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ARTICLE

Perfluoroalkylated Linear Polyglycerols and their Supramolecular Assemblies in Aqueous Solution

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In this study, amphiphiles composed of linear polyglycerols (LPGs) with hydroxyl, methoxy, and ethoxy side groups and end capped with one or two perfluorooctyl chains (R_{f8}) were designed to form supramolecular architectures. The amphiphiles and bolaamphiphiles are capable of self-assembling into spherical and flat micelles as well as nonionic unilamellar vesicles (niosomes) in aqueous solution. The fluorous compartments of the micelles preferentially encapsulate a trifluoromethylated disperse red 1 compared to the unaltered dye. Micellar solutions of methoxy LPG compounds show a transition in water at temperatures of 38 °C (**2**) and 33 °C (**5**) in which reversibly larger agglomerates are formed. Both amphiphiles also form very stable unilamellar vesicles with diameters ranging from 30 nm up to 10 µm, if prepared via thin film hydration method, which remain intact above 38°C.

Introduction

Design and development of functional self-assemblies is one of the major challenges in the field of supramolecular chemistry.[1,2,3] Especially, supramolecular carrier systems have been focused on for delivering of therapeutic molecules in both native and chemically unaltered form.[4,5] The various amphiphilic systems, which have been developed in the last few decades, are broadly divided into two major classes, polymeric and small molecule systems, according to their molecular weight.[6] Depending on their amphiphilicity, both systems result in nanostructures like micelles and vesicles.[7] While micelles can solubilize hydrophobic guests within their hydrophobic cores,[8,9] vesicles can be used to entrap hydrophilic guests dissolved in the enclosed water and can also integrate hydrophobic molecules within the hydrophobic compartments of their membranes.[10] Membranes of vesicles are particularly interesting due to their structural similarity with cell membranes and can be studied as biomimetic membranes or model systems for membrane dynamics.[11,12] Both polymeric and small molecule amphiphiles are inherently advantageous polymeric systems because of their higher loading capacity[13,14] and small molecules for their lipid-like behavior.[6,15] Recently the novel class of hybrid amphiphiles was designed to combine these advantages.[6,8]

Among the many types of amphiphilic block copolymers that have been reported in the literature, polyethylene glycol (PEG) has been widely used as a hydrophilic block because of its biocompatibility and prolonged circulation time in vivo.[16,17] However, PEG offers only two terminal functional groups to perform chemical modifications and is therefore very limited in terms of tuned properties. In contrast, linear polyglycerols (LPGs) are a class of biocompatible, polyhydroxy polymers that could be considered as a multifunctional analog of PEG.[18] LPGs provide great diversity due to their alkoxide side group functionality.[19] This allows for the preparation of LPGs with tunable properties, such as hydrophilicity or lower critical solution temperature (LCST),[20,21] which are useful for the formation of supramolecular carriers.[14,22] Additionally, hydroxyl side groups can be further functionalized subsequently.

Perfluoroalkyl chains have been shown to confer unique properties to amphiphiles.[23] Strong hydrophobic and low van-der-Waals interactions of fluorinated chains dramatically increase the surface activity of fluorinated amphiphiles as well as their tendency to self-assemble in water,[24,25] which is reflected by the much lower CMC values of fluoro-surfactants if compared to equivalent hydrocarbon-surfactants.[26]

A detrimental side effect of the unique fluorocarbon properties that has been identified as a global problem is the persistence of long-chain perfluorocarbon acids in the environment.[27,28] Although their acute toxicity is relatively low, the uncertainties about their environmental fate, pharmacokinetics, and long-term toxicity remain a considerable risk.[29] Due to these knowledge gaps and the unique qualities of perfluorocarbons it appears worthy to expand the knowledge

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about the physicochemical properties and interaction of perfluorocarbons in an aqueous environment. Various fluorinated amphiphiles have been designed to create stable and compartmented supramolecular structures.[30-33] Introducing perfluoroalkyl chains as a hydrophobic segment in amphiphiles is presumed to be beneficial for encapsulating fluorinated drugs.[34,35] Fluorinated PEG amphiphiles, for instance, were shown to bind highly fluorinated sevoflurane through interactions with the internal fluorous phase.[36] Recently, Tucker et al. investigated the influence of surfactant topology on the physicochemical properties of perfluorinated-PEG amphiphiles in water and revealed enhanced kinetic stability for linear and dibranched surfactants.[37] Our group utilized fluorous interactions of perfluoroalkyl chains grafted onto dendritic polyglycerols to form supramolecular architectures.[38–40] We also synthesized fluorinated linear polyglycerol surfactants for droplet-based microfluidics.[41] These triblock copolymers with two large perfluoropolyether units, however, are only soluble in perfluorinated solvents.

