Supramolecular Hydrogels

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Supramolecular Nanofibers and Hydrogels of Nucleopeptides**

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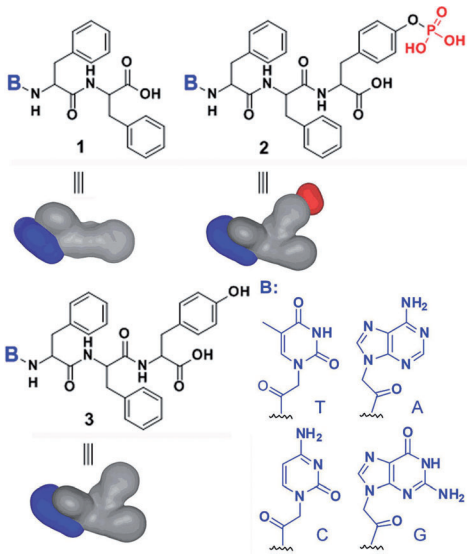
Integration of nucleobases with small peptides generates a novel kind of nucleopeptides as biocompatible and biostable supramolecular hydrogelators. As a class of molecules that contain both nucleobases and amino acids, nucleopeptides bear considerable biological and biomedical importance.[1] Naturally occurring nucleopeptides, such as willardiine-containing nucleopeptides and peptidyl nucleosides, are antibiotics against microorganisms.[2] A number of unnatural nucleobase containing peptides, such as peptide nucleic acids (PNA), have found successful applications in biology and biomedicine (as an analog of DNA).[3,4] Such biological significances render nucleopeptides as attractive targets for heterocyclic chemistry and useful molecules for studying biology, which has achieved considerable success.[5] There is, however, little work to use nucleopeptides for developing novel class of materials.[6] Thus, we decide to explore the potential of nucleopeptides to serve as building blocks for biomaterials. Among many possible choices of the types of materials, we chose to generate hydrogels[7] of nucleopeptides for two simple reasons: 1) supramolecular hydrogels, resulting from molecular self-assembly in water that form entangled nanofibers, have exhibited considerable promises for applications in biomedicine because of the inherent biocompatibility and biodegradability associated with the supramolecular nanofibers;[8] 2) despite their versatility and importance, small nucleopeptides have been hardly explored for hydrogels. Thus, the primary goal of this work is to design, synthesize, and evaluate molecular hydrogelators[7a,b,9] made of nucleopeptides.

Despite the existence of several well-characterized forms of nucleopeptides (chiral nucleopeptides, achiral nucleopseudopeptides, or peptidyl and amino nucleosides),[1b] it is unknown which types of nucleopeptides would be optimal for generating molecular hydrogelators that form nanofibers and hydrogels. Based on the fact that the dipeptide, L-Phe-L-Phe (FF; L-Phe = L-phenylalanine), forms nanotube structures[10] and aromatic rings interact with neighboring nucleobases to stabilize designed DNA structures,[4a] we hypothesize that the conjugation of FF with a nucleobase leads to a molecular hydrogelator. Such a rationale turns out to be valid. As shown in Scheme 1, the connection of a nucleobase (adenine, guanine, thymine, or cytosine) to the dipeptides segment (FF), affords a novel series of nucleopeptides (**1**) as hydrogelators that self-assemble in water to form nanofibers and produce hydrogels at a concentration of 2.0 wt% and a pH value around 5. Molecular mechanics (MM) calculations indicate that the Hoogsteen interactions among nucleobases promote the formation of the nanofibers. The conjugation of tyrosine phosphate to **1** yields another group of nucleopeptides, precursor **2**, which undergoes catalytic dephosphorylation to generate hydrogelator **3** that forms supramolecular nanofibers and hydrogels at low concentrations (2.0 wt%) and physiological pH value. Surprisingly, both **2** and **3** exhibit significant resistance to proteinase K, a powerful digestive enzyme. This result unambiguously confirms the unique advantage of the nucleobase. Moreover, circular dichroism (CD) experiments and rheological measurements indicate that the nucleobases of the nucleopeptidic hydrogelators, after self-assembly, are able to interact with the nucleic acids through Watson–Crick H-bonding. Because nucleobases are an important class of biofunctional motifs, this work not only illustrates the first example of nucleopeptides as hydrogelators made by an enzymatic reaction, but also provides a facile way to explore the potential applications of nucleopeptides as biomaterials, which may lead to a new and general platform to examine specific biological functions (e.g. binding to DNA and RNA) of a dynamic supramolecular system that is able to interact with both proteins and nucleic acids.

