Polymersome Morphologies

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Controlled Shape Transformation of Polymersome Stomatocytes**

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One of the intriguing properties of giant phospholipid unilamellar vesicles (giant liposomes, 1–100 μm) is their ability to readily transform their spherical shape as a response to environmental changes.[1,2] By fluctuating the osmotic conditions, the composition of the lipids, or the temperature,[3] exotic forms, such as starfish, prolate, stomatocyte (cup-like) and discocyte (disc-like) assemblies, can be generated.[4] However, the highly flexible surface membrane of liposomes provides only transient morphologies which cannot easily be captured.

Recently, we reported on the shape transformation of polymeric vesicles (100–500 nm),[7] self-assembled from amphiphilic building blocks of which the hydrophobic domain exhibits a high glass transition temperature (T_g).[5,6] Dialysis of these polymersomes, created in a mixture of water and organic solvents, led to an osmotic pressure difference over the polymersome membrane. A volume decrease of the inner compartment caused by rapid outward diffusion of the organic molecules induced formation of stomatocytes. Upon slow removal of the organic solvent, the solvent-swollen flexible membrane transformed into its usual rigid glassy state, and the shape at that specific moment was trapped. This method is different from traditional polymersome preparations, where the spherical shape is directly captured by quenching in an excess of water.[8] Furthermore, in contrast to the liposomal constructs, the resulting stomatocyte morphology was proven to be stable for at least a year.

Once the polymeric membrane has lost its dynamic behavior under ambient conditions, the vesicular structure is believed to be stably trapped in the currently morphology. We nevertheless envisioned that, if the rigidifying process of the hydrophobic segment could be reversed, polymeric aggregates should in principle be able to shape transform in a similar way to liposomes.

Herein, we experimentally demonstrate a highly controllable procedure to switch the rigidity of the glassy hydrophobic membrane domain of polymeric stomatocytes back to flexible and responsive to the environment. This method allows the shape transformation process to continue to the most energetically favorable morphology. We demonstrate that clear-cut changes in the re-shaping conditions drive the rearrangement of the stomatocyte membrane into a variety of remarkable structures, such as kippahs,[9] oblates, and polymersomes (Figure 1). By taking advantage of the straightforward glass transition of the hydrophobic segment from flexible to rigid, we were able to kinetically entrap transient morphologies at every desired moment. This resulted in interesting insights into the shape change trajectory.

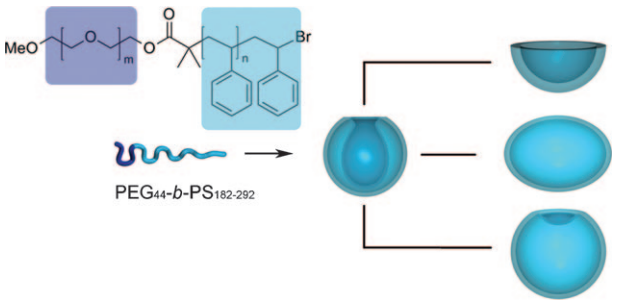

Figure 1. The shape transformation of stomatocytes into three different morphologies, from top to bottom: kippahs, oblates, and polymersomes.

The polymeric vesicles were based on amphiphilic poly(ethylene glycol)-*b*-polystyrene (PEG-*b*-PS) block copolymers. These polymers were synthesized by atom transfer radical polymerization (ATRP) of styrene starting from a PEG_{44}-macroinitiator. The number average degree of polymerization (DP_n) of the PS segment ranged between 182–292 and showed a narrow size distribution (polydispersity index (PDI) of 1.07–1.18; see Table S1, Supporting Information).

