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Dendritic Amphiphiles

Steric Constraints Induced Frustrated Growth of Supramolecular Nanorods in Water

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Abstract: A unique example of supramolecular polymerisation in water based on monomers with nanomolar affinities, which yield rod-like materials with extraordinarily high thermodynamic stability, yet of finite length, is reported. A small library of charge-neutral dendritic peptide amphiphiles was prepared, with a branched nonaphenylalanine-based core that was conjugated to hydrophilic dendrons of variable steric demand. Below a critical size of the dendron, the monomers assemble into nanorod-like polymers, whereas for larger dendritic side chains frustrated growth into near isotropic particles is observed. The supramolecular morphologies observed by electron microscopy, X-ray scattering and

diffusion NMR spectroscopy studies are in agreement with the mechanistic insights obtained from fitting polymerisation profiles: non-cooperative isodesmic growth leads to degrees of polymerisation that match the experimentally determined nanorod contour lengths of close to 70 nm. The reported designs for aqueous self-assembly into well-defined anisotropic particles has promising potential for biomedical applications and the development of functional supramolecular biomaterials, with emerging evidence that anisotropic shapes in carrier design outperform conventional isotropic materials for targeted imaging and therapy.

Introduction

Exploiting supramolecular chemistry and the toolbox of the non-covalent bond is the basis for advancing the field of molecular self-assembly into functional nanostructures.^[1] In water, the key to success in obtaining robust and stable morpholo-

gies lies in the use of hydrogen bonding, Coulombic forces, π - π and dispersive interactions, in combination with solvophobic shielding.^[2] The aqueous supramolecular materials produced are promising for biological and biomedical functions, in the design of delivery vehicles^[3] or supports for regenerative medicine.^[4] A largely underestimated design criterion for the production of supramolecular nanostructures is the combination of attractive and repulsive forces, which have been postulated to be crucial to obtain materials of well-defined shape and size.^[5] In view of biomedical applications, shape anisotropy and a high aspect ratio of nanoscale vehicles is increasingly recognised as a beneficial design parameter that determines the efficacy in targeted imaging and therapeutic applications.^[6] Filament-like micelles have been reported with blood circulation half-times that are up to 10 times larger than those of spherical morphologies;^[7] phagocytosis, as well as margination and adhesion to blood vessel walls are known to reduce residence times in the blood vasculature, and have been shown to be shape dependent;^[8] most recently the Stupp laboratory disclosed the design of shape-dependent targeting of nanostructures to injured vasculature or delivery to tumour tissues.^[9]

We hereby report the self-assembly of neutral amphiphilic dendritic^[10] nonaphenylalanines into supramolecular nanorods in water (Figure 1). By equipping the hydrophobic peptide-based core with tetraethylene glycol (TEG)-functionalised dendrons with increasing size, we impose steric constraints into the 1D self-assembly encoded in the dendritic peptide sequence. By using a wide range of experimental techniques, cryogenic transmission electron microscopy (cryo-TEM), small-

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Figure 1. Schematic representation of the aqueous self-assembly of dendritic nonaphenylalanine amphiphiles into supramolecular nanorods.

angle X-ray scattering (SAXS) and pulsed-field gradient (PFG) NMR spectroscopy, we show that nanorods are obtained in aqueous buffer. Circular dichroism (CD) spectroscopy based investigations of the polymerisation mechanism reveal a process that is non-cooperative in nature and a remarkably high nanomolar binding affinity of the monomers. The combination of both properties leads to very stable, albeit not infinitely long, supramolecular nanorods, which are typically observed for co-operative 1D polymerisations.^[11] Importantly, when the size of the hydrophilic dendron becomes too large, we observe limited 1D growth, in line with morphological investigations: this produces objects that are restricted in size and globular in shape. We have referred to such systems as frustrated growth.^[5] However, whereas the majority of reports, to date, rely on Coulombic repulsive forces, induced frustrated growth of 1D supramolecular nanostructures based on steric constraints remain very rare.^[5a,i,l]