Figure 1 a shows the typical synthetic route exemplified by the process for making the hydrogelators based on adenine.



Scheme 1. Molecular structures and shapes of the hydrogelators and corresponding precursors based on nucleopeptides.

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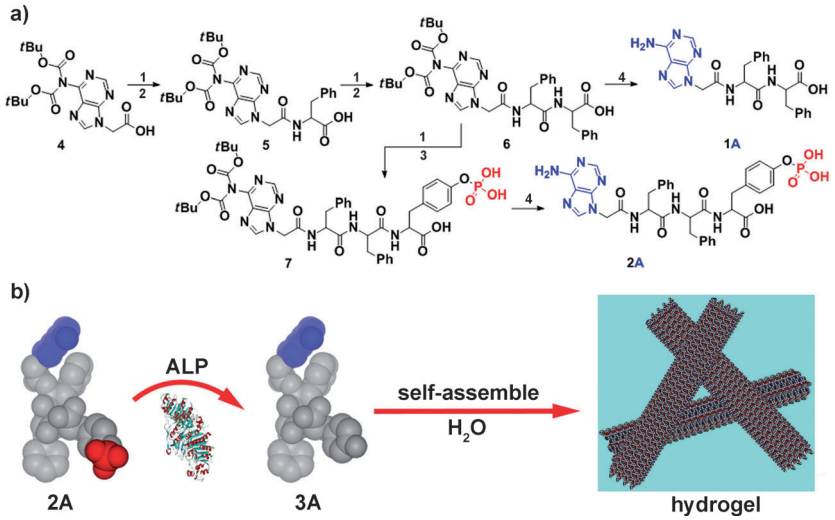


Figure 1. a) Synthetic route of a hydrogelator (**1A**) and a precursor (**2A**) based on adenine. 1) Dicyclohexylcarbodiimid (DCC) and *N*-hydroxysuccinimid (NHS), 2) L-phenylalanine, 3) L-tyrosine phosphate, and 4) trifluoroacetic acid (TFA). b) Dephosphorylation process catalyzed by alkaline phosphatase (ALP) that converts **2A** to **3A** and results in nanofibers and a hydrogel.

Following the procedures reported by Nieddu and co-workers[11] for making nucleobase acetic acids, we first synthesized bis(*tert*-butyloxycarbonyl) (bis-Boc) protected adenine, (N^6-bis-Boc-adenine-9-yl)-acetic acid (**4**). After being activated by *N*-hydroxysuccinimide (NHS), **4** reacts with L-Phe to afford **5**, which undergoes the same NHS activation and phenylalanine coupling to give the key intermediate **6**.

Subsequent removal of the Boc-protecting groups with trifluoroacetic acid (TFA) yields the nucleopeptides (**1A**) in 47% total yield. Encouraged by the observation that **1A**, acting as a hydrogelator, self-assembles to form nanofibers with the diameter of 16 nm (Figure 2a) and results in a hydrogel at the concentration of 2.0 wt% and pH of 5.0, we used the NHS-activated intermediate **6** to react with L-Tyr phosphate to obtain **7**, which forms the precursor **2A** after deprotection of the Boc groups. The dephosphorylation process of precursor **2A** catalyzed by an enzyme (Figure 1b) leads to a translucent hydrogel of nucleopeptide **3A** (Table 1) at physiological pH value. A ^{31}P NMR study confirms that the precursor (**2A**) completely transforms into the hydrogelator (**3A**) in 12 h after the addition of alkaline phosphatase (ALP, see Figure S2 in the Supporting Information), and the TEM images (Figure 2) of the negative stained hydrogel of **3A** reveals nanofibers with a width of 20 nm, confirming that nanofibers of **3A** act as the matrices to sustain the hydrogel (with a storage modulus around 2082 Pa at 2.0 wt%).