Herein we report on the synthesis of LPG amphiphiles and bolaamphiphiles terminated with perfluorooctyl moieties and on their differing aggregation behavior in water. In contrast to known PEG amphiphiles bearing one[36,42,43] or two[42-44] perfluorooctyl units, we use LPG as the hydrophilic unit to introduce tunable hydrophilicity, thermoresponsive properties and render post-functionalization possible.

Experimental

Materials and methods

2*H*,2*H*,3*H*,3*H*-perfluoroundecanoic acid ($HOOC(CH_2)$-R_{f8}), calcein, and disperse red 1 were purchased from Sigma Aldrich. Dialysis was performed in regenerated cellulose tubes from Spectrum Laboratories (Spectra/Por® 6 Dialysis membrane, purchased from Roth, Karlsruhe, Germany). The deionized water used was purified using a Millipore water purification system with a minimum resistivity of 18 MV cm^{-1}. All aqueous solutions for DLS or IFT measurements were filtered with 0.45 µm polytetrafluoroethylene (PTFE) syringe filters.

^1H and ^{19}F NMR spectra were recorded on a Jeol ECX spectrometer operating at 400 MHz and a Bruker Avance 3 spectrometer operating at 700 MHz, respectively, at concentrations of 20–100 mg mL^{-1}. The chemical shifts have been reported in δ (ppm) values and were referenced as indicated. Experiments using electrospray-ionization, time-of-flight, high-resolution mass spectrometry (ESI-TOF-HRMS) were conducted on an Agilent 6210 ESI-TOF mass spectrometer (Agilent Technologies). Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry was performed on a Bruker ultrafleXtreme MALDI-TOF/TOF mass spectrometer using a cyano-4-hydroxycinnamic acid matrix. Absorption spectra were recorded on a LAMBDA 950 UV/Vis/NIR spectrometer (PerkinElmer, USA). A DataPhysics OCA tensiometer (Data Physics Instruments, Germany) was used to carry out surface tension measurements via the pendant-drop method. Dynamic light scattering (DLS) measurements were performed with a Zetasizer Nano-ZS from Malvern Instruments equipped with a 633 nm He–Ne laser. Cryogenic transmission electron microscopy (cryo-TEM) was conducted using a Philips CM12 (100 kV, LaB6-illumination) at a primary magnification of 58300x. Atomic force measurements (AFM) were done with a Nanoscope MultiMode 8 from Bruker that operated in tapping mode. Niosomal encapsulated calcein was visualized with a confocal microscope (Leica SP8, Argon Laser, PMT detector (498-550 nm), Leica Application Suite X Software, Leica, Germany) and by applying an excitation wavelength of 495 nm. All samples were prepared according to our standard methods (see ESI).

Synthesis of amphiphiles

Syntheses of the amphiphiles were performed by coupling N-hydroxysuccinimide (NHS) activated 2H,2H,3H,3H-perfluoroundecanoic acid to the terminal amino functions of linear polyglycerols (LPGs) in THF. The linear polyglycerols LPG(OMe), LPG(OEt), as well as LPG(OEE), which is the precursor for LPG(OH), were prepared in three separate living anionic polymerizations in dry 1,2-dimethoxyethane (DME) at 110 °C. Alkyl ether side group functionalities were introduced using respective oxirane monomers by following the procedures of Hans et al.[45] and Wurm et al.[46] in which 3-(dibenzylamino)-1-propanol served as initiator. In order to build LPGs with two terminal amino groups, the polymerizations were quenched with the mesylated derivative of the initiator instead of water. The addition of 3-(dibenzylamino)propyl methanesulfonate substituted the terminal alcohol with the protected amino function and resulted in symmetric LPG terminal groups. Subsequent deprotection of the dibenzyl termini via hydrogenation produced the respective mono- and diamino LPGs that were coupled with NHS activated 2H,2H,3H,3H-perfluoroundecanoic acid to give the corresponding amphiphiles and bolaamphiphiles shown in Scheme 1. Detailed information of all syntheses including the synthesis of the Disperse Red 1 derivative are given in the Electronic Supporting Information (ESI).

Sample preparation

Aqueous solutions of the amphiphiles were prepared by two different methods. In the direct method the amphiphiles were directly dissolved in appropriate amounts of water. In the thin film hydration method[47] a methanolic solution of the amphiphile was dried to a thin film in a round-bottom flask, which, after complete removal of the remaining solvent at high vacuum, was re-dispersed in pure water and purified by dialysis in regenerated cellulose membrane with 10 kDa MWCO. Solutions prepared by either of these methods were measured by DLS to determine aggregates sizes and thermoresponsive behavior. All samples were filtered through a 0.45 µm PTFE filter and equilibrated at the correct temperature for 5 minutes right before the measurements.

