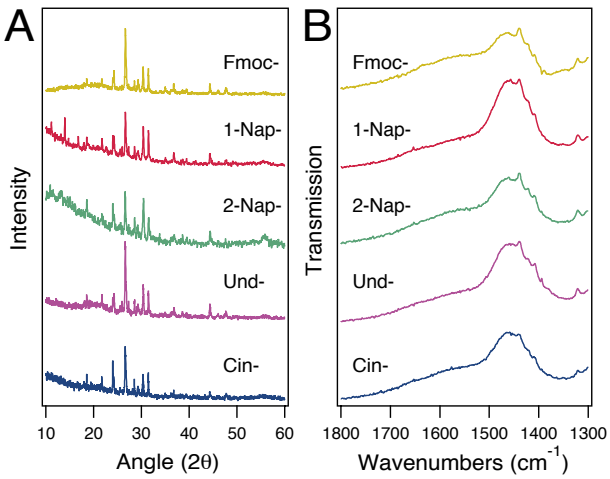


Fig. 5 Characterization of the molecular arrangement of peptides in the fibrils. Peptide concentration: 5 mg mL^{-1}. (A) XRD spectra of the lyophilized peptide hydrogelator fibrils. (B) FTIR spectra of the peptide hydrogelator fibrils measured in D_2O solution.

Conclusions

In summary, we present the first direct measurement of the mechanical properties of individual fibers in peptide hydrogels by AFM imaging and statistical analysis. Our results indicate that the hydrogelator fibrils containing the GFFY motif show low bending rigidity and Young's moduli similar to that of individual collagen fibrils from extracellular matrix. We propose that such mechanical properties may make them of strong gelation capability and suitable for the applications in cell culture and tissue engineering. Based on the structural analysis of the fibrils, we suggest that the intermolecular interaction strength is the deterministic factor for the mechanical stability of individual fibrils. It will be interesting to extend this study to other peptide hydrogelator fibrils and to reveal more general principles for the peptide fibril mechanics. Recent studies have established direct link between the overall mechanical properties of hydrogel substrate and the differentiation of stem cells. [60-62] However, it is largely unexplored whether local mechanical environment provided by individual fibrils also affect the stem cell differentiation. This study paves the way for the understanding of local mechanical properties of substrates to cell behaviors at the single fibril level.

Acknowledgement

We would like to thank Prof. Gaolin Liang and Mr. Anming Tang for their assistance on the Cryo-EM measurement. This work was supported by the National Natural Science Foundation of China (Nos. 11304156, 11334004, 31170813, and 91127026), the Priority Academic Program Development of Jiangsu Higher Education, the Startup Research Funding of NUIST, Jiangsu PhD Gathering Scheme, the Technology Foundation for Selected Overseas Chinese Scholar, and the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry, China.

Notes and references

[a] Jiangsu Engineering Technology Research Centre of Environmental Cleaning Materials, Jiangsu Key Laboratory of Atmospheric Environment Monitoring and Pollution Control, Jiangsu Joint Laboratory of Atmospheric Pollution Control, Collaborative Innovation Center of Atmospheric Environment and Equipment Technology, School of Environmental Science and Engineering, Nanjing University of Information Science & Technology, 219 Ningliu Road, Nanjing, Jiangsu, 210044, P.R. China

[b] Collaborative Innovation Center of Advanced Microstructures, National Laboratory of Solid State Microstructure and Department of Physics, Nanjing University, 22 Hankou Road, Nanjing, Jiangsu, 210093, P.R. China

[#] These authors contribute equally to this work.

† Electronic Supplementary Information (ESI) available: [Detailed experimental procedures and Supplementary Figures.]. See DOI: 10.1039/c000000x/

1. R. H. Pritchard, Y. Y. Huang and E. M. Terentjev, *Soft Matter.*, 2014, 10, 1864-1884.
2. T. M. Svitkina, *Curr. Opin. Cell Biol.*, 2013, 25, 574-581.
3. P. Singh, C. Carraher and J. E. Schwarzbauer, *Annu. Rev. Cell Dev. Biol.*, 2010, 26, 397-419.
4. D. A. Fletcher and R. D. Mullins, *Nature*, 2010, 463, 485-492.