To obtain stomatocytes, the previously reported shape transformation procedure was used.[7] The initial vesicles were prepared by the cosolvent method.[10] The PEG_{44}-*b*-PS_{292} block copolymer was dissolved in a mixture of THF and 1,4-dioxane which are good solvents for both segments. To induce self-assembly of the amphiphiles, ultrapure water, which is a precipitant for PS, was slowly added to the mixture until a content of 50% (in volume) was reached. Upon dialysis of the cloudy suspension against water, the spherical morphology of the vesicles was transformed into bowl- shaped stomatocyte

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polymersomes. To reshape the polymeric stomatocytes, a mobile and permeable vesicular membrane had to be regenerated. THF and dioxane were considered as good candidates for the recovery of a flexible polymer membrane owing to their confirmed plasticizing effect on PS and miscibility with water. Quick addition of THF and dioxane to the stomatocyte solution resulted in gross structural disruption and the formation of small vesicles and large polymer aggregates (Figure S1, Supporting Information). When the organic solvents were gradually introduced by dialysis of the aqueous stomatocyte solution against a mixture of water and organic solvent (1/1, v/v) however, no precipitates were observed by eye.

We reasoned that a greater degree of swelling of the membrane provides a better prospect on successful shape transformation of the vesicular structure. Since the swelling of homo-polystyrene is somewhat higher in THF than dioxane based on comparison of the Hildebrand solubility parameters ($\delta = 16.6$–20.2 $[MPa]^{1/2}$ for homo-PS, $\delta = 18.6$ $[MPa]^{1/2}$ for THF and $\delta = 20.5$ $[MPa]^{1/2}$),[11,7] the ratio between these two organic solvents was chosen in favor of THF (respectively 75:25, v:v). A concentrated aqueous stomatocyte solution was dialyzed against water containing 50% of the predetermined THF:dioxane mixture. Aliquots of 20 μL were withdrawn over time using a pre-set schedule and added at once to a large excess of pure water (1000 μL). This procedure rapidly vitrified the PS segments in order to develop enough rigidity to preserve the morphology at the moment of retraction. More concentrated samples were prepared for cryo-TEM and cryo-SEM analysis (100 μL transformed vesicle solution in 400 μL water). The amount of organic solvent (up to 10% in volume) still present in these more concentrated samples did not hamper immediate vitrification of the transient morphology,[12] as was confirmed with electron microscopy (Figure S2, Supporting Information).

Examination of the quenched PEG_{44}-*b*-PS_{292} block-copolymer assembly solutions by dry-TEM revealed a shape transformation from stomatocyte to a hollow hemisphere or "kippah",[9] as depicted in Figure 2a. The average estimated wall thickness of the structures after 18 h of dialysis was (47 ± 3) nm (number of counted molecules $(n) = 50$). Compared to the initial bilayer membrane of (26 ± 2) nm ($n = 50$), these walls are 81% thicker than a normal vesicle. The increase in thickness, together with the perfectly round shape, indicated formation of the fully collapsed structure. This suggested kippah morphology was confirmed by both cryo-TEM and cryo-SEM analysis (Figure S5, Supporting Information); average estimated wall thicknesses of (57 ± 3) nm ($n = 10$) and (46 ± 4) nm ($n = 30$) were measured respectively, while for unilamellar membranes (29 ± 1) nm ($n = 30$) and (26 ± 4) nm ($n = 10$) were measured. According to cryo-TEM analysis of the membranes, the walls of the hollow hemisphere are 97% thicker than usual. However, it is not assured at this moment whether the two bilayers are merged or merely lying nearby each other.

The high T_g of the PS domain allowed us to kinetically entrap the transient structures and thereby follow the transformation trajectory by TEM (Figure S3a, Supporting Information). The first changes in morphology were observed after one hour, suggesting that at least 30 min were required for the membrane to accomplish the transformation from glassy to flexible. After one hour, the volume of the inner compartment was decreased and the mouth of the stomatocytes opened up (Figure 2a). Cryo-TEM images of the solution

