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## COMMUNICATION

## Photo-responsive self-assemblies based on bio-inspired DNA-base containing bolaamphiphiles

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**Photo-responsive self-assemblies based on bio-inspired bolaamphiphiles with oligo (ethylene oxide) hydrophilic outer segments and a hydrophobic inner segment with thymine were investigated. The morphology of the self-assemblies transformed from ribbon-like structures to spherical structures in water by UV irradiation which was investigated by <sup>1</sup>HNMR, <sup>13</sup>CNMR, DLS and TEM.**

In the last decade, researchers have developed several approaches to create stimuli-responsive nanomaterials, which have the ability to respond in an autonomous way upon exposure to an external stimulus such as heat, chemicals, light. One of the fascinating approaches is responsive self-assembling structures. The design and synthesis of self-assembled structures are of great interest due to their potential utilisation in different applications, for example drug delivery and nanodevices systems.<sup>1,2</sup>

Self-assembly structures are created by amphiphiles which have the ability to self-assemble spontaneously in aqueous solution to form highly ordered structures.<sup>3</sup> One type of amphiphiles that consists of two hydrophilic groups linked by a hydrophobic skeleton is known as bolaamphiphiles.<sup>4</sup> In comparison to conventional amphiphiles, bolaamphiphiles tend to have relatively low solubility and critical micelle concentration (CMC). Like conventional amphiphiles, they aggregate spontaneously in water to form various self-assembly structures including micelles, vesicles and fibres.<sup>5,6</sup>

Some chemistries have been used in the design of responsive molecular systems. For example, imine bond,<sup>6,7</sup> hydrazone bond,<sup>8</sup> Se-N covalent bond<sup>9</sup> were incorporated into amphiphilic molecules to impart their sensitivities to suitable triggers such as heat, chemical and light.

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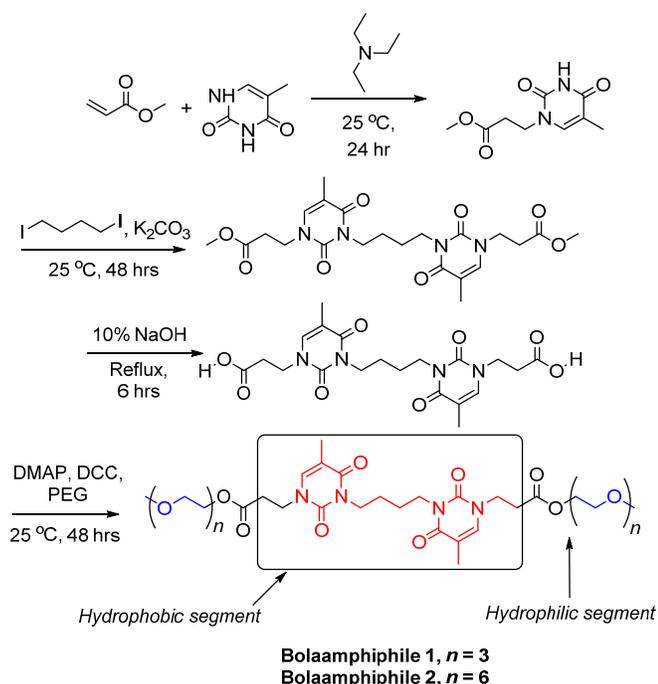
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These chemistries have been incorporated to regulate the transition between a monomeric state and self-assembled aggregates upon exposure to external stimulus.<sup>3,10</sup>

Thymine, one of the nucleic bases in DNA, is a highly sensitive molecule to light<sup>11</sup> via [2π+2π]-cycloaddition reaction upon exposure to UV light.<sup>12</sup> Usually, it forms dimers when irradiated with light of wavelength >270 nm and the dimer can be cleaved using <249 nm UV light.<sup>13,14</sup>

Inspired by previous work done by our group and the desire to utilise this chemistry in an aqueous biological context,<sup>14</sup> herein we report for the first time the synthesis of thymine incorporated water soluble bolaamphiphilic molecules that are capable of self-assembly in water. The structures formed on assembly transform from ribbon-like structures to spherical structures upon exposure to UV light. The bolaamphiphile comprised two thymine molecules separated by a spacer group (butyl group) linked by two oligo(ethylene glycol) with various chain lengths as shown in Scheme 1. Due to the structure of these novel bolaamphiphiles, they are expected to self-assemble leading to close association of thymine groups, allowing the photocyclization of thymine to take place when irradiated by UV light. This approach will provide a promising platform for the development of novel stimuli responsive nanomaterials for drug delivery systems, nanodevices, etc.

Bolaamphiphiles were synthesised through four sequential reactions, synthesis of thymine propanoate, synthesis of bis-butyl-bis-thymines, hydrolysis of bis-thyminylnyl acetate and finally esterification of the bis-thyminylnyl acetate. Two bolaamphiphiles were varied in the length of oligo(ethylene oxide); bolaamphiphile **1** with triethylene oxide and bolaamphiphile **2** with hexaethylene oxide.



