Self-Assembly

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pH-Switchable Ampholytic Supramolecular Copolymers**

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A particularly attractive feature of noncovalent bonds is their adaptive and responsive properties towards external stimuli.[1] Consequently self-assembled systems are able to respond to very specific changes in their environment (optical,[2] mechanical,[3] or biological[4] stimuli), thereby enabling self-healing properties,[3b,5] controlled release of cargo,[2b,6] or emergent behavior such as self-replication.[3a,7] Thus, the use of external stimuli to control the self-assembly of supramolecular polymers has great potential for manipulating the morphology of the materials and thereby their function. This is very appealing due to their many applications in electronics,[1a] sensors,[8] and biomedical technologies.[9] Surprisingly, unlike biological structures, most artificial supramolecular polymers rely either on single-component self-assembled systems or on multicomponent systems that interact in an additive, non-cooperative fashion.[9b,10]

We report here the development of multicomponent supramolecular copolymers in water which are formed in a cooperative fashion as a result of a number of noncovalent interactions involving each of the monomers. pH switching of only one of the components will destabilize the material and cause depolymerization to occur as a sharp response, thus rendering the higher order aggregates multistimuli-responsive, stable at close to physiological pH, and unstable at lower and higher pH values. In a supramolecular engineering approach, the intrinsic stability of the noncovalent copolymers and the pH window for the self-assembly to remain switched on can further be tuned by the affinity of the co-monomers.

Peptide materials such as nanofibres,[11] nanotubes,[3a,12] and artificial β-barrels[13] have attracted much interest for the development of responsive supramolecular systems in water. Inspired by the studies by the research groups of Stupp,[2c,9a,11b,14] Percec,[15] and Matile,[16] we have designed two types of complementary supramolecular co-monomers that are based on small β-sheet-encoded synthons,[14b,17] that is, pentapeptides. The two co-monomers incorporate complementary sites of interaction (pairs of acid/base or cation/anion functionalities) that are embedded in the pentapeptide sequences through alternating hydrophobic/hydrophilic amino acids (Figure 1).[13,18] The critical peptide length for aggregation can be overcome by attaching each of the small peptide strands to a C_3-symmetric scaffold. In addition, the dendritic peptide amphiphile codes for one-dimensional (1D) noncovalent polymerization, which is known to occur for amphiphilic C_3-symmetric supramolecular monomers,[19] and avoids the formation of 2D sheets or micellar aggregates. Our strategy is thereby complementary to the self-assembly of zwitterions developed by Schmuck and co-workers,[1b,20] and the self-assembly of coiled-coil peptide motifs, which are stabilized by Coulombic interactions embedded in polar residues within their hydrophobic interfaces, as reported by Woolfson and co-workers.[21]

We investigated the aggregation behavior of the co-monomers **1a** and **1b** in aqueous solution by using circular dichroism (CD) spectroscopy (Figure 2). CD spectroscopy is a powerful tool to investigate the order and self-assembly of peptide amphiphiles and chiral supramolecular polymers.[14a,22] The CD spectra of the individual co-monomers **1a** and **1b** in 20 mM phosphate buffer (pH 6.1) both exhibited a strong negative CD band at around 198 nm and a very weak positive band at around 210 nm. These CD features are characteristic of a lack of secondary structure, a state usually referred to as a random coil (Figure 2A)[23] and provides a good indication that both anionic and cationic monomers do not spontaneously aggregate in buffered environments (Figure 2B). It should be noted that homopolymerization does not take place since hydrogen bonding cannot overcome the intermolecular repulsive electrostatic forces of the six formal charges per co-monomer, even if the net charge of the co-monomers is reduced by acidifying a solution of **1a** or increasing the pH value of a solution of **1b** (see Figure S1 in the Supporting Information). However, when mixing the two co-monomers in a 1:1 ratio, the CD spectrum changed dramatically and showed a strong positive band at around 195 nm and a strong negative CD band at around 210 nm. The solutions were sonicated for one minute after mixing to avoid

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Supporting information for this article (synthetic procedures, material characterization, as well as full details about the instrumentation and theoretical calculations) is available on the WWW under http://dx.doi.org/10.1002/anie.201303810.

