

Supporting Information

Multifusion-Induced Wall-Superthick Giant Multilamellar Vesicles

Wei Yao, Hanqing Qian, Jialiang Zhang, Wei Wu, Xiqun Jiang*

Experimental section

Materials:

The sodium CS ($M_w = 30000$ g/mol) was purchased from TianPu medicine (China). CS was obtained by dissolved sodium CS in distilled water and acidified with 4 M HCl solution, followed by precipitating with ethanol. The precipitate was then dried in vacuum at 50 °C for 24 h and stored at desiccator. DEA (Adrich) was used as received. Chitosan ($M_w = 5000$) was purchased from Nantong Shuanglin Biological Product Inc. Potassium peroxydisulfate ($K_2S_2O_8$) was recrystallized from deionized water before used. Rhodamine B, Rhodamine 123, Fluorescein isothiocyanate (FITC) and casein labeled with FITC were purchased from Sigma-Adrich and were used as received.

Preparation of CS-PDEA Vesicles

100 mg of CS and 100 μ L DEA was dissolved in 10 mL distilled water in a three-neck flask under magnetic stirring. When the solution became homogeneous and clear, the temperature was raised to 75 °C in argon atmosphere, and 10 mg $K_2S_2O_8$ in 1 mL distiller water was added to the system to initiate the polymerization of DEA monomers. 2 hours later, the reaction system became opaque. The opaque solution was centrifuged at 5000 rpm for 3 to 5 min, a film of electrostatic CS-PDEA complexes was formed under centrifuge tube. Then, the supernate was removed and fresh distilled water was added into the tube. When the solution was vibrated gently, the CS-PDEA vesicles were formed spontaneously.

Fluorescence Labeling of CS-PDEA Vesicles

2 g of sodium CS was dissolved in 15 mL distilled water in a flask under magnetic stirring and pH of the solution was adjusted to 8.0 with 0.4 M NaOH solution. 4 mg Rhodamine B in 100 μ L DMSO was then added into the solution and the mixture was stirred at room temperature for 12 h in the dark. Next, the solution was acidified with 4.7 mL HCl solution (4 M) and the labeled CS was precipitated by adding ethanol into the solution and washed with ethanol to remove the free Rhodamin B. On the other hand, 15 μ L 2-aminoethyl methacrylate

and 1 mg Fluorescamine were dissolved in 0.5 mL acetone and the mixture was stirred for 12 h. Then the acetone was evaporated and the 2-aminoethyl methacrylate labeled with Fluorescamine was collected. Finally, doubly labeled CS-PDEA vesicles were prepared, with 100 mg of Rhodamine B labeled CS, 100 μ l DEA, 10 mg fluorescamin-labeled 2-aminoethyl methacrylate and 10 mL distilled water, by the procedure described above.

Physiochemical Characterizations

The pH measurements were performed with a Delta 320 pH-meter (Merrler Toledo Instruments Co., Ltd., Switzerland). The sizes of the giant vesicles were measured by Malvern (Mastersizer 2000 particles size analyzer). The zeta potential of the nanoparticles was obtained with a Zetaplus (Brookhaven Instruments Corporation). The results were the average of three runs. The molecular weights of CS and PDEA were measured by a gel permeation chromatography system (GPC) equipped with a static light scattering detector (DAWN HELEOS, Wyatt Technology Corporation). Sulfur content of CS and CS-PDEA vesicles were measured by elemental analyser (Elementar German, vario EL II)

Morphological Studies

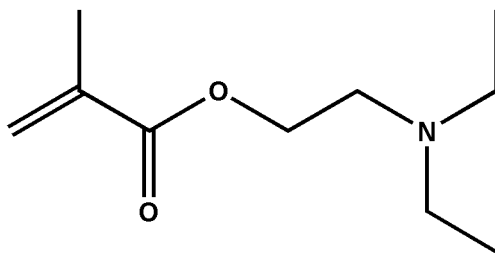
Vesicles were observed by confocal laser scanning microscope (Zeiss German, LSM 710) and fluorescent microscope (OLYMPUS). The membranes of the vesicles were investigated by TEM (JEM-100S, JEOL, Japan). The operating voltage was set at 100 kV. The morphology of giant vesicles was observed by scanning electron microscopy (SEM) (S-4800 Hitachi, Japan) with operating voltage of 3 kV. For TEM observations, giant CS-PDEA vesicles were dealt with vacuum freeze drying and then embedded in epoxy resin and sections with about 70 nm thick were obtained by microtoming. For SEM observation, the CS-PDEA vesicles were freeze-dried. In addition, process of freeze drying including an important procedure, the CS-PDEA vesicles solution was immersed in liquid-nitrogen, it took several seconds for the sample change from liquid to solid.

