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Regular Article

Where are those lipid nano rings?

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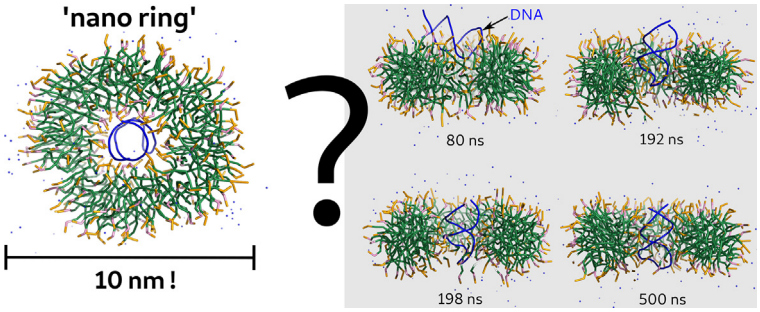
GRAPHICAL ABSTRACT



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ABSTRACT

Highly curved toroidal micelles with diameters as small as 100 nm have been successfully constructed by self-assembly of amphiphilic block copolymers. These structures may have potential applications in gene or drug delivery. Experimental observations suggest that toroidal micelles likely originate from spherical or disc-like micelles which are tricked into forming toroidal micelles upon external stimuli ('smart' materials). Since self-assembly of polymeric and lipid surfactants is guided by the same physical principles, we hypothesize that 'smart' lipid surfactants can be equivalently tricked into forming highly curved toroidal micelles that are tenfold smaller (≃10 nm diameter). Paradoxically, these 'nano rings' have never been observed. Using coarse-grained molecular dynamics (MD) simulations in conjunction with a state-of-the-art free energy calculation method (a string method), we illustrate how a thermo-responsive lipid surfactant is able to form toroidal micelles. These micelles originate from disc-like micelles that are spontaneously perforated upon heat shocking, thereby supporting a longstanding hypothesis on the possible origin of polymeric toroidal micelle phases observed in experiments. We illustrate that kinetically stable 'nano rings' are substantially shorter lived than their tenfold larger polymeric analogs. The estimated life-time (milliseconds) is in fact similar to the characteristic breaking time of the corresponding worm-like micelle. Finally, we resolve the characteristic finger print which 'nano rings' leave in time-resolved X-ray spectra and illustrate how the uptake of small DNA fragments may enhance their stability. Despite a shared kinetics of self-assembly, length scale dependent differences in the life-time of surfactant phases can occur when phases are kinetically rather than thermodynamically stable. This results in the apparent absence or presence of toroidal micelle phases on different length scales. Our theoretical work precisely illustrates that the universality of surfactants nevertheless remains conserved even at different length scales.

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1. Introduction

Directed self-assembly of surfactants plays an important role in the development of novel nanostructures utilized in, for example, gene and drug delivery [1–6]. In particular, toroidal nanostructures are of growing importance due to their unique geometry and potential utility in material fabrication. In recent years a variety of amphiphilic block copolymers have been shown capable to self-assemble into toroidal micelles (e.g.,[7–14]). The diameter of the smallest toroidal micelles formed by amphiphilic polymers is about 100 nm [9].