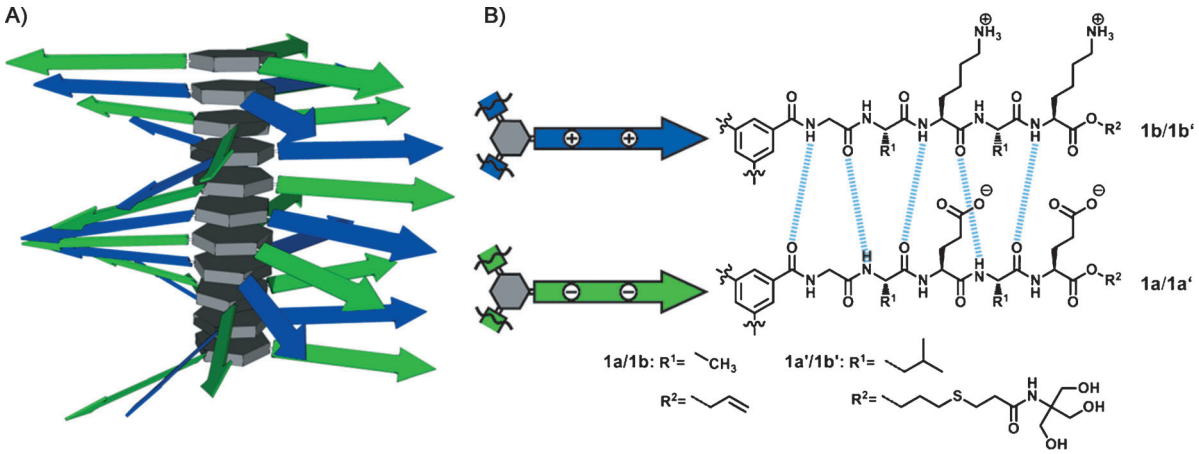

Figure 1. A) Schematic representation of the supramolecular copolymer and B) the anionic/cationic β-sheet-encoded dendritic co-monomers **1a**/**1b** and **1a**′/**1b**′.

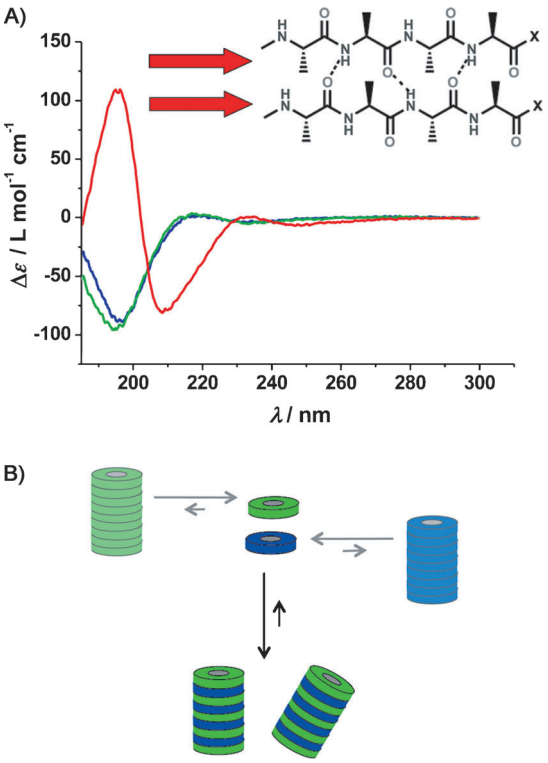

Figure 2. A) CD spectra of solutions of the dendritic co-monomers **1a** (green curve), **1b** (blue curve), and a 1:1 mixture of **1a**/**1b** (red curve). All measurements are performed at dendritic peptide amphiphile concentrations of 0.1 mM in 20 mM phosphate buffer (pH 6.1) at 293 K. B) Schematic representation showing the lack of supramolecular homopolymerization in isolated solutions of **1a** and **1b**, and the β-sheet-directed heteropolymerization of 1:1 mixtures of **1a** and **1b** into supramolecular copolymers.

kinetic traps in the assembly process. The observed bands are both highly characteristic of the secondary structure of a hydrogen-bonded parallel β-sheet (Figure 2A).[24] Mixing the oppositely charged co-monomers in a 1:1 ratio screens the anionic and cationic charges and initiates the supramolecular copolymerization. This is a cooperative process, where attractive Coulomb interactions reinforce parallel β-sheet-encoded hydrogen bonding to form copolymer architectures.