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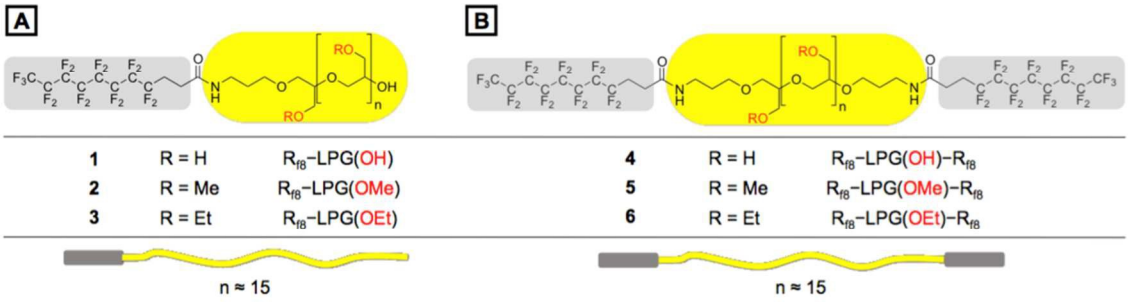

Scheme 1. Chemical structures of (A) mono-fluorinated LPG amphiphiles (**1**-**3**) and (B) di-fluorinated LPG bolaamphiphiles (**4**-**6**) with differing side group functionalities.

For encapsulation experiments, 0.5 mg/mL stock solutions of DR and DR-CF_3 in THF were prepared. In a typical procedure, 600 µL of the stock solution was evaporated and dried, before 3.0 mL of the respective aqueous amphiphile solution (0.1 wt%) was added. The solutions were stirred for 12 h at 1200 rpm and filtered through 0.45 µm PTFE syringe filters to remove unsolubilized dye. To determine the dye loading capacity, the resulting clear, colorless to red aqueous solutions were lyophilized and the residues were re-dissolved in MeOH for UV/vis measurements. Concentrations of the encapsulated dyes were calculated according to Lambert-Beers law[48] using molar extinction coefficients (ε) of 24,500 $L \cdot mol^{-1} \cdot cm^{-1}$ at 479 nm for DR and 20,800 $L \cdot mol^{-1} \cdot cm^{-1}$ at 467 nm for DR-CF_3 of standard calibration curves, which were experimentally obtained from methanolic solutions. The drug loading (DL) content was calculated as the ratio of encapsulated dye to carrier in mol%. All spectra were baseline corrected and the results were averaged from three independent measurements. The resulting assemblies were visualized by cryo-TEM and their mean diameters determined by DLS. For the calcein-loaded vesicles, after evaporation of the methanolic solution of the respective amphiphile (4 mg/mL), the remaining film was rehydrated in 4 mL of 6 mM calcein in MilliQ water to achieve a final amphiphile concentration of 1 mg/mL. Unentrapped calcein was separated from the vesicle solution by dialysis in MilliQ water (MWCO 10 kDa). The calcein-loaded vesicles were visualized by fluorescence microscopy.

Results and discussion

Chemical structure and surface activity

To investigate the self-assembly of LPG-based nonionic fluorous surfactants, different amphiphilic compounds (**1**-**6**) were synthesized. These compounds can be divided into two categories depending on the molecular structure of the LPG derivatives: normal amphiphiles (**1**-**3**) bearing a perfluoralkyl chain at one end and bolaamphiphiles (**4**-**6**) with perflouralkyl chains attached to both ends. All the LPGs were synthesized with the same degree of polymerization to provide a direct comparison of the self-assemblies from the different polymers as shown in Scheme 1. Therefore, different hydrophilicities and thermoresponsive behavior in each category resulted only from the side chain modifications of the LPGs.

The molecular weight of these amphiphiles varies between 1700 and 2500 g mol^{-1} depending on the side chain functionality of the LPGs and the number of perfluoralkyl chains. Consequently, both parameters also define the hydrophilic-lipophilic balance (HLB) of the amphiphiles. HLB values by Griffin, which are classically used for nonionic surfactants, only account for the mass ratio of hydrophilic to hydrophobic moieties.[49] The hydrophilicity differences of LPG side chains are not considered. For example, the OH groups would add less mass than OMe or OEt on the hydrophilic side, although they confer more hydrophilicity to the amphiphile. We therefore provide HLB values according to the group contribution method by Davies[50] that account for each functional group with a numerical value (Table 1). Calculations are specified in Section 4 of the ESI.