The thermodynamic stability of toroidal micelles has been extensively studied using continuum elastic models (e.g., citation [15]) or self-consistent field theory (e.g. citation [16]). The excess free energy of a toroidal micelle is predominantly determined by its bending free energy. Modeling the torus by a circle [17], the bending free energy is given by, $F_b = \frac{\kappa\pi}{R}$, with R being the radius of the torus and κ the elastic bending modulus (See *SI* for further details). The formation of toroidal micelles from cylinder-forming amphiphilic molecules in solution was initially thought to occur through fusion of the micelle's free end caps in an end-to-end fashion [18–20]. For a worm-like or rod-like micelle with contour length L, toroidal micelle formation is thermodynamically favorable when the free energy of its two free end caps, $2F_{cap}$, becomes larger than the bending free energy of the corresponding toroidal micelle, thus $2F_{cap} > \frac{2\kappa\pi^2}{L}$. For micelles undergoing 'chain growth polymerization' toroidal micelle formation will eventually become thermodynamically favorable, because the bending energy stored in the torus vanishes with increasing contour length, $\propto 1/L$. However, ring formation via fusion of the two free ends is opposed by an entropic free energy cost that according to random walk statistics increases with the logarithm of the contour length, $\propto \ln L$ (see *SI* for a detailed explanation). The total free energy barrier against ring formation, F^*_{ring}, is given by, $F^*_{ring} = \frac{2\kappa\pi^2}{L} + c(T)\ln L + F_0$, with $c(T)$ and F_0 being constant terms. Fig. 1a sketches the kinetic free energy barrier against ring formation as a function of L. The plot illustrates the existence of an optimum fibril length at which formation of a toroidal micelle is most likely. However, a growing and sufficiently stiff worm-like or rod-like micelle will overshoot such a regime. Therefore, formation of a ring, despite being thermodynamically favorable, is generally a rare event, albeit less rare in floppy worm-like micelles with attractive free ends [21]. In support of this notion, experimental observations have promoted the hypothesis that highly curved toroids in ABC triblock copolymers are constructed either through elimination of high-energy spherical micelles and/or cylindrical micelle end caps, or through perforation of disc-like micelles [8]. Extensive mesoscopic field-based simulations of single-component amphiphilic diblock copolymer systems prompted that toroidal micelles are formed from growing spherical micelles that suddenly transit into toroidal micelles [22]. A similar transition has been observed in coarse-grained dissipative particle dynamics simulations of triblock copolymers [23]. In these pathways, the micelles do not coalesce, but rather grow radially by attracting copolymers from the solution. Once a critical micelle size is exceeded, copolymers start to flip-flop such that the micelle's core becomes hydrophilic and transits into a toroidal micelle.

Experimental fabrication of highly curved toroidal micelles is commonly based on exploiting the stimuli-responsiveness of polymeric surfactants [8–10]. Stimuli-responsive or 'smart' materials are capable of altering their physical properties upon exposure to external stimuli. Formation of toroidal micelles is actively induced by gradually changing solvent conditions (e.g., the ratio between ethanol and THF) [8,9]. This alters the solubility of the blocks and consequently impairs the thermodynamic stability of a pre-existing spherical or disc-like micelle structure thereby stimulating the subsequent kinetic transition into a toroidal micelle [8,9]. It is still an open question whether also lipids or other small sized amphiphiles can form toroidal micelles via stimuli-directed self-aggregation. Although lipid and polymeric surfactants form similar lyotropic phases [4,5], highly curved lipid toroidal micelle phases have thus far not been experimentally observed. Given the characteristic hydrophobic thickness of lipid self-assemblies [5], being about 4 nm, the lower size limit of a highly curved toroidal micelle would be in the range of about 10 nm – a size of interest for potential applications in the biomedical field. Lipid 'nano rings' could, for example, be envisioned as biocompatible transporters or vehicles of short DNA and RNA fragments. However, it is remains unclear whether highly curved lipid toroidal micelle phases are actually kinetically accessible.

Molecular simulations provide a growing and powerful tool to explore the complex landscape of surfactant self-assembly [24]. Here, we use coarse-grained molecular dynamics (MD) simulations