High-resolution transmission electron microscopy (HRTEM) experiments were performed to further confirm the supramolecular copolymerization of **1a** and **1b** into 1D morphologies (Figure 3, see also Figure S2 in the Supporting

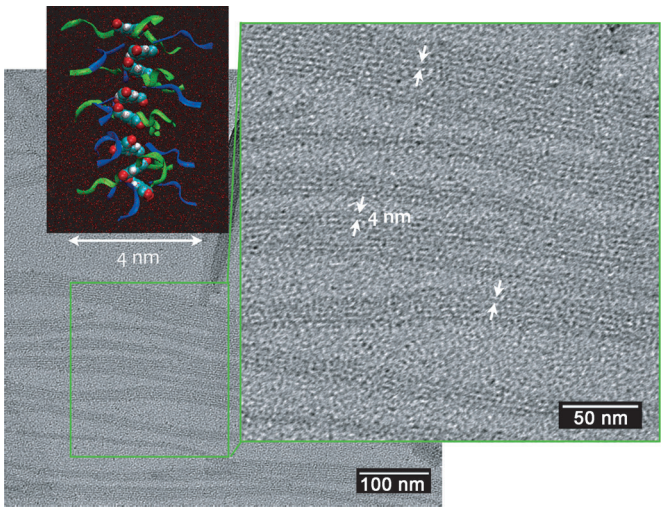

Figure 3. HRTEM images of the self-assembled dendritic peptide amphiphiles **1a** and **1b** [the co-monomers were mixed in a ratio of 1:1 at a total concentration of 0.1 mM in 20 mM phosphate buffer (pH 6.1) and then deposited onto the TEM grid].

Information). After depositing solutions of 1:1 mixtures of the co-monomers onto TEM grids, long anisotropic nanorods could be observed. The 4 nm width of the nanorods correlates well with the diameter of the co-monomers. They appear to aggregate laterally, most likely through templating effects and drying effects on the TEM grid. Remarkably, they could be visualized without the addition of staining agent, which is usually required to enhance contrast in organic soft matter. This is a further indication that the β-sheet-encoded core of the peptidic nanorods is densely packed as a result of the β-sheet-encoded secondary structure.

Cross-polarization magic-angle spinning (CPMAS) ^{13}C NMR spectroscopy was used to identify the β-sheet content in the supramolecular copolymers. It is known for polypeptides in particular that the chemical shift of carbonyl carbon atoms obtained by CPMAS ^{13}C NMR spectroscopy strongly depends on the secondary structure and is, therefore, highly diagnostic.[25] A freeze-dried solution of 1:1 mixtures of co-monomers **1a** and **1b** shows a chemical shift of $\delta = 172 \pm 1$ ppm for the carbonyl carbon atom (see Figure S3 in the Supporting Information), which is in excellent agreement with reported values for β-sheet-ordered oligoalanines, -lysines, and -glutamic acids.[26] Except for the aromatic amide signal, no other carbonyl signal was observed in the low field region; ^{13}C carbonyl shifts would have been expected in this region for random-coil arrangements.[26] Overall, the solution-based CD experiments and CPMAS ^{13}C NMR spectroscopic analysis in the solid state confirm that all the peptide side arms in the supramolecular copolymers are packed into ordered parallel β-sheet conformations.

In addition to experimental evidence, we performed theoretical calculations to correlate the hierarchical copolymerization of **1a**/**1b**. A solvated octameric model consisting of the co-monomer pairs of **1a**/**1b** was investigated by performing a molecular dynamics (MD) simulation (see Figure S4 in the Supporting Information). The supramolecular copolymer maintained a stable 1D columnar conformation throughout the MD simulation, and geometric information about specific intermolecular interactions in the assembly was averaged over the trajectory. Intriguingly, the aromatic core did not form a regular stack, as evident by the erratic values for the stack height as well as the tilt and slip parameters (see Figures S5 and S6 in the Supporting Information).