Amphiphiles **1**, **2**, **4**, and **5** are readily soluble in water, while amphiphiles **3** and **6**, which contain ethoxy-LPG are almost insoluble. The critical aggregation concentrations (CACs) of the amphiphiles were determined from surface tension measurements using the pendant drop method (ESI). Here, CACs varying between 25-45 µM were found for all six amphiphiles. Moreover, a superior surface activity of the fluorinated amphiphiles is reflected by the low surface tension values at their respective CACs.

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Table 1. Molecular weights, HLB values (Davies, calculation in ESI), surface activity, and critical aggregation concentration in aqueous solutions, determined by [a]MALDI-TOF and [b]pendant drop measurements.

Amphi -phile	MW [g mol^{-1}][a]	HLB (Davies)	γ_{CAC} [mN m^{-1}][b]	CAC [10^{-5} mol L^{-1}][b]
1	1700	19	37.2 ± 1.9	4.5 ± 0.9
2	1800	12	24.8 ± 2.5	3.1 ± 0.6
3	2000	10	23.1 ± 1.8	4.4 ± 0.7
4	2200	12	28.0 ± 2.8	3.6 ± 0.7
5	2300	5	18.9 ± 1.9	2.5 ± 0.5
6	2500	3	24.8 ± 1.3	2.9 ± 0.6

Dynamic light scattering (DLS) measurements were performed to determine the hydrodynamic diameters of the aggregates. Diameters less than 10 nm were found for amphiphiles **1**, **2** and **5**, which is comparable to micellar dimensions expected for the respective amphiphilic and bolaamphiphilic systems. In contrast, much larger aggregates of around 200 nm in diameter with a broad size distribution are formed from bolaamphiphile **4**, which indicates that **4** assembles into polydisperse aggregates (Fig. S2, ESI).

^{19}F-NMR study

The aggregation of the amphiphiles was also investigated by ^{19}F-NMR spectroscopy. The experiments were performed at a much higher concentration than the CACs to attain a good signal-to-noise ratio. Figure 1 displays the NMR-spectra of amphiphile solutions (5 wt%) in MeOD-d_4, which is a suitable solvent even at higher concentrations, and in D_2O. In MeOH-d_4, all four ^{19}F-signals, originating from the R_{f8}-perfluoroalkyl groups as indicated, are well resolved (Fig. 1A).

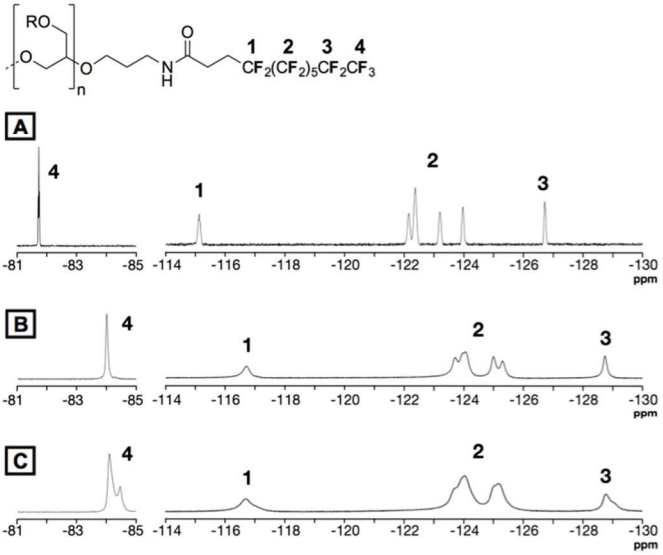

Figure 1. ^{19}F-NMR spectra of (A) amphiphile **1** in MeOD-d_4, (B) amphiphile **1** in D_2O, and (C) bolaamphiphile **4** in D_2O. Spectra of the respective methoxy amphiphiles **2** and **5** show the same trend but with a poorer signal-to-noise ratio due to lower solubility. The chemical shift is referenced to trifluoroacetic acid (TFA) -76.5 ppm.

In D_2O, however, the CF_3 and CF_2 signals are clearly broadened and shifted upfield. Broadening of the signals and the chemical shift imply the formation of aggregates with a fluorous compartment in D_2O (Fig. 1B) and resemble the results from previous studies with perfluorooctyl PEG amphiphiles.[36] Interestingly, bolaamphiphile **4** that also showed narrow single peaks in MeOH-d_4 (Fig. S8, ESI) showed each two signals for the CF_3 group and the adjacent CF_2 group in D_2O (Fig. 1C). The second CF_3 signal has approx. 30% of the main peaks intensity. These findings suggest two different arrangements of the R_{f8} chains of bolamphiphile **4** in water.

