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Tubular Polymersomes: A Cross-Linker-Induced Shape Transformation

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Supporting Information

ABSTRACT: Polymersomes, polymeric vesicles constructed of block copolymers, can undergo a sphere-to-tubule transition under the influence of a chemical modification of the polymeric bilayer. A strain-promoted alkyne−azide cycloaddition (SPAAC) reaction between azide handles inside the hydrophobic domain of the membrane and an excess of a bicyclo[6.1.0]nonyne (BCN)-cross-linker causes the vesicle to stretch in one dimension. Tubular polymersomes up to 2 μm in length can be obtained with this shape transformation. The introduction of a cleavable cross-linker makes this process reversible and opens the way for future drug delivery applications.

Shape transformations of giant vesicles have attracted much attention over the years.[1−4] By adjusting the temperature, pH, osmotic pressure, or composition of the membrane, liposomes have been turned into exotic structures like starfish, pears, stomatocytes, or discocytes.[5−9] The dynamic nature of the phospholipid membrane, however, makes these transitions transient and hinders the application potential of these unusual vesicular morphologies.

Polymersomes, artificial vesicles made out of amphiphilic block copolymers, have emerged as multifunctional nanostructures in the past decade.[10,11] The possibility to tailor the chemical design of the membrane allows for good control over the shape and the stiffness of the vesicle. This has been demonstrated in a number of reports regarding conformational changes of polymeric assemblies.[11−14] In a recent publication we described a shape transition of polymersomes consisting of poly(ethylene glycol)-*block*-polystyrene (PEG-*b*-PS) block copolymers.[15] Dialysis of these polymersomes over an osmotic pressure difference led to a decrease of the inner volume of the vesicles and caused their membranes to deform into a stomatocyte-shaped arrangement. Subsequent quenching with water captured the morphology of the vesicles due to the high glass transition temperature (T_g) of the polystyrene block. This process was proven to be reversible in a controlled way by the introduction of plasticizing agents for the hydrophobic segment of the membrane. It was also shown that a range of different polymeric assemblies like kippahs and oblates could be generated by the kinetic entrapment of transient structures.[16]

In our investigation on the shape transformation of polymersomes we continue to explore the responsiveness of the polymeric membrane toward different stimuli. Herein we show that a change in the chemical composition of the membrane, by means of the addition of a cross-linker, can cause the vesicle to stretch into a tubule. We demonstrate that this shape transformation is highly dependent on the initial design of the block copolymer and on the concentration and nature of the cross-linker. By the introduction of a reducible disulfide bridge inside the cross-linker we show that the shape transition is governed by a kinetic process which can be reversed by cleavage of the disulfide bond.

We discovered the cross-linker-induced shape transformation when we were investigating the stabilization of polymersomes consisting of poly(ethylene glycol)-*b*-poly(styrene-*co*-4-vinylbenzyl azide) (PEG_{44}-*b*-P(S-*co*-4-VBA) polymers by means of a cross-linking reaction with BCN-cross-linker **1** (Figures 1, S2).

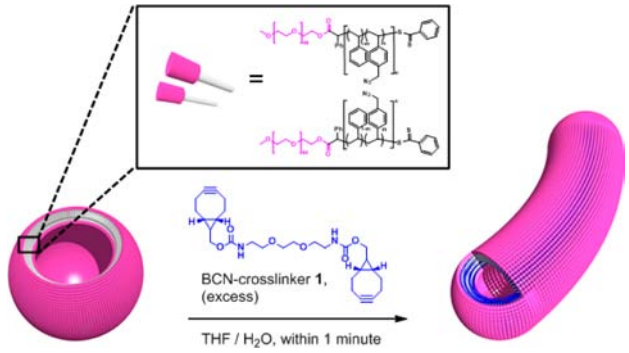

Figure 1. Schematic representation of the shape transformation of polymersomes.

This cross-linking technique is based on a strain-promoted alkyne−azide cycloaddition (SPAAC) reaction.[17,18] The PEG-*b*-P(S-*co*-4-VBA) polymers were obtained out of poly(ethylene glycol)-*b*-poly(styrene-*co*-4-vinylbenzyl chloride) (PEG-*b*-P(S-*co*-4-VBC) polymers, which were prepared via reversible addition−fragmentation chain transfer copolymerization between styrene and 4-vinylbenzyl chloride (4-VBC). The cosolvent method was applied to produce the polymeric vesicles. Block copolymer **P1** (Table 1) was dissolved in THF, and ultrapure water was added at a rate of 1.0 mL/h until a H_2O:THF content of 50% was reached. At this point the solution became cloudy, an indication for the presence of polymersomes. When we added increasing amounts of **1** to this solution we observed an interesting phenomenon (Figure 2). At a 1:1 (BCN/azide) cross-linker concentration the polymeric