5. F. Chiti and C. M. Dobson, *Annu. Rev. Biochem.*, 2006, 75, 333-366.
6. J. Hardy and D. J. Selkoe, *Science*, 2002, 297, 353-356.
7. M. Tanaka, S. R. Collins, B. H. Toyama and J. S. Weissman, *Nature*, 2006, 442, 585-589.
8. J. F. Smith, T. P. Knowles, C. M. Dobson, C. E. Macphee and M. E. Welland, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, 103, 15806-15811.
9. J. Adamcik, J. M. Jung, J. Flakowski, P. De Los Rios, G. Dietler and R. Mezzenga, *Nat. Nanotech.*, 2010, 5, 423-428.
10. T. P. Knowles and M. J. Buehler, *Nat. Nanotech.*, 2011, 6, 469-479.
11. G. Lamour, C. K. Yip, H. Li and J. Gsponer, *ACS Nano*, 2014, 8, 3851-3861.
12. S. Zhang, T. Holmes, C. Lockshin and A. Rich, *Proc. Natl. Acad. Sci. U. S. A.*, 1993, 90, 3334-3338.
13. J. P. Schneider, D. J. Pochan, B. Ozbas, K. Rajagopal, L. Pakstis and J. Kretsinger, *J. Am. Chem. Soc.*, 2002, 124, 15030-15037.
14. Z. Luo and S. Zhang, *Chem. Soc. Rev.*, 2012, 41, 4736-4754.
15. H. M. Wang, C. H. Yang, M. Tan, L. Wang, D. L. Kong and Z. M. Yang, *Soft Matter.*, 2011, 7, 3897-3905.
16. H. M. Wang and Z. M. Yang, *Nanoscale*, 2012, 4, 5259-5267.
17. B. E. Ramakers, J. C. van Hest and D. W. Lowik, *Chem. Soc. Rev.*, 2014, 43, 2743-2756.
18. G. Fichman and E. Gazit, *Acta Biomater.*, 2014, 10, 1671-1682.
19. A. R. Thomson, C. W. Wood, A. J. Burton, G. J. Bartlett, R. B. Sessions, R. L. Brady and D. N. Woolfson, *Science*, 2014, 346, 485-488.
20. D. J. Adams, *Macromol. Biosci.*, 2011, 11, 160-173.
21. D. N. Woolfson, *Biopolymers*, 2010, 94, 118-127.
22. M. Reches and E. Gazit, *Science*, 2003, 300, 625-627.
23. K. L. Morris, L. Chen, J. Raeburn, O. R. Sellick, P. Cotanda, A. Paul, P. C. Griffiths, S. M. King, R. K. O'Reilly, L. C. Serpell and D. J. Adams, *Nat. Commun.*, 2013, 4, 1480.
24. M. Hughes, P. Frederix, J. Raeburn, L. S. Birchall, J. Sadownik, F. C. Coomer, I. H. Lin, E. J. Cussen, N. T. Hunt, T. Tuttle, S. J. Webb, D. J. Adams and R. V. Ulijn, *Soft matter.*, 2012, 8, 5595-5602.
25. A. R. Hirst, S. Roy, M. Arora, A. K. Das, N. Hodson, P. Murray, S. Marshall, N. Javid, J. Sefcik, J. Boekhoven, J. H. van Esch, S. Santabarbara, N. T. Hunt and R. V. Ulijn, *Nat. Chem.*, 2010, 2, 1089-1094.
26. S. Fleming and R. V. Ulijn, *Chem. Soc. Rev.*, 2014, 43, 8150-8177.
27. I. W. Hamley, *Angew. Chem. Int. Ed. Engl.*, 2014, 53, 6866-6881.
28. C. J. Newcomb, S. Sur, J. H. Ortony, O. S. Lee, J. B. Matson, J. Boekhoven, J. M. Yu, G. C. Schatz and S. I. Stupp, *Nat. Commun.*, 2014, 5, 3321.
29. Y. Gao, J. Shi, D. Yuan and B. Xu, *Nat. Commun.*, 2012, 3, 1033.
30. H. Wang, J. Wei, C. Yang, H. Zhao, D. Li, Z. Yin and Z. Yang, *Biomaterials*, 2012, 33, 5848-5853.
31. C. Ren, H. Wang, X. Zhang, D. Ding, L. Wang and Z. Yang, *Chem. Commun.*, 2014, 50, 3473-3475.
32. H. Wang, A. Han, Y. Cai, Y. Xie, H. Zhou, J. Long and Z. Yang, *Chem. Commun.*, 2013, 49, 7448-7450.
33. H. Wang, J. Liu, A. Han, N. Xiao, Z. Xue, G. Wang, J. Long, D. Kong, B. Liu, Z. Yang and D. Ding, *ACS nano*, 2014, 8, 1475-1484.