Scheme. 1 Synthesis route of bolaamphiphiles.

Amphiphilic systems usually aggregate above a specific concentration known as the critical aggregation concentration (CAC). The CACs of the synthesised amphiphilics were determined by fluorescence measurements in aqueous solution using pyrene as a hydrophobic probe.<sup>15-18</sup> The CAC of bolaamphiphiles **1** and **2** were found to be 0.2 and 0.5 mM, respectively as shown in Fig. 1.

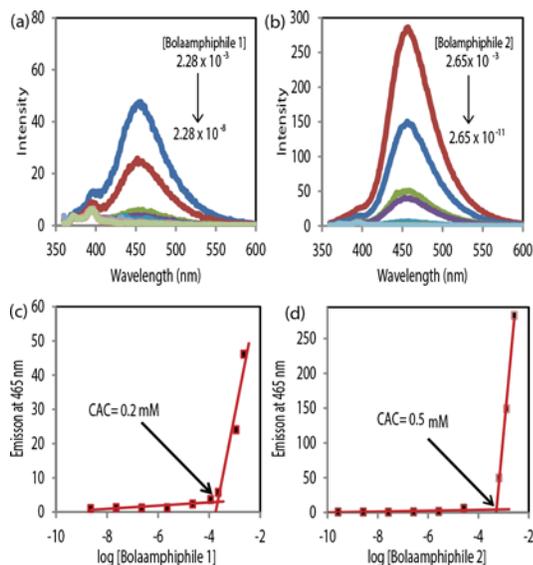


Fig. 1 Fluorescence emission spectra for pyrene as a function of concentration for bolaamphiphile **1** (a) & **2** (b) measured at a fixed excitation wavelength (350 nm), (c) & (d) The emission intensity of pyrene at 465 nm (in arbitrary units) as a function of log [bolaamphiphile **1**] and [bolaamphiphile **2**].

As it is known that  $[2\pi+2\pi]$ -cycloaddition reactions can be monitored by ultraviolet-visible spectroscopy (UV-Vis). This technique depends mainly on the differences between the UV absorption characteristics of the starting olefin and corresponding cyclobutane photo-product. The UV-Visible measurements for the sample before the irradiation showed that the bolaamphiphiles absorbed UV at 289 nm due to the presence of thymine moiety which usually is capable of absorbing UV in that region.<sup>19,20</sup> Both bolaamphiphiles were then irradiated with 302 nm UV for 3 days with energy levels up to  $60 \text{ J}\cdot\text{cm}^{-2}$  to achieve the  $[2\pi+2\pi]$ -cycloaddition between bolaamphiphiles. The absorption at 289 nm was noticeably reduced in bolaamphiphile **1** & **2** by 78% and 96%, respectively (Fig.2). This is an interesting result showing that incorporated thymine moieties photodimerised.

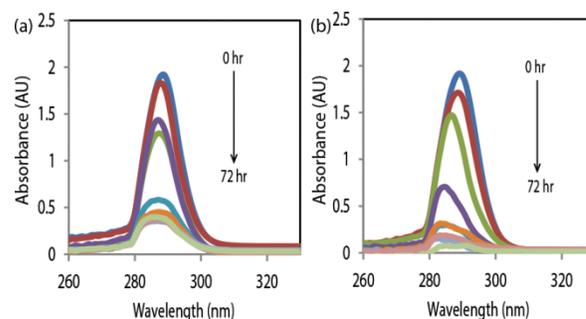


Fig. 2 UV-Vis absorbance spectrum for bolaamphiphile **1** (a) & bolaamphiphile **2** (b) before and after UV-irradiation.

The NMR and IR spectra for both bolaamphiphiles after UV-irradiation showed that both self-assembled structures underwent photochemical conversion via their photoactive thymine units. The <sup>1</sup>H-NMR spectra showed that bolaamphiphiles **1** and **2** were photodimerised at over 60% and almost 100%, respectively. In bolaamphiphile **1**, the spectrum showed two new peaks which correspond to the cyclobutane-CH and -CH<sub>3</sub> protons of cyclobutane isomer at the  $\delta$  0.87 and  $\delta$  3.10 ppm. Similarly, the same first peak - CH<sub>3</sub> protons was detected at  $\delta$  0.87 but cyclobutane-CH peak is believed to overlap another peak around 3-4 ppm due to the presence of water and PEG chain. We confirmed the photo-conversion of bolaamphiphile **2** by <sup>13</sup>C-NMR which indicated the appearance of peaks at  $\delta$  19.0 and 58.5 ppm that correspond to cyclobutane carbons C5 and C6. In addition, the IR band near  $1535 \text{ cm}^{-1}$  spectral region, which has been attributed the C5=C6 stretching mode of the thymine units in the bolaamphiphiles spectrum is missing and the C5-CH<sub>3</sub> stretching band, which occurred at  $768\text{-}769 \text{ cm}^{-1}$  for bolaamphiphiles before irradiation has shifted to a lower wavenumber ( $755\text{-}756 \text{ cm}^{-1}$ ) in both spectra after irradiation. The morphology of the self-assembled structures was characterized by TEM using both cryogenic and staining techniques.<sup>21</sup> The images of both bolaamphiphiles **1** & **2** in water show the formation of ribbon-like structures. The ribbon-like structures in bolaamphiphile **1** are very narrow and they are about  $>1 \mu\text{m}$  long (Fig.3\_a). On the other hand, bolaamphiphile **2** showed relatively broader and flatter structures evidenced by low contrast between the background and the ribbon-like structures in the image obtained in Fig. 3\_b (about 0.1  $\mu\text{m}$  width and  $>1 \mu\text{m}$  long). It is well known that bolaamphiphile can