Therefore, according to the MD simulations, π-π stacking and hydrophobic effects of the small aromatic core do not act as primary stabilizing interactions for the supramolecular copolymerization of **1a**/**1b**. The averaged stack heights (core–core distance) from the MD simulations (ca. 4.5 Å) are significantly greater than the heights determined by gas-phase semiempirical optimizations (ca. 3.5 Å), or indeed the hybrid DFT/MM gas-phase optimizations (ca. 3.8 Å) in the gas phase. Snapshots of the MD simulations show water molecules intercalated between the aromatic cores which elongate the aromatic core–core distances. In contrast to the absence of interactions involving the cores, hydrogen-bonding interactions as well as salt bridges could be observed in the peptide side arms of the aggregates (see the Supporting Information).

These results further support the above proposed mechanism: the self-assembly is induced in a cooperative fashion through a combination of attractive Coulomb forces and parallel β-sheet-directed hydrogen bonding, which are encoded in the supramolecular synthons. Note that we use the term cooperative self-assembly in a broad sense to describe an aggregation process that is favored because of a number of noncovalent interactions originating from each of the co-monomers. As a consequence of a lack of statistical thermodynamic theory for supramolecular 1D copolymerizations, we have not yet been able to quantify the degree of cooperativity involved in the hierarchical multicomponent self-assembly process.[10b,c]

A particularly attractive feature of weakly basic or weakly acidic self-assembled peptide materials is the ability to reversibly change their ionic character by adjusting the pH value and thereby switching the material properties on and off.[9b,10a,d,19c,20,27] The supramolecular copolymers reported here are unique because of their ampholytic character: containing both basic and acidic side groups in the same self-assembled macromolecule. We performed CD experiments at different pH values to investigate the dual responsiveness of the coaggregates based on **1a** and **1b** (Figure 4). The recorded spectra in a medium pH range of pH 6.1–7.0 were characteristic of β-sheet-ordered supramolecular copolymers. Lowering the pH value to 3.7 or

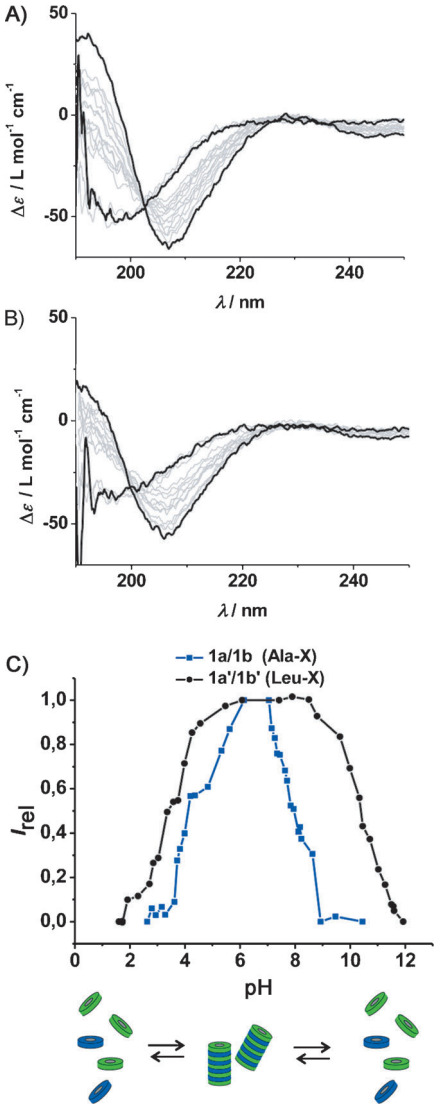

Figure 4. CD spectra of a 1:1 mixture of the co-monomers **1a**/**1b** at variable pH values: A) Basic titration and B) acidic titration (the thick black lines represent the start and end points of the titrations); C) the corresponding normalized data and schematic representation of the pH stability window of the supramolecular copolymers [blue data points = **1a**/**1b** ($\lambda = 207$ nm), black data points = **1a′**/**1b′** ($\lambda = 215$ nm)].

increasing it to 8.6 results in both bands characteristic of parallel β-sheets (at around 195 nm and 210 nm) becoming weaker but they do not disappear. Only at pH 3.6 and pH 8.9 is the negative band at 198 nm that is observed for the isolated solutions of **1a** and **1b** restored, which is characteristic of polypeptides lacking any secondary structure. Unlike conventional, single-component self-assembled weakly acid or weakly basic amphiphilic peptides that aggregate when the pH values are decreased or increased, respectively, the reported ampholytic supramolecular copolymers reversibly assemble at a close to neutral pH value and disassemble under acidic and basic conditions.[28] While the acidic pH switch seems to be defined by the pK_a value of the glutamic acid residues of around 4, the basic switch is about 1.5 pH units lower than expected for isolated lysine side chains. Boden and co-workers have reported similar findings for self-assembling glutamic acid and ornithine-based peptides, where the decrease in the apparent pK_a value of the basic amino acid most likely arises from repulsive Coulomb interactions between neighboring positively charged side chains.[29] Our system is a unique case of a supramolecular coassembly process that is switched on at a physiologically relevant pH range and can be switched off sharply when leaving this pH window.

