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Supramolecular self-assembly of giant polymer vesicles with controlled size

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1. Experimental Section

Synthesis of HBPO-star-PEO. The hyperbranched multi-arm copolymer, HBPO-star-PEO, was synthesized by two-step cationic ring-opening polymerization. At the first step, 3-ethyl-3-oxetanemethanol was quickly added into the solution of $BF_3{\cdot}OEt_2$ in dried CH_2Cl_2 under argon atmosphere (the molar ratio of 3-ethyl-3-oxetanemethanol to $BF_3{\cdot}OEt_2$ is 2:1). The reaction mixture was stirred vigorously for 24h at 10 ^oC to form HBPO precursor. An aliquot of the precursor was withdrawn for characterization. At the second step, the residual HBPO precursor was carefully divided into three parts (the active species was still living in each part), and different amounts of ethylene oxide (EO) were dropwise added into each part of the precursor at a rate of 0.05 ml/min (kept the temperature of the reaction mixture around -20 ^oC). After the addition of EO, the reaction was kept for another 24h under stirring condition around -20 ^oC. Pure water was then added into the reaction system to quench the cationic copolymerization. The products denoted by HBPO-star-PEO were purified using column chromatography with silica gel, and chloroform was used as the elution solvent. The yield of the HBPO-star-PEO was 90%. In this way, a series of hyperbranched multi-arm copolymers of HBPO-star-PEO with the same HBPO core and different PEO arms were obtained. HBPO-star-PEO is a sort of amphiphilic macromolecules with a hydrophobic hyperbranched poly(3-ethyl-3-oxetanemethanol) core (HBPO) and a large amount of hydrophilic poly(ethylene glycol) arms (PEO). The diagrammatic sketch of the HBPO-star-PEO molecule was illustrated in main text (Figure1).

Characterization of HBPO precursor and HBPO-star-PEO. The molecular weight and the polydispersity of the HBPO precursor and the resulting HBPO-star-PEO macromolecules were determined by the size exclusive chromatography (SEC) in *N,N*-dimethylformamide (DMF) relative to polystyrene standards. The degree of branching (DB) of the HBPO precursor was determined by ^{13}C NMR in DMSO-*d6*. The results show that the DB, the number-average molecular weight ($\bar{M}_n$) and the polydispersity index of the HBPO precursor are 0.4, 6,400 and 1.5, respectively. The

molar ratio of EO units in PEO arms to 3-ethyl-3-oxetanemethanol units in HBPO cores was determined by ^{1}H NMR in chloroform-*d1*. The detailed characters of HBPO-star-PEO molecules (HB1-3) are shown in the main text (Table 1). In addition, only a little of the residual hydroxyl groups in HBPO cores of HB1-3 were detected with MAS-solid state ^{13}C NMR (not shown). However, in the previous work of macroscopic molecular self-assembly, the HBPO cores of the HBPO-star-PEO molecules included ~28% residual hydroxyl groups. [1]

Self-assembly procedure of the HBPO-star-PEO. The synthesized HB1-3 samples of 0.1 g were respectively added to 10 ml deionized water under stirring with a magnetic bar in room temperature. Water is the good solvent for PEO arms and the non-solvent for HBPO cores. The appearance of turbidity in the solution indicated the formation of the aggregations, and the solution turbidity increased from HB3, HB2 to HB1.

The observation of the self-assembly morphology. The aggregation morphologies of HB1-3 were directly observed at 20 ^{o}C by the optical microscopy (Leica Dmlp, TMS94) equipped with a Sony digital cameral. If the polymer concentration were less than 100 mg/ml, the solution of HB2 and HB3 would keep the vesicle morphology and size for at least two months around 20 ^{o}C. However, the HB1 vesicles (10 mg/ml) settled from the solution after several hours without stirring. The aggregation morphology was also observed by TEM. For TEM measurement, a drop of the polymer solution was placed onto 400 mesh copper grids coated with parlodion film stabilized with vacuum evaporated carbon. The excess fluid was drained off with filter paper after five minutes. Then a drop of negative stain of 2% aqueous uranyl acetate solution was added to stain the samples for four minutes. The excess stain was removed with filter paper. The samples were examined in an electron microscope (JEOL JEM-100CX II), operating at 100 kv.

