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Corona-Cross-Linked Polymer Vesicles Displaying a Large and Reversible Temperature-Responsive Volume Transition

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Amphiphilic block copolymer (BCP) vesicles (polymersomes) represent an active research area due to their potential for applications such as controlled drug delivery and nanoreactors for controlled chemical reactions.[1,2] Much effort has been dedicated to two important issues. On the one hand, in order to preserve the structural integrity of polymer vesicles under varying conditions, cross-linking of polymer chains has widely been used,[3] like for core−shell micelles.[4] On the other hand, by using stimuli-responsive BCPs, many polymer vesicles could react to environmental changes such as pH,[5] temperature,[6] redox reactions,[7] and light.[8] An easy way to prepare stimuli-responsive BCP vesicles is to choose a hydrophobic block that, in response to a specific stimulus, can become hydrophilic or increase the polarity to shift the hydrophilic−hydrophobic balance toward the vesicles' dissociation in aqueous solution. It is then obvious that if such BCP vesicles are cross-linked, only a volume change would occur since the cross-links prevent polymer chains from molecularly dissolving. It is of fundamental interest to design and develop such structurally stable and dynamically stimuli-responsive materials. In this Communication, by proposing the concept of "soft" coronal cross-linking, we demonstrate a general strategy for polymer vesicles that can undergo a large and reversible stimuli-induced volume change in solution. Recently, McCormick's group reported the first direct assembly in aqueous solution of a thermally responsive vesicle from a double hydrophilic BCP of poly(*N*-(3-aminopropy)methacrylamide hydrochloride) (PAMPA) and poly(*N*-isopropylacrylamide) (PNIPAM).[9] They showed that after vesicle formation at $T >$ LCST of PNIPAM a negatively charged polyelectrolyte, poly(sodium 2-acrylamido-2-methylpropanesulfonate) (PAMPS), could be used to complex with the positively charged PAMPA corona and effectively "lock" the vesicle structure. Although the system featured the capacity of unlocking the vesicles by adding an electrolyte to dissociate the polyelectrolyte complex, in the "locked-in" state, the polyelectrolyte complex is kind of "hard" coronal cross-linking because the cross-linked corona is compact and insoluble and, by confining PNIPAM in the interior, could greatly suppress the swelling of vesicles at $T <$ LCST even though PNIPAM chains became more solvated inside the capsule. The same group later developed similar polymer vesicles using poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA) as the corona and obtained cross-linking with gold nanoparticles formed by reducing $NaAuCl_4$ complexed to amine groups, which still gave no evidence for large swelling of the vesicles at $T <$ LCST of PNIPAM.[10] We would like to propose the concept of "soft" coronal cross-linking, which designates a cross-linking state that allows the polymer vesicle to expand greatly when the membrane becomes soluble in water in response to a stimulus, while still preserving the vesicle structural integrity with no dissolution of polymer chains. In other words, a large reversible volume change can take place under the "locked-in" state of the vesicles. As shown below, such a "soft" coronal cross-linking could be obtained by having a low cross-linking density for polymer chains forming the corona of vesicles.

The "soft" coronal cross-linking design is schematically illustrated in Figure 1. The corona is *slightly* cross-linked. Under the effect of a stimulus such as a temperature change, should the hydrophobic membrane-forming block become soluble in water, the chains are allowed to fully hydrated and expand with the cross-linked corona. The unconstrained hydration of the vesicle wall and cross-linked corona leads to a large volume increase, while the vesicle shape persistence is ensured by the cross-linked chains. To demonstrate the approach, which clearly can be applied to many stimuli, we synthesized a thermally sensitive and corona-cross-linkable P(DMAEMA-*co*-CMA)-*b*-PNIPAM by incorporating coumarin methacrylate (CMA) comonomer units in PDMAEMA (Figure 1). A low extent of coronal cross-linking can be obtained through photoinduced dimerization of a small amount of CMA groups (∼5 mol % with respect to the DMAEMA units).[11] Vesicles with PNIPAM wall were easily prepared by heating an aqueous BCP solution (0.2 mg mL^{-1}) to 40 °C (> LCST of PNIPAM). After cross-linking of the P(DMAEMA-*co*-CMA) corona upon exposure to UV light (15 min, 4 mL of polymer solution, ∼ 500 mW/cm^2 measured at 320 nm in front of the solution), the solution was cooled back to 20 °C (< LCST) with PNIPAM chains recovering the solubility in water. With both polymers in the solvated state at 20 °C, un-cross-linked chains, which inevitably exist owing to the required low cross-linking degree, could diffuse outside of the aggregates upon equilibration. Vesicles were collected by centrifugation and used for characterizations. Using a sample of $P(DMAEMA_{49}$-*co*-$CMA_3)$-*b*-$PNIPAM_{74}$, for which coumarin units in the corona-forming block represent ∼5 mol % ensuring a low cross-linking density, we obtained strong evidence for a large and reversible volume transition of the vesicles, reaching about 700% volume increase upon cooling from 40 to 20 °C (see Supporting Information for details on the synthesis and

