Vesicles

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"Breathing" Vesicles with Jellyfish-like On–Off Switchable Fluorescence Behavior**

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Nowadays, synthetic vesicles serve as excellent model membranes to mimic the dynamic and structural features of cells in a blossoming field coined cytomimetic chemistry, as reviewed by Menger et al.[1] Most studies have been based on lipid vesicles (liposomes). For example, Sackmann and co-workers demonstrated liposome budding and fission induced by heating and osmotic deflation.[2] Menger and co-workers reported the fusion, birthing, foraging, healing, separation, endocytosis, adhesion, and aggregation of liposomes.[3] Ewing and co-workers showed the exocytosis of liposomes.[4] Keating and co-workers observed liposome budding.[5] Polymeric vesicles (polymersomes) have also been used in cytomimetic chemistry. For example, Eisenberg and co-workers described the fusion, fission, and breathing of polymersomes.[6] Our group[7] reported the real-time fusion, fission, and aggregation of giant hyperbranched polymer vesicles. Thus, generally speaking, great progress has been made in cytomimetic chemistry, and synthetic vesicles have proved to be valuable model systems for investigating the mechanisms of these cellular processes, especially in the discrimination of essential membrane activities from protein interference.

Until now, all reported cytomimetic processes have been limited to shape transformations of the vesicle membranes, and no function has been generated during these processes. However, in many cellular processes or living organisms there is a concomitant function expression during membrane deformation. For example, some jellyfish show on–off switchable fluorescence behavior in response to the swelling and shrinkage of the membrane during the breathing process:[8] fluorescence is quenched when the jellyfish breathes in, and strong green fluorescence is emitted when the jellyfish breathes out (Figure 1 A). The molecular basis for this concomitant breathing and light-emitting process is the oxidation- or deoxidation-induced conformation transformation of the membrane-incorporated fluorescent proteins (see section 5 of the Supporting Information). Thus, to push forward the development of cytomimetic chemistry, it is necessary to combine vesicular morphological transformations with dynamic functional changes. That is, cytomimetic vesicle deformation should trigger or be accompanied by the

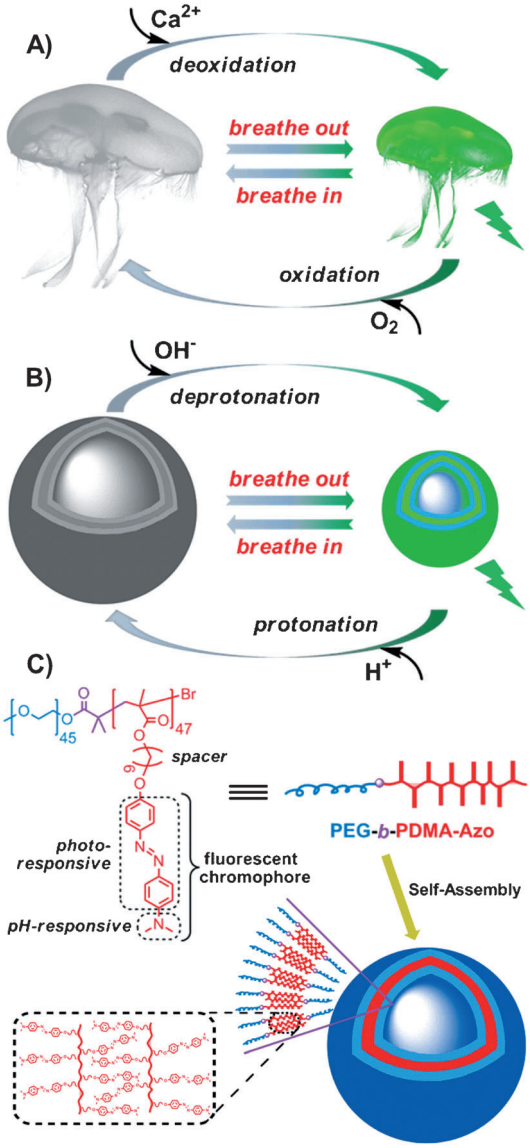

Figure 1. The breathing processes of A) jellyfish and B) vesicles accompanied by highly reversible green-fluorescence quenching and recovery. C) Schematic representation of the amphiphilic diblock copolymer PEG-*b*-PDMA-Azo and the vesicle structure.

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expression of a function, such as the generation of light-emitting signals as seen in jellyfish. To our knowledge, a concomitant morphological and functional transformation in cytomimetic chemistry has not been described to date.

