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Photoresponsive amphiphiles based on azobenzene-dendritic glycerol conjugates show switchable transport behavior[†]

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The synthesis and aggregation behavior of photo-switchable, nonionic dendritic amphiphiles was investigated with regard to transport and release of guest molecules. The correlation between the critical micelle concentration and the switching ability is shown.

Nowadays the concept of drug delivery is of great interest because the application of many drugs suffers from low water-solubility and short half-life in the bloodstream. Because these drugs are only applicable in low concentrations, they have low pharmaceutical potential. The development of new drugs that do provide water-solubility and an adequate residence time is often not practical due to the time and resources needed in development and testing. The use of additives is an alternative to improve the solubility of existing drugs. For example, the hydrophobic antitumor drug Taxol[®] is used in combination with a polyethoxylated castor oil called Cremophor[®]. These additives, however, produce unexpected effects, such as hypersensitivity reactions¹ and peripheral neuropathy.² Thus, recent research has concentrated on the development of biocompatible micelles,³ which are able to incorporate guest molecules and improve their overall solubility in pharmaceutical preparations. Grinstaff and co-workers have reported on dendritic amphiphiles from glycerol, succinic acid and myristic acid.4a,b Our group has recently developed new dendritic amphiphiles based on second generation glycerol dendrons, which form monodisperse micelles and show a significantly higher solubilization compared to other nonionic surfactants.⁵ These dendritic glycerol amphiphiles show exceptionally low critical micelle concentrations (cmc) of $\sim 10^{-6}$ M. In addition to the enhancement of solubility, a triggered release of the guest molecules is highly desired because the pharmaceutical potential of drugs can be further increased and side-effects decreased. Attempts have been made to disrupt micellar structures with different external stimuli like temperature,⁶ pH,⁷ or redox processes.⁸ Most of these methods involve the degradation of the amphiphilic

molecule, which is irreversible or can lead to other undesired effects. Another promising approach is the light-induced isomerization of azobenzenes.⁹ This photo-switch has been used for controlling the expression of genes or the function of proteins¹⁰ as well as in liquid crystals¹¹ or for molecular switches on surfaces.¹²

The thermodynamically favored azobenzene trans isomer can be switched to the *cis* isomer within seconds by light, usually at UV wavelengths of 350 nm. Back-switching to the trans isomer can be achieved either thermally or by light at longer wavelengths. Abe et al. reported on the light-triggered change in micelle formation of an azobenzene derivative substituted with ammonium bromide as a polar head group and a short aliphatic chain.¹³ However, the use of ionic amphiphiles as a polar group is problematic for potential applications like additives for drugs or in solution with various pH values. In this communication, a new class of switchable non-ionic amphiphiles based on glycerol dendrons have been employed, which form highly defined micelles.¹⁴ Furthermore, by choosing different generations of the dendrimer the polarity and the size of the headgroup can be tuned. Due to the large change in geometry of the azobenzene-dendritic glycerol conjugates upon irradiation of light, the aggregation behavior is expected to change significantly, which should lead to lower aggregation (higher cmc) of the amphiphiles in the cis state as compared to the trans isomer (Fig. 1). For comparison we synthesized a polymeric amphiphile made from polyethylene glycol with a molecular weight of 500 g mol⁻¹ (mPEG500).



Fig. 1 Proposed micelle disruption by light at a concentration between cmc (*trans*) and cmc (*cis*).

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Fig. 2 Structures of the four glycerol amphiphiles.

In this case, due to the flexible linear arrangement, less change in the aggregation behavior (cmc) is expected upon photoisomerization for the PEG-based amphiphile.

The photoresponsive amphiphiles were synthesized starting from 4-aminophenol, which was treated under standard diazotation conditions and then reacted with phenol under basic conditions to yield 4,4-dihydroxyazobenzene (see ESI†). The hydrophobic building block was attached to the azobenzene by etherification with the chosen aliphatic chain (C11 and C16). The hydrophilic units, glycerol dendrons of Generation [G2] and [G3], were synthesized as reported previously.¹⁵

Their attachment to the azo switch was carried out by etheration with mesylated acetal-protected glycerol dendrons under basic conditions. Subsequent removal of the acetal protecting groups resulted in the four azobenzene-containing amphiphiles (Fig. 2). The same procedure was carried out for the ether formation of mPEG(500) with the hydrophobic building block.