Yeast Culture and Stain

Pichia cells (X33), a kind of yeast cells, were cultured in YPD solid medium (1% yeast extract, 2% peptone, 2% glucose, 2% agar) at 28 °C. The cells were collected and washed with 0.12 M NaCl solution, and then the cells were dispersed in the 0.12 M NaCl solution. The cell concentration of 10^7 cells per mL was determined by using a cell counting chamber. For staining *Pichia* cells, 1 mg Rhodamine 123 was dissolved in 1 mL DMSO firstly, and then 5 μ L DMSO containing Rhodamine 123 was added in the cell solution. The cells were stained at 30 °C with shaking at 200 rpm.

Cell Encapsulation

After the cells were collected and washed with 0.12 M NaCl solution. 1 mg chitosan ($M_w = 5000$) was added into 1 mL cell solution (10^7 cells per mL) to pre-coat *Pichia* cells and the solution was stirred for 10 minutes. In order to get rid of the residual chitosan, the cell solution was centrifuged (5000 rpm, 3 min) and washed with 0.12 M NaCl solution. After that, 200 μ L solution of *Pichia* cells which were pre-coated by chitosan was added into 1 mL CS-PDEA opaque solution (see preparation of CS-PDEA vesicles). Next, the solution was centrifuged at 5000 rpm for 3 min and the supernate was removed. The film containing stained *Pichia* cells under centrifuge tube was immersed in 1 mL distilled water. When the solution was vibrated gently and CS-PEDA vesicles were formed, *Pichia* cells were encapsulated in the vesicles spontaneously.



Scheme S1 Chemical structure of 2-(diethylamino) ethyl methacrylate (DEA)

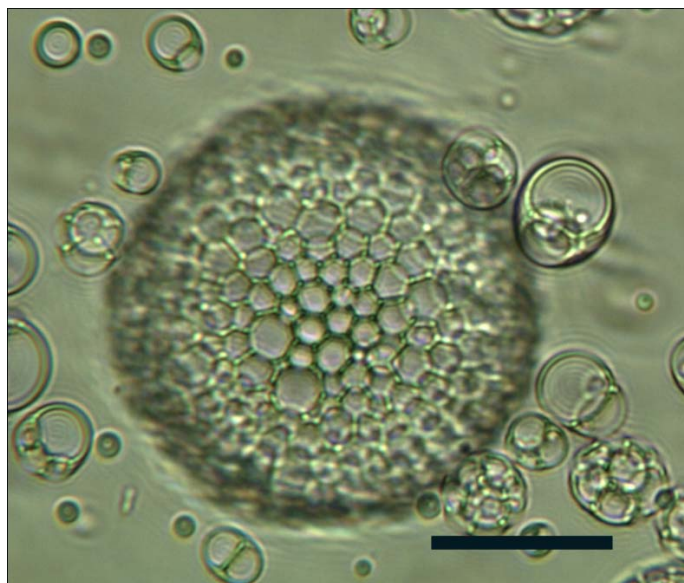


Figure S1 Optical microscopic image of CS-PDEA compound vesicles, the scale bar is 40 μm .