The triggered disassembly of surface-functionalized polycationic scaffolds into low-molecular-weight building blocks is an attractive strategy for the development of oligonucleotide delivery vehicles,[2b,30] where the pH-induced disassembly of the polycationic architecture and the loss of multivalent points of interaction would lead to the decomplexation and release of the oligonucleotide. The triggered disaggregation of the self-assembled polycations could furthermore reduce their toxicity.

To further demonstrate that the pH window where self-assembly is switched on, and therefore the stability of the supramolecular copolymers, can be tuned and manipulated by adjusting the hydrophobicity of the co-monomers, we investigated the aggregation behavior of the more hydrophobic peptide sequences Gly-Leu-Glu-Leu-Glu and Gly-Leu-Lys-Leu-Lys embedded in the co-monomers **1a′** and **1b′**. Analogous to **1a** and **1b**, CD-spectroscopic investigations of the isolated monomer solutions of **1a′** and **1b′** led to CD bands characteristic of random coil structures. A 1:1 mixture of **1a′** and **1b′** in phosphate buffer (pH 6.1) gave rise to a significant change in the CD signature, where the negative CD band at 198 nm shifted to 215 nm and the positive one at around 210 nm disappeared (see Figure S8 A in the Supporting Information). In the case of these significantly more hydrophobic supramolecular aggregates, the addition of 37.5 vol % acetonitrile led to a complete disappearance of the aggregate-specific CD band at 215 nm (see Figure S8 B,C in the Supporting Information).[19a] Combined, these results confirm that **1a′** and **1b′** copolymerize in a neutral buffer; the observed CD signature is shifted relative to that of the **1a**/**1b** copolymers which is most probably due to the sterically more demanding leucine amino acids which lead to a more pronounced twist in the parallel β-sheet secondary structure.[14a] Interestingly, pH-dependent CD measurements show that the distinct shift from the coaggregate to the monomer bands can also be achieved by either increasing or decreasing the pH value. In contrast to the co-assembly of **1a** and **1b**, the pH window where self-assembly is switched on is now increased, and full disassembly is observed at pH 2 and pH 12 (Figure 4; see also Figure S8 D,E in the Supporting Information). The supramolecular copolymers become thermodynamically more stable because of the more pronounced hydrophobic effects of the β-sheet-encoded peptide sequences when six alanine residues in **1a**/**1b** were replaced by six leucine residues in **1a′**/**1b′**. The increased aggregate stability is responsible for the shift in the apparent pK_a values of the oligoglutamic acids in **1a′** and protonated oligolysines in **1b′**. This is in agreement with recent studies by Goldberger and co-workers on linear peptide amphiphiles that show a pH shift in the self-assembly transition depending on the β-sheet propensity of the amino acid sequence in their β-sheet domain.[27a]

In conclusion, we have synthesized β-sheet-encoded anionic and cationic supramolecular co-monomers. These dendritic peptide amphiphiles, when self-assembled from a 1:1 monomer feed ratio, form supramolecular copolymers through a combination of Coulomb attractive forces and hydrogen-bonding interactions. The obtained ampholytic supramolecular materials have been designed for on-off polymerization in response to pH triggers. Unlike most single-component self-assembled peptide materials, the unique hierarchical copolymerization process of our aggregates is operative in a physiologically relevant pH range. Decreasing or increasing the pH value outside of this pH window results in polymerization being switched off and disassembly occurs through the loss of the salt bridges of the peptide side arms. The use of a supramolecular engineering approach enables the pH window to be increased by enhancing the affinity of the supramolecular co-monomers. We are currently exploring applications where multivalency, bioactivity, and release of cargo can be switched on and off in a triggered fashion; responsiveness to physicochemical or biological stimuli is of particular interest for applications in molecular imaging as well as drug and oligonucleotide delivery vehicles.