The formation of the nanofibers and the hydrogel of **1A** or **3A** indicates that the direct attachment of a purine or pyrimidine base to a small peptide is a valid approach to design hydrogelators of nucleopeptides. To examine the generality of this approach, we used the synthetic procedures similar to Figure 1a to produce the nucleopeptides consisting of other nucleobases (**G, T**, or **C**) and examined their capability to form nanofibers and hydrogels. As revealed by TEM (Figure 2), hydrogelators **1G, 1T**, and **1C** self-assemble to form nanofibers with the width of 15, 9, and 10 nm, respectively, and the nanofibers entangle to trap water and result in hydrogels (Table 1) at a concentration of 2.0 wt% and under slight acid condition (pH 5.0).

Like **2A**, precursors **2G** and **2T**, at a concentration of 2.0 wt% and pH 7.4, upon addition of ALP (10 U) turn into hydrogelators **3G** and **3T**, respectively. This enzymatic conversion leads to the formation of nanofibers **3G** and **3T** and results in the corresponding hydrogels shown in Table 1. TEM reveals that the diameters of the nanofibers of **3G** (14 nm) and **3T** (9 nm) are similar to those of the nanofibers of **1G** and **1T**, respectively. At a concentration of 2.0 wt% and pH 7.4, **3C** self-assembles to afford both nanoparticles (11 nm) and short, thin nanofibers (4 nm in diameter and about 200 nm long), but fails to form well-defined nanofiber networks to provide effective matrices that warrant a hydrogel of **3C**.

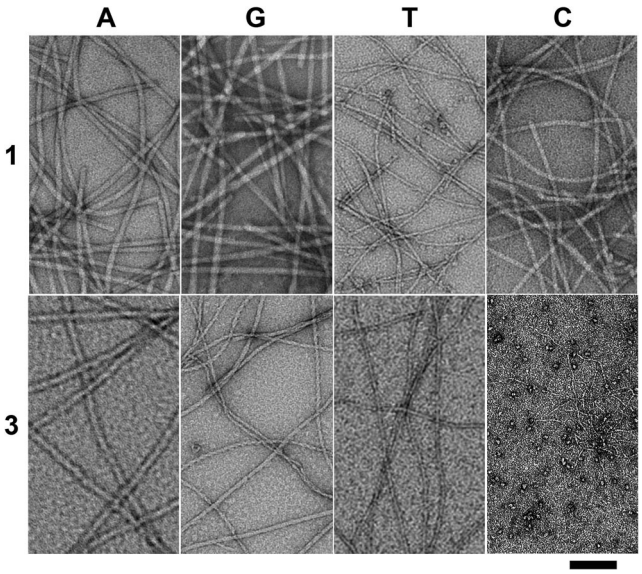
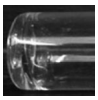
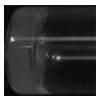
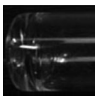
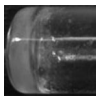
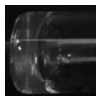
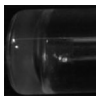
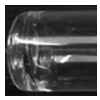
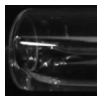


Figure 2. Transmission electron micrographs of the hydrogels formed by **1A**, **1G**, **1T**, **1C**, **3A**, **3G**, **3T** and the solution of **3C** (scale bar = 100 nm).

We measured the rheological properties of the hydrogels to gain further insight on their characteristics. As shown in Table 1, the hydrogel of **1G** exhibits the highest storage modulus (12.6 KPa), the hydrogels of **1A** and **1T** possess relatively high storage moduli of 8.1 and 6.3 KPa, respectively, and the hydrogel of **1C** has the lowest storage modulus (26 Pa). The storage moduli of the hydrogels of **3G** and **3T** are 682 and 2.9 Pa, respectively, indicating that the hydrogel of **3T** possesses much weaker mechanical strength than those of the hydrogels **3A** and **3G** (Table 1). The relatively high

Table 1: The conditions and properties of the nucleopeptidic hydrogelators and corresponding supramolecular nanofibers and hydrogels.