The photoisomerization was examined using simple UV/Vis adsorption spectroscopy. The UV/Vis spectra of the compound G2azoC11 illustrate the light-induced switching (Fig. 3). The absorbance at 350 nm decreased after 60 minutes to 50% of the former intensity, which means approximately half of the *trans* isomers were switched to the *cis* compound. For the compound bearing the next higher glycerol generation, G3azoC11, the photo-stationary state (pss) was increased to 70%. The higher ability to switch can be explained by the lower aggregation number of the micelles bearing [G3] as compared to [G2]. Trappmann *et al.* determined the aggregation



Fig. 3 UV/Vis spectrum of the *trans* (black) and the partially switched (grey) G2azoC11.

Table 1 Characterization of the amphiph	iles
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Compound	PSS ^a (%)	Particle size ^b	PDI	cmc in mol l ⁻¹	cmc ratio cis/trans
G2azoC11	50	9.2 nm	0.2	<i>trans</i> : 4×10^{-5}	2
G3azoC11	70	7.2 nm	0.08	<i>trans</i> : 7×10^{-6} <i>cis</i> : 8×10^{-5}	11
G2azoC16	20	10.2 nm	0.02	trans: 6×10^{-6}	—
G3azoC16	50	9.4 nm	0.1	trans: 4.4×10^{-6}	2
mPEGazoC11	10	205 nm	0.28	cis: 8.5×10^{-5} trans: 3.1×10^{-5} cis: 3.0×10^{-5}	
^a Photostationary state. ^b DLS by intensity, for spectra see ESI.†					

number of amphiphiles with a similar structure: glycerol dendrons connected to an aliphatic chain by an aromatic moiety.¹⁴ The resulting spherical micelles of [G3] contained only a third of the molecules compared to the micelles of the [G2] amphiphile, which indicates [G3] amphiphiles are more loosely packed due to the larger size of the headgroups.

The [G3] micelles are more defined even though the amphiphile head group is larger, which leads to more space in the interior.¹⁴ This phenomenon led, in our case, to less steric hindrance of azobenzene switching. Thus, the ability to switch from *trans* to the *cis* isomer was higher. The same phenomenon occurred with the examined C16 amphiphiles, where the pss increased from 20% for [G2] to 50% for [G3]. The pss was lower than for the amphiphiles with a C11 chain as a hydrophobic unit, which can be explained by a higher aggregation number and inhibition due to more densely packed micelles (Table 1).

These observations were further supported by the particle size measurements from dynamic light scattering (DLS). The micelles formed by G2azoC11 had a diameter of 9.2 nm, whereas compound G3azoC11 with the higher PG dendron formed more defined aggregates with 7.2 nm. The size of the G3azoC11 micelles is in excellent agreement with the theoretical value of 7.3 nm of two molecules in a row for the diameter. In addition, the size distributions revealed well-defined particles because the intensity curve showed a monomodal behavior. The molecular weights of the resulting micelles can also be measured by static light scattering. For the trans-G3azoC11 an average molecular weight of the micelles of 98.1 \pm 6.8 kDa was determined, which indicated there were 66 ± 5 molecules per micelle. After switching to the *cis* isomer by light a higher molecular weight was measured. According to the SLS measurements the aggregates were then composed of 97 \pm 14 molecules. Taking into account the resulting mixture of cis and trans isomers which form at the pss, the higher aggregation number suggests a formation of ill-defined, mixed micelles.¹⁶ The change in geometry during the switching process should also affect micelle stability and, accordingly, the critical micelle concentration (cmc), which was determined by the pendant drop method. The cmc for G2azoC11 was doubled to 8 \times 10 $^{-5}$ M upon irradiation with UV light. The effect of isomerization was much higher for the G3azoC11, where the cmc increased by a factor of ten. The large influence