Thermoresponsive behavior

In general, LPGs exhibit different LCSTs depending on the side chain functionality. Therefore, the thermoresponsive behavior of the synthesized amphiphiles was studied by measuring the aggregate size at different temperatures using DLS. As mentioned earlier, the ethoxy derivatives (**3** and **6**) resulted in cloudy solutions even at room temperature. In contrast, solutions of the hydroxyl derivatives (**1** and **4**) did not show any clouding up to 60 ˚C. The methoxy derivatives (**2** and **5**), however, produced cloudy solutions around 40 ˚C. DLS measurements at different temperatures disclosed a sudden jump in size with narrow size distributions at 38 ˚C and 33 ˚C for **2** and **5**, respectively, which was completely reversible for several cycles as shown in Figure 2. Altogether, the thermoresponsive behavior of the different amphiphiles correlates well with LCST temperatures of other LPGs reported in the literature.[19,20] The increase in the size is thought to be due to the collapse of LPG(OMe) chains at higher temperatures that results in an agglomeration of smaller aggregates. This could be visualized by fluorescence microscopy of an aqueous solution of compound **5** with encapsulated rhodamine. Here, fluorescent agglomerates were observed only above 40 ˚C (Fig. S3, ESI).

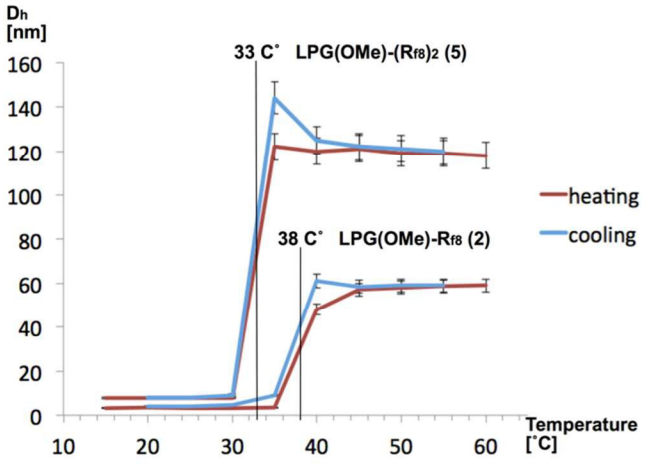

Figure 2. Temperature dependent reversible agglomeration of LPG(OMe)-R_{f8} (**2**) and LPG(OMe)-(R_{f8})$_2$ (**5**) aggregates in solution (0.1 wt%) determined by DLS.

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Furthermore, the size of these agglomerates clearly depends on the concentration of the solutions both, below and above the cloud point (Figure 3).

The susceptibility of the amphiphiles' aggregation to different parameters like concentration and temperature motivated a study on the influence of the sample preparation method. Hereby, the methoxy derivatives (**2** and **5**) clearly display size differences of the aggregates prepared by either of the different methods. DLS measurements indicated much larger aggregates in the size range of 300-400 nm when the thin film hydration method was used, while the direct dissolution method yielded aggregates of less than 10 nm in diameter. DLS size measurements at elevated temperatures indicated even a slight decrease in the assemblies' sizes for the thin film preparation, which contrasts the steep increase in size for the assemblies originating from the direct solution (Fig. 2). This discrepancy emphasizes a difference in the aggregation process caused by either preparation method.