As inspired by jellyfish, we report herein a smart "breathing" polymeric vesicle with reversible on–off switchable fluorescence behavior. The polymeric vesicles were prepared through the aqueous self-assembly of an amphiphilic diblock copolymer consisting of many fluorescent chromophores of dimethylaminoazobenzene (DMA-Azo groups). They exhibited pH-responsive breathing behavior involving the swelling and shrinkage of the vesicles. Furthermore, in analogy with jellyfish, the fluorescence is quenched when the vesicles breathe in, whereas a strong green fluorescence is emitted when the vesicles breathe out (Figure 1 B). The jellyfish-like breathing and light-emitting behavior could be induced reversibly many times by the alternate addition of HCl and NaOH. Mechanistic investigations showed that this behavior originates from reversible conformation transformations of the DMA-Azo chromophores as induced by protonation or deprotonation.

We first attempted to synthesize the azobenzene-containing amphiphilic diblock copolymer by using brominated poly(ethylene glycol) (PEG-Br) as the macroinitiator to initiate the atom-transfer radical polymerization (ATRP) of readily obtained 4-(4-dimethylaminophenylazo)phenyl acrylate (monomer **1**; see Scheme S1 in the Supporting Information). Unfortunately, the block copolymer was not formed owing to the low polymerization activity and great steric bulk of monomer **1**. To overcome this problem, we introduced a long alkyl-chain spacer (hexyl) into monomer **1** and replaced the acrylate group with methacrylate. The resulting highly reactive monomer 6-(4-(4-dimethylamino phenylazo)-phenoxy)hexyl methacrylate (monomer **2**; see Schemes S2 and S3 and Figures S1–S3 in the Supporting Information) underwent ATRP with PEG-Br to give the well-defined target amphiphilic diblock copolymer PEG-*b*-PDMA-Azo ($M_n = 21.4$ kDa, $M_w/M_n = 1.07$; see Figures S4 and S5) with thermotropic liquid-crystalline behavior (see Figure S6). Furthermore, the diblock copolymers tend to self-assemble in water as a result of their amphiphilic nature. The critical aggregation concentration (CAC) was determined to be approximately 0.04 mg mL^{-1} (see Figure S7) by measurement of the UV absorbance at 317 nm of azobenzene chromophores in the diblock copolymers at increasing concentrations.

The morphology and size of the aggregates were investigated by atomic force microscopy (AFM), transmission electron microscopy (TEM), and dynamic laser scattering (DLS) measurements. The AFM image in Figure 2 A shows spherical particles with a diameter-to-height ratio larger than 20, which indicates a thin-layered and collapsed vesicle structure.[9] The presence of the concave feature (white arrows in the inset of Figure 2 A) in the deformed particles indicates a possible hollow structure of the spherical particles. Moreover, the contrast difference between the particle skin and the inner pool in the TEM image of the self-assembled structures shows that these particles are unilamellar vesicles (Figure 2 B).[10] The hollow structure of the vesicles was also

