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Supporting Information

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Shaping Giant Membrane Vesicles in 3D-Printed Protein Hydrogel Cages

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Experimental Procedures

Preparation of BSA Solution

4.2g bovine serum albumin (BSA) (Lyophilized powder, A7030, Sigma Aldrich) and 1.62 mL of DMSO (18v/v%) were added to 20 mM HEPES buffer to make up a total volume of 9 mL solution. The mixture was centrifuged (20000g) for 15min to remove impurities and foam before use. 85mM rose bengal (Sigma Aldrich, 330000) was prepared separated. The BSA photoresist (420g L^{-1}) was prepared by mixing BSA resin and rose bengal at the ratio of 9:1v/v.

3D BSA Hydrogel printing

3D BSA hydrogel printing was processed with the Nanoscribe Photonic Professional (Nanoscribe GmbH). 3D structures were design with Solidwork. The parameters were defined with Describe. If without specifying, the following parameters were used, laser power: 50mW (100%), scan speed: 30000μm s^{-1}, slicing distance: 0.5μm, hatching distance: 0.2μm. All structures were printed with 63x NA1.4 objective in silicone isolator chamber (Thermo Fisher Scientific, 0717104) pasted on round glass coverslip (Diameter= 30mm, thickness #1.5).

During printing, the chambers were covered a small coverslip to avoid strong evaporation. After fabrication, structures were rinsed with Phosphate Buffered Saline (PBS) buffer (pH7) to remove the excess BSA resin and photoresist.

Swelling Studies

Five repeat free-form solid cuboids (14x14x15μm) with slicing distance 0.2–0.8μm were fabricated with different laser power (30mW-50mW) and scan speed (10000-30000μm s^{-1}). The swelling of structures were observed at different pHs (5-11) using confocal microscope. Structures swelling were studied from low pH to high pH. Structures were equilibrated in different pH solution for 10 minutes before they were transferred for imaging. Areas of the cubes were measure with Fiji (Analyze particles). Then the swelling ratios were calculation as A_{pH}/A_{pH5}, where A means area. Confocal imaging was performed on a commercial Zeiss LSM 780 laser scanning microscope, using a water immersion objective (C-Apochromat, 40 × /1.2W, Zeiss). Samples were excited with the 561 nm laser.

GUVs preparation

Giant unilamellar vesicles (GUVs) were produced by electroformation in PTFE chambers with Pt electrodes according to the published protocol[20] with minor changes. Six microliter of lipid mixture (1mg/mL in chloroform) was spread onto two Pt wires and dried in a desiccator for 30 min. The chamber was filled with 350μL of an aqueous solution of sucrose (~ 300mOsm kg^{-1}). An AC electric field of 1.5 V (RMS) was applied at a frequency of 10Hz for 1.5h, followed by 2Hz for 0.25 h. Unless otherwise stated, vesicles composed of DOPC, containing additional 0.5mol% Atto655-DOPE, were electroformed in an aqueous solution of sucrose iso-osmolar compared to imaging buffer (~ 300mOsm kg^{-1}). For the phase separation, GUVs were prepared from mixtures of DOPC, SM (18:0), and cholesterol (2:2:1) plus 0.2mol% Atto655-DOPE and 0.3mol%NBD-DSPE.

GUVs trapping and shaping

3D structures were exchanged into pH5 PBS buffer (~294mOsm kg^{-1}) for 10min. Then, 20 μL or more of the GUV suspension (without-diluted) were added on top of the printed structures in the imaging chambers. Samples were incubated for at least 0.5 h at room temperature. After GUVs sinking down and diffusing inside the traps, samples were transferred for imaging. Then, samples were gently equilibrated into pH11 PBS buffer for 10min to reach the maximal swelling. The deformation of the trapped GUVs were imaged with confocal microscopy. To

avoid bursting the GUVs during deformation, the osmolality of pH11 PBS buffer (~307mOsm kg^{-1}) was slightly higher than sucrose solution inside GUVs.

