Photoresponsive supramolecular polymers

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We report multi-modal-control over the assembly behaviour of a first-generation molecular motor bola-amphiphile in water by light, pH and the choice of counter-ions. These findings open up opportunities for the development of materials that reconfigure enabling complex functions in response to different stimuli.

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4.1 Introduction

The variety of noncovalent interactions between assembled units found in nature provides a basis for designing artificial supramolecular systems. The intrinsic dynamics, tunability of properties and stimuli responsiveness of synthetic supramolecular systems is controlled by the precise design of organic molecules, mesogenic materials, polymers, and a variety of organic-inorganic hybrid composites, allowing modulation of e.g. assembly and functionality. Recent advances in supramolecular chemistry and soft materials design have enabled the creation of various supramolecular systems responsive to external stimuli, especially in aqueous media. Encoding specific responses to different stimuli into self-assembling systems is a promising approach to achieve more complex dynamic and adaptive behaviour. Besides making use of multi-stimuli responsive polymers or responsive organic-inorganic composites, it has also been demonstrated that supramolecular assemblies of small organic molecules in water can be controlled independently by external stimuli like light, pH, and ions. Notably, a few of these systems can be controlled by various external stimuli simultaneously. For example, the supramolecular assemblies of polyanionic dendritic peptide amphiphiles, reported by Besenius et al., were controlled systematically by pH and ionic strength. Among the various stimuli, light offers a non-invasive way with high spatial as well as temporal precision for manipulating the structures of molecular assemblies. However, implementing responsiveness to other stimuli into photo-responsive supramolecular systems based on organic molecules to realize multi-modal control over their assembly in water remains highly challenging.

Taking advantage of rotary motors as light-driven multistate switches, we have previously developed a co-assembling supramolecular system, composed of a second-generation molecular motor and a lipid (1,2-dioleoyl-sn-glycero-3-phosphocholine), allowing upon irradiation, transformations from nanotubes into vesicles and multi-lamellar vesicles (in a fully reversible cycle) whereby comparable packing parameters were identified for both structures (1/2<P<1). In the present study, multi-modal control over the self-assembly morphology of a first-generation molecular motor is reported using light, pH and ions as triggering elements. This class of molecular motors offers advantages due to the distinct geometric differences between cis- and trans-isomer, leading to more pronounced changes of the packing parameter upon irradiation, and the absence of thermal back-isomerization. In the present study, the morphology of the molecular motor bola-amphiphile (MBA) aggregates could be switched between sheet-like structures and a mixture of micelles and vesicles upon irradiation (Figure 4.1). By contrast, morphology transitions from sheet-like assemblies to discs and ultimately micelles were found with increasing pH. Moreover, the
addition of NaCl resulted in the formation of vesicles, while CaCl₂ led to macroscopic aggregates.

Figure 4.1. Schematic illustration of the photo-isomerization of MBA and multi-modal control over its assembly behaviour.

4.2 Results and Discussion

4.2.1 Molecular design and synthesis

The structures of trans- and cis-isomers of MBA are shown in Figure 4.1. Although the rotation of first-generation motors passes through four states, the metastable trans-isomer is not discussed in this study due to its short half-life (t_{1/2} < 30 s) at room temperature. Hence, trans-MBA in this paper refers to the stable trans-isomer. Stable trans- and metastable cis-isomer could be interconverted by irradiation with 312 nm and 365 nm light, respectively, while the stable cis-isomer could be obtained through thermal helix inversion from the metastable cis-isomer with a half-life of ~20 h. The stable and metastable cis-isomer are expected to have the same assembly morphology, due to their related geometry. Therefore, we mainly focus on the photo-induced morphology transition between stable trans-MBA and metastable cis-MBA in the present study. Two carboxylic acid groups are connected to the motor core through alkyl-linkers whose relative orientation significantly changes upon cis-trans isomerization, potentially leading to distinct differences in self-assembly behaviour. The carboxylic acid functionality has recently also been used as the end group on a motor amphiphile forming electrostatic interactions with counter-ions, such as Ca²⁺ to allow the formation of macroscopic string micro-actuators. In the present study, we use this group as a cation binding as well as pH-responsive unit to gain additional handles for multi-modal control over MBA’s assembly behaviour in addition to photochemical cis-trans isomerization. The synthesis and
characterization of trans-MBA and stable cis-MBA are summarized in Scheme 4.1. Stable trans-1 and stable cis-1 were synthesized following our group’s published method.\textsuperscript{20,21}