Sample	**1A**	**1G**	**1T**	**1C**	**3A**	**3G**	**3T**	**3C**
concentration [wt%]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
pH	5.0	5.0	5.0	5.0	7.4	7.4	7.4	7.4
optical images								
width of nanofibers [nm]	16	15	9	10	20	14	9	5[a]
critical strain [%]	1.0	0.8	1.2	0.6	0.4	2.0	8.0	–
storage modulus G′ [Pa]	8090	12613	6346	26	2082	682	2.9	–
IC_{50} [μM][b]	>500	>500	>500	>500	>500	>500	>500	>500

[a] These thin nanofibers have low quantity and coexist with nanoparticles, thus fail to result in a hydrogel. [b] Concentration required for 50% inhibition of cell viability.

storage moduli of hydrogels of **1A**, **1G**, **3A**, and **3G** may stem from purine bases that favor the formation of Hoogsteen base pair,[12] in addition to strong π–π interactions of purine nucleobases that contain two fused five- and six-membered heterocyclic rings. Moreover, the lower storage moduli of the hydrogels of **3** relative to those of the corresponding hydrogels of **1** suggest that the presence of tyrosine may reduce the efficiency of the noncovalent interactions required for the stabilization of self-assembled nanostructures, thus resulting in a relatively weak viscoelastic property of those hydrogels.

Furthermore, the addition of an oligomeric deoxyadenosine (A10) to the hydrogel of **1T** or **3T** results in a more stable hydrogel (see Figures S3 and S6 in the Supporting Information), as demonstrated by the increase of storage modulus (G′) from 6.3 KPa (of hydrogel **1T**) to 14.3 KPa (of the hydrogel of **1T** and A10), or from 2.9 Pa (of hydrogel **3T**) to 12.0 Pa (of the hydrogel of **3T** and A10; see Figure S6 in the Supporting Information). This result suggests that Watson–Crick interactions between the self-assembly of **1T** (or **3T**) and A10 favor molecular aggregation and enhance the mechanical strength of the hydrogels. To further examine Watson–Crick H-bonding between complementary nucleobases among the hydrogelators, we use hydrogelators of **1T** and **1A** (or **3T** and **3A**) to prepare a mixed hydrogel and find that the storage modulus (G′) increases from 6.3 KPa (of hydrogel **1T**) to 18 KPa (of the hydrogel of **1T** and **1A**), or from 2.9 Pa (of hydrogel **3T**) to 150 Pa (of the hydrogel of **3T** and **3A**). The mixed hydrogel of the mismatched nucleobases (i.e. **1T** and **1G** or **1T** and **1C**) exhibits, however, little increase of the storage moduli (see Figure S8 in the Supporting Information) in comparison to that of hydrogel **1T**. These results indicate that these nucleopeptidic hydrogelators preserve Watson–Crick interactions of the nucleobases.

We used circular dichroism (CD) to study the superstructures of these nanofibers of self-assembled nucleopeptides in the gel phase. The hydrogels of **1** have the common feature of β-sheet structure according to the CD spectra with a positive peak near 195 nm and a negative peak around 210 nm (see Figure S3 in the Supporting Information), suggesting that these nucleopeptides arrange into β-sheet-like configurations. The hydrogels of **3A**, **3G**, and **3T** display the common feature of CD spectra with a positive peak near 195 nm and a negative peak around 210 nm, which also suggests that the nucleopeptides adopt β-sheet-like configurations. The CD spectrum of the **3C** solution exhibits a positive peak near 203 nm and a negative peak around 215 nm, which red-shifts relative to the signals of typical β-sheet configuration. The red-shifted signal related to β-sheet-like configurations is likely associated with a structure, which is twisted relative to the standard planar β-sheet structure, agreeing with the fact that the increase in β-sheet twisting causes disorder and results in short nanofibers and nanoparticles, which leads to weak mechanical strength.[13] Overall, the signals of β-sheet structures (i.e. transitions at 195–225 nm) of **1** are stronger than those of **3**, following the trend that the storage modulus of **1** is larger than that of **3.** The CD signals with broad bands around 300 nm among the hydrogels **1** and **3** likely originate from the formation of mesophases of hydrogelators because they locate far from the chromophoric absorption region (ca. 270 nm) of the hydrogelators (see Figure S4 in the Supporting Information).