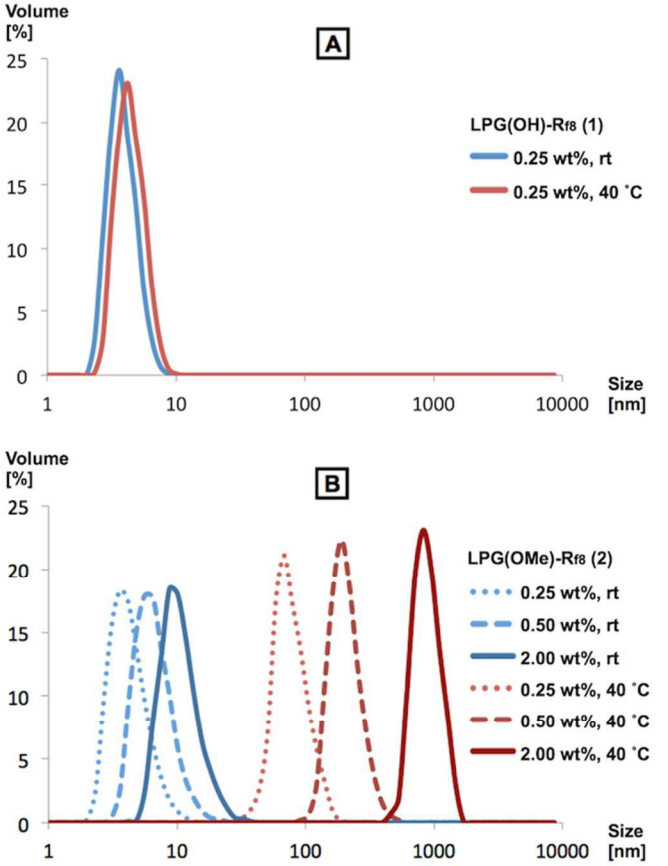

Figure 3. Temperature and concentration dependent aggregate sizes determined by DLS, exemplified for the hydrodynamic radii of (A) the hydroxy-amphiphile **1** showing the same aggregate size, independent of temperature and concentration and (B) the methoxy-amphiphile **2** showing a clear dependancy on the concentration both, below and above the cloud point.

Cryo-TEM and AFM measurements

Cryo-TEM was performed to investigate the morphology of the different aggregates in solution. These studies indicated that amphiphile **1** with hydroxyl side chains assembles into uniform globular aggregates irrespective of the preparation method (Fig. 4A, 4B). The size of these aggregates was determined to be 5 ± 1 nm. In contrast, bolaamphiphile **4** with its hydroxyl side chains forms two different morphologies consisting of smaller, partially elongated micelles (< 10 nm) and larger circular aggregates (100-300 nm) as shown in Figure 4C. The larger circular aggregates did not show an elevated contrast at their edges. Together with the absence of wrinkles these structures are reminiscent of stiff, flat arrangements of amphiphiles found in bicelles. This was supported by AFM measurements, where the height of the layers was determined to be 5.7 ± 0.2 nm as shown in Figure 4D.

In this respect, LPG(OH) amphiphiles **1** and **4** behave similarly to the corresponding PEG amphiphiles, which form micelles (12 nm) when only one perfluorooctyl chain is attached and larger aggregates (132 nm) when two perfluorooctyl chains are present.[43] However, our measurements clearly identified the larger aggregates to be flat circular bicelles instead of spherical nanoaggregates.[43]

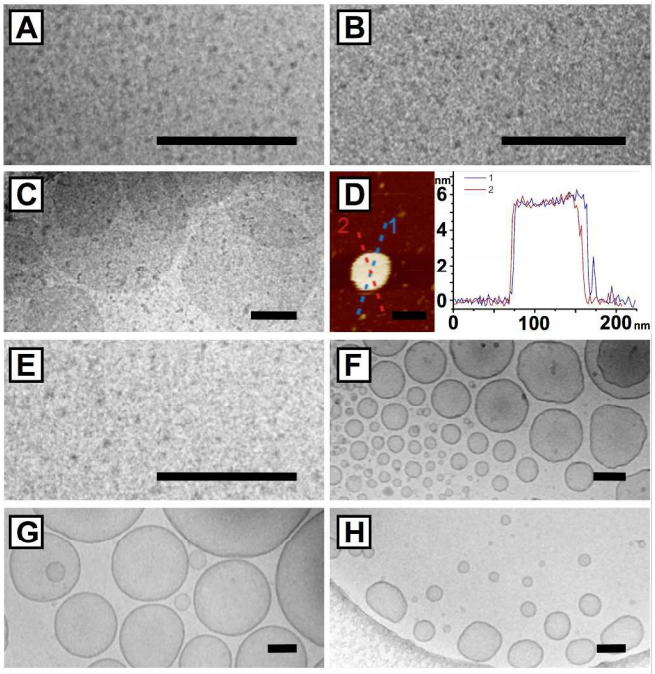

Figure 4. Cryo-TEM images (A, B, C, E, F, G, H) and an AFM image (D) of aqueous amphiphile solutions (0.1-0.25 wt%): Micelles of hydroxyl amphiphile **1** prepared by dissolution (A) and film hydration (B); bicelles of hydroxyl bolaamphiphile **4** (C) and its determined height (D); micelles of methoxy amphiphile **2** prepared by dissolution (E) and vesicles prepared by film hydration (F); vesicles of methoxy bolaamphiphile **5** prepared by dissolution (G) and prepared by film hydration (H). All scale bars denote 100 nm.

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Cryo-TEM experiments were also performed with methoxy LPGs **2** and **5**, which aggregation behavior was already indicated to be sensitive to the sample preparation method by DLS measurements. We found that amphiphile **2** forms micellar aggregates of 3.5-6 nm in diameter, if prepared by the direct dissolution method, similar to its hydroxy analog **1** (Fig. 4E). If prepared according to the thin film hydration method, a completely different morphology, i.e., vesicles formed. (Fig. 4F) The size of these vesicles varies from a few tens to hundreds of nanometers. Interestingly, the bola-analog **5** only forms vesicles regardless of the preparation method (Fig. 4G and 4H).