form ribbon- or rod-like structures due to the nature of the chemical structure.<sup>22</sup>

Upon UV-irradiation, each bolaamphiphile solution showed interesting morphologies that are different from the ones obtained before being exposed to UV-light. The ribbons that were evident for both bolaamphiphiles in water prior to irradiation were found to be disrupted and transformed to spherical structures after irradiation (Fig.3-c&d). The average diameter of the spherical structures in bolaamphiphile **1** was approximately 10 nm whereas in bolaamphiphile **2** it was approximately 100-200 nm. It is worth noting that the stain technique was used to confirm the morphology of the spherical structures due to poor contrast for these structures in cryo-TEM (see ESI).

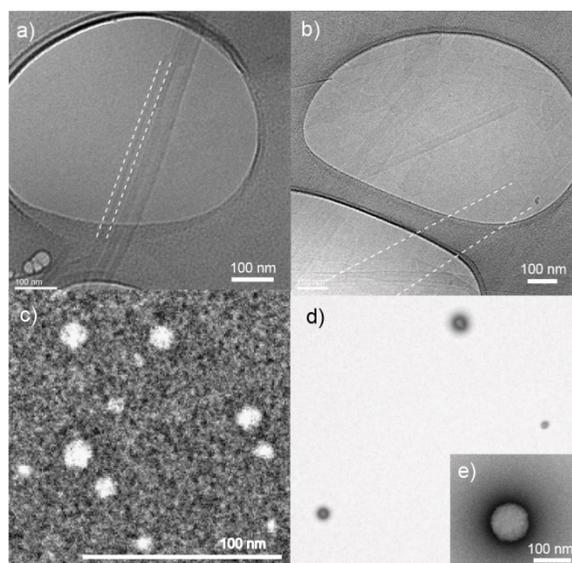


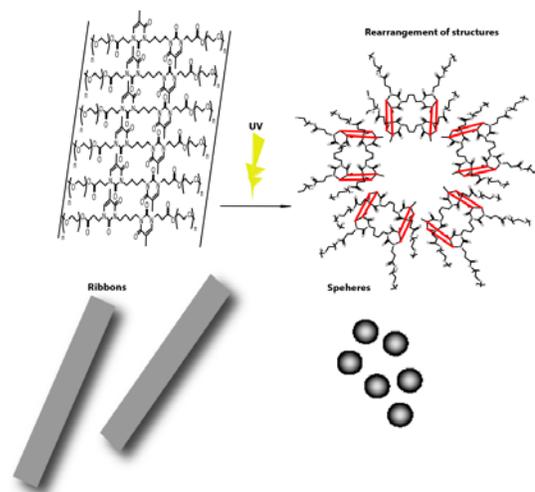
Fig. 3 Cryo-TEM images of bolaamphiphile **1** (a) & bolaamphiphile **2** (b) before irradiation and TEM with staining images obtained for bolaamphiphile **1** (c) & bolaamphiphile **2** (d) after irradiation.

The transformation found in the shapes upon irradiation could be rationalised on several grounds. Upon the irradiation adjacent thymines started to photodimerise and cyclobutane structures formed. Therefore, the packing of the molecules is disrupted and dimers of two molecules with a bent structure form where hydrophilic oligo (ethylene oxide) chains are on the same side (U-shaped structures). This bending might have led to the formation of the spherical self-assembled structures after irradiation as shown in Scheme 2.

Dynamic light scattering (DLS) was used to characterise the size of the self-assembled structures. DLS data (provided in ESI) showed that the self-assembled structures formed by the two bolaamphiphiles before irradiation have a larger size than the detection limit of the instrument used. However, the irradiated samples showed average sizes of 100 nm and 215 nm. This supports the findings obtained by TEM.

In conclusion, we have reported an example of a switchable bolaamphiphilic system based on light-sensitive thymine molecule. The bolaamphiphile can aggregate into highly ordered ribbon-like structures in water and can transform the aggregation morphology

into spherical structures in response to UV light. There are many possible applications of these new materials. Experiments to evaluate their application in drug delivery, photorheological fluids, and in sensing systems will be conducted in future studies.



Scheme 2 Proposed mechanism of self-assembly of bolaamphiphiles after UV-irradiation at 302 nm.

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