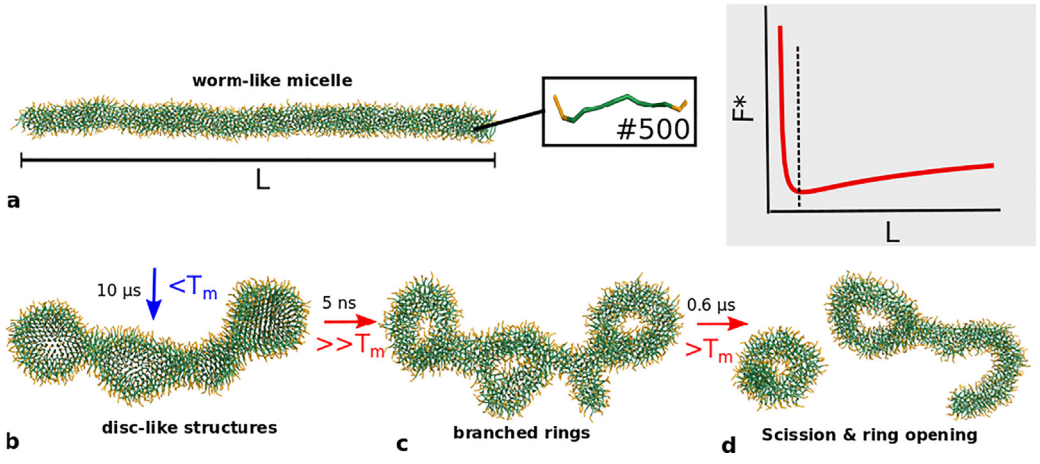

Fig. 1. Temperature induced formation of lipid toroidal micelles. (a) A worm-like micelle faces a kinetic barrier against ring formation via self-folding and fusion of the two free end caps ($F*$) that increases with growing length L. (b) Cooling of a fluid worm-like micelle below T_m (273 K) results in the formation of a fibril consisting of disc-like structures. (c,d) A short subsequent heat shock (400 K for 5 ns) induces a sudden disc to torus transition. The formed branched toroidal structure is subsequently simulated at 360 K for 600 ns of simulated time. The unstable junctions between the toroidal micelles result in scission and 'ring opening'. The lipid surfactant in this example features two hydrophilic phosphatidylcholine headgroups (colored orange) connected to the ends of a hydrophobic chain (colored green) with a chain melting temperature, $T_m \simeq 295$ K. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in conjunction with a state-of-the-art free energy calculation method (a string method) to shed some light into the complex kinetics and free energy landscape of stimuli-directed self-aggregation. We illustrate the example of a thermo-responsive 'smart' lipid surfactant that forms hexagonal shaped disc-like micelle structures below the chain melting temperature (T_m), which are tricked into forming 10 nm-sized toroidal micelles ('nano rings') rather than worm-like-micelles upon heat shocking ($\gg T_m$). Finally, we illustrate that kinetically stable 'nano rings' are substantially shorter lived than their tenfold larger polymeric analogs.

2. Methods

Molecular dynamics (MD) simulations were performed performed with the GROMACS simulation package [25] version 2019, unless stated otherwise, using the MARTINI coarse-grained (CG) model for bio-molecular simulations version 2.2. The MARTINI model is parametrized on representing thermodynamic properties such as partitioning free energies of alkanes in water [26]. The modeled lipid surfactant – a single chain bolaamphiphile [27–29] – mimicks a saturated hydrophobic chain consisting of 32 CH_2 units (B-block) connected to two hydrophilic zwitterionic phosphocholine (PC) headgroups on both ends (A-block) [30]. Its symmetric A–B–A architecture is in fact equivalent to that of a Pluronic – a widely used family of thermo-responsive symmetric triblock copolymers manufactured by BASF [31–33]. A detailed description of all simulation techniques, including force-field parameters, used in this study can be found in the extended method section within the *SI*.

Minimum free energy pathways were resolved using a density-field based string method implemented in GROMACS version 4.6.4 (see *SI* and citation [34] for further details on this method). Reported simulation times have been corrected for the about four times faster diffusion of the coarse-grained model in comparison to atomistic simulations by multiplication by a constant factor of four [26]. The simulation system was coupled to a constant temperature bath using the 'V-rescale' algorithm with a relaxation time of 1.0 ps. The time step used in the simulation was 20 fs. The dielectric constant in the simulations was $\epsilon_r = 15$. The neighbour-list was updated every 10 simulation steps. The pressure was isotropically coupled to 1 bar (Berendsen pressure coupling) with a relaxation time of 1.0 ps. A shifted cutoff of 1.2 nm was used for both Coulomb and Lennard-Jones (LJ) interactions. Interactions were gradually scaled to zero beyond 0 nm (Coulomb) and 1.0 nm (LJ) respectively.

The replica exchange simulations were performed using GROMACS version 4.5.7. The replica exchange frequency was 2 ps. In the solvent free replica exchange simulations a Langevin thermostat was used with a friction coefficient of 0.25 ps^{-1}. All solvent free simulations were performed in the *NVT* ensemble. Further details on the simulation setups and settings can be found in the *SI*.

3. Results

3.1. Thermo-responsive formation of toroidal micelles

In a first step, we study the effect of cooling a pre-constructed worm-like micelle consisting of 500 surfactant molecules below the phase transition temperature T_m. The MARTINI model is able to qualitatively describe the fluid-gel phase transition in lipid membranes [35,36]. Fig. 1b illustrates that the transition towards the gel phase enforces the formation of disc-like structures, which are especially pronounced at the two free ends of the worm-like micelle. We will study these structures in closer detail in a later section. Motivated by an earlier hypothesis on the origin of toroidal micelles [8], we investigate whether these disc-like structures have a propensity to transit into toroidal micelles upon an external stimulus. To this aim, we apply a short 5 ns heat shock ($\gg T_m$). Indeed, heat shocking enforces a rapid transition into branched toroidal micelles (Fig. 1b). However, these branched toroidal micelles subsequently split off into forming separate, isolated 'rings' after simulation at a lower temperature (360 K). Interestingly, these observations indicate that a lipid connection between the formed 'rings' – a four-branch or four-junction – is evidently not thermodynamically stable (Fig. 1d). This behavior stands quite in contrast to the behavior of polymeric surfactants observed in experiments [37,8] and mesoscopic simulations [38,39] as well as the behavior observed for ionic surfactants in molecular simulations [21]. Furthermore, we observe that a formed four-junction can alternatively escape into a three-junction via 'opening' of one ring [40], Fig. 1d. We extensively study these scission mechanisms and their relative probabilities of occurrence in close detail in Fig. S2.