Min oscillation in vesicles

1. Proteins

The plasmids for the expression of His-MinD[28], His-EGFP-MinD[32] and His-MinE[28] have been described previously. His-MinD, His-EGFP-MinD and His-MinE were purified according to the published protocols. In brief, proteins were expressed in *E. coli BL21* (DE3) pLysS and further were purified via Ni-NTA affinity purification. Then proteins were further purified using gel filtration chromatography in storage buffer (50mM HEPES, pH 7.25, 150mM KCl, 10% Glycerol, 0.1mM EDTA). Proteins were quick-frozen and stored in aliquots at -80°C until further use.

2. Proteins Encapsulation in vesicles

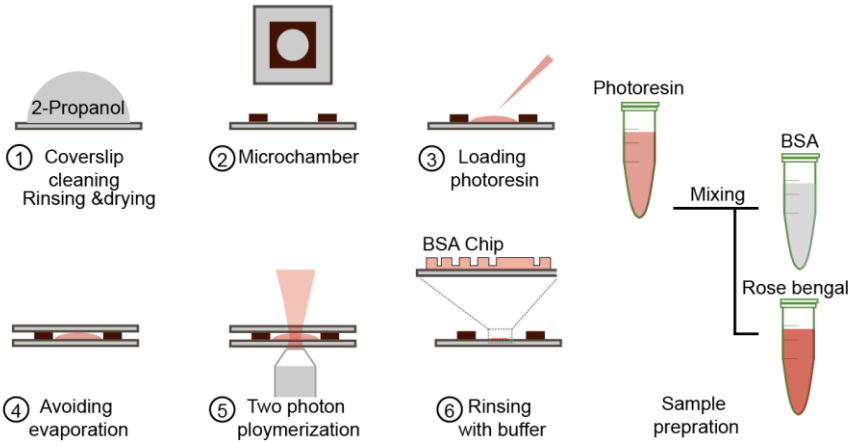
Min system was encapsulated in vesicles by emulsion transfer (the cDICE method[31]) according to the published protocol[30b]. Briefly, both inner and outer solution contain Min protein buffer (25mM tris-HCl (pH 7.5), 150mMKCl and 5mM MgCl2). In addition, the solution encapsulated in the GUVs contained 1.5µM MinD, 1.5µM eGFP-MinD, 3µM MinE, 5mM ATP, v/v 15% iodixanol (from OptiPrep™, Sigma Aldrich) and an oxygen scavenger system (3.7U ml^{-1} pyranose oxidase, 90U/ml catalase, 0.8% glucose. Osmolarity of encapsulated solution was about 560mOsm kg^{-1}, measured with Fiske® Micro-Osmometer Model 210). As the GUV-surrounding solution, Min protein reaction buffer and 200 mM glucose were used to match the osmolarity of the inner solution.

The lipid we used is DOPC (1,2-Dioleoyl-sn-glycero-3-phosphocholine, Avanti Polar Lipids, Inc.) and DOPG (1,2-Dioleoyl-sn-glycero-3-phosphoglycerol, Avanti Polar Lipids, Inc.) (Both 25mg ml^{-1} in chloroform) in a ratio of 4:1. The lipids were mixed in a silicon oil (5 cST) and mineral oil (sigma-aldrich. M5904) mixture (ratio, 4:1).

Then the inner solution was loaded into a 1 mL syringe, which was then placed into a syringe pump system (neMESYS base 120 with neMESYS 290N) and connected through tubing to a glass capillary (100µm inner diameter). 700µl of outer solution was pipetted into a spinning cDICE chamber, followed by approximately 5ml of the lipid-in-oil mixture. The capillary tip

was then immersed in the oil phase and the inner phase injected at a flow rate of 50µl h^{-1} for 15 minutes. The vesicles were withdrawn from the cDICE chamber with a micropipette.

