Supporting information for:

The bis-urea motif as a tool to functionalize self-assembled nanoribbons

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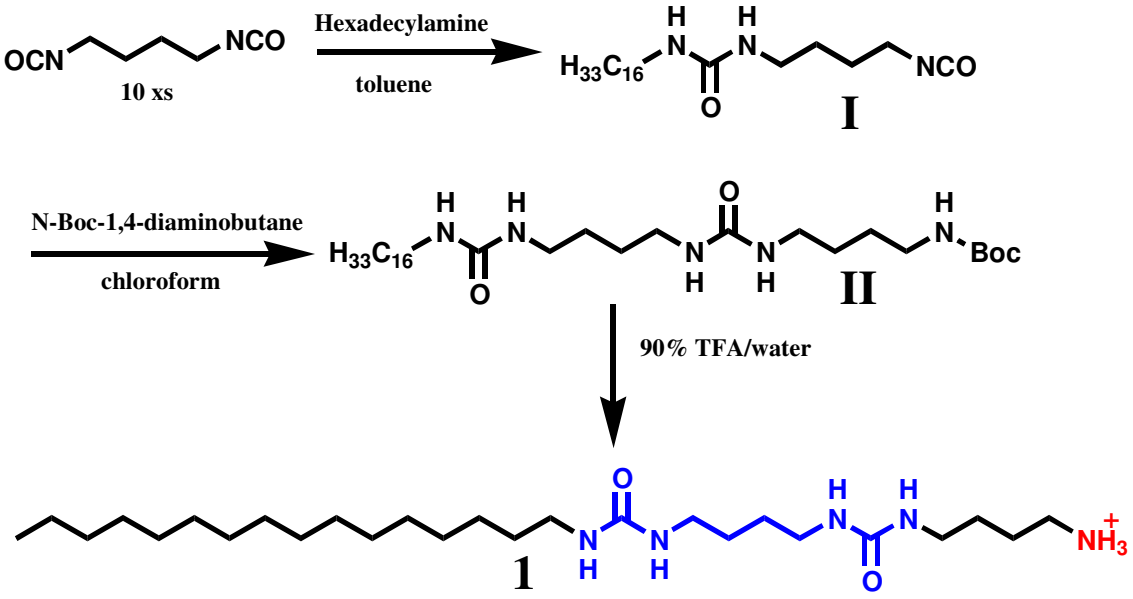
I - Surfactant synthesis

Surfactant **1** was synthesized starting from 1,4-diisocyanobutane, which was coupled to hexadecylamine to form synton I. Purification by precipitation from and subsequent washing with hexane of I, was followed by coupling to mono Boc-protected 1,4-diaminobutane. After precipitation from and washing with diethyl ether, the Boc-group of II was removed by deprotection in 90% TFA/water solution, which yielded the desired surfactant **1**. Before use **1** was freeze dried from glacial acetic acid.

^{1}H NMR ($CDCl_3/CD_3OD$): δ 0.9 (CH_2CH_3, 3H, t); 1.2-1.9 ($(CH_2)_{14}CH_3$, 28H, $NHCH_2(CH_2)_2CH_2NH$, 8H m); 2.9 (CH_2NH_3, 2H, m); 3.1 (CH_2NH, 8H, m); NH not visible due to H-D exchange.

MALDI-TOF-MS: m/e 470.44 (M^+); 492.25 (M^+ -H + Na)



Scheme 1. Synthesis of **1**.

II - Cryo-TEM preparation

The cryo-TEM images of the ribbons were obtained by applying small aliquots (3 mL) of a 5 mg/mL aqueous ribbon suspension to Quantifoil grids (R2/2 Quantifoil Jena) within the environmental chamber (relative humidity 100%) of the Vitrobot™ both at 24 °C and 50 °C. Excess liquid was blotted with filter paper using an automatic blotting device within the environmental chamber of the Vitrobot™. The grid was subsequently shot through a shutter into melting ethane placed just outside the environmental chamber. The vitrified specimens were stored under liquid nitrogen and observed at -170°C (Gatan 626 cryo holder) in a Philips CM12 microscope. Micrographs were taken at 120 kV using low dose conditions.