Experimental Section

The ^{13}C CP-MAS NMR spectrum was collected at 125.8 MHz (Bruker Avance 500 spectrometer) using a commercial Bruker 2.5 mm double resonance MAS probe spinning at 25 kHz, applying a π/2 pulse length of 3.5 μs, a recycle delay of 10 s, a CP contact time of 3.5 ms, and 16384 transients.[31] CD spectra were recorded on a J-815 (JASCO) spectrometer. The HRTEM measurements were carried out on a Libra 200 FE TEM (Zeiss) equipped with a field emission gun operating at 200 kV.

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[1] a) T. Aida, E. W. Meijer, S. I. Stupp, *Science* **2012**, *335*, 813 – 817; b) T. Fenske, H.-G. Korth, A. Mohr, C. Schmuck, *Chem. Eur. J.* **2012**, *18*, 738 – 755; c) F. B. L. Cougnon, J. K. M. Sanders, *Acc. Chem. Res.* **2012**, *45*, 2211 – 2221; d) E. Krieg, B. Rybtchinski, *Chem. Eur. J.* **2011**, *17*, 9016 – 9026; e) R. J. Wojtecki, M. A. Meador, S. J. Rowan, *Nat. Mater.* **2011**, *10*, 14 – 27; f) J.-M. Lehn, *Chem. Soc. Rev.* **2007**, *36*, 151 – 160.

[2] a) T. Hirose, F. Helmich, E. W. Meijer, *Angew. Chem.* **2013**, *125*, 322 – 327; *Angew. Chem. Int. Ed.* **2013**, *52*, 304 – 309; b) S. K. M. Nalluri, J. Voskuhl, J. B. Bultema, E. J. Boekema, B. J. Ravoo, *Angew. Chem.* **2011**, *123*, 9921 – 9925; *Angew. Chem. Int. Ed.* **2011**, *50*, 9747 – 9751; c) T. Muraoka, C.-Y. Koh, H. Cui, S. I. Stupp, *Angew. Chem.* **2009**, *121*, 6060 – 6063; *Angew. Chem. Int. Ed.* **2009**, *48*, 5946 – 5949.

[3] a) J. M. A. Carnall, C. A. Waudby, A. M. Belenguer, M. C. A. Stuart, J. J.-P. Peyralans, S. Otto, *Science* **2010**, *327*, 1502 – 1506; b) P. Cordier, F. Tournilhac, C. Soulié-Ziakovic, L. Leibler, *Nature* **2008**, *451*, 977 – 980.

[4] R. J. Williams, A. M. Smith, R. Collins, N. Hodson, A. K. Das, R. V. Ulijn, *Nat. Nanotechnol.* **2009**, *4*, 19 – 24.

[5] S. Burattini, B. W. Greenland, D. H. Merino, W. Weng, J. Seppala, H. M. Colquhoun, W. Hayes, M. E. Mackay, I. W. Hamley, S. J. Rowan, *J. Am. Chem. Soc.* **2010**, *132*, 12051 – 12058.

[6] a) A. Samanta, M. C. A. Stuart, B. J. Ravoo, *J. Am. Chem. Soc.* **2012**, *134*, 19909 – 19914; b) C. Kim, S. S. Agasti, Z. Zhu, L. Isaacs, V. M. Rotello, *Nat. Chem.* **2010**, *2*, 962 – 966; c) R. Klajn, M. A. Olson, P. J. Wesson, L. Fang, A. Coskun, A. Trabolsi, S. Soh, J. F. Stoddart, B. A. Grzybowski, *Nat. Chem.* **2009**, *1*, 733 – 738.

[7] a) D. H. Lee, J. R. Granja, J. A. Martinez, K. Severin, M. R. Ghadiri, *Nature* **1996**, *382*, 525 – 528; b) T. Tjivikua, P. Ballester, J. Rebek, *J. Am. Chem. Soc.* **1990**, *112*, 1249 – 1250.

[8] M. Yin, J. Shen, W. Pisula, M. Liang, L. Zhi, K. Müllen, *J. Am. Chem. Soc.* **2009**, *131*, 14618 – 14619.