Supplement figures



Scheme S1. Schematics of 3D printing BSA hydrogel

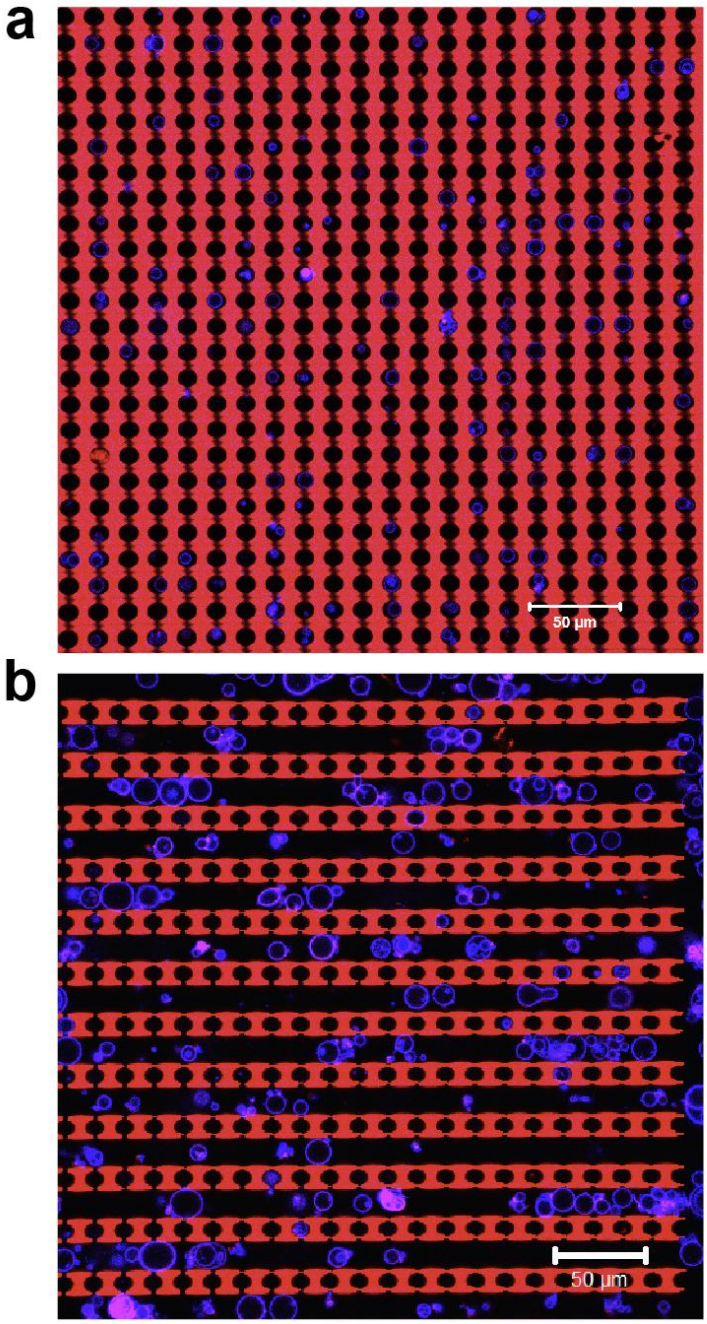


Figure S1. Overviews of the GUVs trapped in a.) The individual trap chip (22x 25 array) and b.) The group trap chip (11 channels), scale bar 50µm. GUVs were produced with DOPC and labelled with 0.5mol% Atto655-DOPE.