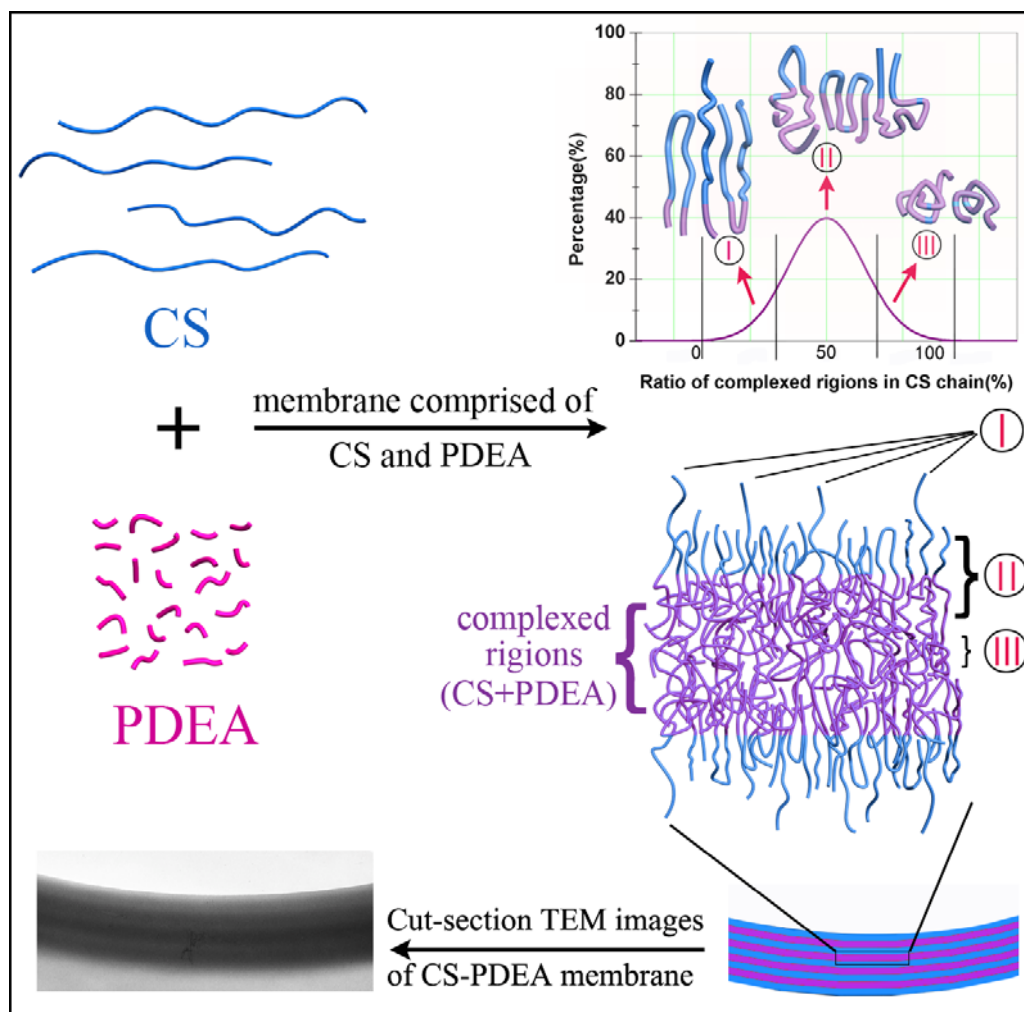


Figure S2 Schematically showing the electrostatic complexation between CS and PDEA, and possible membrane structure of CS-PDEA vesicles. Based on element analysis, we obtained that the mole ratio of monosaccharide of CS to DEA of PDEA in the vesicles is about 2. Considering the random complexation between CS ($M_w = 30$ kDa) and PDEA ($M_n = 2$ kDa), the number of complexed PDEA chains in individual CS chain is hypothesized to be a normal distribution, as shown in the top of Figure, that is, less monosaccharides in CS molecule are complexed by DEA (part I), near half of monosaccharides in CS molecule are complexed by DEA (part II) and almost completely complexed molecules (part III). Combined with the thickness of hydrophilic and hydrophobic layers measured by TEM, the hydrophilic segments of amphiphilic molecules (part I, II) constitute the hydrophilic regions in the vesicle wall. It should be noted that the thickness (44 nm) of hydrophilic region in multilamellar vesicles measured by TEM corresponds to the total thickness of two hydrophilic surfaces in two leaflets. For hydrophobic layer, together with the almost completely hydrophobic molecules (part III) which exist between two leaflets, the hydrophobic segments of amphiphilic molecules (part I, II) constitute the hydrophobic layer. This is consistent with the TEM result that hydrophobic thickness (68 nm) is thicker than hydrophilic layer (44 nm).