2. Self-assembly Mechanism Section

Self-assembly interactions. The HBPO-star-PEO molecule has a hyperbranched hydrophobic core and large population of hydrophilic PEO arms, so the aggregation of HBPO-star-PEO molecules driven by the hydrophobic interaction in water is well expected. On the other hand, there are a lot of hydroxyl groups in HBPO-star-PEO molecules, therefore the formation of hydrogen bonds is another potential interaction for the self-assembly. Variable temperature FTIR was used to detect the hydrogen bonds in the resulting dried vesicles. Variable temperature FTIR was conducted on Bruker Equinox 55 under N_2 from 25 ^{o}C to 175 ^{o}C, and the vesicle solution was coated on the KBr crystal wafer for the measurement. The samples were carefully dried before the measurement. The results are provided in figure S1. The peaks of methylene or methyl groups (around 2900 cm^{-1}) of HBPO cores were kept in the same intensity and used as inner standards, and the intensity of hydroxyl groups (in the range of 3000-3750 cm^{-1}) were referenced to the signal of methylene or methyl

groups. With increasing temperature from 25 °C to 175 °C, the peak of the hydroxyl groups gradually becomes narrower and shifts to the high wavelength side, which clearly indicates that a certain amount of hydroxyl groups have formed hydrogen bonds in the dried vesicles originated from the self-assembly of HBPO-star-PEO molecules. The peak of hydroxyl groups in the vesicles at 25 °C is not very wide in the FTIR spectrum compared with that of the macroscopic self-assembly tubes, which indicates that the hydrogen bonds in the vesicles are not so strong as those in macroscopic tubes.[1]

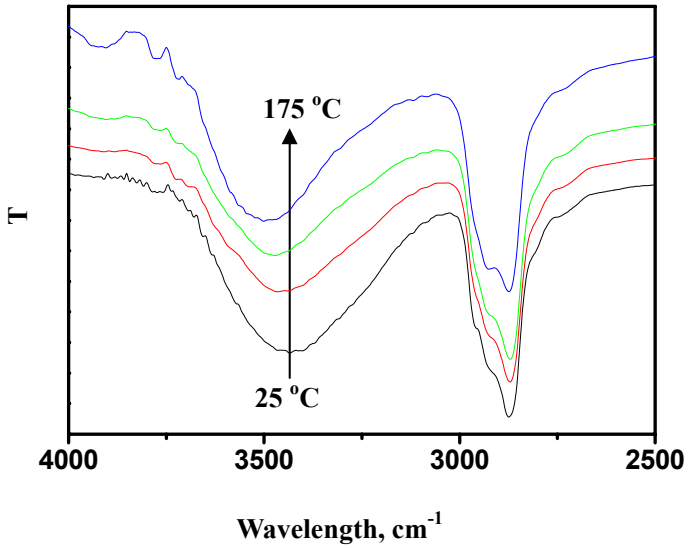

Figure S1. Variable temperature FTIR spectra of dried giant vesicles from HBPO-star-PEO molecules. The temperature of the related curve from the bottom to the top is 25 °C, 65 °C, 105 °C and175 °C respectively.

NMR spectroscopy is one of the most often employed methods to investigate hydrogen bonds in solution. [2-4] Variable-temperature ^{1}H NMR measurements conducted at 25 °C, 50 °C and 70 °C were carried out to detect the hydrogen bonds in the in the solutions of HB1-3 vesicles.. Figure S2 demonstrates a typical temperature dependence ^{1}H NMR spectrum of HB3 vesicles. The assignment of ^{1}H NMR data is as following: proton signals assigned to HBPO cores (peaks in the range of 0.7 to 1.5 ppm assign to C*H*$_3$C*H*$_2$- group in HBPO core, peaks in the range of 3.1 to 3.4 ppm assign to -C*H*$_2$-O- groups in HBPO core); the proton signal assigned to PEO arms (peaks in the range from 3.4 to 3.8 ppm, -C*H*$_2$-O- groups). All chemical shifts are referenced to the signal for C*H*$_3$ groups. The increase of the solution temperature leads to upfield shifts of the peaks of -C*H*$_2$-O- groups both in HBPO cores and PEO arms (Fig. S2b, S2c and S2d). We consider that the destruction of the hydrogen bonds leads to the consistent upfield shift of the -C*H*$_2$-O- proton with the increase of temperature.