4.2.2 Isomerization behavior in solution

The photo-responsive behaviour was studied by UV/Vis absorption and \textsuperscript{1}H NMR spectroscopy. Irradiation of the trans-isomer with 312 nm light resulted in a decrease of the absorption bands at 286 nm and 312 nm with concomitant formation of a new band around 350 nm, indicating the formation of the metastable cis-isomer (Figure 4.2a). After irradiating for 18 min, no further changes were observed, meaning that the photostationary state (PSS) had been reached. Subsequent irradiation with 365 nm light induced the opposite spectral changes caused by the back-isomerization to trans-MBA (Figure 4.2b). An isosbestic point at 329 nm was observed during both irradiation processes, confirming the clean formation of metastable cis- and trans-isomer, respectively. As shown in the \textsuperscript{1}H NMR spectra in Figure 4.2c and d, irradiation of a sample of trans-MBA with 312 nm light induced the formation of a new set of signals (e.g. Hα = 6.55 ppm, Hβ = 1.21 ppm) belonging to the metastable cis-isomer. The cis:trans ratio at PSS was found to be 63:37 (Figure 4.2d). Subsequent irradiation at 365 nm led to full recovery of the initial spectrum (Figure 4.2e), demonstrating that the cis-trans isomerization of MBA can be precisely controlled by light.
Figure 4.2. Changes in UV/Vis absorption spectra (a) over the course of irradiating a sample (1.0 x 10^-5 M in degassed MeOH) of trans-MBA to PSS at 25 °C using 312 nm light and (b) during subsequent irradiation to PSS with 365 nm light. 1H NMR spectra (CD3OD, 25 °C, 400 MHz) of trans-MBA (2.8 x 10^-3 M) (c) before and (d) after irradiation to PSS with 312 nm light for 45 min at 5 °C and (e) after subsequent irradiation to PSS with 365 nm light for 15 min.

4.2.3 Control over the assembly by light

To investigate the critical aggregation concentration (CAC) of trans-MBA a Nile Red fluorescence assay (NRFA), which probes the internal hydrophobicity of assemblies, was performed. The results revealed a CAC of 4.0 x 10^-6 M for trans-MBA (Figure 4.3). The structure of aggregates of trans-MBA in water was imaged using cryogenic transmission electron microscopy (cryo-TEM) to accurately capture their solution-state morphology (See Experimental Section 4.5.5). Since self-assembly of trans-MBA is sensitive to pH and ionic strength (vide infra), irradiation experiments were carried out in sodium borate buffer (pH = 9.3, 0.1 M). As shown in Figure 4.4a, trans-MBA was found to form sheet-like
assemblies (P≈1). After irradiating with 312 nm light for 10 min to form metastable cis-MBA, vesicles with a diameter of around 20 nm and micelles with 5–6 nm diameter were observed in addition to sheet-like structures (Figure 4.4b). As a comparison, we studied the aggregate morphology of stable cis-MBA, whose CAC (1.0 × 10⁻⁵ M, Figure 4.3) was distinct from that of trans-MBA (4.0 × 10⁻⁶ M), implying possible different assembly morphologies between trans and cis-isomers. Indeed, the stable cis-isomer showed the formation of micelles with 5–6 nm diameter (P≤1/3) (Figure 4.10). Due to its related geometry, metastable cis-MBA is expected to have the same assembly structure as stable cis-MBA. Therefore, the presence of micelles in the sample of trans-MBA after irradiation (mixture with metastable cis-MBA) can be attributed to the self-assembly of the metastable cis isomer. The formation of vesicles (1/2<P<1), we tentatively assigned to the co-assembly of both stable trans- and metastable cis-molecules. The remaining sheet-like assemblies (Figure 4.11) hint at an excess of self-assembling trans-MBA present in the mixture. This was supported by ¹H NMR measurement revealing a 6% share of metastable cis-MBA in the mixture (Figure 4.8b). Upon subsequent irradiation with 365 nm light for 10 min (metastable cis-MBA to trans-MBA isomerization), the original sheet-like assemblies were recovered, albeit with some small vesicles remaining in the mixture (Figure 4.4c). ¹H NMR, however, revealed that there was no remaining cis-MBA (Figure 4.8c). This phenomenon had also been observed in earlier studies in our group.¹⁵ It was found that molecular motors could recover their original assembly morphologies only upon being subjected to conditions allowing them to reorganize. We therefore facilitated reorganisation by repeating the assembly preparation process (heating-cooling cycle) using the sample shown in Figure 4.4c. Indeed, after this treatment, only sheet-like structures were observed (Figure 4.4d).