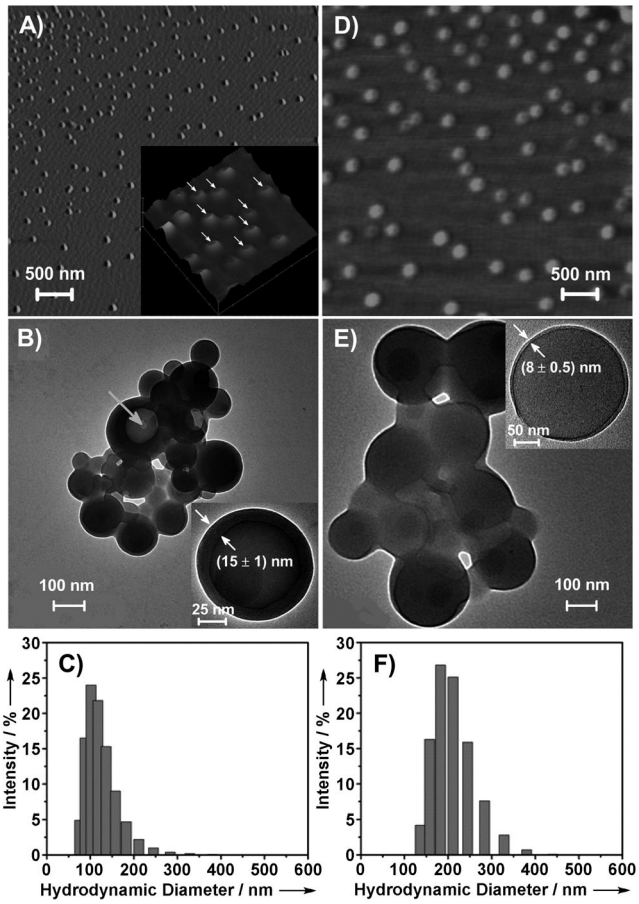

Figure 2. A) AFM image, B) TEM image, and C) DLS profile of the self-assembled vesicles in the initial state (pH 7). D) AFM image, E) TEM image, and F) DLS profile of the vesicles at pH 4. The inset in (A) is a 3D image of the vesicles deformed by the external force of the AFM tip. The insets in (B) and (E) are magnified images.

confirmed by the holes observed in the particles (gray arrow in Figure 2 B; see also Figure S8). The average size of the vesicles according to the AFM and TEM images is approximately 120 nm, which is consistent with the hydrodynamic diameter (D_h) of approximately 123 nm determined by DLS (Figure 2 C). The vesicles are flexible and can deform along the force direction under the pressure of the AFM tip even in a dried state (see Figure S9).[11] They are also very stable and can be kept in water without any change in either morphology or size for at least half a year. The vesicles are also stable in phosphate-buffered saline (PBS) buffer (pH 7.4) at 37 °C according to the real-time DLS analysis (see Figure S10).

The molecular packing structure in the vesicles was further studied by TEM and UV spectroscopy. The average vesicle-wall thickness, as determined from TEM images on the basis of 60 vesicles, is approximately 15 nm. The PDMA-Azo chain in PEG_{45}-*b*-P(DMA-Azo)$_{47}$ was calculated to be about 12 nm if it adopts a rigid conformation. Therefore, the diblock copolymers are thought to pack in an interdigitated arrangement to form a bilayer structure. As further evidence for this packing mode, the UV absorption spectrum of the vesicles in water showed a pronounced blue shift when compared to that obtained in the good solvent THF (Fig-