Results and Discussion

Synthesis

The synthesis of dendritic amphiphiles **1.D₃**, **2.D₄** and **2.D₈** (Scheme 1) is based on the facile, high yielding and gram-scale preparation of 9-fluorenylmethoxycarbonyl (Fmoc)-triphenylalanine allyl ester through Fmoc solid-phase peptide synthesis. After removal of the protecting group, the triphenylalanine allyl ester was coupled to benzenetricarbonyl chloride to build the nonaphenylalanine hydrophobic and dendritic core of the self-assembly unit in a convergent methodology. Thiol–ene radical addition^[12] to the allyl ester moiety was subsequently performed. Prior to attaching the peripheral dendrons, the addition of mercaptopropionic acid led to the key electrophilic building block **1**, whereas the addition of aminoethanethiol led to a nucleophilic building block **2**. Using standard peptide coupling reagents, we finally attached the water-solubilising dendrons with an increasing number of TEG chains per peptide side arm. Newkome dendron **D₃**,^[13] with three TEG chains, was coupled to core **1** to obtain **1.D₃**. Dendrons **D₄** and **D₈**, based on the 2,2-bis-methylolpropionic acid (bis-MPA) dendritic scaffold and appended with four and eight TEG chains, respectively, were coupled to molecule **2** to obtain the amphiphilic dendrimers **2.D₄** and **2.D₈**.

Morphological investigations

The three supramolecular monomers **1.D₃**, **2.D₄** and **2.D₈** were highly water soluble. Using cryo-TEM, we were able to visualise the supramolecular polymers formed by the self-assembly of monomers **1.D₃** and **2.D₄** in aqueous buffer (20 mM phosphate buffer at pH 7.4): anisotropic objects with a thickness of about 5.5 nm and contour lengths in the order of 100 nm can be observed at concentrations of 5 mg mL^{−1} (Figure 2). The former

Scheme 1. Chemical structures of the supramolecular monomers **1.D₃**, **2.D₄** and **2.D₈**: the dendritic nonaphenylalanine hydrophobic cores **1** and **2** are equipped with TEG-functionalised dendrons of variable size (**D₃**, **D₄** and **D₈**).

Figure 2. Cryo-TEM micrographs for the self-assembled dendritic peptide amphiphiles **1.D₃** (A, B) and **2.D₄** (C, D) in 20 mM phosphate buffer (pH 7.4), with monomer concentrations of 5 mg mL⁻¹ and vitrified at 293 K, at magnifications of 45 × 10³ (A and C; scale bar 200 nm) and 60 × 10³ (B and D; scale bar 100 nm).

value agrees with the expected thickness based on the length of the stretched out hydrophobic side arm of 3.1 nm for building block **1.D₃** and 3.3 nm for **2.D₄**. This confirms that 1D polymerisation takes place to yield rigid, non-flexible cylinders, which we refer to as nanorods. However, cryo-TEM investigations did not allow us to extract concentration-dependent contour lengths of the rods; one limitation in the case of monomer **1.D₃** appeared to be the tendency for the self-assembled nanorods to form densely packed films during vitrification in sample preparation. We therefore turned to synchrotron SAXS to complement the microscopic data with a detailed insight into the morphologies of the supramolecular assemblies of

1.D₃, **2.D₄** and **2.D₈** in aqueous solution. Figure 3 summarises the concentration-dependent scattering profiles of all dendritic peptides in 20 mM phosphate buffer (pH 7.4). In the case of **1.D₃** and **2.D₄**, the hallmark of rigid cylindrical structures—a power-law regime of $I \propto q^{-1}$ at intermediate q values—can be observed in the SAXS profiles. These scattering curves are described with a form factor for homogeneous cylinders (Figure 3A and B), with a cross-sectional diameter that agrees well with the thickness observed in the cryo-TEM micrographs. Towards lower q values the SAXS profiles tended to level off, which allowed us to obtain an estimate for the length, L , of the nanorods. Upon reducing the **1.D₃** and **2.D₄** monomer concentrations from 0.5 to 0.125 mM, we observed a shortening of the nanorods. This dilution-induced decrease in the average molecular weight of the two different polymers is expected for an equilibrium-type supramolecular polymer.^[11] By resolving the lengths of the nanorods, differences in supramolecular polymers obtained from monomers with the smallest and medium-sized dendron, **1.D₃** and **2.D₄**, respectively, become apparent. At a concentration of 0.5 mM, dendron **1.D₃** gives rise to SAXS profiles that can be fitted with a nanorod contour length of $L = 63 \pm 6$ nm, which is reduced to $L = 35 \pm 4$ nm by dilution to 0.125 mM (Figure S3A in the Supporting Information). The cross-sectional diameter (d_{CS}) of 5.6 ± 0.4 nm agrees very well with the thickness observed in the cryo-TEM micrographs. In the case of **2.D₄**, the same monomer concentrations led to profiles that corresponded to nanorods of $L = 46 \pm 5$ and 36 ± 4 nm, respectively, with $d_{CS} = 6.1 \pm 0.4$ nm (Figure S3B in the Supporting Information). The concentration-dependent SAXS profiles of the dendritic amphiphile equipped with the largest hydrophilic peripheral dendron, **2.D₈**, show a plateau in the small q regime, which is indicative of very small dimensions (Figure 3C). These scattering profiles are best described with a form factor for a cylinder with an elliptical cross-section, which yields lengths, L , ranging from 1.8 ± 0.2 to 2.2 ± 0.2 nm (Figure S3B in the Supporting Information). Thus, the SAXS data of **2.D₈** provides the first indication that self-assembly is dominated by frustrated growth. Much smaller nanostructures are therefore obtained compared with the anisotropic rod-like assemblies of **1.D₃** and **2.D₄**.