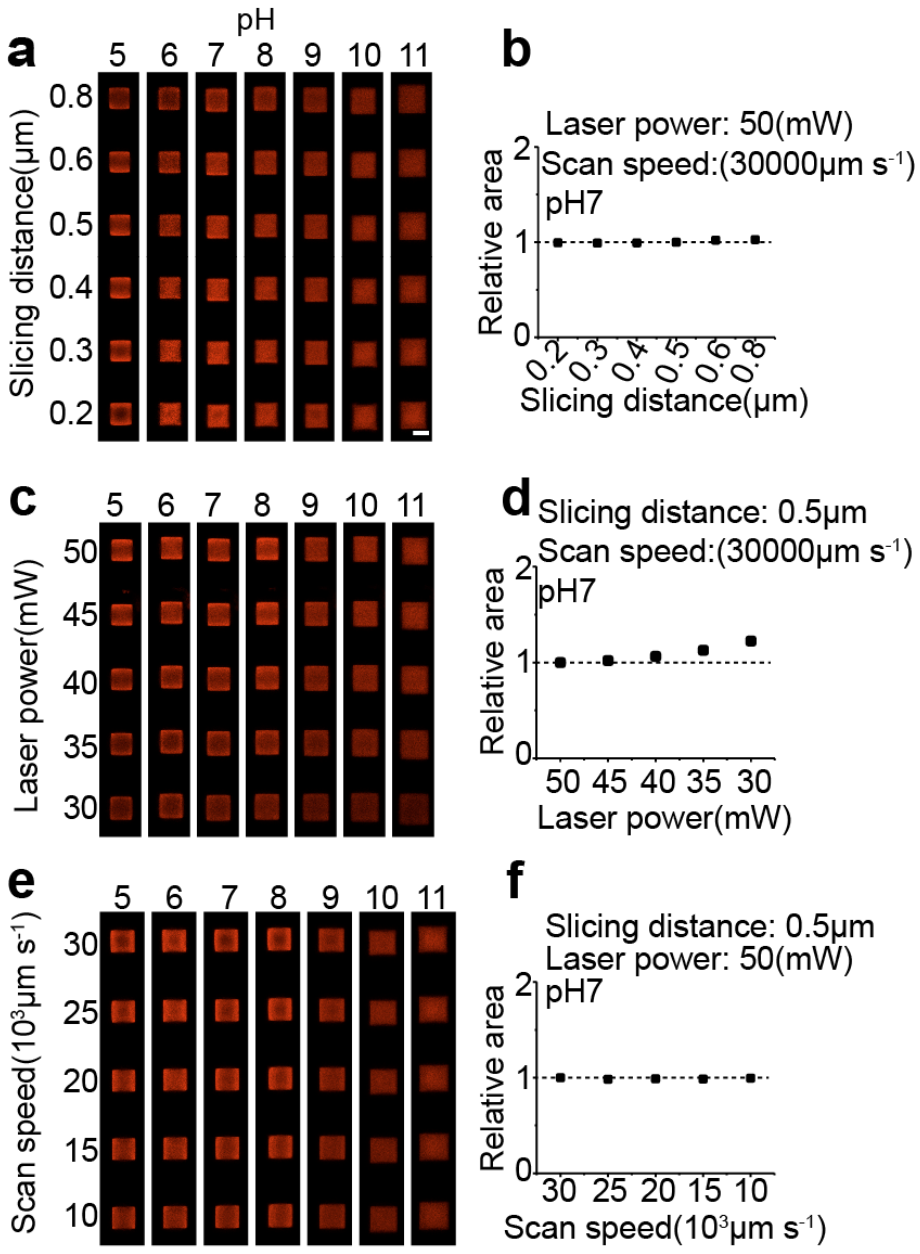


Figure S2. pH-dependent swelling of 3D printed cubic hydrogels fabricated with different fabrication parameters a.) pH responsive swelling of hydrogel cube with different slicing distances from 0.2 to 0.8μm (Laser power: 50mW, Scan speed: 30000μm s^{-1}), scale bar 10μm. b.) relative area of structures printed with different slicing distance at pH7. The slicing distance will not influence printing size of the structures. c.) pH responsive swelling of hydrogel cube with different laser power from 30 to 50mW (Slicing distance: 0.5μm, Scan speed: 30000μm s^{-1}). d.) Relative area of structures printed with different laser power at pH7. Printing with laser power lower than 40mW increased structure size. e.) pH responsive

swelling of hydrogel cube with different laser scan speed from 10000 to 30000μm s^{-1} (Slicing distance: 0.5μm; laser power: 50mW). f.) Relative area of structures printed with different scan speed at pH7. The relative area calculated with A/A_0, where A_0 means the area of structure printed with fabrication parameters (Slicing distance: 0.5μm, Laser power: 50mW, Scan speed: 30000μm s^{-1}).