Similar to other nucleobase-containing small molecules that bind with nucleic acids through Watson–Crick interaction,[14] hydrogelators **1T** or **3T** also bind to oligomeric deoxyadenosine (e.g. A10), which results in distinctive changes in the CD spectra. For example, comparing to the CD of hydrogel **1T**, the CD of the **1T**–A10 mixed gel (see Figure S5 in the Supporting Information) exhibits the decreased ellipticity of positive bands at 192 and 228 nm and negative bands around 205, 247, and 287 nm. The CD spectrum of the **3T**–A10 mixed gel (see Figure S5 in the Supporting Information) shows that the addition of A10 both changes the intensity of the bands at 195 and 205 nm and creates a new band at 303 nm that possibly is a result of the conformational change of the self-assembled structures of **3T** induced by A10.[14a–c] In addition, compared to the solution of **1T** or **2T**, the CD spectra of the mixed solution of A10 with hydrogelator **1T** (or precursor **2T**) shows slight changes in the band shape, indicating the relatively weak interaction in the solution state (see Figure S5 in the Supporting Information).

We also used molecular mechanical (MM) calculations to evaluate noncovalent interactions[15] and simulate the width of the nanofibers of **1**. As shown in Figure 3a, the simulated widths of the nanofibers are 15, 16, 9, and 11 nm for nucleopeptides **1A**, **1G**, **1T**, and **1C**, respectively, which correlate well with experimental observations (Figure 2).

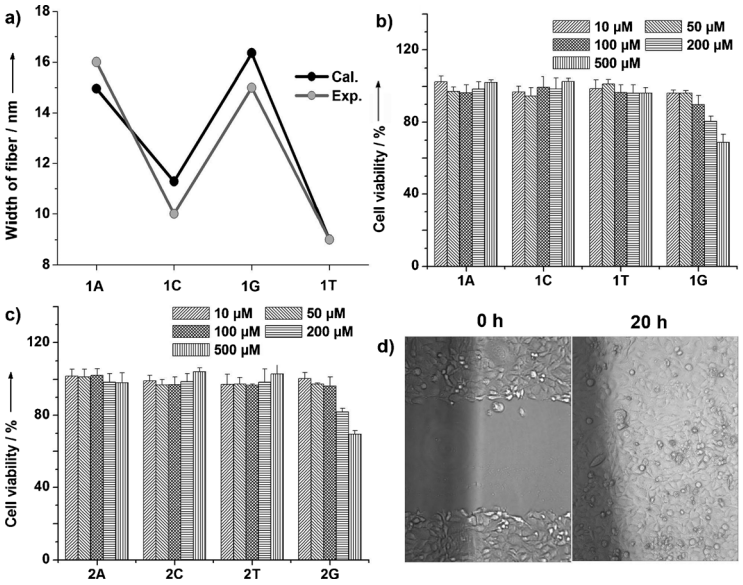


Figure 3. a) Width of fibers of hydrogels **1A**, **1C**, **1G** and **1T** based on transmission electron micrographs (in gray) and molecular mechanical calculations (in black). Cell viability test for 72 h of b) **1A**, **1C**, **1T**, and **1G** and c) **2A**, **2C**, **2T**, and **2G**. d) Optical images of HeLa cells on the surface 0 and 20 h after creation of scratchs in the presence of hydrogel **3T** (by adding 27.7 mM **3T** to the media).