In a following step, we study the disc-to-torus transition in close detail by performing extensive temperature replica exchange (T-REM) simulations of a smaller aggregate (144 molecules) over a temperature ladder ranging from 280 K to 450 K. The size of this aggregate corresponds to the size of the isolated toroidal micelles formed in Fig. 1. Replica exchange simulations allow for an extensive sampling of structure space. We conduct these simulations both in implicit and explicit solvent conditions [41,26]. The obtained temperature versus enthalpy curve suggests a phase transition temperature T_m of about 330 K for the implicit solvent model and 295 K for the explicit solvent model (see Fig. S3 and S4). Indeed, disc-like micelles are formed below T_m, see Fig. 2a. The central region of such a disc-like micelle is comprised of hexagonal close-packed lipid tails whereas its edge consists of a belt of intertwined surfactants thereby effectively shielding its hydrophobic edge (Fig. 2a). In fact, this observed structure somewhat resembles the proposed structure of a "hockey puck" micelle [42]. A closer look at the disc's overall structure reveals that such a disc is not circular but rather hexagonally shaped, see Fig. 2a. This can be explained by realization of a low energy configuration, i.e. a hexagonal packing, upon gelation. The cross-sectional side view of the disc reveals that the surfactants adopt a strongly bent conformation near the edge of the disc, see Fig. 2b. Since such a bent configuration reduces the unfavorable hydrophobic surface of the disc's edge, we hypothesize that a remaining degree of chain flexibility below T_m may be essential for disc formation. Indeed, increasing the chain stiffness of the surfactant's hydrophobic mid part (shown in green color), i.e. modeling this part as a stiff rod, effectively impairs disc formation in our simulations and rather results into an alternative helical fibril structure, in agreement with previous grid-based Monte Carlo simulations [43,44] (see Fig. S5). Finally, it is important to emphasize that the surfactant's special architecture, i.e. the bolaform, is crucial for disc stability. For sake of illustration, we performed simulations of a stable pre-formed disc at 275 K where we 'sliced' the bola surfactants in half through the center of their hydrophobic tail region, thereby yielding two regular lipid surfactants (see Fig. S6). Consequently, the disc rapidly loses its structural integrity and morphs into a regular worm-like micelle despite conservation of the overall hydrophobic and hydrophilic volume fraction. This clearly indicates that 'bolafication' is essential for the disc's stability. To this end, we emphasize that rationalisation of the disc's free energy and concomitant stability by elastic models (e.g., [45]) is not straightforward due to the crystalline nature of the disc in the gel phase.

Above T_m, the structural space is dominated by toroidal micelles. Exploring the formation of toroidal micelles in detail reveals that toroidal micelles indeed result from spontaneous 'perforation' of disc-like micelles above T_m, see Fig. 2c. Therefore, our