[9] a) J. B. Matson, R. H. Zha, S. I. Stupp, *Curr. Opin. Solid State Mater. Sci.* **2011**, *15*, 225 – 235; b) A. R. Hirst, B. Escuder, J. F. Miravet, D. K. Smith, *Angew. Chem.* **2008**, *120*, 8122 – 8139; *Angew. Chem. Int. Ed.* **2008**, *47*, 8002 – 8018.

[10] a) M. D. Segarra-Maset, V. J. Nebot, J. F. Miravet, B. Escuder, *Chem. Soc. Rev.* **2013**, DOI: 10.1039/C1032CS35436E.; b) T. F. A. de Greef, M. M. J. Smulders, M. Wolffs, A. P. H. J. Schenning, R. P. Sijbesma, E. W. Meijer, *Chem. Rev.* **2009**, *109*, 5687 – 5754; c) Z. Chen, A. Lohr, C. R. Saha-Möller, F. Würthner, *Chem. Soc. Rev.* **2009**, *38*, 564 – 584; d) R. J. Mart, R. D. Osborne, M. M. Stevens, R. V. Ulijn, *Soft Matter* **2006**, *2*, 822 – 835.

[11] a) R. V. Ulijn, A. M. Smith, *Chem. Soc. Rev.* **2008**, *37*, 664 – 675; b) G. A. Silva, C. Czeisler, K. L. Niece, E. Beniash, D. A. Harrington, J. A. Kessler, S. I. Stupp, *Science* **2004**, *303*, 1352 – 1355; c) S. Zhang, *Biotechnol. Adv.* **2002**, *20*, 321 – 339; d) J. D. Hartgerink, E. Beniash, S. I. Stupp, *Science* **2001**, *294*, 1684 – 1688.

[12] M. R. Ghadiri, J. R. Granja, L. K. Buehler, *Nature* **1994**, *369*, 301 – 304.

[13] N. Sakai, J. Mareda, S. Matile, *Acc. Chem. Res.* **2008**, *41*, 1354 – 1365.

[14] a) E. T. Pashuck, H. Cui, S. I. Stupp, *J. Am. Chem. Soc.* **2010**, *132*, 6041 – 6046; b) L. C. Palmer, Y. S. Velichko, M. Olvera de La Cruz, S. I. Stupp, *Philos. Trans. R. Soc. A* **2007**, *365*, 1417 – 1433; c) H. A. Behanna, J. J. J. M. Donners, A. C. Gordon, S. I. Stupp, *J. Am. Chem. Soc.* **2005**, *127*, 1193 – 1200.

[15] a) M. Peterca, M. R. Imam, C.-H. Ahn, V. S. K. Balagurusamy, D. A. Wilson, B. M. Rosen, V. Percec, *J. Am. Chem. Soc.* **2011**, *133*, 2311 – 2328; b) V. Percec, A. E. Dulcey, V. S. K. Balagurusamy, Y. Miura, J. Smidrkal, M. Peterca, S. Nummelin, U. Edlund, S. D. Hudson, P. A. Heiney, H. Duan, S. N. Magonov, S. A. Vinogradov, *Nature* **2004**, *430*, 764 – 768.

[16] a) S. Litvinchuk, H. Tanaka, T. Miyatake, D. Pasini, T. Tanaka, G. Bollot, J. Mareda, S. Matile, *Nat. Mater.* **2007**, *6*, 576 – 580; b) S. Litvinchuk, G. Bollot, J. Mareda, A. Som, D. Ronan, M. R. Shah, P. Perrottet, N. Sakai, S. Matile, *J. Am. Chem. Soc.* **2004**, *126*, 10067 – 10075.

[17] J. J. L. M. Cornelissen, J. J. J. M. Donners, R. de Gelder, W. S. Graswinckel, G. A. Metselaar, A. E. Rowan, N. A. J. M. Sommerdijk, R. J. M. Nolte, *Science* **2001**, *293*, 676 – 680.

[18] H. Frauenrath, E. Jahnke, *Chem. Eur. J.* **2008**, *14*, 2942 – 2955.

[19] a) P. Besenius, G. Portale, P. H. H. Bomans, H. M. Janssen, A. R. A. Palmans, E. W. Meijer, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 17888 – 17893; b) M. K. Müller, L. Brunsveld, *Angew. Chem.* **2009**, *121*, 2965 – 2968; *Angew. Chem. Int. Ed.* **2009**, *48*, 2921 – 2924; c) K. J. C. van Bommel, C. van der Pol, I. Muizebelt, A. Friggeri, A. Heeres, A. Meetsma, B. L. Feringa, J. van Esch, *Angew. Chem.* **2004**, *116*, 1695 – 1699; *Angew. Chem. Int. Ed.* **2004**, *43*, 1663 – 1667.