34. Y. Gao, M. J. C. Long, J. F. Shi, L. Hedstrom and B. Xu, *Chem. Commun.*, 2012, 48, 8404-8406.
35. W. Zheng, J. Gao, L. Song, C. Chen, D. Guan, Z. Wang, Z. Li, D. Kong and Z. Yang, *J. Am. Chem. Soc.*, 2013, 135, 266-271.
36. J. B. Matson and S. I. Stupp, *Chem. Commun.*, 2012, 48, 26-33.
37. M. C. Giano, Z. Ibrahim, S. H. Medina, K. A. Sarhane, J. M. Christensen, Y. Yamada, G. Brandacher and J. P. Schneider, *Nat. commun.*, 2014, 5.
38. I. Cherny and E. Gazit, *Angew. Chem. Int. Ed. Engl.*, 2008, 47, 4062-4069.
39. C. Yan and D. J. Pochan, *Chem Soc Rev*, 2010, 39, 3528-3540.
40. J. Raeburn, A. Z. Cardoso and D. J. Adams, *Chem. Soc. Rev.*, 2013, 42, 5143-5156.
41. Y. Li, M. Qin, Y. Cao and W. Wang, *Sci. China Phys. Mech. Astron.*, 2014, 57, 849-858.
42. K. Y. Lee and D. J. Mooney, *Chem. Rev.*, 2001, 101, 1869-1879.
43. A. M. Kloxin, C. J. Kloxin, C. N. Bowman and K. S. Anseth, *Adv. Mater.*, 2010, 22, 3484-3494.
44. Z. Yang, G. Liang, L. Wang and B. Xu, *J. Am. Chem. Soc.*, 2006, 128, 3038-3043.
45. A. Mahler, M. Reches, M. Rechter, S. Cohen and E. Gazit, *Adv. Mater.*, 2006, 18, 1365-1370.
46. A. M. Smith, R. J. Williams, C. Tang, P. Coppo, R. F. Collins, M. L. Turner, A. Saiani and R. V. Ulijn, *Adv. Mater.*, 2008, 20, 37-41.
47. Y. Li, Y. Ding, M. Qin, Y. Cao and W. Wang, *Chem. Commun.*, 2013, 49, 8653-8655.
48. B. Ding, Y. Li, M. Qin, Y. Ding, Y. Cao and W. Wang, *Soft Matter.*, 2013, 9, 4672-4680.
49. Y. Ding, Y. Li, M. Qin, Y. Cao and W. Wang, *Langmuir*, 2013, 29, 13299-13306.
50. C. Ou, J. Zhang, X. Zhang, Z. Yang and M. Chen, *Chem. Commun.*, 2013, 49, 1853-1855.
51. T. P. Knowles, A. W. Fitzpatrick, S. Meehan, H. R. Mott, M. Vendruscolo, C. M. Dobson and M. E. Welland, *Science*, 2007, 318, 1900-1903.
52. I. Usov and R. Mezzenga, *ACS Nano*, 2014, 8, 11035-11041.
53. J. C. Wang, M. S. Turner, G. Agarwal, S. Kwong, R. Josephs, F. A. Ferrone and R. W. Briehl, *J. Mol. Biol.*, 2002, 315, 601-612.
54. T. P. Knowles, J. F. Smith, A. Craig, C. M. Dobson and M. E. Welland, *Phys. Rev. Lett.*, 2006, 96, 238301.
55. Z. Yang, G. Liang, Z. Guo, Z. Guo and B. Xu, *Angew. Chem. Int. Ed. Engl.*, 2007, 46, 8216-8219.
56. Z. M. Yang, K. M. Xu, Z. F. Guo, Z. H. Guo and B. Xu, *Adv. Mater.*, 2007, 19, 3152-3156.
57. Z. Yang, G. Liang, M. Ma, Y. Gao and B. Xu, *Small*, 2007, 3, 558-562.
58. S. Xiao, S. Xiao and F. Grater, *Phys. Chem. Chem. Phys.*, 2013, 15, 8765-8771.
59. H. Ndlovu, A. E. Ashcroft, S. E. Radford and S. A. Harris, *Biophys. J.*, 2012, 102, 587-596.
60. A. J. Engler, S. Sen, H. L. Sweeney and D. E. Discher, *Cell*, 2006, 126, 677-689.
61. J. Swift, I. L. Ivanovska, A. Buxboim, T. Harada, P. C. Dingal, J. Pinter, J. D. Pajerowski, K. R. Spinler, J. W. Shin, M. Tewari, F. Rehfeldt, D. W. Speicher and D. E. Discher, *Science*, 2013, 341, 1240104.
62. J. H. Wen, L. G. Vincent, A. Fuhrmann, Y. S. Choi, K. C. Hribar, H. Taylor-Weiner, S. Chen and A. J. Engler, *Nat. Mater.*, 2014, 13, 979-987.