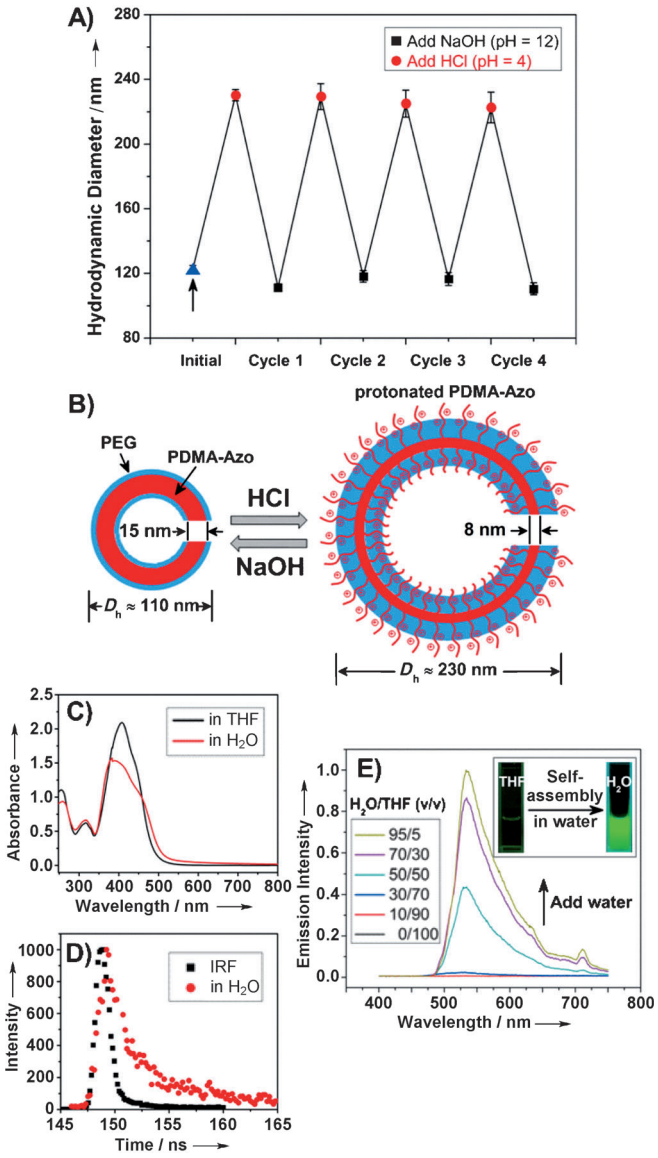

Figure 3. A) Reversible size change of the vesicles as detected by DLS upon the alternate addition of HCl (pH 4) and NaOH (pH 12). Black bars represent the mean values ($n=4$). B) Schematic representation of the pH-induced reversible "breathing" process of the vesicles upon the protonation and deprotonation of PDMA-Azo blocks in response to the pH value of the solution. C) UV/Vis spectra of PEG-*b*-PDMA-Azo in THF and in H_2O ($c=0.05$ mg mL^{-1}). D) Fluorescence decay curve of the vesicles in H_2O ($c=0.20$ mg mL^{-1}) with excitation at 380 nm and detection at 536 nm after proper deconvolution of the instrument response function (IRF). E) Steady-state fluorescence spectra of the copolymer in H_2O/THF mixed solvents with different volume ratios ($c=0.20$ mg mL^{-1}) at an excitation wavelength of 380 nm. The insets in (E) are fluorescent images of the solutions.

ure 3C). This result indicates that the π-conjugated DMA-Azo chromophores form an H-type aggregate in the vesicles.[12] However, since the UV absorption band is relatively broad and weak, the DMA-Azo groups in the vesicles may be arranged in a less optimal mode of H-aggregation. On the basis of these data, we propose the vesicular self-assembly mechanism shown in Figure 1C.

The groups of Eisenberg,[13] Lecommandoux,[14] Klok,[14] Armes,[15] and others[16] have done excellent work on the development of stimuli-responsive vesicle systems. The polymeric vesicles obtained in this study demonstrated a pH-induced "breathing" behavior. They are pH-responsive since the DMA-Azo groups have a pK_a value of about 3.6.[17] At an initial pH value of 7, the average vesicle diameter is about 120 nm, and the vesicle-wall thickness is about 15 nm. When the pH value decreased to 4, AFM showed a diameter of approximately 236 nm (Figure 2D), TEM a diameter of approximately 240 nm (Figure 2E), and DLS a diameter of approximately 233 nm (Figure 2F). Thus, a remarkable expansion of the vesicle volume by a factor of about 7 occurs. Meanwhile, the vesicle-wall thickness was halved to approximately 8 nm according to the TEM image (Figure 2E). Furthermore, when the pH value was increased to 12, the vesicles shrunk to nearly their initial size (ca. 112 nm), and their walls reverted back to their original thickness (ca. 15 nm). This vesicle expansion and shrinkage accompanied by the thinning and thickening of the vesicle walls is reversible for at least four cycles upon the alternate addition of HCl and NaOH to switch the pH value between 4 and 12 (Figure 3A). The vesicle morphology also remained constant during the four cycles according to the TEM (see Figure S11) and AFM images obtained afterwards (see Figure S13). Such highly reversible pH-induced swelling and shrinkage of the vesicles is reminiscent of the reported "breathing" behaviors of vesicle systems.[13b,16b] In this case, the acid-induced swelling can be regarded as a "breathing in" process, whereas the base-induced shrinkage is a "breathing out" process.

The "breathing" mechanism of the vesicles can be explained as shown in Figure 3B. The vesicles possess a compact or thick bilayer wall structure at pH 7 or 12. When the pH value is decreased to 4, the PDMA-Azo segments are protonated (about a 33% degree of protonation) and change from hydrophobic to hydrophilic. As a result, the interchain electrostatic repulsive force compels the vesicles to expand to some extent to lower their interaction free energy. This expansion corresponds to vesicle swelling, the so-called "breathing in" process. Meanwhile, spreading of the protonated PDMA-Azo chains from the vesicle core to the corona leads to thinning of the vesicle wall. However, the vesicles are not disassembled at pH 4 owing to the protonation degree of the PDMA-Azo chains of only 33%. Inversely, when the pH is increased to 12, a deprotonation process occurs. Subsequently, the electrostatic repulsive force in the vesicles decreases and finally disappears, and the PDMA-Azo chains spreading in the vesicle coronae become hydrophobic again and retract back into the vesicle cores. Thus, vesicle shrinkage and vesicle-wall thickening occur; the vesicles revert back to their original state in the "breathing out" process.