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Table 1. Investigation of Polymer Composition on the Final Morphology of the Polymersomes

polymer	linker	type	azide cont.[a]	morphology[b]
P1	**1**	PEG_{44}-*b*-P(S_{128}-*co*-4-VBA_{29})	18%	tubular
P2	**1**	PEG_{44}-*b*-P(S_{108}-*co*-4-VBA_{16})	13%	tubular
P3	**1**	PEG_{44}-*b*-P(S_{133}-*co*-4-VBA_{8})	5%	spherical
P4	**1**	PEG_{44}-*b*-P(4-$VBA)_{29}$-*b*-PS_{95}	23%	tubular
P5	**1**	PEG_{44}-*b*-PS_{95}-*b*-P(4-$VBA)_{29}$	23%	micellar
P6	**1**	PEG_{44}-*b*-P(S_{128}-*co*-4-VBC_{29})	0%	spherical
P2	**4**	PEG_{44}-*b*-P(S_{108}-*co*-4-VBA_{16})	13%	spherical[c]

[a]Percentage of 4-vinylbenzyl azide monomers in the hydrophobic block. [b]Morphology of polymersomes 1h after adding a 10:1 (BCN/azide) cross-linker concentration of **1**. [c]Polymersomes degraded after 15h.

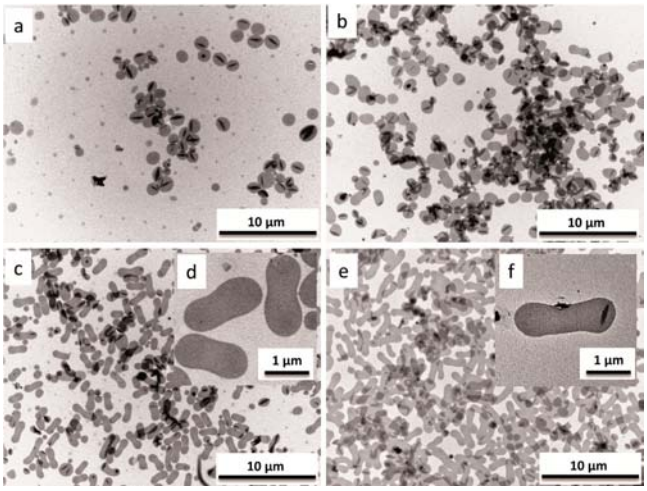

Figure 2. TEM images of polymersomes after addition increasing amounts of **1**. (a) 1:1, (b) 2:1, (c) 4:1, (e) 10:1 (BCN/azide) cross-linker concentrations. The inset images (d) and (f) represent close-ups of the polymersomes in (c) and (e), respectively. The TEM images were recorded after the polymersomes had been dialyzed against THF for 24h.

vesicles retained their spherical morphology with an average size of 600 nm (Figures 2a, S5). However, when we raised the amount of **1** to a 2:1 (BCN/azide) concentration, the polymersomes started to stretch in one dimension (Figure 2b). This elongation continued up to a 10:1 (BCN/azide) cross-linker concentration, when the polymersomes reached an average length of 2 μm (Figures 2e,f, S5). Adding more cross-linker did not result in longer vesicles (Figure S5).

To analyze the inner structure of the tubular polymersomes we studied the assemblies with cryogenic scanning electron microscopy (Cryo-SEM, Figure 3a) and cryogenic transmission electron microscopy (Cryo-TEM, Figure 3b). Both techniques confirmed the hollow lumen of the vesicles and revealed a membrane diameter of 25−35 nm, which did not significantly differ from the noncross-linked polymersomes.

We further examined the hydrodynamic volume of the vesicles using dynamic light scattering (DLS). Interestingly, the volume did not change after cross-linking, implying that the shape transformation is not the result of osmosis or a fusion process (Figure S6a).

We also determined the cross-linking density of the different polymersomes in Figure 2 by adding an excess of a dansyl-azide linker **2** (Figure 4b) to the dialyzed samples of cross-linked

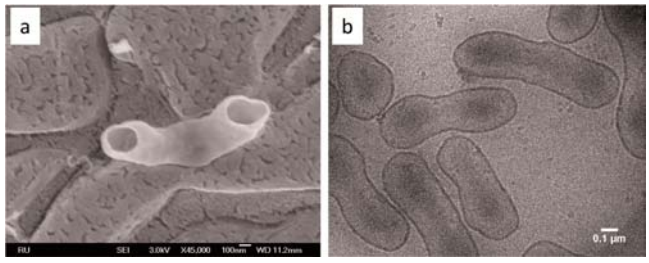

Figure 3. (a) Cryo-SEM and (b) -TEM images of tubular polymersomes.