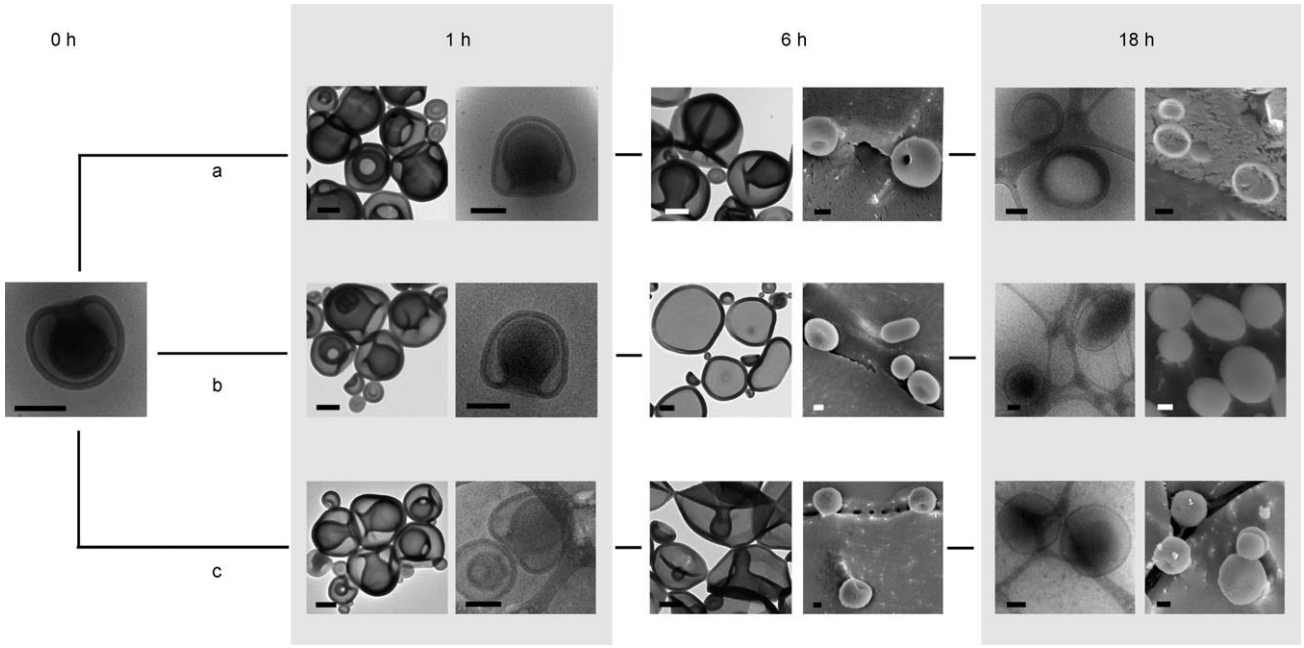

Figure 2. Shape transformation of stomatocytes of PEG-*b*-PS block copolymers over time illustrated by dry-TEM (left images at 1 h and 6 h), cryo-TEM (0 h, right images at 1 h, left images at 18 h) and cryo-SEM images (right images at 6 h and 18 h). The stomatocytes were dialyzed against a mixture of water and organic solvent (1/1, v/v) using three different ratios of THF to dioxane by volume: a) 75:25, b) 50:50, and c) 25:75. All scale bars: 200 nm. Larger images are depicted in Figure S4 and S5, Supporting Information.

after 1 h verified that the observed difference in morphology is a consequence of actual shape change and not an artifact resulting from the sample preparation procedure. Further investigation of the process showed a simultaneous flattening and widening of the vesicular structure after 2 h, followed by re-contraction of the membrane forming the entrance in 6 h. Eventually the stable morphology was reached, which in this case amounted to the formation of hollow hemispherical domes.

We envisioned that dialysis of the stomatocytes in an aqueous solution containing an excess of THF compared to dioxane caused such a great degree of swelling of the PS domain that the membrane became fairly weak and not only permeable to organic solvents but to water as well. This, in combination with osmotic pressure, finally led to collapse into the kippah morphology.

When the re-shaping conditions were slightly modified by performing the dialysis with an almost equal amount of THF:dioxane (50:50, v:v) while retaining the water to organic solvents ratio (1/1, v/v), a completely different concluding morphology was discerned (Figure 2b). Although TEM micrographs of the dried sample withdrawn after 18 h displayed kippahs at first sight, the deviation of a perfectly round shape and the lighter irregularity in the middle of the structures were suggesting a different shape. Cryogenic electron microscopy proposed an oblate structure, which verified that membrane structures analyzed by normal TEM suffered from drying effects which caused them to collapse.