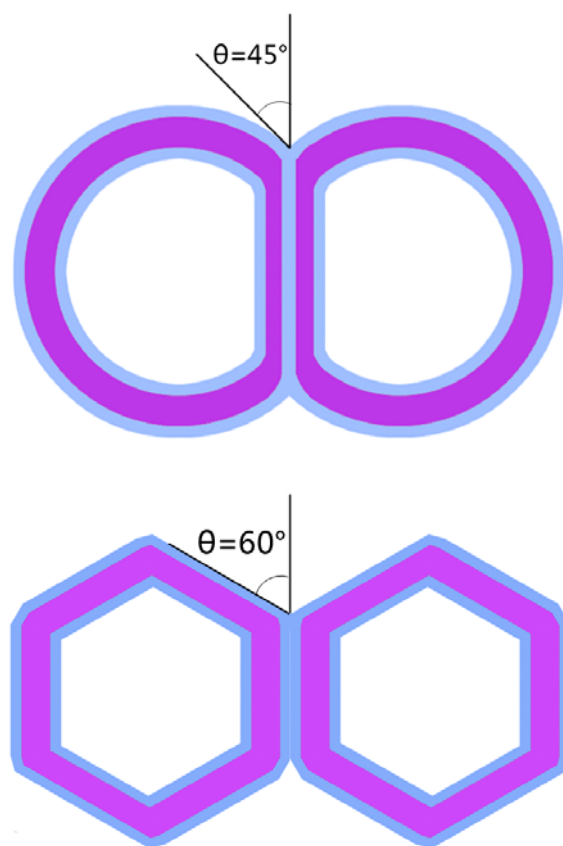


Figure S3 Schematic geometry of adhering vesicles. θ , angle of contact.

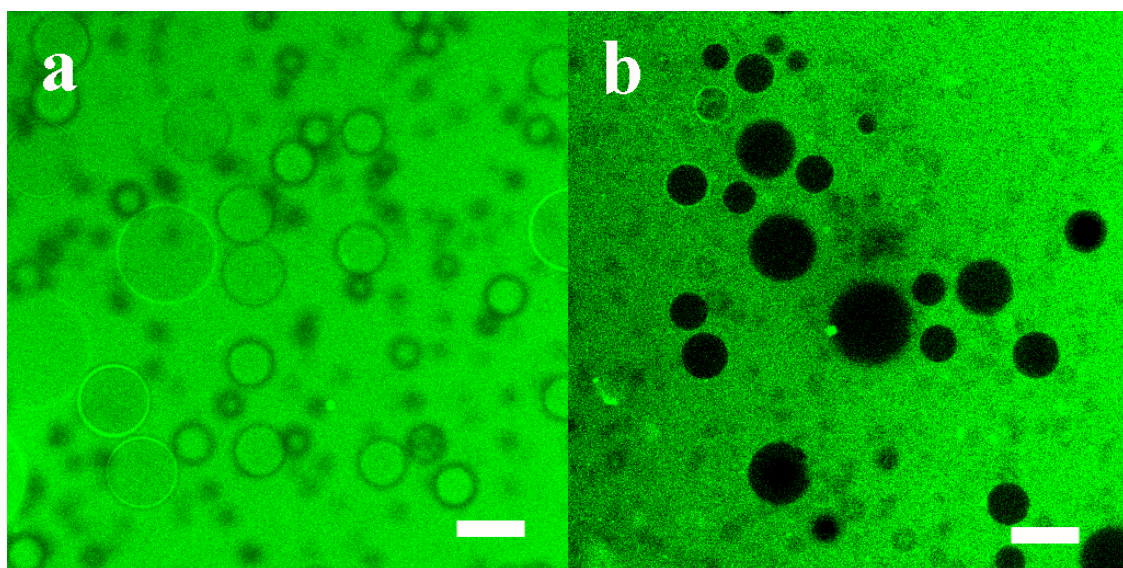


Figure S4. CLSM images of CS-PDEA vesicles. a) Addition of free FITC; b) Addition of FITC-labeled casein. Scale bars represent 50 μm .