According to the simulations, the widths of the nanofibers in hydrogels of **1A** and **1G** are likely a result of Hoogsteen base pair formation[12,16] by adenine or guanine nucleobases (see Figures S10 and S11 in the Supporting Information). In addition, MM calculations support the formation of β-sheet-like structures.

To verify the biocompatibility of the hydrogelators, we added hydrogelator **1** or precursor **2** to the culture of HeLa cells and measured the proliferation of the cells. According to the MTT assay shown in Figure 3, after being incubated with a hydrogelator (**1A**, **1T**, or **1C**) at a concentration of 500 μM or a precursor (**2A**, **2T**, or **2C**) for 72 h, the cell viability remains at 100%. Although the cell viability decreases slightly when they are incubated with 500 μM **1G** or **2G** for 72 h, the concentration required for 50% inhibition (IC_{50}) is still >500 μM. These results prove that nucleopeptides **1**, **2**, and, **3** are biocompatible. We also used a simple wound-healing assay[17] to examine the capability of the nanofibers and hydrogels of **3** to serve as a material in which cell–matrix interactions are maintained. As shown in Figure 3d, the presence of the hydrogel of **3T** in the cell culture has little inhibitory effect on the migration of the cells, which further supports the biocompatibility of **3.**

Besides biocompatibility, biostability is also an essential requisite for a biomaterial. Thus, we examine the stability of hydrogelators by incubating them with proteinase K, a powerful protease that hydrolyzes a wide range of peptidic substrates and cleaves the peptide bond adjacent to the carboxyl group of aliphatic and aromatic amino acids with blocked alpha amino groups.[18] As shown in Figure 4, more than 85% of **2T**, **3T**, or **3A**, more than 70% of **2A** or **3C**, and above 50% of **2C** remain after 24 h of the incubation with proteinase K; more than 40% of **2G** or **3G** remain after 4 h of the incubation with proteinase K. Although less than 10% of **1T** or Nap-FFY[19] remain after 4 h of incubation with proteinase K (see Figure S13 in the Supporting Information), the excellent or fair resistance to enzymatic digestion, exhibited by the nucleopeptides **2** and **3**, confirms the unique advantage of nucleobases. Because of their high resistance to proteases, the hydrogel formed by hydrogelator **3T**, **3A**, or **3C** promises to serve as new biomaterial for applications that require long-term biostability. In addition, this result suggests that the incorporation of nucleobase may be an effective approach for improving the biostability of other small peptidic hydrogelators.

In conclusion, this work demonstrates the generation of a new type of hydrogelators based on conjugates of nucleobases and short peptides that self-assemble in water to afford supramolecular hydrogels upon a pH- or enzymatic trigger, and introduces a new, simple, and general approach for developing soft, biocompatible materials from nucleopeptides. This work provides a facile way to explore new applications of nucleopeptides as functional biomaterials because of the facile incorporation of bioactive peptides or molecular recognition motifs[20] into nucleobases.

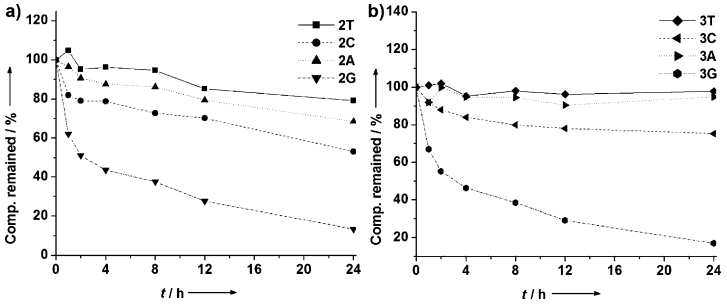


Figure 4. Time-dependent course of the digestions of hydrogelators a) **2T**, **2C**, **2G**, and **2A**, and b) **3T**, **3A**, **3G**, and compound **3C** by proteinase K.

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[1] a) G. N. Roviello, D. Musumeci, E. M. Bucci, C. Pedone, *Mol. Biosyst.* **2011**, *7*, 1073; b) G. N. Roviello, E. Benedetti, C. Pedone, E. M. Bucci, *Amino Acids* **2010**, *39*, 45.