III - IR experiments

An IR-transparent silicon waver, which was coated with a 3 nm thin gold layer to increase adsorption to the surface, was used to align the ribbon aggregates. While placed under a 45 degree angle, a droplet of 5 mg/mL ribbon solution was moved down the waver using gravity and a stream of nitrogen. This procedure was repeated 3 times. AFM confirmed alignment on a 10x10 micrometer length scale, although local 3x3 nm regions appeared to be better aligned. When determining the angle of variation in alignment between two extremes, an angle α of ~ 20º-25º could be constructed (figure 1). If one would assume perfect alignment with no variation, a 60% reduction, as found by IR when measured perpendicular to the alignment direction, results in an orientation of the reduced vibrations within the ribbon structure at an angle of 22º. This nicely matches the variation found in the alignment of the sample. It can therefore be concluded that based on this variation a larger reduction of 60% can not be expected.

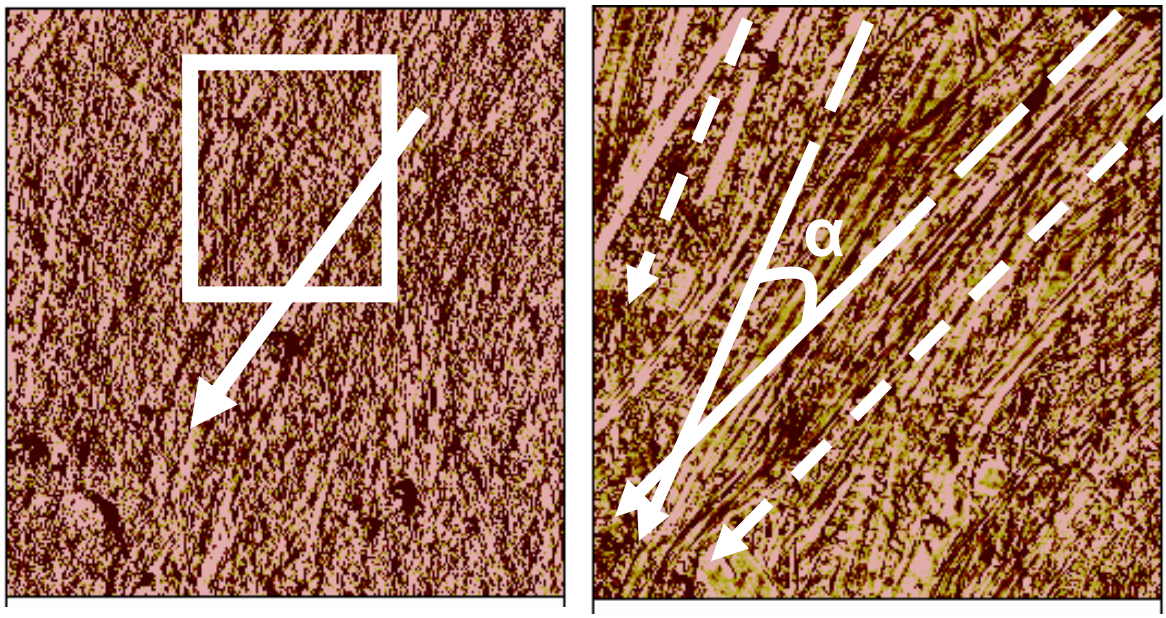


Figure 1. AFM amplitude images showing general and local alignment indicated by the arrow at different length scale. (Left) 10x10 μm, the white square indicates the enlarged region, (right) 3x3 μm with white arrows indicate the variation of two extreme orientations.