Figure 4.3. Nile Red fluorescence assays for determination of the critical aggregation concentrations of trans-MBA and stable cis-MBA (concentration: 1.4 × 10⁻⁷–2.8 × 10⁻³ M).
Figure 4.3

Indeed, after this treatment, only sheet assembly preparation allowing them to reorganize. We therefore facilitated reorganisation by repeating the process (heating–cooling cycle) using the sample shown in Figure 4.4. Nile Red fluorescence assays for determination of the critical aggregation concentrations of both stable and metastable isomer. The formation of vesicles (1⁄2<P<1), we tentatively assigned to the coexistence of sheets and planar micelles with 5nm diameter (P≤1/3). After irradiating with 312 nm light for 10 min (Figure 4.4a), the original sheet assembly of the metastable cis-MBA isomer showed the formation of vesicles with a diameter of around 20nm and micelles with 5nm diameter (P≈1). After irradiating with 365 nm light for 10 min (Figure 4.4b), it was found that molecular motors dissociation of the carboxylic acid group. Thus, the ratio of molecules with larger head group area rises with increasing pH. Based on the equation of molecular packing parameters for amphiphiles, this increase of head group area leads to a decrease of the packing parameter, causing the self-assembly to change from sheets to micelles. In our case, the morphology transformations of trans-MBA were also in accordance with this analysis.

4.2.4 Control over the assembly by pH

Besides reversible control by irradiation, the assembly structure of trans-MBA also showed significant changes upon altering pH. As shown in Figure 4.5, trans-MBA formed sheet-like structures (P=1) at pH 8.8 (Figure 4.5a) and disc-like structures (1/2<P<1) with a diameter of 30–40 nm and a thickness of 2–3 nm at pH 9.8 (Figure 4.5b), as well as tiny micelles (P≤1/3) with a diameter of 4–5 nm at pH 11 (Figure 4.5c). Therefore, as pH goes up structures with increasingly smaller packing parameters are formed, which we attribute to the increasing concentration of deprotonated molecules at higher pH of the solution. It has been demonstrated that the head group area of long-chain fatty acids increases significantly upon dissociation of the carboxylic acid group. Thus, the ratio of molecules with larger head group area rises with increasing pH. Based on the equation of molecular packing parameters for amphiphiles, this increase of head group area leads to a decrease of the packing parameter, causing the self-assembly to change from sheets to micelles. In our case, the morphology transformations of trans-MBA were also in accordance with this analysis.
sample at pH 11 to decrease the pH to 9.4, the morphology changed from micelles to vesicles and sponge-like structures as congeries of vesicles (Figure 4.5d). In this case, however, both pH as well as the concentration of NaCl were altered and the observed transformation of morphology could be the result of changing either one or both of these conditions.

Figure 4.5. Cryo-TEM images of trans-MBA (7.0 × 10⁻³ M) in aq. NaOH solution with (a) pH = 8.8, (b) pH = 9.8, (c) pH = 11 and (d) after adjusting its pH to 9.4 by adding an aq. HCl stock solution starting from pH 11. (blue: upright discs, yellow: discs at an angle with the substrate, white: micelles, black: contaminants)