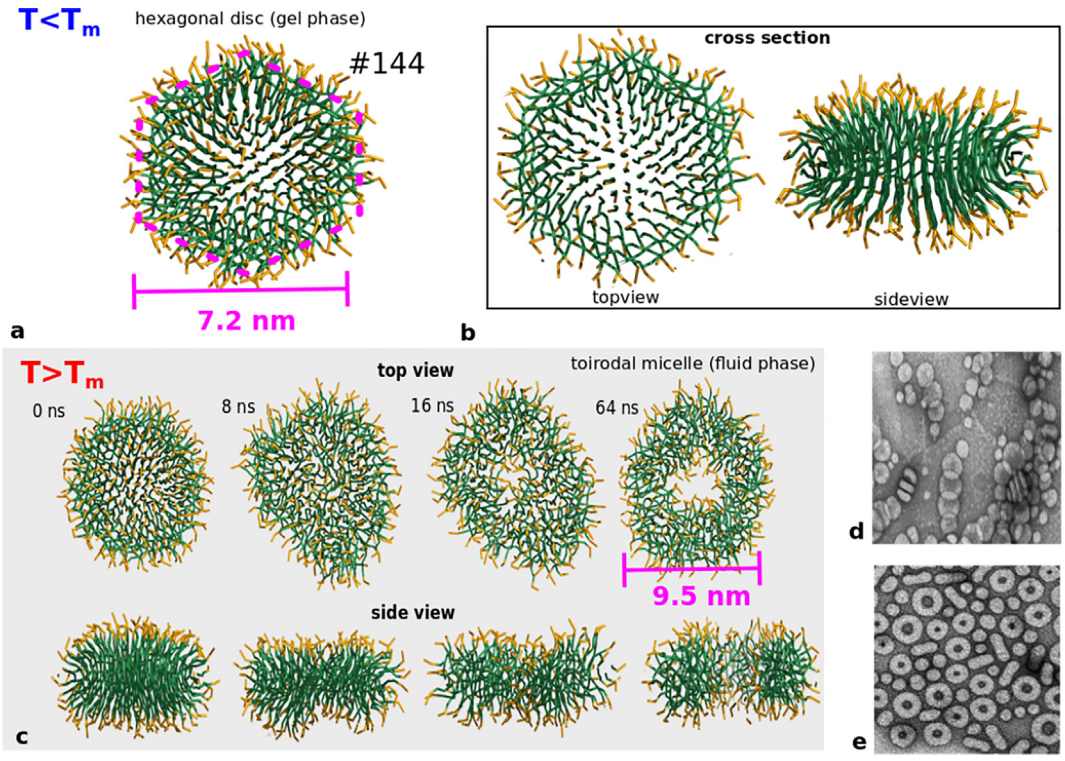

Fig. 2. Disc-like and toroidal micelles. (a) Example of a hexagonal disc formed within the molecular dynamics simulations below T_m. (b) The edge of the disc consists of a belt of intertwined surfactants (top view). Note that the surfactants adopt a bent conformation close to the disc's edge (side view). (c) Heating a disc in the gel phase up to 360 K induces a rapid transition toward a toroidal micelle. (d,e) Transmission electron microscopy (TEM) imaging of discs (d) and toroidal micelles (e) formed in ABC tri-block copolymer solutions (Adapted from Cui et al. [8])..

simulations support the hypothesis that toroidal micelles in ABC triblock copolymer systems directly originate from the perforation of preceding disc-like micelles upon an external stimulus (e.g., change in solvent conditions)[8], see Fig. 2d,e. Our simulations suggest the following mechanism: Upon melting the disc loses structural integrity. Elongation of the disc into a worm-like micelle would increase the interfacial length of the disc's unfavorable edge. Therefore, the circular shape of the disc remains rather conserved upon melting. Since the disc's edge is relatively hydrophobic, it tends to shield itself from the water phase by 'escaping' into a toroidal micelle. Worm-like micelles are rarely observed in the high temperature regime. These worm-like micelles do not result from 'ring opening' but rather from the formation of propeller-shaped micelles consisting of two smaller discs slightly above T_m (see Fig. S3). These discs are seemingly too small to allow a perforation mechanism into a toroidal micelle. Thus, melting of a disc-like micelle proceeds via different competitive kinetic pathways. Toroidal micelles are formed if the disc-to-torus transition proceeds at a faster rate – a kinetic trap. Here, a well-defined overall circular disc shape likely enforces formation of a toroidal micelle. Therefore, the more circular disc-like structures formed at the ends of the frozen worm-like micelle are expected to have an increased propensity to transit into toroidal micelles, see Fig. 1b.

3.2. Kinetic stability of the formed toroidal micelle

Opening of the torus is characterized by a scission event and is associated with the formation of two free hemispherical end caps. To this end, we calculated the free energy barrier against scission using a state-of-the-art density-field based string method [34]. This method resolves a thermodynamically reversible reaction pathway of minimal free energy, i.e. the most likely pathway, to transit from a reactant into the product state, in our example being a (i) toroidal micelle, and (ii) an open toroidal micelle respectively (see *SI* for further details).