During cryogenic preparation, the vesicles are pushed together by chance to induce the formation of the densest membrane stacks, which are visible as equidistant striations (Fig. S4, ESI). Such structures can be used for an accurate size determination of the membrane layers using Fourier transformation as well as averaged densities along the membranes profiles. An interval of 8 ± 0.5 nm was found that represents the distance between adjacent membrane layers and therefore the thickness of the entire membrane including those areas that are occupied by the methoxy-LPG head groups, which seem to contribute no contrast and are thus invisible in the electron micrographs (Fig. S5, ESI).

Despite all these findings from the complementary combination of AFM, DLS, and cryo-TEM experiments, two questions still remained, i.e., about the thermodynamically stable morphology of amphiphile **2** and what are the origins of the contradicting sizes for bolaamphiphile **5** found in DLS (< 10 nm) and cryo-TEM (vesicles).

Table 2. Hydrodynamic diameters D_h of 0.1 wt% aqueous amphiphile solutions at room temperature and 40 °C and their transition temperature determined by dynamic light scattering (DLS).

	Directly dissolved in water			Thin film hydration	
Amphi-phile	D_h at rt [nm]	Cloud point	D_h 40 °C [nm]	D_h at rt [nm]	D_h 40 °C [nm]
1	4.6 ± 0.2	-	4.5 ± 0.1	4.4 ± 0.3	4.5 ± 0.2
2	3.2 ± 0.3	38°C	48.8 ± 1.7	242 ± 80	229 ± 28
4	178 ± 5	-	179 ±14	211 ± 15	203 ± 14
5	7.8 ± 1.0	33°C	121 ± 4	443 ± 101	398 ± 83

Aging experiments revealed that the aggregates emerged from the direct solutions of amphiphile **2** and **5** increased in size over time. Thus, diameters > 50 nm were found for bolaamphiphile **5** after 1 week and for amphiphile **2** after 2 months (Fig. S6, ESI). Cryo-TEM showed vesicles for both these aged solutions. With this, the vesicular arrangement turned out to be the stable morphology of methoxy derivatives resulting from the sample preparation method also explains size discrepancies upon heating by DLS. Amphiphile **2** forms micellar aggregates that are smaller than 10 nm in diameter by using the direct dissolution method. Heating the sample above the cloud point leads to a collapse of the polymeric chains, induces further agglomeration of the micelles, and thus increases the size of the aggregates. On the other hand, the same amphiphile forms vesicles if prepared by the thin film hydration method. But now, heating the sample leads to a collapse of polymeric chains and shrinkage of the vesicles. All hydrodynamic diameters of the assemblies as well as the cloud points are summarized in Table 2.

Supramolecular aggregates as carriers

The newly synthesized fluorinated amphiphiles should be able to improve the solubility of fluorinated drugs in their fluorophilic compartments if the interactions between perfluoroalkyl groups and the guest molecules are efficient. To test this with amphiphiles **1**, **2**, **4,** and **5** encapsulation experiments were performed using the hydrophobic dye (Disperse Red 1: DR) and a trifluoromethylated analog (DR-CF_3) (Fig. 5).

The dye loading capacity varies considerably with the amphiphiles as well as the fluorination of the dye. A better encapsulation was observed for DR-CF_3 over DR by 3 out of 4 amphiphiles that formed micellar aggregates. The exception, bolaamphiphile **5**, which only forms vesicles quickly after solvation, encapsulated the same amount of DR and DR-CF_3. Looking at the individual dyes, the loading capacity of DR was always below 0.5 mol% and varied as a function of the relative hydrophobicity of the amphiphiles. In the case of DR-CF_3, the non-polar nature of the dye is further increased by the trifluoromethyl group and results in a further decrease of water solubility.

DR-CF_3 could be loaded on amphiphile **1** up to 3.9 mol% (10 mg/g amphiphile), which formed the most uniform and stable micelles. Compared to the non-fluorinated DR with only 0.16 mol% suggests that the trifluoromethylation leads to increased interaction with the fluorous compartment of the aggregates and to a 20-times higher encapsulation.