The so-prepared PEG-*b*-PDMA-Azo vesicles also demonstrate interesting fluorescence properties. PEG-*b*-PDMA-Azo is nonfluorescent in the good solvent THF owing to the non-emissive nature of azobenzene.[18] However, the fluorescence of the copolymer solution was enhanced remarkably by the addition of water (Figure 3E). The higher the water content, the stronger was the fluorescence intensity. The

fluorescence was enhanced by a factor of about 130 at a water volume ratio of 95%. The introduction of water leads to vesicular self-assembly, and the formation of vesicles triggers the enhanced fluorescence. Evidently, this diblock copolymer has the characteristics of aggregation-induced emission (AIE) systems.[19] In good agreement with the fluorescence analyses, the photographs in the inset of Figure 3E clearly show that the solution of the diblock copolymer in THF hardly exhibits any apparent fluorescence, whereas the aqueous vesicular solution presents strong green fluorescence. As further evidence, Figure 3D shows that the vesicles in water have a lifetime of about 2.2 ns. In contrast, owing to its extremely weak fluorescence, the fluorescence lifetime of PEG-*b*-PDMA-Azo in THF could not be determined.

The AIE effect of the obtained vesicles can be explained in terms of three factors. First, the intramolecular vibrational or torsional motions of DMA-Azo groups are greatly restricted after vesicle formation as a result of the stacking of hydrophobic PDMA-Azo chains, which is beneficial to the enhancement of fluorescence efficiency.[19] Second, the *trans*-to-*cis* photoisomerization is hindered to some extent in the vesicles, since the azobenzene chromophores are confined in the bilayer membranes. Thus, the efficiency of nonradiative relaxation is greatly reduced, which leads to the strong fluorescence.[20] Third, as mentioned above, although the DMA-Azo chromophores form H-aggregates in the vesicles, they are relatively disordered or twisted, which is also beneficial to the enhancement of fluorescence.

Next, we explored the fluorescence properties of the vesicles in response to the pH-induced "breathing" process. In the UV spectrum of the vesicular solution (Figure 4A), an enhanced absorption band around 560 nm (black arrow) was observed at pH 4, and this band disappeared again as the pH value was adjusted to 12. Accordingly, the solution also changed from brown at pH 4 to orange at pH 12 (inset in Figure 4A). The orange solution exhibited strong green fluorescence, whereas only very weak fluorescence signals were observed from the brown solution (Figure 4B). Figure 4C shows the corresponding fluorescence spectra for these processes. Upon the addition of HCl, the fluorescence intensity of the vesicular solution gradually decreased; it was lower than the initial value by a factor of approximately 4.5 at pH 4. The fluorescence intensity then increased gradually as the pH value was increased to 12 by the addition of NaOH. The fluorescence quenching and recovery of the vesicular solution can be recycled reversibly many times by the alternate addition of HCl and NaOH (Figure 4D). Thus, the vesicle system exhibits a pH-induced fluorescence quenching and recovery in response to the "breathing" process. The fluorescence is turned off when the vesicle breathes in at pH 4, and the fluorescence is turned on when the vesicle breathes out at pH 12. This behavior is very similar to the breathing process of a jellyfish (Figure 1A).

The central question is the mechanism for the pH-dependent fluorescence properties. First, we believe the fluorescence quenching under acidic conditions is related to protonation and the formation of charge-transfer complexes between the vesicle-forming polymers. The protonated DMA-Azo chromophores ($DMAH^+$-Azo) have a high elec-