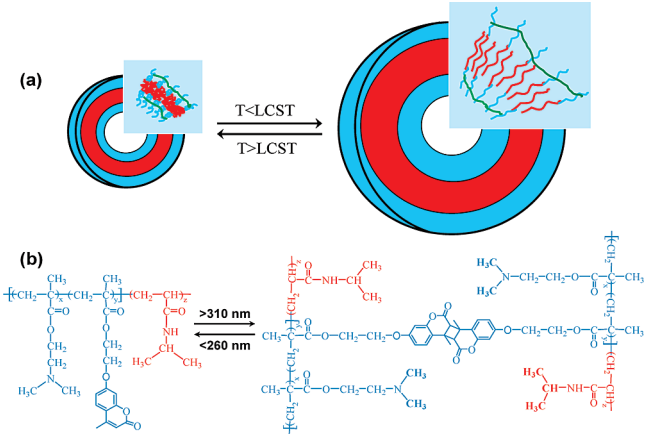

Figure 1. (a) Schematic illustration of the "soft" coronal cross-linking that allows for a large volume change. (b) Chemical structure of the diblock copolymer containing photo-cross-linkable coumarin groups.

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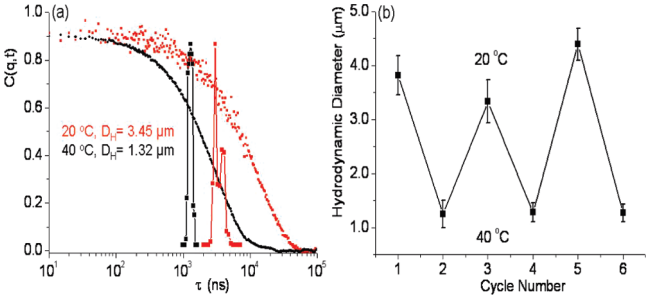

Figure 2. (a) Autocorrelation functions and relaxation time distributions (CONTIN) for the vesicle solution at 20 °C (red line) and 40 °C (black line). (b) Reversible size change of the vesicles at the two temperatures.

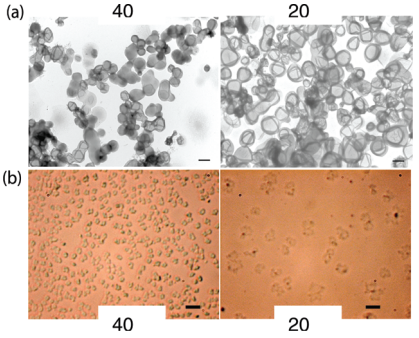

Figure 3. (a) TEM and (b) optical micrographs of vesicles at 20 and 40 °C in the dry state. All scale bars are 2 μm.

characterizations including ^{1}H NMR, UV−vis, DLS, and TEM). Figure 2a shows the typical change of the size distribution of vesicles in solution as determined by DLS. For this measurement, the apparent hydrodynamic diameter (D_H) increased from ~1.3 to 3.5 μm. The fully hydrated vesicles at 20 °C also resulted in a drop in light scattering intensity due to the reduced refractive index difference between the vesicles and the medium. By changing the solution temperature between 20 and 40 °C, the large volume variation of the vesicles is totally reversible. Figure 2b shows three cycles of temperature-induced change in the apparent D_H (the error bars were calculated from five independent measurements). The cross-linking provides a size memory effect that, upon heating to 40 °C, allows the vesicles to undergo a contraction driven by the dehydration of PNIPAM.

Being aware of the uncertainty on the large D_H values as measured by DLS, careful microscopic observations were made, and the results confirmed unambiguously the large volume transition occurred in solution. Figure 3a shows TEM images (unstained) obtained by casting the solution at 20 or 40 °C on a copper grid warmed up at the same temperature. The diameters averaged over >40 particles are about 1.5 μm at 40 °C and 3 μm at 20 °C. This corresponds to a volume increase of 700%. Such a big size difference in the dry state must reflect very different volumes in solution (the DLS data in Figure 2a indicate >900% increase in the apparent hydrodynamic volume). The large vesicles also made it possible to observe their uniform size change directly on an optical microscope, as can be seen from the reflection optical micrographs in Figure 3b (the apparently smaller sizes of vesicles, as compared to those viewed by TEM, could be caused by the encapsulation of the Nile Red dye).