The shape rearrangement of stomatocytes into the oblate morphology was initiated after 30 min of dialysis (Figure S3b, Supporting Information). The opening of the stomatocytes expanded in 1 h as a result of internal volume decrease, and the transformation continued with a certain degree of flattening and thereby widening of the entire construct in 2 h. Although the changes are somewhat less drastic compared to the shape transformation in a more THF-rich environment, a similar path seemed to be pursued. However, the sample at 6 h suddenly showed a very different morphology as observed by EM, which could be described as a red blood cell-like structure of which only one side is dented (Figure 2b). The uniconcave discocytes finally transformed into an oblate shape through minute inflation of the structures whereby the indent disappeared.

To further address the involvement of THF in the shape transformation, a batch of stomatocytes was dialyzed in a 50% aqueous solution mixed with less THF than dioxane, 25:75 in volume (Figure 2c). The first observable shape changes only just occurred after two hours (Figure S3c, Supplementary Information). After 6 h, TEM and cryo-SEM images showed a tremendous decrease in depth and diameter of the stomatocyte's cavity. This process continued until virtually the whole membrane was unfolded, as corroborated by cryo-TEM and cryo-SEM analysis. Hence, plasticizing the membrane with an aqueous organic solvent mixture containing less THF than dioxane leads to growth of the inner compartment volume and thereby re-inflation of the membrane.

Investigation of the shape transformation of stomatocytes prepared from PEG_{44}-*b*-PS_{182} block copolymers revealed a similar transformational pathway as for PS_{292}, which indicates that the shape change procedure is well reproducible with various PEG–PS polymeric building blocks.

Giant liposomes are capable to transform from every possible morphology into another and are never locked in a specific shape. To explore our shape transformation procedure, conventional polymersomes self-assembled from PEG_{44}-*b*-PS_{198} were exposed to water and 50% of THF:dioxane, 50:50 in volume. As shown in Figure 3, the spherical

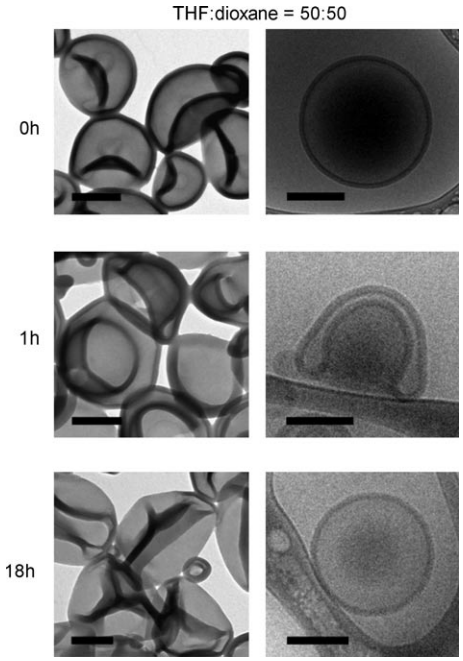

Figure 3. Dry-TEM (left) and cryo-TEM (right) micrographs that illustrate the shape transformation of polymersomes self-assembled from PEG-*b*-PS block copolymers over time. The polymersomes were dialyzed in water/(THF:dioxane), 50/50 (=50:50) by volume. All scale bars: 200 nm.

vesicles initially remodeled into stomatocytes. The first changes occurred between 30 min and 1 h, when the internal volume decreased almost to a minimum and caused the membrane to fold inwards (Figure S6, Supporting Information). The wide opened stomatocytes subsequently transmuted back into perfectly round polymersomes. Remarkably, this most energetic favorable, spherical structure is different from the oblates that were obtained in the similar dialysis experiment with stomatocytes. Therefore, the assembly morphology at the start of the experiment has a significant influence on the transformation process and its final outcome.

Inspired by the continuous shape transformations of liposomes, we have shown that vesicles constructed of a membrane with a glassy and robust hydrophobic segment are no longer merely destined to just attain the spherical morphology. Upon gradual introduction of plasticizing organic solvent molecules, the membrane becomes permeable and responsive to the environment in a controlled approach. Rapid quenching of the membrane enables the entrapment of desired, transient structures. The degree of flexibility intro-

duced to the system has an influence on the final morphology. Although the fluid dynamics of the organic solvent and water molecules through the vesicular membrane are not yet fully understood, numerous combinations of initial polymer assemblies and solvent compositions are currently being examined to controllably create unusual, yet stable morphologies.

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