4.2.5 Control over the assembly using counter-ions

In order to explore a possible new handle for control over the assembly morphologies of trans-MBA, the counter-ion effect of Na⁺, K⁺, Ca²⁺, and Zn²⁺ on the self-assembly structures formed by trans-MBA was studied by Nile Red fluorescence assay. Therefore, the blue shift of Nile Red in trans-MBA dicarboxylate solution (5.0 × 10⁻⁶ M) was determined in the presence of various concentrations of the chloride salts (1.0 × 10⁻⁶–5.0 × 10⁻¹ M) (Figure 4.6). A gradual blue shift was observed with enhanced concentrations of NaCl, KCl, ZnCl₂ and CaCl₂, reflecting an increase of the internal hydrophobicity of the trans-MBA assemblies. Interestingly, the blue shift induced by increasing the concentration of CaCl₂ was significantly larger compared to other chloride salts, indicating that Ca²⁺ ions induce
aggregate formation of trans-MBA more effectively. Based on the results of NRFA, Ca$^{2+}$ and Na$^{+}$ were chosen for additional studies of the control of assembly morphology. Vesicles with diameters of 100–200 nm were observed in aq. 0.1 M NaCl solution (Figure 4.7a) contrasting with the micelles observed in samples at high pH discussed earlier. The addition of NaCl likely reduces the repulsion between the deprotonated head groups due to charge screening. As a consequence, the decrease in head group area results in an increase of the packing parameter of MBA leading to the observed change in assembly structure. However, in 0.1 M aq. CaCl$_2$ solution trans-MBA formed macroscopic precipitates. In order to study the assembly structure in more detail, a sample of trans-MBA dicarboxylate at a lower CaCl$_2$ concentration ($5.0 \times 10^{-3}$ M) was characterized by cryo-TEM; micrometre-sized aggregates were observed as shown in Figure 4.7b. The presence of Ca in these aggregates was confirmed by Energy-dispersive X-ray (EDX) analysis (Figure 4.7c). The preferential induction of aggregate formation observed for Ca$^{2+}$ may be related to the stronger interaction of Ca$^{2+}$ with carboxylate groups.

**Figure 4.6.** Blue shift of Nile Red in trans-MBA solutions ($5.0 \times 10^{-6}$ M) containing various concentrations ($1.0 \times 10^{-6}$–$5.0 \times 10^{-1}$ M) of NaCl, KCl, CaCl$_2$, and ZnCl$_2$.

**Figure 4.7.** Cryo-TEM images of trans-MBA carboxylates ($7.0 \times 10^{-3}$ M) in (a) 0.1 M NaCl solution, and (b) $5.0 \times 10^{-3}$ M CaCl$_2$ solution and (c) in situ elemental mapping of C (red) and Ca (green) of trans-MBA carboxylates CaCl$_2$ solution.
4.3 Conclusion

In summary, we realized multi-modal control over the assembly behaviour of a first-generation molecular motor in water. Transitions between sheet-like structures and a mixture of micelles and vesicles, indicating large changes in packing parameter, could be controlled by light. Dramatic changes of assembly morphology from sheet-like assemblies to discs and ultimately micelles were achieved by adjusting pH. Furthermore, the addition of NaCl led to the formation of vesicles, while CaCl₂ induced macroscopic aggregates. To the best of our knowledge, this is the first example of multi-responsive assembly of a molecular motor-based bola-amphiphile in water. This study significantly enhances our understanding of the self-assembly behaviour of multi-responsive systems in water and thereby paves the way to future applications like adaptive materials, delivery systems or supramolecular materials capable to perform various distinct tasks.