To corroborate the morphological characterisation by electron microscopy and SAXS, we performed PFG-NMR spectroscopy experiments.^[14] PFG spin-echo techniques provide diffusion coefficients for molecules and their assemblies that are related to their hydrodynamic radii, and therefore, shapes and sizes. The quantitative analysis of the PFG-NMR spectroscopy decay curves for solutions of either monomeric or self-assembled polymeric **1.D₃**, **2.D₄** and **2.D₈** in various solvent conditions was performed by using exponential or biexponential fits,^[15] or a stretched exponential distribution model (see the Supporting Information).^[16] The supramolecular polymerisation of **1.D₃**, **2.D₄** and **2.D₈** was analysed in buffered D₂O, in CD₃CN/D₂O (Figure S4 in the Supporting Information) and in mixtures of CD₃OD/D₂O (Figure 5 and Figure S5 in the Supporting Information). Organic solvents, such as acetonitrile and methanol, are known to disrupt supramolecular polymerisation in water by diminishing the hydrophobic shielding of the hydrogen-

Figure 3. SAXS profiles (symbols) and fits with a form factor describing rigid, homogeneous cylinders (lines) for the dendritic peptide amphiphiles **1.D₃** (A) and **2.D₄** (B), and a form factor to describe elliptical cylinders for **2.D₈** (C); all scattering profiles were collected in 20 mM phosphate buffer at pH 7.4 at 293 K at monomer concentrations of 0.5 (top data points), 0.25 and 0.125 mM (bottom data points).

bonding amide sequences.^[5e,17] In a 3:1 CD₃CN/D₂O solvent composition, self-assembly does not take place and all three monomers have similar diffusion coefficients between 2.1×10^{-10} and 2.2×10^{-10} m² s⁻¹. These results correspond to globular objects with a hydrodynamic radius of 1.9 ± 0.2 nm.

In buffered D₂O, significant differences become apparent: first, the diffusion coefficients for self-assembled polymeric **1.D₃** and **2.D₄** are $1.29 \pm 0.13 \times 10^{-11}$ and $1.26 \pm 0.13 \times 10^{-11}$ m² s⁻¹, respectively. These are one order of magnitude smaller than those in the mixture of CD₃CN/D₂O. The size of the nanorod-like aggregates was calculated by using a modified Stokes–Einstein equation (see the Supporting Information) with the Tirado and Garcia de la Torre model, which holds for aspect ratios of $2 < L_H/d_H < 30$ (in which d_H and L_H are the hydrodynamic diameter and length, respectively, of the rod-like object).^[18] Based on the expected molecular thickness of the dendritic amphiphiles, we estimate $d_H = 9.8$ nm for **1.D₃** and $d_H = 10.6$ nm for **2.D₄**, to obtain an average nanorod length of $L_H = 67 \pm 7$ nm for **1.D₃** and $L_H = 65 \pm 9$ nm for **2.D₄**, both at 1 mM monomer concentrations. This trend is in agreement with the cryo-TEM and SAXS data, with which slightly longer polymers were observed for **1.D₃** compared with those of **2.D₄**. The second difference for the measured diffusion coefficients in buffered D₂O compared with the CD₃CN/D₂O solvent mixture is the value obtained for self-assembled **2.D₈** $D = 2.90 \pm 0.29 \times 10^{-11}$ m² s⁻¹, which is 2.3 times larger than that of polymeric **2.D₄**. Here, the Tirado and Garcia de la Torre model breaks down because $L_H/d_H < 2$. Instead, we used the conventional Stokes–Einstein equation for globular particles to determine a hydrodynamic radius, R_{Hr} , of 8.4 ± 0.8 nm.^[19]

