

Morphological control and molecular recognition by bis-urea hydrogen bonding in micelles of amphiphilic tri-block copolymers†

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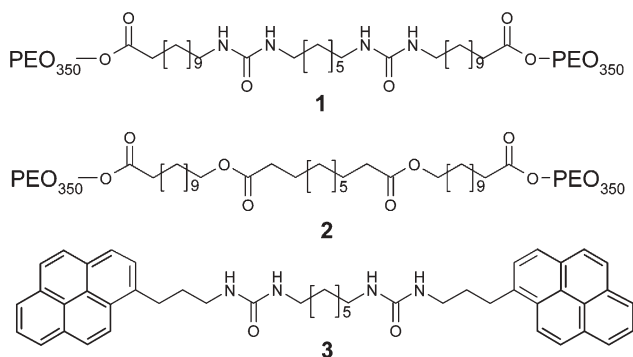
Received (in Cambridge, UK) 24th May 2005, Accepted 9th August 2005

First published as an Advance Article on the web 8th September 2005

DOI: 10.1039/b507171b

Hydrogen bonding between urea groups of amphiphilic tri-block copolymers considerably affects their self-assembly in water, which results in a strong modification of morphology and viscosity of aqueous solutions; the hydrogen bonding motif in these amphiphilic copolymers allows molecular recognition of small molecules with complementary hydrogen bonding units.

Molecular recognition *via* hydrogen bonding in water is a topic with high relevance to biological systems, however it has been investigated rather infrequently in synthetic systems.¹ The urea group is a strong hydrogen bonding unit and the cooperativity of two urea groups is a powerful motif in supramolecular chemistry. Bis-urea groups have been used as “hard blocks” in thermoplastic elastomers,^{2–4} furthermore they also may act as hosts for small molecules which contain complementary bis-urea units.⁵ Bis-ureas have also been shown to form gels in organic solvents⁶ or even in water.⁷



Here we combine the concepts of hydrogen bonding in water and molecular recognition using the bis-urea motif's hydrogen bonds. For this purpose we designed and synthesized amphiphilic tri-block copolymer **1**. In order to minimize the competing interactions of urea with water and polyethylene oxide (PEO),⁸

the bis-urea groups were placed at the centre of the apolar block, and were separated from a PEO block by an aliphatic spacer. For comparison, a reference block copolymer (**2**) was prepared, which lacks the urea groups but has exactly the same hydrophilic : hydrophobic ratio (weight fraction of PEO (w_{PEO}) = 0.55). Synthesis of tri-block copolymers **1** and **2** was performed starting from commercially available polydisperse PEO with $M_n = 350 \text{ g mol}^{-1}$ using a multistep synthetic strategy and were fully characterized (see ESI†). Both compounds were dissolved in water to form micelles. Critical micelle concentrations (CMC) were determined by using pyrene as a fluorescent probe.⁹ From a plot of the ratio of band intensities $I_1 : I_3$ in the fluorescence spectrum *vs.* the concentration of block copolymer, the CMC was taken as the inflection point on the curve. CMC values were determined to be $1.9 \times 10^{-6} \text{ M}$ for **1** and $2.5 \times 10^{-6} \text{ M}$ for **2**.†

The hydrogen bonding between urea groups of **1** in water was established with FT-IR on 1 wt% aqueous solutions (Fig. 1). The presence of strong, narrow bands in the spectrum of **1** at 3338 cm^{-1} (N–H), 1608 cm^{-1} (amide I) and 1578 cm^{-1} (amide II) indicated that urea groups in **1** are strongly hydrogen bonded.^{8,10}

Even though the critical micelle concentrations of both copolymers were comparable, the hydrogen bonds of urea groups strongly affect the ability of the material to be solubilized in water and the morphology of the resulting aggregates. Whereas homogeneous solutions of **2** were readily obtained by stirring, sonication was required for solubilization of urea-containing polymer **1**. The