4.4 Acknowledgements

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4.5 Experimental Section

4.5.1 Materials and Methods

All commercial reagents were purchased from Acros, Aldrich, TCI or Merck and were used as received. All solvents used in the reactions were dried using an MBraun SPS-800 solvent purification system or purchased from Acros, except for MeOH. Analytical TLC was performed on Merck silica gel 60 F254 plates and visualization was accomplished by UV light. Solvents for spectroscopic studies were of spectrophotometric grade (UVASOL Merck). Aq. stock solutions (0.1 M) of NaOH and HCl were prepared freshly using Milli-Q water. Nile Red stock solution (1.0 × 10⁻³ M) was prepared in EtOH. Column chromatography was performed on a Reveleris X2 Flash Chromatography system. NMR spectra were recorded at 25 °C on Varian AMX400 or Agilent 400-MR (¹H: 400 MHz, ¹³C: 101 MHz) spectrometers. Chemical shifts (δ) are expressed relative to the resonances of the residual non-deuterated
solvent for $^1$H [CDCl$_3$: $^1$H(δ) = 7.26 ppm] and $^{13}$C [CDCl$_3$: $^{13}$C(δ) = 77.0 ppm]. Absolute values of the coupling constants are given in Hertz (Hz), regardless of their sign. Multiplicities are abbreviated as singlet (s), doublet (d), doublet of doublets (dd), triplet (t), triplet of doublets (tt), quartet (q), multiplet (m), and broad (br). High-resolution mass spectrometry (HRMS) was performed on an LTQ Orbitrap XL spectrometer with ESI ionization. All reactions were performed under anhydrous conditions under a N$_2$ atmosphere.

The photo-responsive behaviour was studied by steady-state absorption and $^1$H NMR spectroscopy. CD$_3$OD and MeOH were degassed by bubbling argon for 30 min prior to use in the photoisomerization experiments followed by NMR and UV-vis absorption spectrometry. UV-vis spectra were recorded on a Hewlett-Packard HP 8543 Diode Array in a 1 cm path length quartz cuvette. Irradiation of trans-MBA in degassed MeOH were carried out at 25 °C using a Spectroline hand-held UV lamp with LONGLIFE™ filter (8-watt model) positioned at a distance of 5 cm from the sample.

4.5.2 Synthesis and characterization

Trans-MBA

A solution of trans-1 (100 mg, 0.287 mmol) in DMF (2 mL) was added to methyl-11-bromoundecanoate (321 mg, 1.15 mmol), tetrabutylammonium iodide (414 mg, 1.15 mmol) and Cs$_2$CO$_3$ (374 mg, 1.15 mmol), and the reaction mixture was stirred at 80 °C for 16 h. De-ionized (DI) water (15 mL) was added and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic phases were dried over Na$_2$SO$_4$ and concentrated in vacuo. The crude product was purified by flash column chromatography (SiO$_2$, pentane/EtOAc 19/1) to afford the corresponding ester as a colorless oil. To a solution of aq. NaOH (0.3 mL, 4 M), THF (3 mL) and MeOH (3 mL) was added the ester. The reaction mixture was heated at reflux for 2 h then concentrated in vacuo. DI water (10 mL) and aq. HCl (1 M) was added to adjust pH < 7. The aqueous phase was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (30 mL), dried over Na$_2$SO$_4$ and concentrated in vacuo. The crude product was purified by column chromatography (SiO$_2$, pentane/EtOAc 8/2) to afford trans-MBA as a colourless solid (93 mg, 0.13 mmol, 45%). $^1$H NMR (400 MHz, CDCl$_3$) δ 6.54 (s, 2H), 4.03 – 3.90 (m, 4H), 2.89 (p, J = 6.3 Hz, 2H), 2.59 (dd, J = 14.1, 5.6 Hz, 2H), 2.39 – 2.28 (m, 10H), 2.20 – 2.11 (m, 8H), 1.87 – 1.78 (m, 4H), 1.63 (m, 4H), 1.51 (m, 4H), 1.36 – 1.26 (m, 20H), 1.09 (d, J = 6.4 Hz, 6H).$^{13}$C NMR (101 MHz, CDCl$_3$) δ 180.0, 156.6, 142.7, 142.0, 134.1, 131.4, 120.8, 111.2, 68.7, 42.5, 38.7, 34.3, 29.9, 29.8, 29.7, 29.7, 29.5, 29.4, 26.6, 25.0, 19.5, 19.0, 16.5. HRMS (FTMS – n ESI) [M-H]$^-$, calculated: 715.49431; found: 715.49701.