In summary, PFG-NMR spectroscopy measurements confirmed morphological characterisations derived from concentration-dependent SAXS experiments: in the case of the dendritic peptide amphiphiles **2.D₈**, containing the largest hydrophilic side chains, frustrated growth is operational. Most likely, the equilibrium constant to describe supramolecular polymerisation is less favourable than those of monomers **1.D₃** and **2.D₄**. Apparently, the formation of anisotropic nanorods is in-

hibited by steric constraints, and objects of globular shape are obtained in the case of **2.D₈**. In contrast, the smaller dendritic side chains allow self-assembly into nanorod-like assemblies with aspect ratios of up to 6.8 for self-assembled **1.D₃** and up to 6.1 for **2.D₄**.

Mechanistic insights

We set out to perform CD spectroscopy to probe the secondary structure of the supramolecular polymers in water, and to further perform polymerisation and depolymerisation studies by using a chemical denaturant (e.g., methanol). The goal was to investigate the thermodynamic stabilities of the aqueous equilibrium polymers and correlate these parameters to their morphologies. The three different monomers give rise to induced CD bands in their self-assembled state in water that are significantly higher than those of the molecularly dissolved or monomeric state in mixtures of H₂O/CH₃OH (Figure 4 and Figures S7 and S8 in the Supporting Information). In the case of **2.D₈**, the induced CD effects are less pronounced than those for self-assembled **1.D₃** and **2.D₄** (Figure S7 in the Supporting Information), which indicates that the formation of ordered morphologies is hampered by the large dendritic side chains, and is in agreement with SAXS and PFG-NMR spectroscopy experiments.

For solutions of **1.D₃** and **2.D₄**, we performed detailed titration experiments with methanol, which depolymerises the equilibrium-based supramolecular assemblies. The organic solvent is able to act as a chemical denaturant, disrupt self-assembly and reduce the size of the polymers in water by diminishing hydrophobic shielding of the hydrogen-bonding amide sequences.^[5e,17] The stability of the polymers is remarkably high and more than 20 vol % of methanol is needed to observe a decrease in the induced CD effects, which is indicative of the disassembly of ordered aggregates (Figure 4B and Figure S9 in the Supporting Information). Importantly, we could confirm that the loss of secondary structure observed by CD spectroscopy correlated with a decrease in the hydrodynamic size of

Figure 4. A) CD spectra in 25 mM mixtures of phosphate buffer (pH 7.4)/CH₃OH (0–100 vol%) at 293 K for dendritic peptide amphiphile **1.D₃** (0.125 mM, the bottom spectrum was recorded in pure buffer and the top spectrum in pure methanol). B) The fitted dimensionless degree of aggregation (0 refers to the molecularly dissolved state and 1 to a fully polymerised system, monitored at the CD band of $\lambda = 203$ nm) of **1.D₃** and **2.D₄** at 0.125 mM concentrations; data points were extracted from the CD spectra in A) and Figure S7A in the Supporting Information.

the polymer in solution (Figure 5). The PFG-NMR spectroscopy echo decay curves clearly show larger diffusion coefficients as the fraction of deuterated methanol in D₂O increases from 0 to 100%, which indicates disassembly of polymeric **1.D₃**. Using the obtained diffusion coefficients, we plotted the hydrodynamic radius, R_H , for self-assembled **1.D₃** as a function of the fraction of CD₃OD in D₂O (Figure 5B). The hydrodynamic radius, R_H , of 14.3 nm in pure deuterated water was slightly reduced to $R_H = 12.9$ nm at 25 vol% CD₃OD. Only at CD₃OD fractions of ≥ 50 vol% did the R_H decrease considerably to 3.6 nm and finally 1.3 nm in pure CD₃OD; these are likely to be very small oligo- and monomeric species, respectively.