IV - UV-vis experiments

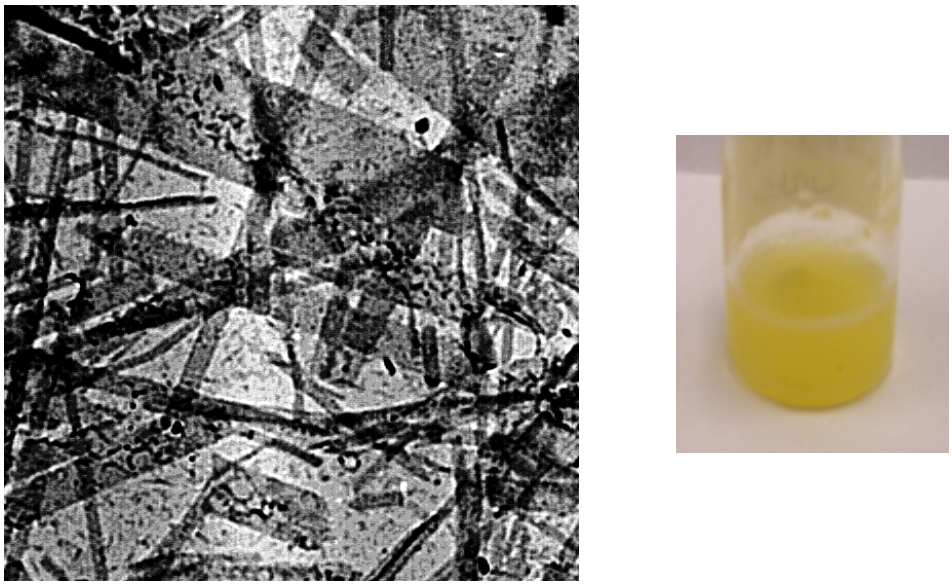


Figure 2. TEM image (right) of a dried ribbon suspension (left) in which 6 mol% dye molecules **2** were incorporated.

For the incorporation of both bis-urea functionalized dyes **2** and **3** within the ribbon structure, a 5 mg/mL (8.5 mmol/L) solution of bis-urea surfactant **1** was prepared in chloroform, to which 6 mol% of **2** or **3** was added resulting in a stock solution. For UV-vis measurements, these solutions were diluted with chloroform until a maximum absorption of 1 was reached. The aqueous aggregate suspension was formed by adding water to the chloroform stock solutions, followed by evaporation of the organic solvent, resulting in a yellow turbid aqueous suspension; TEM measurements on a dried sample confirmed the presence of ribbons (figure 2). Water was added to reach a final concentration of 5 mg/mL surfactant **1**. UV-vis spectra of the resulting suspensions were taken using the same dilution ratio as used for the chloroform solutions. As described in the manuscript text, a large blue shift could be observed for the λ_{max} of 2 and 3 in the aqueous ribbon suspension. For the CTAB measurement 6 mol% of **2** was added to an aqueous 5 mg/mL CTAB solution and heated to 70 ºC while stirring. After cooling a clear orange solution was obtained, which was diluted with water until a maximum absorption of 1 was reached; in this case no blue shift was observed.

To investigate the origin of the blueshift, UV-vis experiments were performed using both dye molecules **2** and **3,** in which the solvent polarity was gradually increased going from chloroform to methanol and subsequently to water containing 10 vol% methanol. Increasing polarity from chloroform to methanol caused a small blueshift to 390 nm (figure 3, left). A subsequent decrease of the absorption band was observed upon increasing the water content to 70 vol%. However, at a water content of 60 vol% a second absorption peak appeared at 340 nm whereas the absorption intensity at 390 nm became largely reduced. Increasing the water content up to 10 vol% resulted in an increase of the 340 nm and a complete reduction of the 390 nm absorption, accompanied by an increase in scattering (figure 3, right). In general three possible explanations are given for a blueshift in azobenzene systems: (i) cis-trans isomerisation, (ii) polarity effects of the solvent[(1)] and (iii) H-aggregation. The half live of the cis-isomer at room temperature is in the order of 1 second.[(2)] Since the blue shift in the measured samples was a permanent effect, this explanation can be ruled out. The small shift of 15 nm to 390 nm is likely to be caused by an increase in polarity; however, the sudden large blueshift of 50 nm, going from 70% to 60% methanol/water, is probably due to additional aggregation. This idea is further strengthened by the increase of scattering and decrease in absorption.