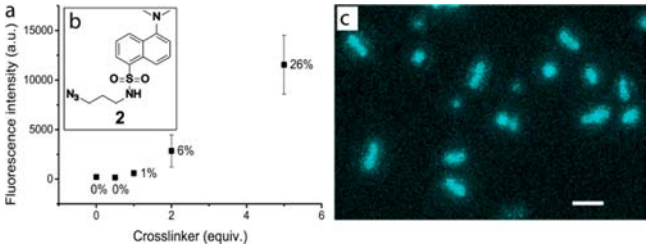

Figure 4. (a) Fluorescence intensity plot of cross-linked polymersomes (Figure 2a−c,e) labeled with fluorophore **2**. The percentages next to the data points represent the amount of labeled BCN moieties, which is the inverse of the cross-link density. (b) Chemical structure of dansyl-azide linker **2**. (c) Confocal fluorescence image of tubular polymersomes (Figure 2e) labeled with fluorophore **2**. The scale bar represents 2 μm.

vesicles. Any BCN moiety that had not reacted with an azide handle inside the polymersomes could in this way be targeted by the dansyl-azide linker. This allowed us to measure the relative amount of monofunctionalized **1** using fluorescence spectroscopy (Figure 4a). For a quantitative assessment of the cross-linking density we applied a correlation curve in which we plotted the degree of functionalization of block copolymer **P1** with a dansyl-BCN linker (Figure S3) against the fluoresence intensity. This showed us that the cross-linking density remained very high (>94%) when up to 2.0 equiv of cross-linker **1** were added to the polymersomes. Only in case when a 5-fold excess of **1** was being used did the vesicles reveal an increased fluorescence intensity (Figure 4c). However, the cross-linking density was still 74% in this situation.

In order to get a better insight in the mechanism of the shape transformation, we synthesized block copolymers **P2** and **P3**, in which respectively 13% and 5% of the styrene monomers were functionalized with azide handles (Table 1). While polymersomes consisting of **P2** polymers changed into a tubular shape upon reaction with an excess of cross-linker **1** (Figures 5a, S8), polymeric vesicles containing **P3** polymers remained spherical under similar conditions (Figures 5b, S9). Furthermore, block copolymers **P4** and **P5** were prepared in which the location of the 4-vinylbenzyl azide monomers in the hydrophobic block was modified. When the azides were positioned next to the hydrophilic PEG block, as in **P4**, tubular polymersomes were produced upon addition of an excess of **1** (Figures 5c, S10). However, when they were located toward the middle of the hydrophobic domain, as in **P5**, the vesicles were converted into wormlike micellar structures (Figures 5d, S11). This proves that not only the concentration of the azide handles but also the location is crucial for the shape transformation. An explanation for these observations can be given by the area-

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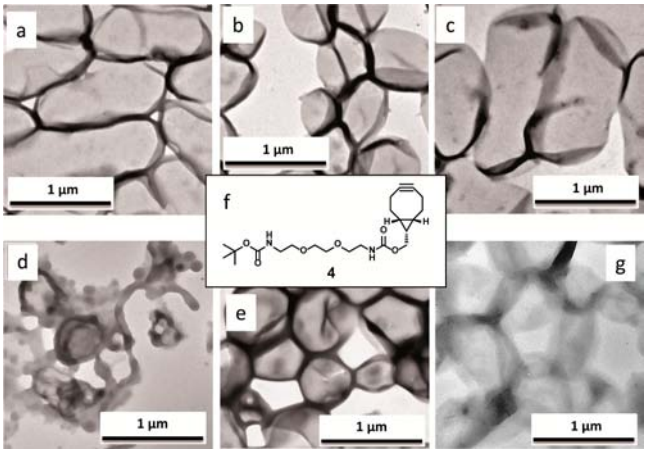

Figure 5. TEM images of polymersomes, consisting of block copolymers (a) **P2**, (b) **P3**, (c) **P4**, (d) **P5**, and (e) **P6**, after addition of 5.0 equiv of cross-linker **1**. (f) Chemical structure of linker **4**. (g) TEM image of polymersomes, consisting of block copolymer **P2**, after addition of 5.0 equiv of linker **4**.