[20] G. Gröger, W. Meyer-Zaika, C. Böttcher, F. Gröhn, C. Ruthard, C. Schmuck, *J. Am. Chem. Soc.* **2011**, *133*, 8961 – 8971.

[21] a) F. Thomas, A. L. Boyle, A. J. Burton, D. N. Woolfson, *J. Am. Chem. Soc.* **2013**, *135*, 5161 – 5166; b) J. M. Fletcher, R. L. Harniman, F. R. H. Barnes, A. L. Boyle, A. Collins, J. Mantell, T. H. Sharp, M. Antognozzi, P. J. Booth, N. Linden, M. J. Miles, R. B. Sessions, P. Verkade, D. N. Woolfson, *Science* **2013**, *340*, 595 – 599.

[22] a) P. A. Korevaar, S. J. George, A. J. Markvoort, M. M. J. Smulders, P. A. J. Hilbers, A. P. H. J. Schenning, T. F. A. De Greef, E. W. Meijer, *Nature* **2012**, *481*, 492 – 496; b) L. Whitmore, B. A. Wallace, *Biopolymers* **2008**, *89*, 392 – 400; c) P. Jonkheijm, P. van der Schoot, A. P. H. J. Schenning, E. W. Meijer, *Science* **2006**, *313*, 80 – 83.

[23] W. C. Johnson, I. Tinoco, *J. Am. Chem. Soc.* **1972**, *94*, 4389 – 4390.

[24] S. Brahms, J. Brahms, G. Spach, A. Brack, *Proc. Natl. Acad. Sci. USA* **1977**, *74*, 3208 – 3212.

[25] G. Floudas, H. W. Spiess, *Macromol. Rapid Commun.* **2009**, *30*, 278 – 298.

[26] H. R. Kricheldorf, D. Mueller, *Macromolecules* **1983**, *16*, 615 – 623.

[27] a) A. Ghosh, M. Haverick, K. Stump, X. Yang, M. F. Tweedle, J. E. Goldberger, *J. Am. Chem. Soc.* **2012**, *134*, 3647 – 3650; b) F. Versluis, I. Tomatsu, S. Kehr, C. Fregonese, A. W. J. W. Tepper, M. C. A. Stuart, B. J. Ravoo, R. I. Koning, A. Kros, *J. Am. Chem. Soc.* **2009**, *131*, 13186 – 13187; c) H. G. Börner, H. Schlaad, *Soft Matter* **2007**, *3*, 394 – 408; d) H. Dong, S. E. Paramonov, L. Aulisa, E. L. Bakota, J. D. Hartgerink, *J. Am. Chem. Soc.* **2007**, *129*, 12468 – 12472.

[28] D. W. P. M. Löwik, E. H. P. Leunissen, M. van den Heuvel, M. B. Hansen, J. C. M. van Hest, *Chem. Soc. Rev.* **2010**, *39*, 3394 – 3412.

[29] A. Aggeli, M. Bell, L. M. Carrick, C. W. G. Fishwick, R. Harding, P. J. Mawer, S. E. Radford, A. E. Strong, N. Boden, *J. Am. Chem. Soc.* **2003**, *125*, 9619 – 9628.

[30] a) A. Barnard, D. K. Smith, *Angew. Chem.* **2012**, *124*, 6676 – 6685; *Angew. Chem. Int. Ed.* **2012**, *51*, 6572 – 6581; b) S. P. Jones, N. P. Gabrielson, D. W. Pack, D. K. Smith, *Chem. Commun.* **2008**, 4700 – 4702; c) D. Joester, M. Losson, R. Pugin, H. Heinzelmann, E. Walter, H. P. Merkle, F. Diederich, *Angew. Chem.* **2003**, *115*, 1524 – 1528; *Angew. Chem. Int. Ed.* **2003**, *42*, 1486 – 1490.

[31] M. Khan, V. Enkelmann, G. Brunklaus, *J. Am. Chem. Soc.* **2010**, *132*, 5254 – 5263.