[2] a) M. E. Azzam, I. D. Algranat, *Proc. Natl. Acad. Sci. USA* **1973**, *70*, 3866; b) R. F. Hector, B. L. Zimmer, D. Pappagianis, *Antimicrob. Agents Chemother.* **1990**, *34*, 587; c) M. Itaya, I. Yamaguchi, K. Kobayashi, T. Endo, T. Tanaka, *J. Biochem.* **1990**, *107*, 799.

[3] a) P. E. Nielsen, M. Egholm, R. H. Berg, O. Buchardt, *Science* **1991**, *254*, 1497; b) G. Haaima, A. Lohse, O. Buchardt, P. E. Nielsen, *Angew. Chem.* **1996**, *108*, 2068; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1939.
[4] a) E. T. Kool, *Acc. Chem. Res.* **2002**, *35*, 936; b) P. I. Pradeepkumar, C. Hobartner, D. A. Baum, S. K. Silverman, *Angew. Chem.* **2008**, *120*, 1777; *Angew. Chem. Int. Ed.* **2008**, *47*, 1753; c) E. T. Kool, J. C. Morales, K. M. Guckian, *Angew. Chem.* **2000**, *112*, 1046; *Angew. Chem. Int. Ed.* **2000**, *39*, 990.
[5] P. E. Nielsen, *Chem. Biodiversity* **2010**, *7*, 786.
[6] a) R. Iwaura, K. Yoshida, M. Masuda, M. Ohnishi-Kameyama, M. Yoshida, T. Shimizu, *Angew. Chem.* **2003**, *115*, 1039; *Angew. Chem. Int. Ed.* **2003**, *42*, 1009; b) T. Shimizu, R. Iwaura, M. Masuda, T. Hanada, K. Yase, *J. Am. Chem. Soc.* **2001**, *123*, 5947.
[7] a) L. A. Estroff, A. D. Hamilton, *Chem. Rev.* **2004**, *104*, 1201; b) Z. M. Yang, H. W. Gu, D. G. Fu, P. Gao, J. K. Lam, B. Xu, *Adv. Mater.* **2004**, *16*, 1440; c) K. Y. Lee, D. J. Mooney, *Chem. Rev.* **2001**, *101*, 1869; d) S. W. Choi, Y. Zhang, Y. N. Xia, *Angew. Chem.* **2010**, *122*, 8076; *Angew. Chem. Int. Ed.* **2010**, *49*, 7904.
[8] a) S. Kiyonaka, K. Sada, I. Yoshimura, S. Shinkai, N. Kato, I. Hamachi, *Nat. Mater.* **2004**, *3*, 58; b) G. A. Silva, C. Czeisler, K. L. Niece, E. Beniash, D. A. Harrington, J. A. Kessler, S. I. Stupp, *Science* **2004**, *303*, 1352; c) S. Toledano, R. J. Williams, V. Jayawarna, R. V. Ulijn, *J. Am. Chem. Soc.* **2006**, *128*, 1070; d) R. V. Ulijn, D. N. Woolfson, *Chem. Soc. Rev.* **2010**, *39*, 3349; e) C. Valery, M. Paternostre, B. Robert, T. Gulik-Krzywicki, T. Narayanan, J. C. Dedieu, G. Keller, M. L. Torres, R. Cherif-Cheikh, P. Calvo, F. Artzner, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 10258; f) A. Wada, S. Tamaru, M. Ikeda, I. Hamachi, *J. Am. Chem. Soc.* **2009**, *131*, 5321; g) C. M. Micklitsch, P. J. Knerr, M. C. Branco, R. Nagarkar, D. J. Pochan, J. P. Schneider, *Angew. Chem.* **2011**, *123*, 1615; *Angew. Chem. Int. Ed.* **2011**, *50*, 1577.