Stable cis-MBA
Stable cis-MBA was synthesized from stable cis-1 by using the same method and scale as described for trans-MBA in section 3.2. The crude product was purified by flash column chromatography (SiO₂, pentane/EtOAc 8/2) to afford stable cis-MBA as a colourless solid (87 mg, 0.12 mmol, 42%). ¹H NMR (400 MHz, CDCl₃) δ 6.53 (s, 2H), 3.96 – 3.80 (m, 4H), 3.32 (p, J = 6.7 Hz, 2H), 3.03 (dd, J = 14.5, 6.3 Hz, 2H), 2.40 – 2.29 (m, 6H), 2.24 (s, 6H), 1.73 (m, 4H), 1.63 (m, 4H), 1.50 – 1.24 (m, 30H), 1.07 (d, J = 6.7 Hz, 6H). ¹³C NMR (400 MHz, CDCl₃) δ 179.6, 155.6, 141.8, 140.6, 135.6, 130.0, 122.2, 111.5, 68.4, 41.5, 37.7, 33.7, 29.3, 29.0, 28.9, 28.7, 28.5, 25.7, 24.3, 20.2, 18.5, 14.0. HRMS (FTMS – n ESI) [M-H]⁻, calculated: 715.49431; found: 715.49454.

4.5.3 Nile red fluorescence assay.¹⁸a,b

A stock solution of Nile Red (1.0 × 10⁻³ M in EtOH) was diluted with trans-MBA and stable cis-MBA solutions to a final concentration of 2.5 × 10⁻² nM. Concentrations of trans-MBA and stable cis-MBA were ranging from 1.4 × 10⁻⁷ to 2.8 × 10⁻³ M. Sample solutions were excited at 550 nm wavelength and the emission spectra were recorded from 580–750 nm by using a JASCO FP6200 spectrofluorometer. Blue shifts were calculated by subtracting the emission wavelength of Nile Red in Milli-Q water from the emission wavelength of the samples. Blue shifts were plotted against MBA concentrations to determine critical aggregation concentrations. The results revealed CACs of 4.0 × 10⁻⁶ M and 1.0 × 10⁻⁵ M for trans-MBA and stable cis-MBA.

4.5.4 NMR study

The molecules are dissolved very well in deuterated methanol, as suggested by the clear signals consisting of sharp peaks without broadening in the obtained ¹H NMR spectra (Figure 4.2c-e). It was not possible to obtain a clear spectrum of an identical sample in D₂O, because the concentration used for the ¹H NMR study was far beyond the CAC (4.0 × 10⁻³ mM) in aqueous medium. Therefore we freeze-dried samples used in cryo-TEM studies and subsequent dissolved the sample in deuterated methanol to measure the ¹H NMR spectra (Figure 4.8).
Stable cis-MBA was synthesized from stable cis-1 by using the same method and scale as described for trans-MBA in section 3.2. The crude product was purified by flash column chromatography (SiO$_2$, pentane/EtOAc 8/2) to afford stable cis-MBA as a colourless solid (87 mg, 0.12 mmol, 42%).

$$\text{H NMR (400 MHz, CDCl}_3 \delta 6.53 (s, 2H), 3.96–3.80 (m, 4H), 3.32 (p, J = 6.7 Hz, 2H), 3.03 (dd, J = 14.5, 6.3 Hz, 2H), 2.40–2.29 (m, 6H), 2.24 (s, 6H), 1.73 (m, 4H), 1.63 (m, 4H), 1.50–1.24 (m, 30H), 1.07 (d, J = 6.7 Hz, 6H).}$$

$$\text{C NMR (400 MHz, CDCl}_3 \delta 179.6, 155.6, 141.8, 140.6, 135.6, 130.0, 122.2, 111.5, 68.4, 41.5, 37.7, 33.7, 29.3, 29.0, 28.9, 28.7, 28.5, 25.7, 24.3, 20.2, 18.5, 14.0.}$$

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The molecules are dissolved very well in deuterated methanol, as suggested by the clear signals consisting of sharp peaks without broadening in the obtained $^1$H NMR spectra (Figure 4.2c–e).

I t was not possible to obtain a clear spectrum of an identical sample in D$_2$O, because the concentration used for the $^1$H NMR study was far beyond the CAC (4.0 x 10$^{-3}$ mM) in aqueous medium. Therefore we freeze-dried samples used in cryo-TEM studies and subsequently dissolved the sample in deuterated methanol to measure the $^1$H NMR spectra (Figure 4.8).