Concentration dependence ^{1}H NMR experiments were also conducted to detect the hydrogen bonds in HB1-3 vesicle solutions. The samples used in the experiment were

further purified by precipitation in normal hexane. In general, H-bonded groups display the concentration-dependent chemical shifts and line widths that reflect the intermolecular H bonding. Figure S3 demonstrates a concentration dependence ^{1}H NMR spectrum of HB3 vesicles. All chemical shifts are referenced to the signal for CH_3 groups. As the concentration of HB3 in aqueous solution increased from 5mg/ml to 50 mg/ml, a downfield chemical shift and narrowing of the line width of the proton signal of -CH_2-O- groups in both HBPO cores and PEO arms were observed. However, the proton signals of CH_3-CH_2- group retaining its chemical shift and line width at all concentrations were independent of the solute concentration. The magnetic environments of the -CH_2-O- groups are deshielded as a result of H-bonding associations, leading to downfield chemical shifts. The sharpening of the line width also results from the H-bonding association due to slower exchange and/or relaxation on the proton NMR timescale. The protons of CH_3-CH_2- group do not form hydrogen bonds, therefore no dependence of the related proton signals on the concentration can be observed.

In summary, the temperature dependence ^{1}H NMR experiment shows an upfield chemical shift of the -CH_2-O- proton with increasing of temperature. The concentration dependence ^{1}H NMR experiment shows a downfield chemical shift and narrowing of the line width of the proton signal of -CH_2-O- groups with the increase of solute concentration. These results suggest that -CH_2-O- groups in both HBPO cores and PEO arms form hydrogen bonds in the self-assembled HB1-3 vesicle solution.

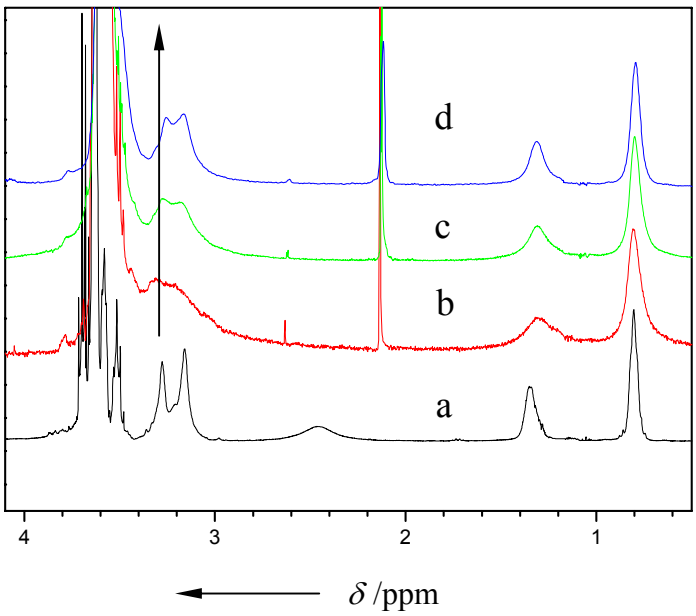

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Figure S2. Variable temperature ^{1}H NMR spectrum of HBPO-star-PEO molecules assembled in D_2O. (a) HBPO-star-PEO dissolved in $CDCl_3$; (b), (c), (d) HBPO-star-PEO in D_2O, the temperature in (b), (c) and (d) is 25^{o}C, 50 ^{o}C and 70 ^{o}C respectively.

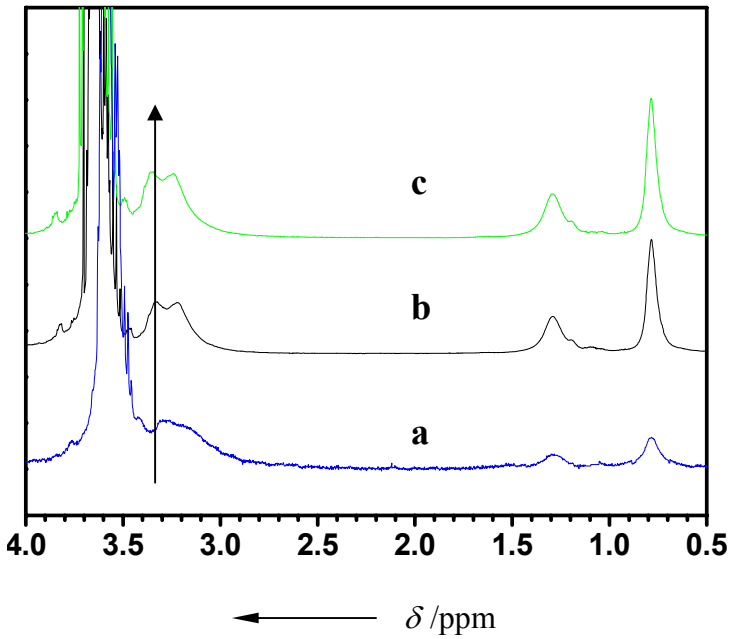

Figure S3. Concentration dependence ^{1}H NMR spectrum of HBPO-star-PEO molecules assembled in D_2O. The polymer concentration of (a), (b) and (c) is 5, 25 and 50 mg/ml respectively.