As mentioned above, the expansion upon cooling and the contraction upon heating of the vesicles going through the LCST of PNIPAM were accompanied by a drop and rise of the scattering intensity, respectively. This allowed us to monitor the kinetics of these processes. For the expansion experiment, the vesicle solution equilibrated at 40 °C was quickly moved to the sample holder of DLS held at either 20 or 25 or 30 °C, and the scattering intensity at 90° was collected immediately. The results in Figure 4a show that the expansion process was completed within about 30 s at 20 °C, 40 s at 25 °C, and 65 s at 30 °C. Considering that it took the solution a certain amount of time to reach the final temperature below LCST (about 55, 43, and 35 s for reaching 20, 25, and 30 °C, respectively), the process is very fast. Understandably, the lower the temperature, the faster was the hydration of PNIPAM. By cooling the solution to 20 °C, the expansion of vesicles was over before the solution reached the final temperature. Likewise, the kinetics of the contraction process upon heating from either 20 or 25 or 30 to 40 °C was revealed by the increase in scattering intensity. The results in Figure 4b also pointed to a fast process. In this case, since the final temperature above LCST was the same, the time required for the

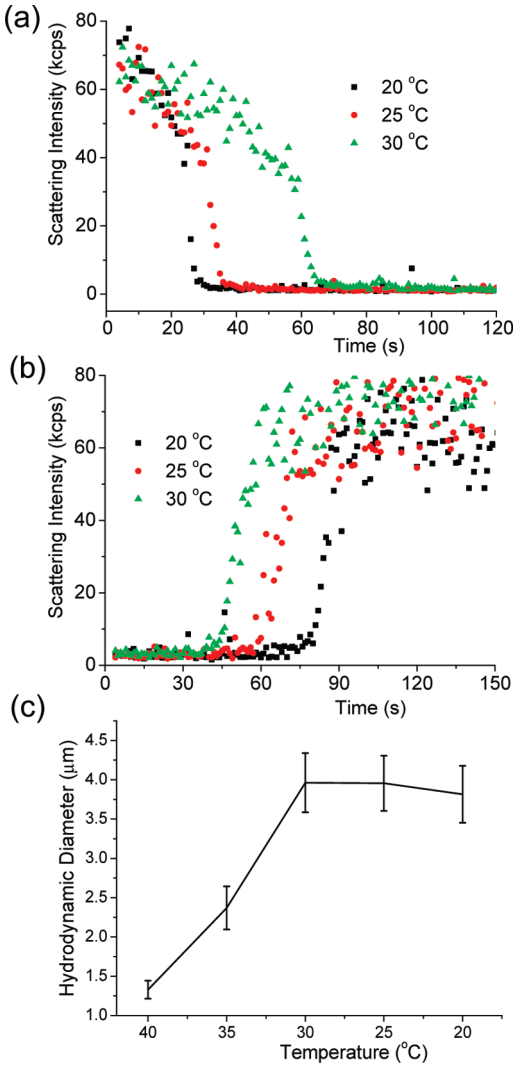

Figure 4. (a) Scattering intensity (at 90°) of a vesicle solution (1.5 mL, 0.2 mg/mL) vs time upon cooling from 40 °C (>LCST) to various tempeartures below LCST. (b) Scattering intensity of the same vesicle solution vs time upon heating from various temperatures below LCST to 40 °C. (c) Plot of the equilibrium size of vesicles vs temperature.

solution to reach it from below LCST (about 92, 65, and 52 s for heating from 20, 25, and 30 °C, respectively) appeared to determine the speed of vesicle contraction. The process was the fastest with the solution heated from 30 to 40 °C (~50 s) because of the smallest temperature difference (less time required for thermal equilibrium). The speeds and magnitude for the swelling and contraction of the vesicles are similar to those of PNIPAM