The λ_{max} values of **2** and **3** mixed with bis-urea surfactant dissolved in chloroform/methanol or with aqueous CTAB solution remains at 405 nm. After addition of water and evaporation of organic solvents, due to the high order of the ribbons, matching dye molecules are all “locked in” in a similar fashion promoting uniform H-aggregation, resulting in a single blue-shifted absorption. Non-matching dyes on the other hand, do not fit the ribbon lattice and are therefore less tightly bound resulting in more freedom to move. The larger freedom to move allows phase separation of the dye molecules within the ribbon structure causing more molecules to aggregate and therefore a slightly larger blueshift when comparing **3** with **2**. CTAB molecules form micelles, which are much smaller in diameter resulting in less scattering and a clear solution. The dye molecules, due to their hydrophobic nature are taken up in the apolar inner core of the micelle. No aggregation occurs in this part of the micelle resulting in no change of the λ_{max} compared to apolar solvents. Because **3** showed a residual absorption maximum at 405 nm

when incorporated in the ribbon suspension, we speculate that a part of the dye molecules is taken up into the hydrophobic inner part of the ribbon. It is however apparent that both water insoluble dyes have been incorporated into the ribbon structure given the similarity of the resulting spectra when compared to the spectra of the neat dye molecules in methanol/water mixtures (<60% methanol).

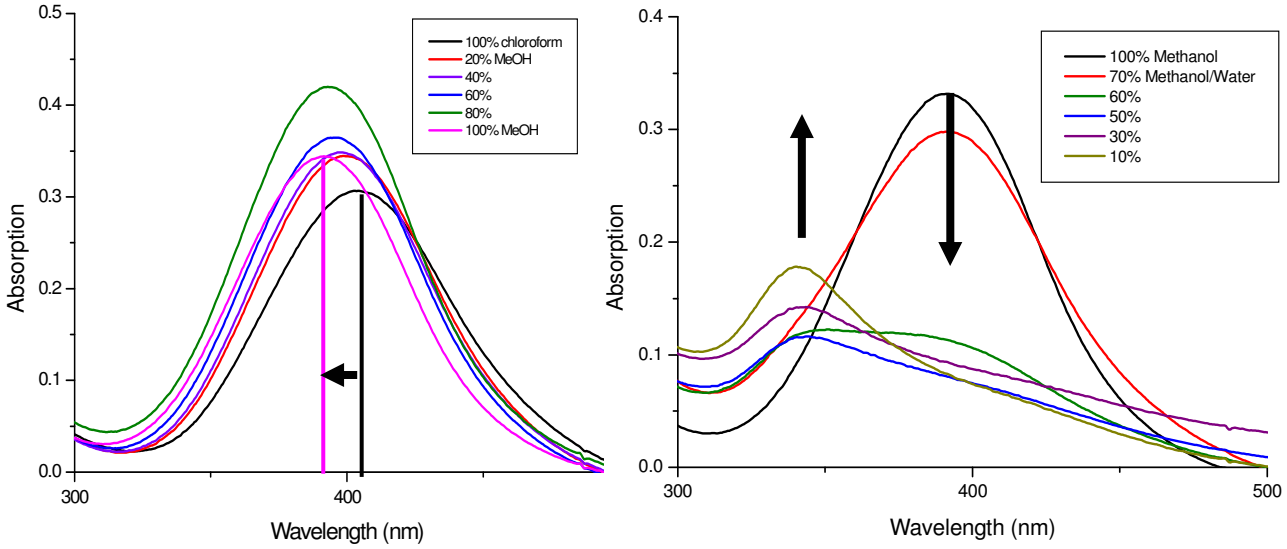


Figure 3. UV-vis spectra of **2** dissolved in increasing polar solvent from (left) chloroform to methanol and (right) subsequently to water containing 10 vol% methanol. The arrows mark the small blue-shift to 390 nm from chloroform to methanol, the increasing blueshifted absorption at 340 nm and the decreasing 390 nm absorption going from 100% methanol to 10% methanol/water. The spectra were corrected for scattering.