To extract thermodynamic parameters to describe supramolecular polymerisation, we used an equilibrium model adapted to solvent-dependent polymerisations to fit the CD titration curves for **1.D₃** and **2.D₄** (Figure 4B and Figure S9 in the Supporting Information).^[20] In this generalised model, the self-assembly process is described by sequential addition equi-

Figure 5. A) Echo decays of molecularly dissolved and self-assembled dendritic peptide amphiphile **1.D₃** (1 mM) in different solvent compositions of CD₃OD in D₂O, at 295 K. Symbols represent the experimental data points and grey lines are results obtained by the exponential fits. B) Hydrodynamic radii, R_H , of dendritic peptide amphiphile **1.D₃** and its self-assembled supramolecular polymers in different solvent compositions of CD₃OD in D₂O (grey line serves as a guide to the eye).

libria of monomers with an equilibrium constant K_e . The model expresses potential non-linear and cooperative effects in polymerisation by using a cooperativity parameter, $\sigma = K_n/K_e$, based on the equilibrium constant, K_n , that is operative in the nucleation regime. The Gibbs free energy of monomer association is assumed to be linearly dependent on the volume fraction of CH₃OH.^[20a,b] Global non-linear least-squares curve fittings obtained are in good agreement with the experimental data of the supramolecular polymerisations of **1.D₃** and **2.D₄** (Figure 4B). These highlight cooperativity parameters close to unity and suggest the absence of a nucleation event in polymerisation. This is significant because the presence of polymers with very large molecular weight, which are in equilibrium with monomeric species, is one of the hallmarks of nucleated and cooperative supramolecular polymerisations.^[11]

Instead, an isodesmic or non-nucleated model describes the data very well, and we obtain Gibbs free energy values of $\Delta G^0 = -44 \pm 2 \text{ kJ mol}^{-1}$ for **1.D₃** and $\Delta G^0 = -40 \pm 1 \text{ kJ mol}^{-1}$ for **2.D₄**. These values correspond to equilibrium constants, K_{eq} , of 7×10^7 and $1.4 \times 10^7 \text{ M}^{-1}$ for **1.D₃** and **2.D₄**. Notably, in the case of **2.D₈**, we were unable to obtain reliable depolymerisation curves because the induced CD effects in the self-assembled polymeric state were too weak due to the less efficient packing of the hydrophobic peptide core. Therefore, we could not determine the monomer binding affinity, which was likely to be orders of magnitude smaller than those of **1.D₃** and **2.D₄**, and led to frustrated growth induced by the larger dendritic side chains.

For aqueous supramolecular polymers, the affinities we have obtained in the case of **1.D₃** and **2.D₄** are some of the highest reported to date.^[11c,21] Similarly high values are only achievable when using large hydrophobic and π -conjugated molecules, such as naphthalene and perylene bisimides, porphyrin derivatives,^[11c] cucurbituril-based inclusion complexes,^[22] and monomers with fluorinated side chains, as recently reported by the group of Rybtchinski.^[23]

Based on the equilibrium constants obtained, we can calculate the weight-average degrees of polymerisation, DP_w , by using an isodesmic model (see the Supporting Information).^[11] By assuming an approximate oligophenylalanine-rich inter-monomer distance of 0.35 nm in the ordered supramolecular polymer,^[24] the estimated concentration-dependent average contour lengths for the nanorods are $L_w = 176 \pm 90$ and 62 ± 32 nm for **1.D₃**, and $L_w = 78 \pm 17$ and 27 ± 7 nm for **2.D₄**, at monomer concentrations of 1 and 0.125 mM at 293 K. These values match the experimental data of 67 ± 7 nm at 1 mM and 35 ± 4 nm at 0.125 mM for **1.D₃**, and 65 ± 9 nm at 1 mM and 36 ± 4 nm at 0.125 mM for **2.D₄**, obtained from PFG-NMR spectroscopy and SAXS experiments.