4.5.5 Cryo-TEM study.

Sample Preparation.
In order to study the influence of irradiation on the assembly structure of MBA a dispersion of trans-MBA (7.0 × 10^{-3} M) in sodium borate buffer (pH = 9.3, 0.1 M) was heated at 80 °C for 30 min and then cooled down to room temperature to form ordered assemblies (Step 1). Then the solution was placed in a quartz cuvette (1 mm path length) and irradiated with 312 nm light for 10 min using a Spectroline hand-held UV lamp with LONGLIFE™ filter (8-watt model) (Step 2), followed by irradiation with 365 nm light for 10 min using the same lamp (Step 3). Finally, solutions after 312 nm and subsequent 365 nm irradiation were heated at 80 °C for 30 min and then cooled down to room temperature again (Step 4). 20 µL samples taken after each step were used for cryo-TEM measurements immediately. 100 µL solutions after Steps 1, 2 and 3 were freeze-dried and used in NMR studies (Figure 4.8).

Solutions of stable cis-MBA used in cryo-TEM studies were prepared following the same method as described for trans-MBA. The self-assembly morphology of stable cis-MBA is shown in Figure 4.10.

Assembly morphology transitions induced by pH were studied by adding varying amounts of an aq. NaOH stock solution (0.1 M) to an aq. solution of trans-MBA (7.0 × 10^{-3} M). Sample solutions at pH 8.8, pH 9.8 and pH 11 were heated at 80 °C for 30 min and cooled down to room temperature. A sample of pH 9.4 was prepared by adding an aq. HCl stock solution (0.1 M) to a sample solution of pH 11. The pH of the samples was determined using a Mettler-Toledo SevenEasy™ pH meter with Inlab hydrofluoric electrode.

In order to study the assembly morphology of trans-MBA as a function of the counter-ions, an aqueous dispersion of trans-MBA (7.0 × 10^{-3} M) with 2.0 equiv. of NaOH was heated at 80 °C for 30 min and cooled down to room temperature to afford the sample solution. An aq. NaCl (1.0 M) or CaCl₂ (5.0 × 10^{-2} M) stock solution was added to the sample solution to achieve the indicated chloride salts concentrations.

Characterization.

A few µL of each sample solution were placed on holey carbon coated copper grids (Quantifoil 3.5/1, Quantifoil Micro Tools, Jena, Germany). Grids with sample were vitrified in liquid ethane (Vitrobot, FEI, Eindhoven, The Netherlands) and transferred to a FEI T20 cryo-electron microscope equipped with a Gatan model 626 cryo-stage operating at 200 kV. Micrographs were recorded under low-dose conditions with a slow-scan CCD camera.
In order to study the influence of irradiation on the assembly structure of MBA a dispersion of trans-MBA (7.0 × 10⁻³ M) in sodium borate buffer (pH = 9.3, 0.1 M) was heated at 80 °C for 30 min and then cooled down to room temperature to form ordered assemblies (Step 1). Then the solution was placed in a quartz cuvette (1 mm path length) and irradiated with 312 nm light for 10 min using a Spectroline handheld UV lamp with LONGLIFE™ filter (8-watt model) (Step 2), followed by irradiation with 365 nm light for 10 min using the same lamp (Step 3). Finally, solutions after 312 nm and subsequent 365 nm irradiation were heated at 80 °C for 30 min and then cooled down to room temperature again (Step 4). 20 µL samples taken after each step were used for cryo-TEM measurements immediately. 100 µL solutions after Steps 1, 2 and 3 were freeze-dried and used in NMR studies (Figure 4.8).

Solutions of stable cis-MBA used in cryo-TEM studies were prepared following the same method as described for trans-MBA. The self-assembly morphology of stable cis-MBA is shown in Figure 4.10.

Assembly morphology transitions induced by pH were studied by adding varying amounts of an aq. NaOH stock solution (0.1 M) to an aq. solution of trans-MBA (7.0 × 10⁻³ M). Sample solutions at pH 8.8, pH 9.8 and pH 11 were heated at 80 °C for 30 min and cooled down to room temperature. A sample of pH 9.4 was prepared by adding an aq. HCl stock solution (0.1 M) to a sample solution of pH 11. The pH of the samples was determined using a Mettler-Toledo SevenEasy™ pH meter with Inlab hydrofluoric electrode.