V - XRD measurements

Grazing incident XRD on horizontally deposited ribbons, showed a main reflection at 5.7 nm (Figure 4, left) also confirming the ribbon thickness derived from cryo-TEM images. Powder X-ray diffraction on a dried gelled sample of **1** at 40 mg/mL ribbon showed 2 main first order reflections at 4.5 Å and 3.9 Å (Figure 4, right). The 4.5 Å reflection is attributed to the repeating distance of the alkyl chains in the hydrogen bonding direction, i.e. in the length direction of the ribbons. The 3.9 Å reflection can

correspond to the other dimension in the lattice of the intercalating hydrocarbon chains. These distances fit the proposed molecular model and support a close packing of the surfactant molecules in the ribbons.

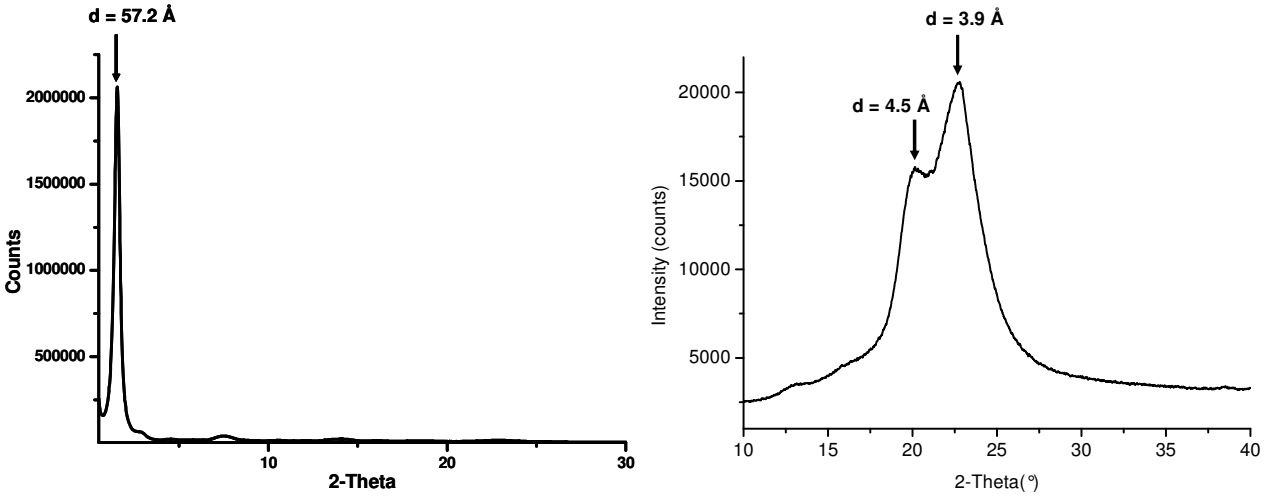


Figure 4. (Left) Grazing incident XRD on horizontally deposited ribbons and (right) powder X-ray diffraction pattern of a dried gelled sample of **1** at 40 mg/mL ribbon.

VI - Incorporation of biotin-streptavidin

For the incorporation of bis-urea functionalized biotin (**4)** into the ribbon structure a 1 mg/mL solution of bis-urea surfactant **1** in chloroform/methanol (9:1 v/v) was prepared. 10 mole% of **4** was added to the solution and mixed. After addition of water the organic solvent was evaporated, while stirring, until the temperature rose above 80 ºC. After cooling to room temperature a white viscous turbid suspension was formed. For the deposition of the biotin fuctionalized ribbons on the TEM grid, a 200 mesh carbon coated grid was placed on 20 µL droplet of the prepared ribbon suspension and left for 15 minutes. Subsequently the grid was transferred to droplets of PBS buffer (pH 7.4)(2 times 5 min), block buffer (Aurion BSA-c™)(1 time 5 min) and again PBS buffer (2 time 5 min). Next, the grid was transferred to a streptavidin-gold solution (Aurion 25 nm gold particles) for 60 minutes, which was diluted 2 times with PBS buffer. This was followed by 6 wash steps on droplets of PBS for 5 minutes, followed by two wash steps on droplets of milli-Q water (2 times 5 min). Finally the excess water was blotted using a

filter paper, after which the grids were examined on a JEOL JEM 2000 FX microscope at 80 kV. The same procedure was followed for the blank sample, i.e. the sample in which **4** was not mixed in.