Conclusion

Self-assembly into well-defined nanosized anisotropic particles in water has great potential for biomedical applications, particularly in view of recent reports that anisotropic shapes in the carrier design are able to outperform conventional isotropic spherical materials. The design of a series of three dendritic and charge-neutral TEG-functionalised peptide amphiphiles was reported. Their core was based on a dendritic nonaphenylalanine equipped with hydrophilic dendrons of varying size. When using the smaller dendrons, we observed the self-assembly of monomers **1.D₃** and **2.D₄** into nanorods of up to 67 ± 7 and 65 ± 9 nm. In the case of **2.D₈**, functionalised with the largest dendron, we observed frustrated growth into small and near isotropic particles with a lower degree of secondary order: the most bulky hydrophilic side chains inhibited self-assembly encoded into the hydrophobic peptidic core, and consequently, self-assembly was frustrated by steric constraints. In contrast, nanorods were obtained for monomers **1.D₃** and **2.D₄**. These were characterised in detail by cryo-TEM, SAXS and PFG-NMR spectroscopy. For **1.D₃** and **2.D₄**, the observed morphologies were in good agreement with mechanistic insights

obtained from fitting CD-monitored polymerisation/depolymerisation curves with an expanded Goldstein and Stryer model. A low degree of cooperativity, and resulting isodesmic growth based on free energy contributions of $-44 \pm 2 \text{ kJ mol}^{-1}$ for **1.D₃** and $-40 \pm 1 \text{ kJ mol}^{-1}$ for **2.D₄**, led to degrees of polymerisation and contour length distributions that matched the experimentally determined size of the nano-objects. In summary, we reported a unique example of non-cooperative supramolecular polymerisation in water that produced materials with extraordinarily high thermodynamic stabilities and nanomolar affinities, yet finite sizes. Based on their design, the materials do not grow infinitely long and have potential as delivery vehicles for imaging and therapy.

Experimental Section

Detailed synthetic procedures, material characterisation, and full details about the instrumentation and methods can be found in the Supporting Information. CD spectra were recorded on a J-815 (JASCO) instrument. All spectra were recorded at 20 °C with monomer concentrations of 0.125 or 0.0625 mM in 25 mM phosphate buffer (pH 7.4) by using quartz cells with a path length of 1 mm. Samples for cryo-TEM were prepared by deposition of a few microliters of 5 mg mL^{-1} solutions of dendritic peptide amphiphiles on glow-discharged holey carbon-coated grids (Quantifoil 3.5/1, Quantifoil Micro Tools, Jena, Germany). After excess liquid was blotted at 100% humidity and 22 °C, the grids were vitrified in liquid ethane (Vitrobot, FEI, Eindhoven, The Netherlands). The vitrified specimens were mounted in a liquid-nitrogen-cooled Gatan 626 cryo-holder (Gatan Inc., Pleasanton, CA) and inserted in the electron microscope. Low-dose images were recorded with a Gatan 4 K slow-scan charge-coupled device (CCD) camera (Pleasanton, CA) on a Philips CM 120 electron microscope (FEI, Eindhoven, The Netherlands) equipped with a LaB₆ tip operated at 120 kV. SAXS experiments were performed at the European Synchrotron Radiation Facility (ESRF) in Grenoble, France, at the high brilliance beamline ID02. An X-ray energy of 12.46 keV and sample-to-detector distances of 1.5, 3 and 10 m were used to cover a q range of $0.07 < q < 4.486 \text{ nm}^{-1}$, in which q was the magnitude of the scattering wave vector. The samples were injected into a polycarbonate capillary at a temperature of 20 °C. The scattering data were corrected for background scattering, detector response and primary-beam intensity fluctuations. The instrument scattering vector was calibrated by using a silver behenate standard. The SAXS profiles were analysed by using the software package SasView (<http://www.sasview.org/>). Best fits were obtained for solutions of **1.D₃** and **2.D₄** with a form factor developed for homogeneous cylinders. This described the scattering profiles of the supramolecular polymers in terms of a contour length, L , and a cross-sectional radius, r_{cs} . Best fits for **2.D₈** were obtained with a form factor for elliptical cylinders, which described the scattering data in terms of a length, L ; a minor axis, r_{mi} ; and a major axis, r_{ma} . The ¹H NMR spectroscopy diffusion experiments were performed on a Bruker 400 MHz Avance NMR spectrometer. The measurements were carried out with a liquid-state probe head (DIFF 30, Bruker) by using a maximum gradient strength, G , of 4 T m^{-1} , at a constant temperature of $T = 295 \text{ K}$. Self-diffusion coefficients of the amphiphiles were measured by PFG-NMR spectroscopy by using a stimulated echo sequence with gradient pulses. Signal analysis was performed on the area of the ¹H signal of the TEG side chains.

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Keywords: amphiphiles • nanomaterials • polymers • self-assembly • supramolecular chemistry

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