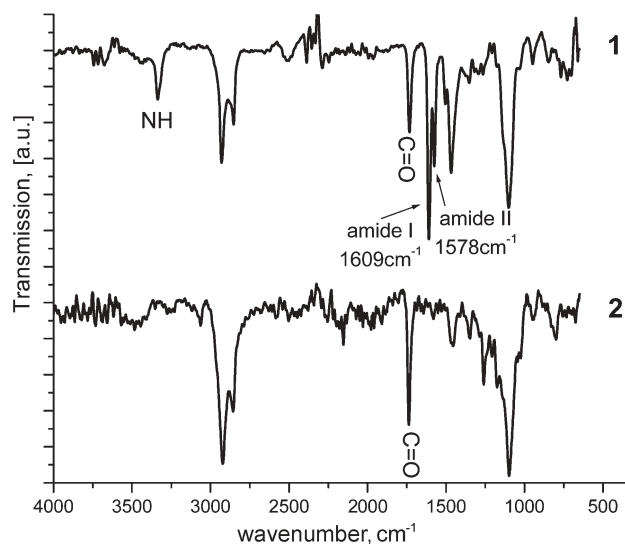


Fig. 1 FT-IR spectra of 1 wt% H₂O solution of **1** and **2**.

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† Electronic supplementary information (ESI) available: Synthetic procedures, cryo-TEM procedure, CMC determination. See <http://dx.doi.org/10.1039/b507171b>

morphology of the micellar aggregates of both compounds was investigated by cryo-TEM on 1 wt% solutions. For a fair comparison, both samples were sonicated for 1 h and equilibrated for 24 h prior to the measurements. As shown in Fig. 2a and 2b both **1** and **2** form cylindrical micelles in water, however the length of the cylinders is noticeably different. Micelles of **1** are short and straight ($d_{\text{core}} = 9 \text{ nm}$, $\ell = 100 \text{ nm}$), whereas the micelles of **2** are narrower, considerably longer ($d_{\text{core}} = 7 \text{ nm}$, $\ell > 1 \mu\text{m}$) and show some curvature.

Remarkably, despite the much shorter micelles, the viscosity of a 1 wt% solution of **1** is much higher than for a similar solution of **2**. Measured 1 h after sonication, the specific viscosities of **1** and **2** were determined to be 6.41 and 0.21, respectively. Further observation of **1** shows that the viscosity slowly increases with time after sonication, whereas the viscosity is constant for **2**.

Unfortunately, attempts to quantitatively determine the viscosity of solutions of **1** as a function of time failed due to the thixotropy of the sample, indicating significant interaction of the rods¹¹ combined with slow kinetics of micellar scission/recombination.¹² The viscosity of **2** was constant and not dependent on shearing history, in line with considerably faster kinetics.

To investigate the effect of sonication on the rod-like aggregates we conducted dynamic light scattering (DLS) measurements. DLS of a 0.5 wt % sample of **1**, which was sonicated for 60 min, stored for 24 h, and subsequently diluted to 0.05 wt% under vigorous stirring showed the presence of polydisperse large aggregates ($R_h = 20 \text{ nm} - 10 \mu\text{m}$). However, after sonication for 10 min., the aggregates were smaller and less polydisperse ($R_h = 15 - 400 \text{ nm}$). This shows that sonication is indeed able to disrupt the aggregation of rods, however, the system returns to its original aggregated state on a time scale of days due to slow dynamics. Similar treatment of solutions of **2** did not lead to a large reduction in aggregate size. Above 37 °C both compounds precipitate from solution.¹³ However, **2** dissolves and forms a clear micellar solution upon recooling, whereas **1** requires sonication, confirming the strong differences in the dynamics of both compounds. At concentrations above 3 wt%, freshly sonicated aqueous solutions of **1** have extremely high viscosities, whereas solutions of **2** easily flow even at 10 wt%. Comparison of 1 and 3 wt% solutions of **1** (Fig. 2a and 2c, respectively) shows that apart from the higher density of rods at 3 wt%, there is no change in morphology, suggesting that the high viscosity is due to overlap of rods[‡] rather than from formation of physical crosslinks.