In order to study the assembly morphology of trans-MBA as a function of the counter-ions, an aqueous dispersion of trans-MBA (7.0 × 10⁻³ M) with 2.0 equiv. of NaOH was heated at 80 °C for 30 min and cooled down to room temperature to afford the sample solution. An aq. NaCl (1.0 M) or CaCl₂ (5.0 × 10⁻² M) stock solution was added to the sample solution to achieve the indicated chloride salts concentrations.

**Characterization.** A few µL of each sample solution were placed on holey carbon coated copper grids (Quantifoil 3.5/1, Quantifoil Micro Tools, Jena, Germany). Grids with sample were vitrified in liquid ethane (Vitrobot, FEI, Eindhoven, The Netherlands) and transferred to a FEI T20 cryo-electron microscope equipped with a Gatan model 626 cryo-stage operating at 200 kV. Micrographs were recorded under low-dose conditions with a slow-scan CCD camera.

**Figure 4.10.** Cryo-TEM image of stable cis-MBA (7.0 × 10⁻³ M) in sodium borate buffer (pH = 9.3, 0.1 M). (Micelles were pointed out with arrows for clearance, and not all the micelles were pointed.)

**Figure 4.11.** Cryo-TEM image of trans-MBA (7.0 × 10⁻³ M) in sodium borate buffer (pH = 9.3, 0.1 M) after 312 nm irradiation for 10 min. (white: micelles, yellow: vesicles, black: sheets)

**Note on the PH effect on photoresponsive assembly**

We performed the photoisomerization process on sample solutions of different pH = 8.8, 9.8, and 11. As shown in Figure 4.12a, sheet-like aggregates were found in the solution of trans-MBA at pH 8.8. After 312 nm irradiation for 10 min, vesicles were observed as a mixture with the sheet-like assemblies (Figure 4.12b). After a subsequent photoirradiation with 365 nm light for 10 min, the sheet-like structures were reformed with some small vesicles remaining in the mixture (Figure 4.12c). This was nearly identical to the result observed in sodium borate buffer. In addition, trans-MBA showed formation of disc-like structures in the sample solution at pH = 9.8 (Figure 4.12d). There was no significant morphological transformation observed after irradiating an identical sample with 312 nm light (Figure 4.12e) and subsequent irradiation with 365 nm light (Figure 4.12f).
Furthermore, micellar structures were observed in the solution of *trans-MBA* at pH = 11 (Figure 4.12g), which showed no obvious assembly transformations following irradiation with 312 nm and 365 nm light (Figure 4.12h-i). Based on these studies, we found that the self-assembly behaviour of MBA molecules is sensitive to pH variation. Photoisomerization induced assembly transformation was only observed in lower pH regimes (either pH 8.8 or borate buffer with pH 9.3). However, at higher pH, MBA showed no significant morphological transformation upon irradiation.

*Figure 4.12.* Cryo-TEM images of *trans-MBA* (7 mM) in aq. NaOH solution with (a–c) pH = 8.8, (d–f) pH = 9.8, (g–i) pH = 11 (a,d,g) before and (b,e,h) after irradiation with 312 nm for 10 min, (c,f,i) as well as after subsequent 365 nm irradiation for 10 min, respectively.
Furthermore, micellar structures were observed in the solution of trans-MBA at pH = 11 (Figure 4.12), which showed no obvious assembly transformations following irradiation with 312 nm and 365 nm light (Figure 4.12-13). Based on these studies, we found that the self-assembly behaviour of MBA molecules is sensitive to pH variation. Photoisomerization induced assembly transformation was only observed in lower pH regimes (either pH 8.8 or borate buffer with pH 9.3). However, at higher pH, MBA showed no significant morphological transformation upon irradiation.

Figure 4.12. Cryo-TEM images of trans-MBA (7 mM) in aq. NaOH solution with (a–c) pH = 8.8, (d–f) pH = 9.8, (g–i) pH = 11 (a,d,g) before and (b,e,h) after irradiation with 312 nm for 10 min, (c,f,i) as well as after subsequent 365 nm irradiation for 10 min, respectively.

4.6 References