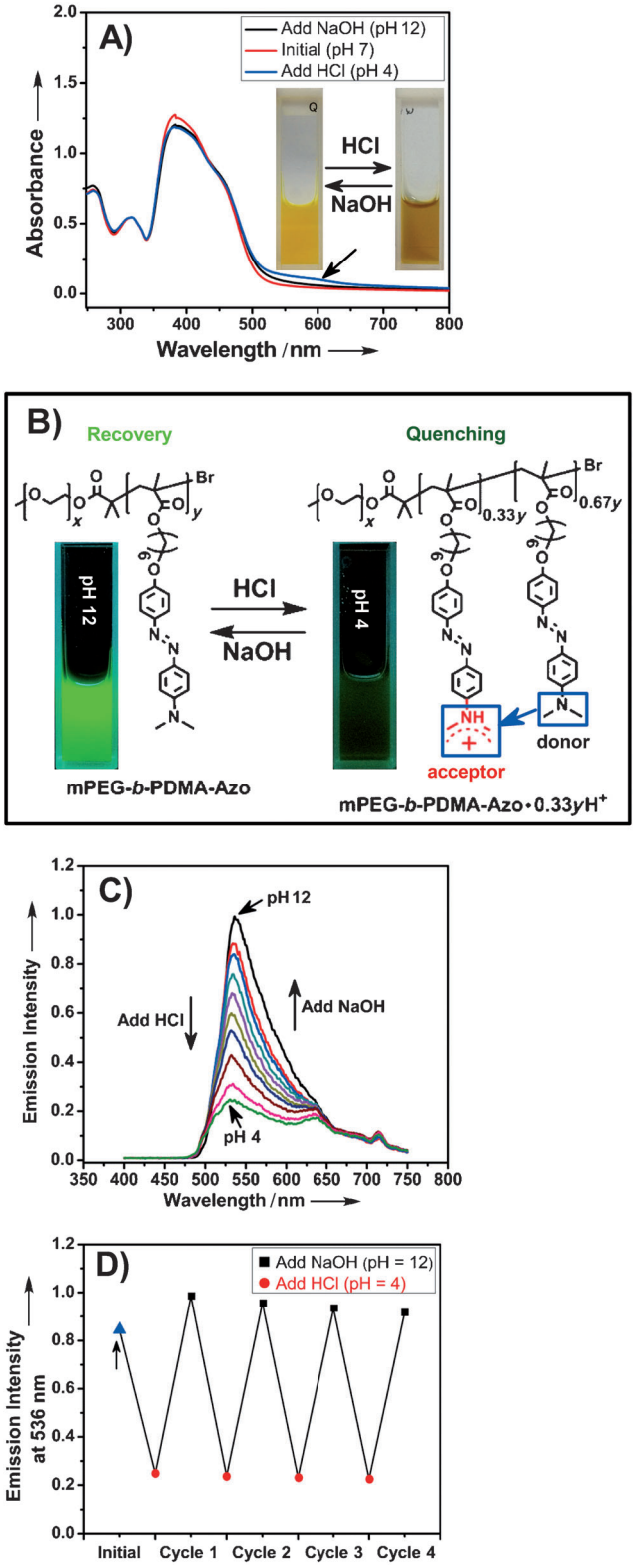

Figure 4. A) UV/Vis spectra of the vesicles at pH 4, 7, and 12 ($c = 0.05$ mg mL^{-1}). The inset shows photographs of the vesicular solution at pH 4 (right) and 12 (left). B) Schematic representation of the protonation and deprotonation of PEG-*b*-PDMA-Azo in the vesicles by the alternate addition of HCl and NaOH. Fluorescent images of the vesicular solution at pH 4 (right) and 12 (left) are also shown. C) Steady-state fluorescence spectra of the vesicular solution at pH values between 4 and 12 at an excitation wavelength of 380 nm. D) Reversible pH-induced change in the fluorescence of the polymer at 536 nm upon the alternate addition of HCl (pH 4) and NaOH (pH 12; $c = 0.20$ mg mL^{-1}).

tron-accepting ability, whereas the unprotonated DMA-Azo chromophores have an electron-donating ability. Therefore, the polymers tend to form charge-transfer complexes based on donor–acceptor interactions (Figure 4B); the formation of these complexes leads to fluorescence quenching.[21] The occurrence of such a mechanism involving complex formation was proved by UV/Vis and steady-state-fluorescence measurements of a solution of the PEG-*b*-PDMA-Azo monomer in THF upon the addition of TFA (see Figure S16). The fluorescence quenching of the vesicles under acidic conditions might also be related to the increase in conformational freedom of the polymers in the vesicles as a result of protonation. Both vesicle swelling and vesicle-wall thinning occur under acidic conditions, and thus the restriction of azobenzene chromophores in the vesicle core decreases. As is characteristic of AIE system, such a change in the molecular packing of the vesicles will result in fluorescence quenching.

In summary, we have prepared a novel class of polymeric vesicles that exhibit a pH-induced "breathing" behavior accompanied by jellyfish-like on–off switchable fluorescence through the aqueous self-assembly of a diblock copolymer of PEG-*b*-PDMA-Azo. During the "breathing in" process under acidic conditions, swelling of the vesicles and thinning of the vesicle walls are accompanied by fluorescence quenching as a result of the protonation process. Inversely, during the "breathing out" process under alkaline conditions, shrinking of the vesicles and thickening of the vesicle walls are accompanied by recovery of the strong green fluorescence as a result of the deprotonation process. The occurrence of changes in the light-emitting properties of the polymer upon membrane deformation during the breathing process is reminiscent of the breathing behavior of jellyfish. Therefore, we believe we have developed a smart vesicle system with controllable and dynamic functions. More importantly, this study may extend cytomimetic chemistry from the mere morphological transformation of membranes to a combination of cytomimetic morphology with the concomitant expression of a function.

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