Having shown that in the aggregates of **1** hydrogen bonds between bis-urea groups effectively modify micellar properties, we set out to use this system for the investigation of molecular recognition by hydrogen bonding in water. To probe the self-complementarity of the hydrogen bonding motif within the micellar aggregates, we designed and synthesized molecule **3**.[†] Due to hydrogen bonding, **3** is strongly aggregated in organic solvents. As a result, a strong excimer band at 480 nm is observed in the fluorescence spectrum. The intensity of the excimer emission depends on the ability of the solvent to compete with intra- and intermolecular hydrogen bonding to separate the pyrene groups.[†] However, if urea–urea hydrogen bonds are absent, the intensity of excimer emission is a measure of the microviscosity of the environment, since it depends on the probability that pyrene groups of a single molecule of **3** come together during the lifetime

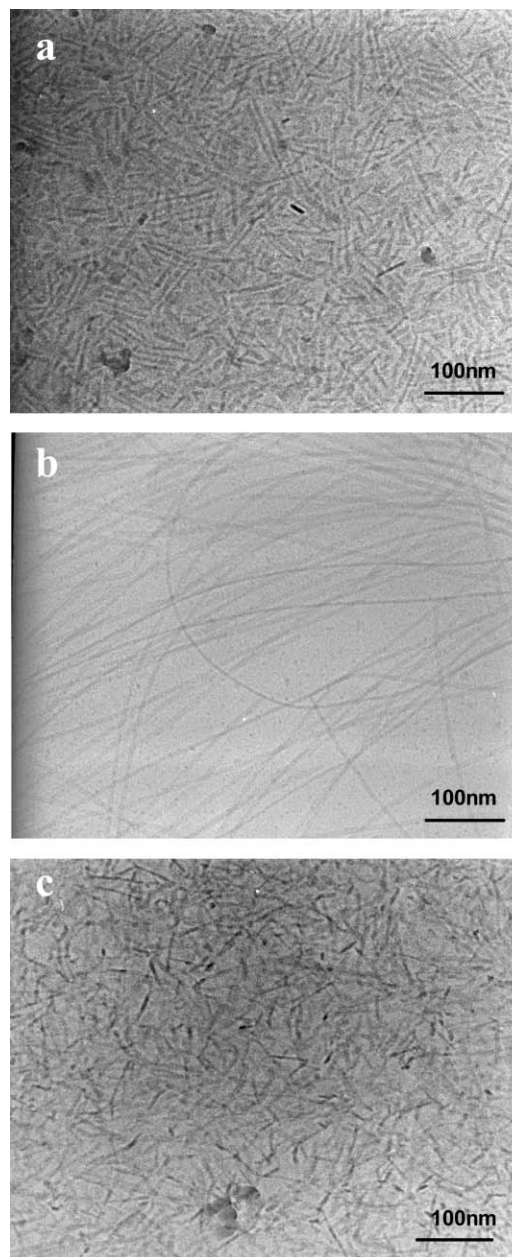


Fig. 2 a, b: Cryo-TEM images of 1 wt% micellar solutions of **1** and **2** respectively; c: cryo-TEM images of a 3 wt% solution of **1**.

of the excited state.¹⁴ Compound **3** was brought to a molecularly dissolved state by titrating a chloroform solution with trifluoroacetic acid (TFA) until the ratio between excimer and monomer emission was constant ($I_E/I_M = 0.18$; 15% TFA) (Fig. 3). These conditions were used to premix **3** with polymer **1** or **2**. After premixing and slow drying, a micellar solution was formed by adding water to the dry mixture followed by sonication. Cryo-TEM did not reveal any changes in micellar morphology upon addition of **3**. Fig. 3 shows the fluorescence spectra of **3** in 1 wt% aqueous solutions of **1** and **2** as well as in 15% TFA in chloroform. Excimer fluorescence was completely absent in micelles of **1** whereas a clear band with relative intensity $I_E/I_M = 0.34$ was observed in the micellar solution of **2**. Due to the strong tendency of **3** to aggregate, excimers can be formed either intramolecularly