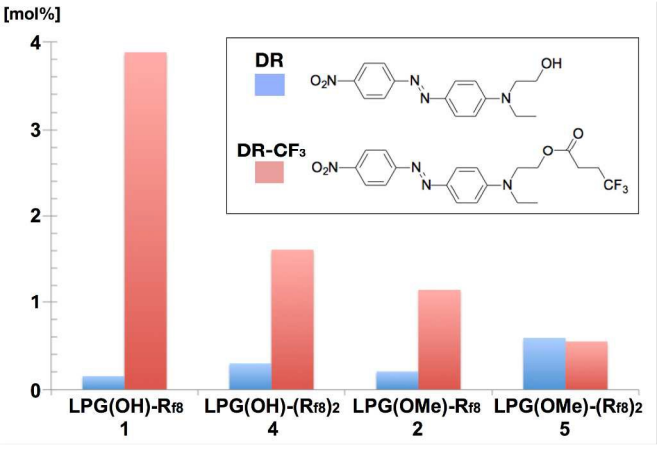

Figure 5. Dye loading content in mol% of amphiphiles (x-axis) for disperse red (DR) and trifluoromethyl disperse red (DR-CF_3). The numeric values are summarized in Table S2 in the ESI.

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Vesicles are also widely used as supramolecular carriers. Their formation is usually observed for amphiphiles with HLB values between 4 and 8.[51] Therefore, methoxy bolaamphiphile **5** with a HLB value of 5 (Table 1) should typically form vesicles, as observed. Surprisingly, also the methoxy amphiphile **2** forms stable vesicles, highlighting this amphiphile with its rather hydrophilic HLB value of 12 as an exception to the rule.

To test the ability of the vesicles from amphiphiles **2** and **5** to encapsulate hydrophilic molecules within their aqueous compartments, calcein encapsulation experiments were performed. Calcein is a hydrophilic fluorescent dye that is used to characterize the formation and stability of vesicles. Calcein encapsulation was performed as described in the experimental part and the formation of vesicles by amphiphiles **2** and **5** was investigated by fluorescence microscopy. As shown in Figure 6 the encapsulation of calcein in the aqueous interior of the vesicles could easily be visualized by fluorescence microscopy. Due to the magnification limit of the microscope, vesicles smaller than 300 nm are not visible by this method. On the other hand, giant vesicles sized 1 to 10 µm that had formed from amphiphiles **2** and **5** are readily displayed on the fluorescence microscopic images in Figure 6.

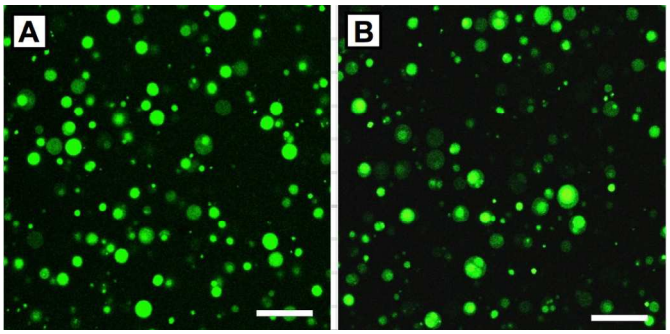

Figure 6. Fluorescence microscopy images of calcein-loaded vesicles of bolaamphiphile **5** (A) directly after preparation and (B) stable at 45 ˚C. Both scale bars denote 25 µm.

The giant vesicles have been stable for at least 2 months. As shown in Figure 6B, even above their cloud points the calcein-loaded vesicles of methoxy amphiphiles **2** and **5** stayed intact and did not break and burst release the calcein. Therefore, these giant vesicles may be interesting model systems for cytomimetic chemistry.

Conclusions

We reported on the synthesis and supramolecular assembly of a group of LPG-based nonionic fluorous amphiphiles and bolaamphiphiles. The aggregation behavior of these amphiphiles was investigated by surface tension measurements, NMR spectroscopy, cryo-TEM, AFM, and DLS. All amphiphiles show similar CACs. In contrast, the size of their aggregates varied depending on the molecular structure and sample preparation method. Cryo-TEM and AFM experiments revealed how the nature of the LPG side chains influences the aggregation behavior in this group. Amphiphiles containing LPGs with hydroxyl side groups (**1** and **4**) form globular micelles or flat circular bicelles, respectively. Amphiphiles **2** and **5** with methoxy LPG side groups form micellar aggregates and vesicles depending on the preparation method. Additionally, they exhibit reversible thermoresponsive agglomeration, when heated above 38 ˚C. The micelles formed from amphiphiles **1**, **2** and **4** were shown to solubilize considerably more trifluoromethylated Disperse Red 1 than the unmodified dye, which indicates a fluorophilic interaction with the perfluoroalkyl core of these micelles. Vesicles from the methoxy-LPGs **2** and **5** were used to encapsulate calcein and their stability above their clouding temperature was demonstrated. In conclusion, the fluorinated polyglycerol amphiphiles reported here exhibit a self-assembly and thermoresponsive behavior that may be useful for the design of supramolecular aggregates and thus extend the toolbox for rational amphiphilic architectures.

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Notes and references

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