difference-elasticity (ADE) model which describes the energy of a fluid vesicle (eq 1).[2,19]

$$H_{ADE} = \kappa\left(\frac{1}{2}\int dA(C_1 + C_2 - C_0)^2 + \frac{\alpha}{2}\frac{\pi}{AD^2}(\Delta A - \Delta A_0)^2\right) \quad (1)$$

In eq 1 C_1 and C_2 represent the curvatures along the two principal directions of the membrane, ΔA_0 is the difference in area between the two monolayers when they are in a relaxed state, ΔA is the actual geometrical differential area between the two monolayers, κ represents the local bending modulus, α is the ratio between the local and nonlocal bending moduli, D is the diameter and C_0 the spontaneous curvature of the bilayer membrane. The latter parameter can be induced either by a different chemical environment on both sides of the membrane or by a different chemical composition of the two monolayers. The addition of an excess of **1** to the azide-functionalized polymersomes creates a gradient of cross-linker over the membrane. The azides close to the hydrophilic outer shell experience a higher local concentration of **1** than the azides attached to the inner leaflet of the polymersome. This subtle difference generates an asymmetry in the cross-link density of the membrane and can lead to a spontaneous curvature of the bilayer. Together with the fact that the total mass of the vesicle membrane increases and as a result the tension between the polymers, this can cause the polymersome to deform into a tubular shape. The short reaction time of the shape transformation (<1 min) shows that this process is most likely kinetically controlled.

To verify whether the actual SPAAC reaction was required for the tubule formation, we prepared polymersomes consisting of block copolymers **P6** in which the azide groups were replaced with chloride functionalities. Due to the insensitivity of BCN toward chlorides, addition of an excess of **1** did not produce cross-linked vesicles. At the same time the shape transformation did not occur as only spherical polymersomes could be recovered (Figures 5e, S12). The necessity of the cross-link reaction for the shape transition was furthermore validated by the addition of 5.0 equiv of linker **4** (Figures 5f, S3) to the vesicles. Because **4** contained only one BCN moiety per molecule a covalent connection between two polymers could not be established. Again the morphology of the polymersomes did not change upon addition (Figures 5g, S13), although the vesicles eventually degraded when the dispersion was stirred for a prolonged time (15h).

Giant liposomes are known to transmute back into their thermodynamically most favored conformation when they are no longer exposed to external stimuli. Since our tubular polymersomes are formed via a kinetic entrapment pathway we wondered whether the vesicles would adopt a spherical morphology once the covalent bond between the polymers was cleaved. To investigate this thermodynamic relaxation process we synthesized cross-linker **5** containing a reducible disulfide bridge (Figures 6a, S3). Adding a 10:1 (BCN/azide)

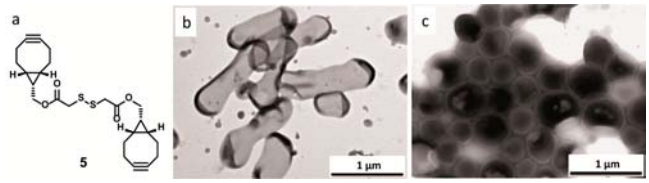

Figure 6. (a) Chemical structure of cross-linker **5**. TEM images of thermodynamic relaxation of tubular polymersomes before (b) and after (c) addition TCEP·HCl.

concentration of this cross-linker to polymersomes consisting of **P2** block copolymers afforded tubular vesicles as expected (Figure 6b). In order to cleave the disulfide bridge we used an excess of tris(2-carboxyethyl)phosphine hydrochloride (TCEP·HCl). Much to our satisfaction, the polymeric vesicles were completely transformed back into their original spherical configuration after only 30 min (Figure 6c). DLS data confirmed that the shape transition was not being caused by an osmotic pressure difference since the hydrodynamic volume of the vesicles remained constant during the relaxation process (Figure S6b).

In conclusion, we have developed a novel shape transformation technique for the preparation of tubular polymersomes, based on the chemical alteration of a polymeric bilayer. We have shown that the shape transition strongly depends on the architecture of the block copolymer and the concentration of the cross-linker. We have also demonstrated that the elongation can be reversed by the application of a cross-linker with a cleavable disulfide bond. The high degree of control over the shape transformation and the advantages of anisotropic particles over spherical particles make these polymersomes interesting objects as possible carriers for drug delivery.[20−23]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The authors declare no competing financial interests.

Notes

The authors declare no competing financial interest.

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