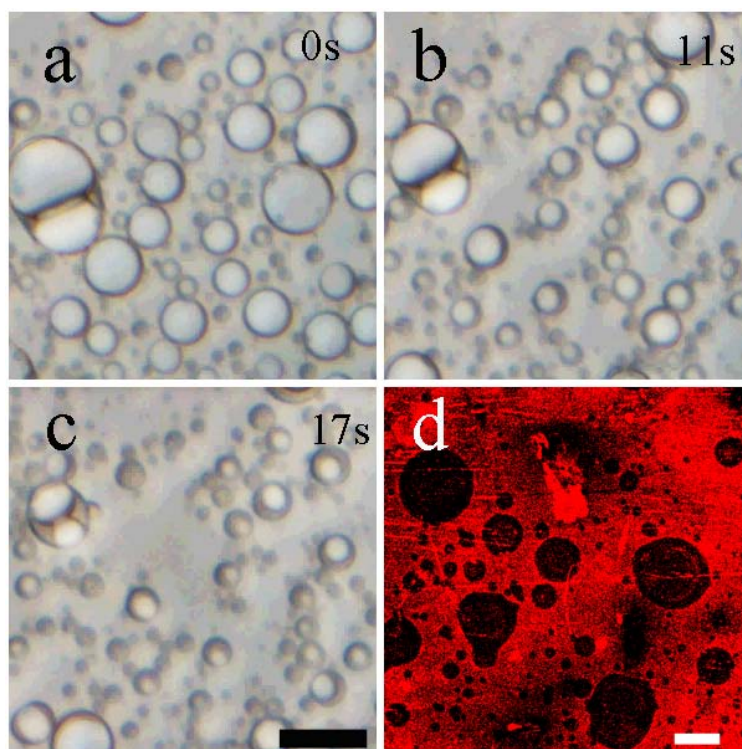


Figure S5. (a)-(c) Optical microscope images of CS-PDEA vesicles in response to casein ($M_w = 20000$). Scale bar represents $100 \mu\text{m}$, the number in each image denotes the elapsed time; (d) CLSM image of CS-PDEA vesicles after Dextran ($M_w = 3000$) which was labeled with Rhodamine B was added. Scale bar represents $50 \mu\text{m}$.

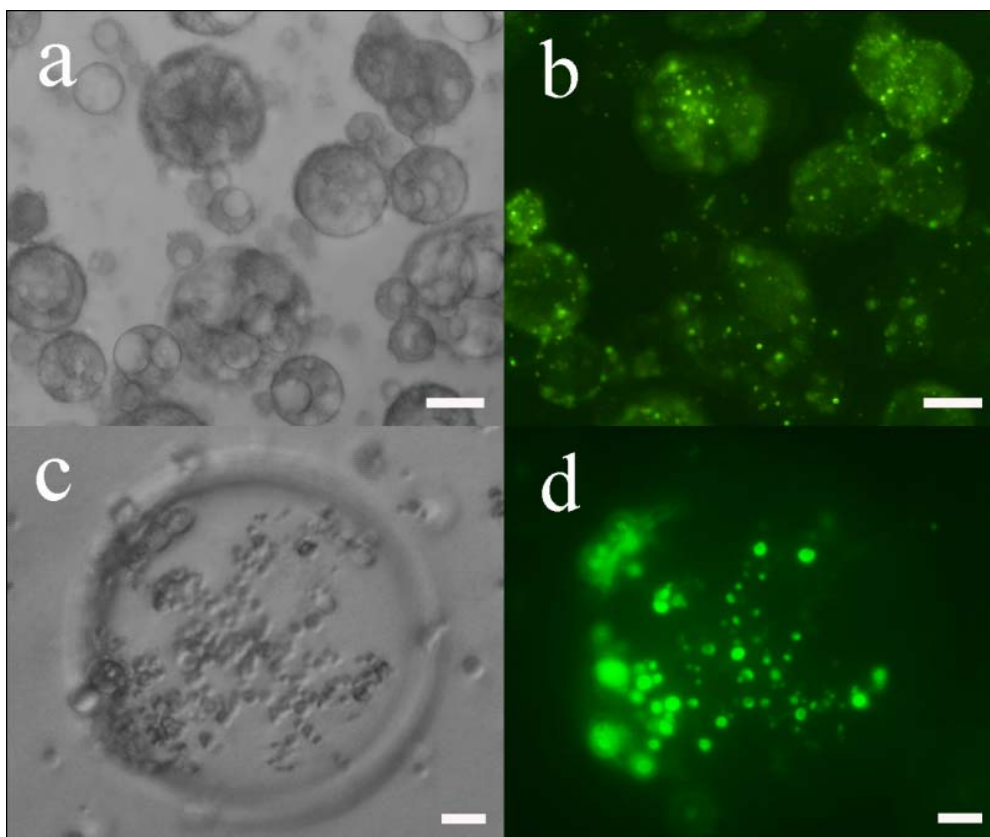


Figure S6 . Optical microscope images of pichia cells encapsulated by CS-PDEA vesicles. (a) and (c) Bright-field images of encapsulated pichia cells; (b) and (d) Fluorescence images of pichia cells in CS-PDEA vesicles. Scale bars of (a) and (b) are 40 μm , scale bars of (c) and (d) are 10 μm .