The resolved pathway of minimal free energy is illustrated in Fig. 3. Notably, the observed scission mechanism is similar – but in a reverse direction – to the observed mechanism of forming the first fusion intermediate in biological membrane fusion [34]. The structure associated with the free energy barrier features a single lipid which connects the two formed free ends. Fig. 3 illustrates a scission barrier of 21 k_BT for a toroidal micelle (144 molecules). The rate of scission k is given by, $k = Ae^{\frac{\Delta F^*}{k_BT}}$, with A being the kinetic prefactor. Therefore, the average life-time τ of a toroidal micelle is $\tau = 1/k$. The scission and fusion rates of hemifusion intermediates in lipid membranes, which are closely related to the scission and fusion of micelles, have been determined from coarse-grained simulations using Markov state modeling (see *SI* in Ref. [48]). From this study we can obtain an estimation of the kinetic prefactor A, being about $10^{-10}s^{-1}$. This estimate includes a factor of $1/4$ to correct for the four times faster diffusion of the used coarse-grained model [26]. In such case, within the accuracy of coarse-grained models, the corresponding average lifetime of a toroidal micelle would be 0.13 s. Our main finding, however, is that the scission barrier of the highly curved toroidal micelle and worm-like micelle remain rather similar, being 21 versus 26 k_BT. This implies that 'ring opening' and subsequent fractionation of the corresponding worm-like micelle are expected to occur on a rather similar time-scale. For worm-like micelles formed by small (ionic) surfactants, characteristic breaking times are of the order of milliseconds as being determined by rheological experiments [49], but may extend up to seconds or minutes for lecithin worm-like micelles at equilibrium [50]. Since time-resolved X-ray scattering experiments enable a time resolution of only a few milliseconds [51]

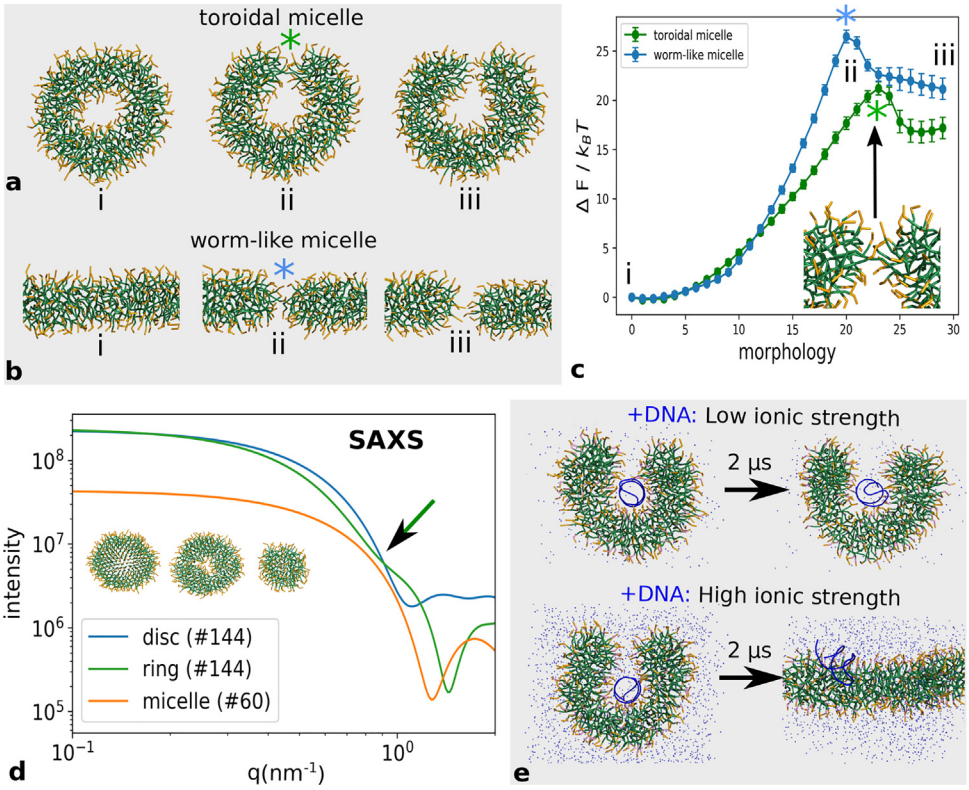

Fig. 3. Free energy barrier against scission. (a,b) Resolved reaction pathway of ring opening (a) and scission of a worm-like micelle (b): (i) The reactant state, (ii) the intermediate corresponding to the resolved free energy barrier, (iii) the product state. (c) Free energy along the resolved scission pathway. (d) X-ray spectrum resolved [46,47] for a disc-like micelle in the gel phase ($< T_m$), a toroidal micelle, and a micelle consisting of 60 surfactants only. The toroidal micelle is characterized by a pronounced 'buckle' at around 1 nm^{-1} (green arrow). (e) Uptake of a 12-base pair polyelectrolyte (a DNA fragment) by an open micelle (#144) comprised of 50 % cationic and 50 % zwitterionic lipid surfactants at low ionic strength (0.1 M NaCl) and high ionic strength (1 M NaCl). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

detection of toroidal micelles with these techniques must be possible even with the toroidal micelles being highly transient in nature. In particular, since toroidal micelles leave a pronounced fingerprint within X-ray and SANS spectra (see Fig. 3d and Fig. S7).