[9] a) M. George, R. G. Weiss, *Acc. Chem. Res.* **2006**, *39*, 489; b) P. Terech, R. G. Weiss, *Chem. Rev.* **1997**, *97*, 3133; c) Q. G. Wang, Z. M. Yang, X. Q. Zhang, X. D. Xiao, C. K. Chang, B. Xu, *Angew. Chem.* **2007**, *119*, 4363; *Angew. Chem. Int. Ed.* **2007**, *46*, 4285; d) Z. Yang, G. Liang, Z. Guo, B. Xu, *Angew. Chem.* **2007**, *119*, 8364; *Angew. Chem. Int. Ed.* **2007**, *46*, 8216; e) X. M. Li, J. Y. Li, Y. A. Gao, Y. Kuang, J. F. Shi, B. Xu, *J. Am. Chem. Soc.* **2010**, *132*, 17707.
[10] a) C. H. Görbitz, *Chem. Eur. J.* **2001**, *7*, 5153; b) C. H. Görbitz, *Chem. Commun.* **2006**, 2332; c) C. H. Görbitz, *Chem. Eur. J.* **2007**, *13*, 1022.
[11] A. Porcheddu, G. Giacomelli, I. Piredda, M. Carta, G. Nieddu, *Eur. J. Org. Chem.* **2008**, 5786.
[12] a) K. Araki, I. Yoshikawa in *Low Molecular Mass Gelators: Design*, *Self-Assembly*, *Function*, Vol. 256, Springer, Berlin, **2005**, p. 133; b) J. T. Davis, *Angew. Chem.* **2004**, *116*, 684; *Angew. Chem. Int. Ed.* **2004**, *43*, 668.
[13] a) M. C. Manning, M. Illangasekare, R. W. Woody, *Biophys. Chem.* **1988**, *31*, 77; b) N. W. Sreerama, R. Woody in *Circular Dichroism: Principles and Applications* (Eds.: N. Berova, K. Nakanashi, R. W. Woody), Wiley-VCH, New York, **2000**, p. 601.
[14] a) J. Arigon, C. A. H. Prata, M. W. Grinstaff, P. Barthelemy, *Bioconjugate Chem.* **2005**, *16*, 864; b) G. M. Dong, L. R. Zhang, L. H. Zhang, *Helv. Chim. Acta* **2003**, *86*, 3516; c) G. Godeau, J. Bernard, C. Staedel, P. Barthelemy, *Chem. Commun.* **2009**, 5127; d) R. Iwaura, F. J. M. Hoeben, M. Masuda, A. Schenning, E. W. Meijer, T. Shimizu, *J. Am. Chem. Soc.* **2006**, *128*, 13298; e) P. G. A. Janssen, J. Vandenbergh, J. L. J. van Dongen, E. W. Meijer, A. Schenning, *J. Am. Chem. Soc.* **2007**, *129*, 6078.
[15] S. L. Mayo, B. D. Olafson, W. A. Goddard, *J. Phys. Chem.* **1990**, *94*, 8897.
[16] S. Sivakova, S. J. Rowan, *Chem. Soc. Rev.* **2005**, *34*, 9.
[17] L. G. W. Rodriguez, J. L. Guan in *Cell Migration: Developmental Methods and Protocols*, Vol. 294 (Ed.: J. L. Guan), Humana Press, Totowa, NJ, **2004**, p. 23.
[18] a) D. Brömme, K. Peters, S. Fink, S. Fittkau, *Arch. Biochem. Biophys.* **1986**, *244*, 439; b) G. L. Liang, Z. M. Yang, R. J. Zhang, L. H. Li, Y. J. Fan, Y. Kuang, Y. Gao, T. Wang, W. W. Lu, B. Xu, *Langmuir* **2009**, *25*, 8419.
[19] a) Z. Yang, G. Liang, B. Xu, *Acc. Chem. Res.* **2008**, *41*, 315; b) Y. Zhang, Y. Kuang, Y. A. Gao, B. Xu, *Langmuir* **2011**, *27*, 529.
[20] H. J. Schneider, *Angew. Chem.* **2009**, *121*, 3982; *Angew. Chem. Int. Ed.* **2009**, *48*, 3924.