The molecular packing model for the vesicle. It is difficult to find out the packing model of HBPO-star-PEO molecules in the resulting vesicle wall. We used ^{1}H NMR to monitor the self-assembly process in order to attain the information of molecular packing. HB1-3 samples were first dissolved in the good solvent of acetone-*d6*, and then D_2O as the selective solvent was continually added into the solution to induce the self-assembly. The control experiment proved that HB1-3 also aggregated into giant vesicles by adding water into the polymer acetone solution (keep the volume ratio of water to acetone identical). The solution-state ^{1}H NMR data were recorded continually when various amount of D_2O was added into the HB1-3 acetone-*d6* solutions. Figure S4 displays the typical ^{1}H NMR data of HB3 molecules self-assembling in the water and acetone mixture. The ^{1}H NMR data illustrated in figure S4 shows that the proton signals assigned to HBPO cores (peaks in the range of 0.7 to 1.5 ppm assign to CH_3CH_2- group in HBPO core, peaks in the range of 3.1 to 3.4 ppm assign to -CH_2-O- groups in HBPO core) trail off with the addition of D_2O, while the proton signals assigned to PEO arms (peaks in the range from 3.4 to 3.8 ppm) keep strong all the time. The result clearly demonstrates that HBPO cores are shielded by PEO arms in the self-assembled vesicle walls. On the other word, HB1-3 vesicle wall has a sandwich-like structure consisting of two outer hydrophilic PEO lamellae and an inner hydrophobic HBPO lamella. It is necessary to point out that the HBPO proton signals become much weakened instead of disappearing when

HBPO-star-PEO molecules self-assemble into vesicles in water. We attribute the residual HBPO proton signals to the existence of HBPO-star-PEO molecules with longer PEO arms in the self-assembly system, and these HBPO-star-PEO molecules dissolve in the water and do not take part in the self-assembly process.

The wall thickness of the vesicle is another important parameter to deduce the molecular packing mechanism. Table 1 in main text shows the wall thicknesses for HB1-3 vesicles measured by TEM (negative staining). HB2 and HB3 vesicles have the wall thickness of 5±2 nm similar to that of lipid vesicles (liposomes) and much narrower than that of the reported crew-cut micelles from conventional block copolymers (about 25nm), however the molecular weights of HB2 and HB3 are much greater than those of lipids, so the molecular volumes of HB2 and HB3 would be tightly compressed and packed if the vesicle wall adopted a bilayer structure as liposome. It is well known that the condensed structure needs much more energy, so monolayer structure is more favorable than a bilayer structure for HB2 and HB3 vesicles. In fact, a similar vesicle wall thickness with a supposed monolayer structure had been found in the aggregations of a commercial triblock copolymer of PEO_5-PPO_{68}-PEO_5. [5] In addition, we [1] have reported the inhomogenous lamella in the macroscopic molecular self-assembly of HBPO-star-PEO with a bigger HBPO precursor (the molecular weight of HBPO precursor is 8,560), and the HBPO lamella has a thickness of 10±2 nm. The lamella thickness of macroscopic tubes strongly supports that HB2 and HB3 vesicles adopt a monolayer structure in the vesicle walls because the wall thickness of HB2 and HB3 vesicles is of 5±2 nm and the molecular weight of HBPO precursor is 6,400. The wall thickness of HB1 vesicles is about twice as wide as that of HB2 and HB3 vesicles, and HB1-3 samples have same HBPO cores, so a bilayer structure is reasonable for HB1 vesicles on the premise that the HB2 and HB3 vesicles should have a monolayer structure.