4. Discussion

Previous SANS experiments suggested that zwitterionic lipid bolaamphiphiles are restricted to form small non-spherical micelles above T_m in solution, while forming worm-like fibrils of microscopic size below T_m [52]. However, it is important to emphasize that the estimated free energy of the two end caps, being $\simeq 20\ k_BT$, is subject to the condition that the two end caps are kept in a close proximity to each other thereby making the reverse fusion reaction likely [34]. In contrast, the size distribution of worm-like micelles in solution is the outcome of a balance between the effective fusion and scission rate *free in solution* [53–58,21,59]. Free in solution, zwitterionic micelles are additionally subject to strong hydration repulsion which opposes close proximity and thereby hinders rapid coalescence of micelles via consecutive fusion events. Hence, the end cap free energy and concomitant scission barrier derived in molecular simulations via free energy calculations (e.g., citations [60,61]) rather reflects an *intrinsic* free energy which may not trivially translate into the expected size distribution free in solution.

Fluid worm-like micelles are often formed by ionic surfactants under high ionic strength [51,49]. Under this condition micelles become attractive [51]. Consequently, the rate of fusion becomes substantially larger than the rate of scission. This enables growth of worm-like micelles up to a size of micrometers. For zwitterionic surfactants at a low concentration, the characteristic time of micelle fusion (the inverse fusion rate) may in fact be smaller than the characteristic breaking time (the inverse scission rate) of a worm-like micelle, despite a positive intrinsic free energy of its free end caps. Thus, zwitterionic surfactants may require a much larger hydrophobic tail to form fluid worm-like micelles than ionic surfactants at high ionic strength because the concomitant decrease in fusion rate must be compensated by a larger breaking time [62]. In contrast, below the chain melting temperature T_m, the characteristic breaking time becomes extremely large (up to days [62]) explaining the stability of lipid worm-like micelles or fibrils in the gel phase [62,29]. It is noteworthy that a large kinetic free energy barrier against fusion is also a key reason for biological membrane fusion requiring the presence of fusion proteins to actively bring the membranes in close proximity, despite the fact that fusion of vesicles is thermodynamically highly favorable [63]. The size distribution of worm-like micelles in solution is determined by both the excess free energy of the end caps, which is largely dictated by the effective packing shape of constituent surfactants [64], and the effective free energy barrier against micelle coalescence, which is dictated by hydration repulsion [51]. Finally, the kinetics and rate of fusion will be significantly faster if fusion is facilitated by 'branching' and subsequent sliding of worm-like micelles rather than solely end-to-end fusion [50].

4.1. Size matters

It is important to emphasize that the overall shape of the open torus at the scission barrier is very similar to that of the closed torus (see Fig. 3a). This suggests that the scission barrier mainly

depends on the elastic energy associated with the formation of the two hemispherical free end caps, since the excess bending free energy of the torus itself is largely conserved. This directly explains why both the barrier against ring opening and the scission barrier of the corresponding worm-like micelle are of similar magnitude (see Fig. 3c). In a highly curved polymeric toroidal micelle, the diameter of both the torus and its constituent tube will be about tenfold larger [9]. Consequently, the constituent tube of the torus increases in stiffness. This increases the concomitant elastic energy associated with forming the two hemispherical free end caps when undergoing scission. We argue that the scission barrier likely scales by a factor of 10^3 with increasing length-scale (see *SI* for a detailed explanation). Polymeric toroidal micelles are thus highly metastable due to their tenfold larger scale (see *SI* for further details).

Lipid liposomes or vesicles, in contrast to lipid toroidal micelles, will self-heal after spontaneous poration. The closing of the pore is enforced by a force at the interface of a formed pore (a line tension). Thus, liposomal formulations can have a shelf life of months or perhaps even years despite the fact that spontaneous membrane pore formation faces a free energy barrier of *only* several tens of k_BT [65]. Even if a toroidal micelle is thermodynamically stable, i.e. the free energy of the two end caps is larger than the bending energy of the torus, ring opening does not yield a restoring force that would promote closing of the torus. Self-healing would thus require the introduction of an additional driving force that opposes unbending of the open torus.