EDXA on the gold covered ribbons has been performed using the TEM EDX detector. Bright field images showed gold particles on the ribbons. When the scanning area was positioned away from the gold particles, no gold reflections could be observed (figure 5A). When the scanning area was positioned on top of the gold particles clearly all reflections characteristic for gold could be observed (figure 5B). When the scanning area was focused on a single gold particle the gold reflections intensified (figure 5C). The observed reflections of copper originate from the copper grid. EDX measurements were performed on a FEI Tecnai 120 kV microscope.

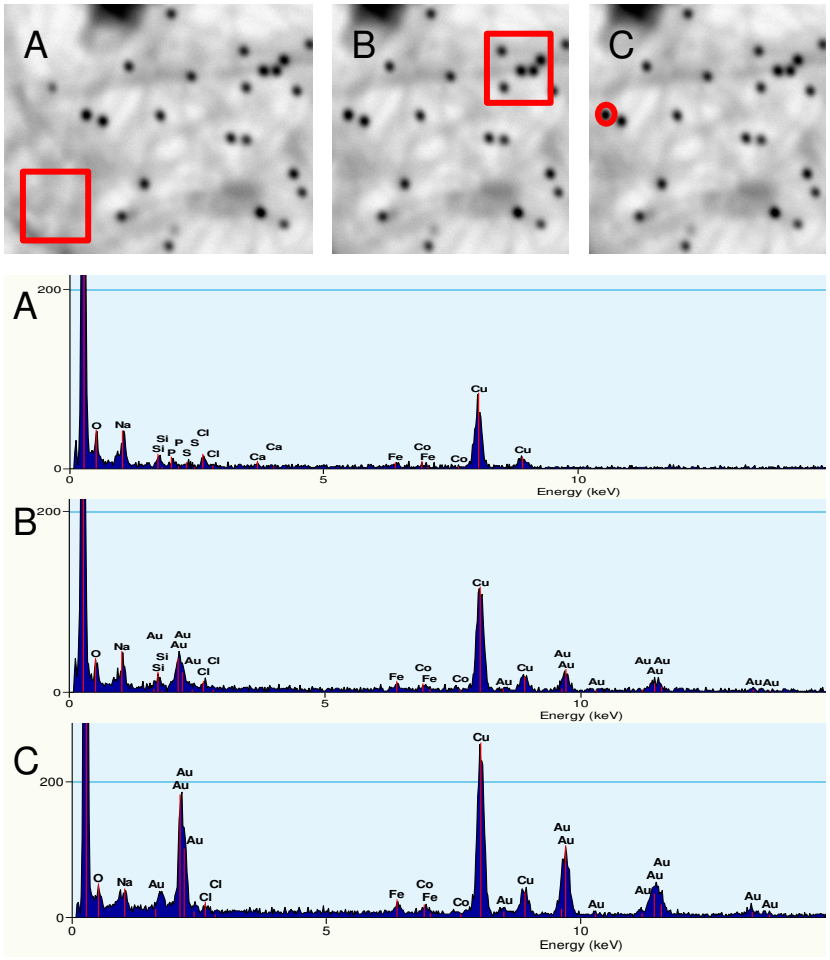


Figure 5. EDXA of 25 nm streptavidin labelled gold particles attached to biotin functionalized ribbons, **A.** bright field image and corresponding EDXA with red square indicating the scanning area where gold

particles are absent, **B.** Same as **A**, with red square indicating scanning area where gold particles are present, **C.** Same as **B** only scanning area reduced to spot focused on a single gold particle.

(1) (a) Reeves, R. L.; Harkaway, S. A. In *Micellization, Solubilization, and Microemulsions*, Mittal, K. L. Ed., *Plenum Press: New York* **1977**, *Vol.2*, 819-834. (b) Corrin, M. L.; Klevens, H. B. Harkins, W. D. *J. Chem. Physics* **1946**, *14*, 480-486.

(2) King, N. R.; Whale, E. A.; Davis, F. J.; Gilbert, A.; Mitchell, G. R. *J. Mater. Chem* **1997**, *7*, 625-630.