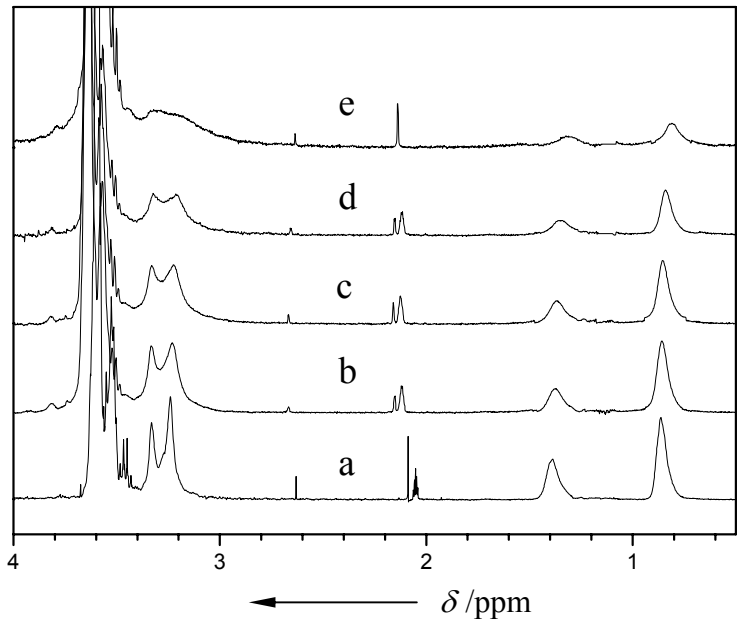

Figure S4. ^{1}H NMR spectrum of HBPO-star-PEO molecules assembled in acetone-*d6* and D_2O mixture. D_2O content (vol%): (a) 0%; (b) 44%; (c) 54%; (d) 64%; (e) 100%.

Small-Angle X-ray Scattering (SAXS) is designed to further prove the lamellar structure in the HB1-3 vesicle wall. SAXS intensities for the samples were obtained on a Philips PW 1700 X-ray diffractometer equipped with a Kratky small-angle X-ray camera. The measurements were performed with a Cu Kα radiation source operated at 60 kV and 30 mA. The scattering intensities were corrected for absorption, background scattering, and incident X-ray fluctuations of the samples. A desmearing procedure was introduced to correct the scattering intensities. At first, dilute HB1-3 vesicle solutions (5mg/ml) were used as samples. However, we could not obtain the lamellar long period because of the weak code of the samples in the SAXS pattern. The lower power of the instrument and the disturbance of the water in the samples lead to the failure of the SAXS measurement. Subsequently, more concentrated vesicle solution (100 mg/ml) was used as the samples. Fortunately, we obtained the scattering peak in the SAXS measurements (Figure S5), although the peaks were relatively weak and imperfect. Figure S5 strongly supports that the HB1-3 vesicles possess the lamellar-type arrangement in the external vesicle walls. The lamellar thickness of the samples measured by SAXS is 5.6 nm for HB3 vesicles, 5.3 nm for HB2 vesicles and 11.1 nm for HB1 vesicles, which fits well with the wall thickness of HB1-3 vesicles determined by TEM (Table 1 in main text). However, we have not obtained the discriminable scattering peaks corresponding to HBPO and PEO lamella, which probably ascribes to the similar electron density between the HBPO and PEO lamella.[1] We wish to carry out the measurement of synchrotron SAXS to get a better SAXS pattern with high resolution whenever it is available.

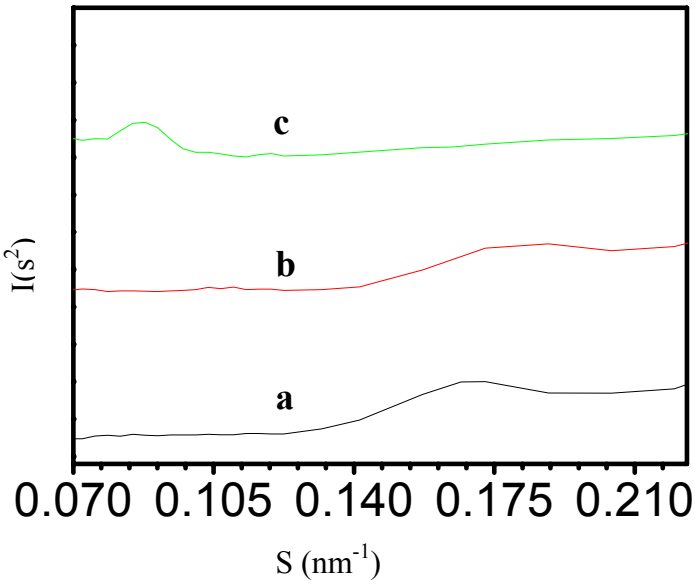

Figure S5. Desmeared and Lorentz corrected SAXS intensities for HB1-3 vesicle samples. (a) HB3 vesicles. (b) HB2 vesicles. (c) HB1 vesicles. The polymer concentration is 100 mg/ml.