Interestingly, our simulations suggest that incorporation of a 12-base pair polyelectrolyte – a DNA fragment – within the central cavity of an open toroidal micelle comprised of 50 % cationic and 50 % zwitterionic lipid surfactants counteracts the release of bending energy at low ionic strength (0.1 M NaCl), see Fig. 3e. The uptake and incorporation of such a DNA fragment is driven by a gain in the electric potential energy when residing within the central cavity of the torus (see Fig. S8). In contrast, high ionic strength (1 M NaCl) enables complete opening of the toroidal micelle because of electrostatic screening and concomitant stiffening of the micelle [66]. This observation is analogous to DNA's well-known ability to stabilize inverted hexagonal phases in stacked positively net charged membrane systems [67]. In contrast, at high ionic strength, the electrostatic screening is too strong and the torus completely opens (see Fig. 3e). Notably, the open ring is metastable even if its bending free energy is compensated (see Fig. 3b) since closing of the ring faces a small free energy barrier against the combination of the two end caps (see Fig. 3a). However, DNA-mediated stabilization of 'nano rings' requires that: (i) The toroidal micelle can exist below the denaturation temperature of DNA, (ii) the formed DNA-micelle complex must be kinetically or thermodynamically restricted from forming alternative phases such as, for example, hexagonally stacked rods or micelles. It is uncertain whether such a phase regime is experimentally accessible.

5. Conclusions

We illustrated how a thermo-responsive lipid surfactant can be kinetically tricked into forming highly curved toroidal structures. Our free energy calculations illustrate that the scission barrier of toroidal micelles, despite being under a high curvature stress, is in fact rather similar to that of worm-like micelles. Consequently, the lifetime of a highly curved toroidal micelle is similar to the breaking time of its corresponding micelle at equilibrium. Our work provides a unique molecular glance into the complex kinetics of stimuli-responsive self-aggregation on a nano scale. Despite seemingly similar kinetics of structure formation on different length scales, scale dependent differences in the life-time of formed phases can arise when the phases are kinetically rather than thermodynamically stable. Consequently, lipid 'nano rings' have a lifetime of likely milliseconds to seconds, quite in contrast to the highly metastable toroidal micelles formed by block copolymers. This explains the paradoxical absence of toroidal micelle phases at a tenfold smaller length scale.

We now consider it plausible that lipid 'nano rings' have already been fabricated within experiments but have thus far escaped experimental detection. We therefore advocate experimental revisiting of previously studied thermo-responsive lipid bola amphiphile systems (e.g., citations [30,52]) with state-of-the-art time-resolved X-ray scattering techniques (e.g., citation [51]). Of related interest is the observed thermodynamic preference of lipid bola amphiphiles to form disc-like structures below T_m. The presence of disc-like structures may very well explain the regular thickness undulations observed in AFM experiments in fibrils formed by lipid bola amphiphiles below T_m [43]. Isolated lipid discs could alternatively precipitate from solution and form fibrils via repetitive stacking of discs analogous to their observed behavior in block copolymer mixtures [8]. Repetitive stacking of slightly tilted discs may explain the observation of fibrils with a somewhat helical appearance as has been observed in transmission electron microscopy of hydrogels formed by lipid bola amphiphiles [30,68]. Fitting high-resolution SANS and SAXS sprectra of these fibril structures with alternative models based on disc structures in either a 'chained' (see Fig. 1A) or stacked conformation may yield novel insights into the packing and self-organization of lipid Pluronic analogs below T_m.

Finally, the stability and lifetime of 'nano rings' could be increased by introducing a restoring force which opposes unbending of the torus. Incorporation of a short double-stranded DNA fragment or another poly-electrolyte within the torus can compensate the unfavorable bending energy stored within a positively net charged toroidal micelle at low ionic strength. Because of their versatile self-organization, bolaamphiphiles have already illustrated a great potential in the field of drug delivery [28]. Lipid 'Nano rings' stabilized by DNA fragments may have interesting applications in, for example, gene delivery technologies [69,5]. Their small size ($\simeq$ 10 nm) as well as their responsive nature upon external stimuli such as, for example, temperature and ionic strength may be advantageous for the transfection of small DNA fragments in cells.

CRediT authorship contribution statement

Laura Josefine Endter: Writing - original draft, Methodology, Investigation, Formal analysis. **Herre Jelger Risselada:** Conceptualization, Writing - original draft, Investigation, Methodology, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jcis.2020.11.038.

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