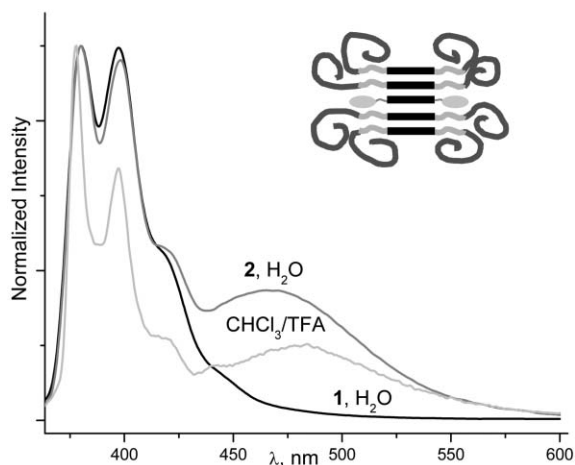


Fig. 3 Fluorescence spectra of 10^{-6} M solution of pyrene clicker **3** in $\text{CHCl}_3/15\%$ TFA, 1 wt% H_2O solution of **1** and **2**, $\lambda_{\text{ex}} = 337$ nm.

or intermolecularly. While it is not possible to distinguish between these types of excimers in micelles of **2**, the complete absence of excimer fluorescence in micelles of **1** is clear evidence that the pyrene moieties are held apart from each other and cannot come together during the lifetime of the excited state. Since the block copolymers **1** and **2** differ only in the presence of bis-urea groups, the distinct difference in excimer emission is attributed to the effect of bis-urea hydrogen bonding. We believe that **3** is incorporated into the hydrogen bonded urea stacks of **1** in an unfolded conformation, and that it hydrogen bonds with the urea groups of **1** keeping the pyrene moieties apart (Fig. 3, inset).

The results outlined above indicate that the urea groups have a strong effect on nearly every aspect of amphiphile assembly in water. The morphology, rheology and host-guest chemistry of **1** are completely different from its non-hydrogen bonding analogue **2**. Although direct structural information is lacking at the moment, the observations allow us to construct a general picture of the aggregates, and of the way hydrogen bonding affects their behavior. Hydrogen bonding between urea groups constrains the packing of the hydrophobic part of the molecules (they become more stretched), resulting in an increase in the core diameter from 7 to 9 nm. As a consequence, the interfacial area per chain is reduced.¹¹ The PEO chains of **1** are therefore also forced to stretch, and accumulation of steric strain limits the length of the micelles (Fig. 4).

A similar effect was observed by Stupp *et al.* in mushroom shaped aggregates of asymmetric tri-block copolymers.¹⁵ Partial dehydration of PEO may accompany stretching, which results in the observed lower solubility of **1**. The thixotropy and slow recovery of viscosity after sonication of **1** indicate that the kinetics of self-assembly of **1** in water are much slower than either self-assembly of **2**, or of bis-urea derivatives in less polar media.⁵ Even though the kinetics of self-assembly of **1** in water are not understood completely, these observations point to a cooperative role of hydrogen bonding, hydrophobic effects and steric shielding by PEO in slowing down self-assembly kinetics.

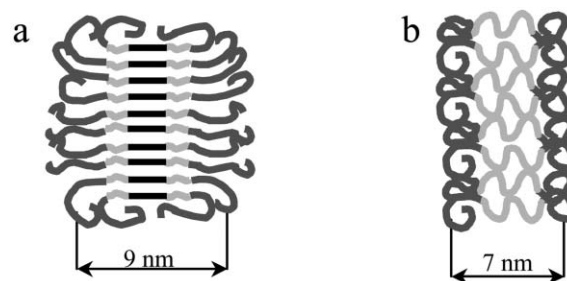


Fig. 4 a, b: Schematic representation of micelle formation in **1** and **2** respectively, indicating steric hindrance by PEO chains to the growth of the rod-like micelles.

In conclusion, by introducing the urea hydrogen bonding motif as a highly specific supramolecular synthon into amphiphilic tri-block copolymers, the nanoscopic structure and self-assembly kinetics of the micelles are strongly modified. Moreover, it was shown that the self-complementarity of bis-urea hydrogen bonding may be used for non-covalent functionalization of the micelles. To our knowledge this is the first example of non-covalent micellar functionalization in water using bis-urea groups, thereby creating opportunities for future applications in such diverse fields as target specific drug delivery¹⁶ or catalyst immobilization.¹⁷

We thank Dr Aissa Ramzi, University Utrecht, for help with DLS.

Notes and references

‡ A very rough estimate of the volume fraction (ϕ) of rods at which they start to overlap is: $\phi = (d/\ell)^2$, corresponding to a 4% solution for $d = 20$ nm (core + corona) and $\ell = 100$ nm.

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