3. The aggregation morphology

Figure S6 displays two additional giant vesicles through the self-assembly of HB1 molecules in water.

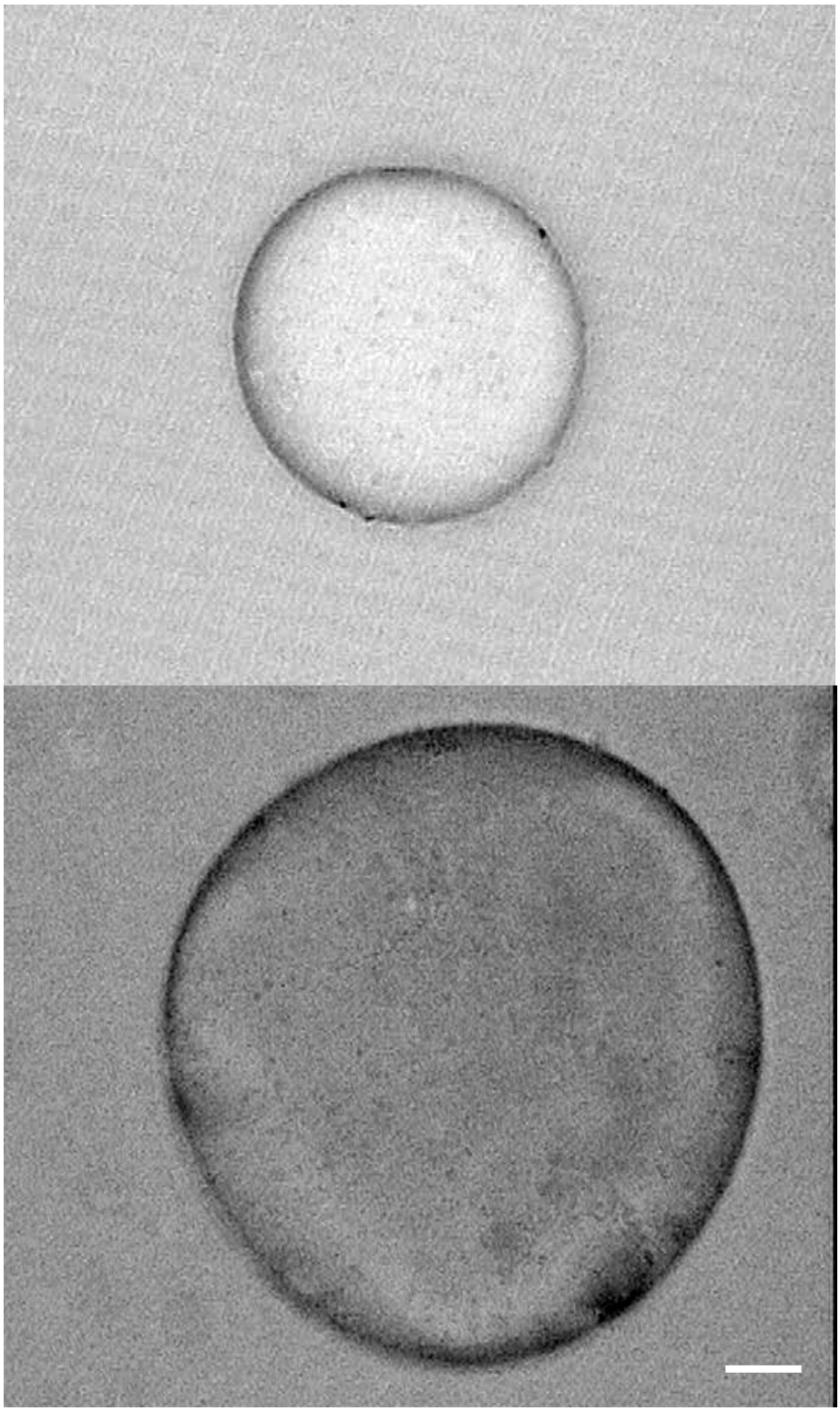

Figure S6. The giant HB1 vesicles. The scale bar represents 25 μm

4. Reference

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