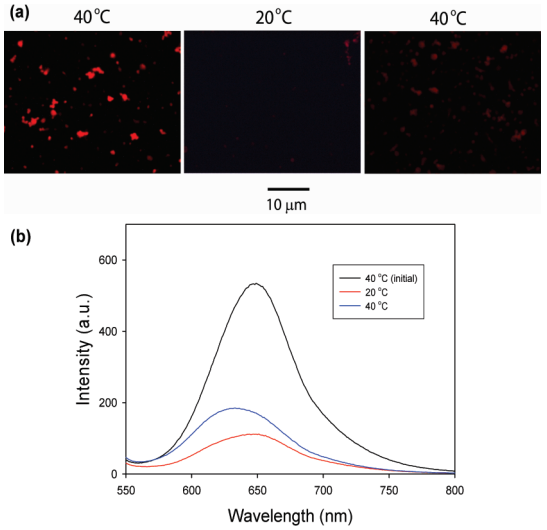

Figure 5. (a) Fluorescence photomicrographs of samples cast from a vesicular solution equilibrated with Nile Red at 40 °C, cooled to 20 °C, and heated back to 40 °C (from left to right). (b) Fluorescence emission spectra of Nile Red (λ_{ex} = 530 nm) recorded from the same solution subjected to the temperature changes.

microgels with voids.[12] This implies that the hydration and dehydration of PNIPAM in the vesicles determine their volume transition process. The preserved structural integrity at $T <$ LCST should contribute to the fast contraction of the vesicles at $T >$ LCST, since the process involves no aggregation of dissolved polymer chains and is driven by the dehydration of an ensemble of polymer chains already linked together. Because of the fast kinetics, the size decrease on heating to 40 °C was found to be the same regardless of the heating rate. This feature is in sharp contrast with non-cross-linked chains, for which the formation of vesicles at $T >$ LCST involves the aggregation of dissolved chains and the relatively slow process is highly sensitive to the heating rate.[9,10] Figure 4c shows the apparent D_H of the vesicles at vartious temperatures around the LCST of PNIPAM. It is worth being noted that despite the light cross-linking, the vesicles were structurally stable. The thermally induced expansion and contraction could be repeated many times with similar kinetics.

The fast stimulus-induced volume transition of polymer vesicles is of interest for controlled delivery applications. A test was conducted. Figure 5a shows the fluorescence photomicrographs recorded with samples cast from a vesicular solution of P(DMAEMA$_{49}$-*co*-CMA$_3$)-*b*-PNIPAM$_{74}$ equilibrated with Nile Red (a hydrophobic dye) at 40 °C, cooled to 20 °C, and reheated to 40 °C. Although the low resolution of the optical microscope could not show the location of dye molecules, their encapsulation by hydrophobic PNIPAM at 40 °C is clear. Upon cooling to 20 °C, the expansion of vesicles with the full hydration of PNIPAM basically quenched the fluorescence of loaded dye molecules, and the large vesicles became invisible due to the lack of fluorescence emission. The fluorescence quenching is due to aggregation of dye molecules in an aqueous medium. Upon heating back to 40 °C, the contraction of vesicles with the dehydration of PNIPAM allowed part of dye molecules to be reloaded, which made the vesicles become noticeable again. Figure 5b shows the fluorescence emission spectra of Nile Red (λ_{ex} = 530 nm), recorded from the same solution subjected to the temperature change. The spectral changes corroborates with the observation (the shift of the emission maximum reflects changes in the polarity of the environment).

Before concluding, two sets of experiments conducted are worth being mentioned. First, by virtue of the reversible photo-cross-linking reaction (Figure 1),[11] photo-de-cross-linking of the vesicles under UV of $\lambda < 260$ nm led to their disintegration at 20 °C due to the dissolution of polymer chains. This photocontrolled "unlocking" of vesicles has the similar effect to the dissociation of the polyelectrolyte complex corona by adding an electrolyte in the solution.[9] Second, we also synthesized BCP samples with coumarin moieties either on the PNIPAM block or on both PDMAEMA and PNIPAM blocks to prepare vesicles with "soft" membrane cross-linking or vesicles with cross-linked corona and wall. In those cases, the thermally induced size changes are much smaller. Membrane cross-linking limits the expansibility of hydrated PNIPAM chains.

In conclusion, we have obtained polymer vesicles that can undergo large, reversible, and fast volume transition in aqueous solution in response to temperature change, while preserving the vesicle structure. We have proposed the concept of "soft" coronal cross-linking as a general designing strategy for such polymer vesicles. The key is to have a lightly cross-linked corona that is capable of both retaining the vesicle structure and allowing the swelling of vesicle membrane when it becomes soluble in water. Polymers whose water solubility can be switched by other stimuli such as pH and light can also be employed to design the vesicles. This achievement is of fundamental interest not only for stimuli-controlled release of loaded guest molecules but also for other possible applications. For instance, it is conceivable that such a large deformation associated with the volume expansion and contraction of polymer vesicles could be explored to generate mechanical energy to carry out a work in solution.

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Supporting Information Available: Synthesis and characterization details, ^{1}H NMR, TEM, and UV−vis results. This material is available free of charge via the Internet at http://pubs.acs.org.

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