can be explained by the increasing effectiveness of switching. The cmc values for the two C16 compounds in the *trans* state were 2.5×10^{-5} M and 4.4×10^{-6} M for G2azoC16 and G3azoC16, respectively, which are lower than the corresponding C11 amphiphiles. For the [G2] moiety there was no detectable difference in cmc upon irradiation of light (350 nm). Corresponding UV/Vis adsorption measurements revealed not only the above-mentioned low pss of 20%, but also a fast thermal back-switching with a half-life of two hours. Thus, the resulting change was too small to be measurable. In the case of the G3azoC16 the cmc doubled upon photo-switching, which corresponds, perhaps noncoincidentally, to the same pss as G2azoC11 of 50%.

We also tested azobenzenes substituted with a linear polyether function, namely mPEG with a molecular weight of 500 g mol⁻¹ that was chosen because the molecular weight is similar to the [G2] PG dendrons and should provide similar solubilization properties. We expected no change in cmc due to the long flexible PEG chain which could more easily compensate the isomerization of the neighboring azobenzene. In fact, the ability to switch was very weak: roughly 10% of the molecules were switched to the *cis* state. The cmc was also negligibly affected upon irradiation. DLS measurements showed large and presumably ill-defined aggregates with sizes of ca. 200 nm. We suppose that the packing of the mPEGazoC11 molecules is too dense for efficient switching because of their linear geometry, which is also reflected by their tendency to form large ill-defined aggregates that are known for nonionic surfactant micelles.¹⁷ Again, steric hindrance restricted the isomerization.

The solubilization of a water-insoluble dye, Nile Red, was also undertaken to further illustrate the effect of switching. At an amphiphile concentration slightly above the cmc of the *trans* G3azoC11, solid Nile Red was added to two vials. One was kept in the dark, the other was irradiated with 350 nm light for 24 hours with continuous stirring. After removal of the excess Nile Red by filtration, the resulting solution of the sample which was kept in dark remained red indicating the solubilization of the dye (Fig. 4). The UV/Vis spectrum of the solution exhibited typical adsorption bands for Nile Red, whereas the other sample, which was kept under 350 nm was the normal color of the azobenzene moiety. Here no adsorption bands for Nile Red were seen in the UV/Vis



Fig. 4 Left: UV/Vis spectra of the light-attenuated solubilization of Nile Red. An uptake of the dye can be seen in the sample which was kept in dark (grey), whereas the switched sample showed no adsorption of the dye (black). Right: solubilization of Nile Red in a 1×10^{-5} M solution of G3azoC11. The left vial was kept in the dark, right vial was kept under 350 nm irradiation.

spectrum because of isomerization to the *cis* isomer, which resulted in an increase in the cmc above the chosen concentration of 1×10^{-5} M. We conclude, based on these observations, that a light attenuated uptake of the dye has occurred. To exclude the possibility of photo-bleaching of the dye, a solution of Nile Red in methanol was kept under 350 nm irradiation for the same time and resulted in no decrease of Nile Red adsorption.

In this work we have shown the synthesis of a new class of light switchable dendritic amphiphiles. All four of the PG-based amphiphiles formed well-defined spherical micelles and offered a higher ability to undergo trans-cis photoisomerization than the corresponding linear mPEG amphiphile, which formed rather large ill-defined aggregates. The size of the micelles influenced the photo-stationary state: the smaller the micelles the higher the pss. These findings are based on the larger empty space between the molecules in smaller micelles which improve the photo response better than densely packed larger aggregates. Because the critical micelle concentration is effectively changed by light, these amphiphiles can be used for controlling the solubilization and encapsulation of a guest molecule. The effectiveness of the switching in modulating the aggregation behavior of the amphiphiles is highly influenced by the pss. A high pss led to a larger change in the cmc and a larger overall effect on the solution behavior. In summary, a new nonionic switchable delivery system has been developed, which will be investigated for controlled release of active agents.

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