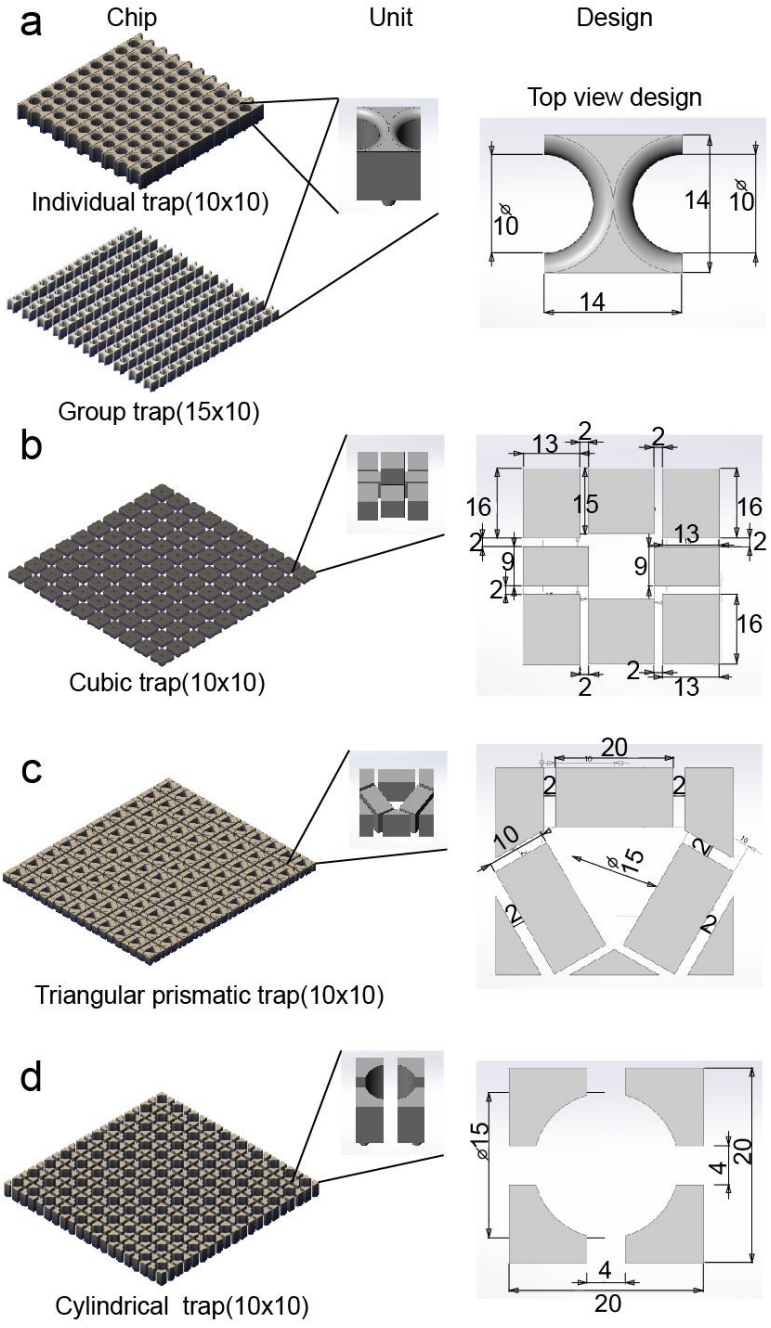


Figure S3. Schematic designs and dimensions of 3D protein hydrogel traps a.) Individual traps with 10x10 array in Fig. 1a. and group traps in Fig. 1b ,c, Fig.3 . b. and Fig.6.) Cubic traps with a 10x10 array in Fig.2f and Fig. 4. c.) Triangular prismatic traps with a 10x10 array in Fig. 4 and Fig. 5. d.) 10x10 cylindrical traps array in Fig. 4. If without specifying, all the structures used in this research are freestanding designs with pillars as supports (Diameters 2μm, Height 2μm)

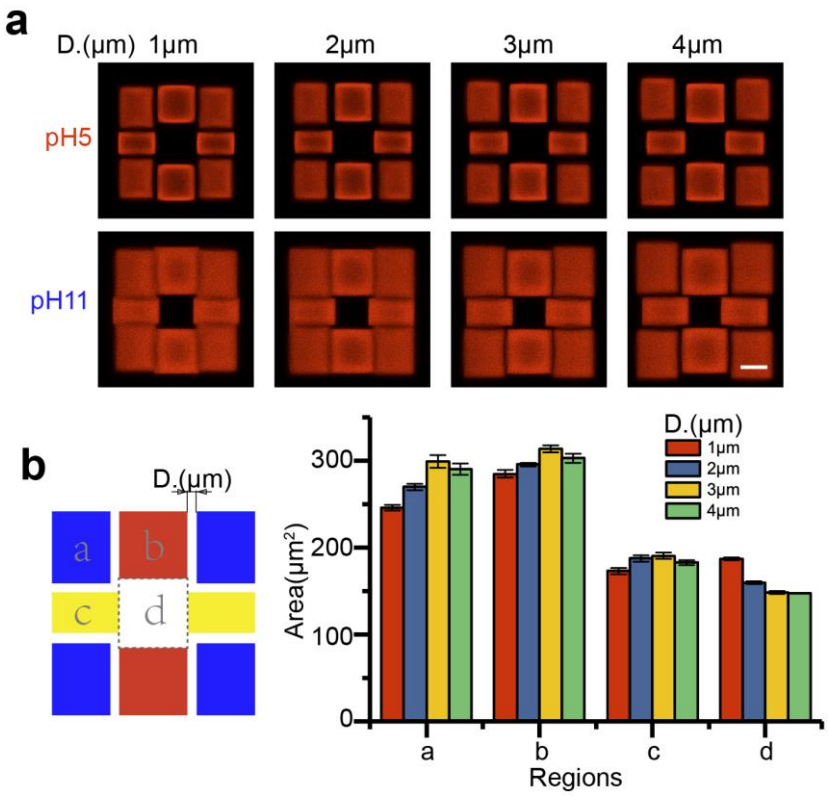


Figure S4. Distances between modules influence structure swelling capability. a.) pH responsive swelling of cubic traps with different distances between modules, scale bar, 10μm. b.) Area swelling of different regions in the cubic trap with different distances, when pH was changed from 5 to 11. Module b and c are freestanding structures with pillar supports. Module a were cuboid without pillar supports.

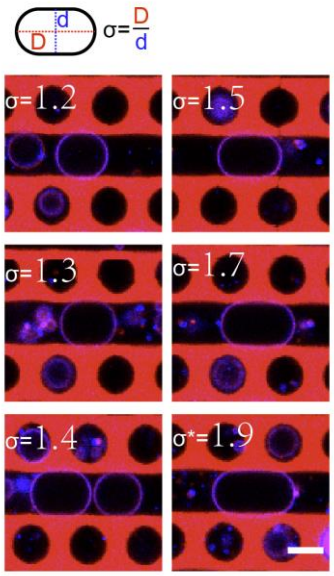


Figure S5. Swelling hydrogel compressing GUVs with different aspect ratios σ (width compared to length), scale bar 10μm. The deformation of GUVs were processed in the group trap chip. Due to the size difference, GUVs with different aspect ratio can be obtained from the swelling compression. * GUV (Diameter>15μm) trapped in the chip had been compressed at pH5.

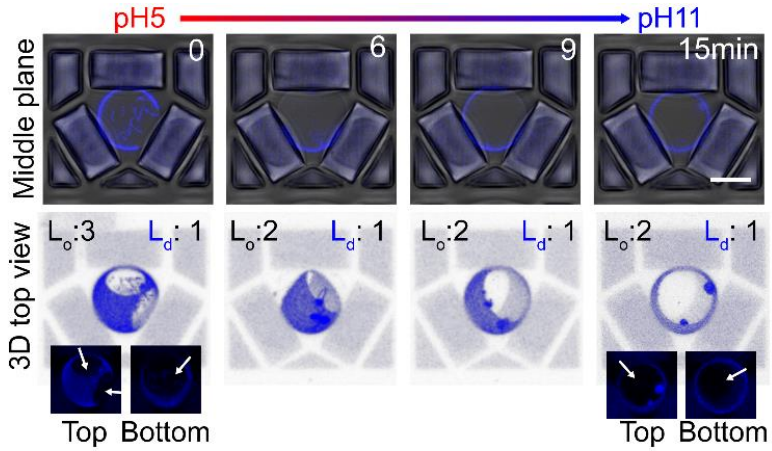
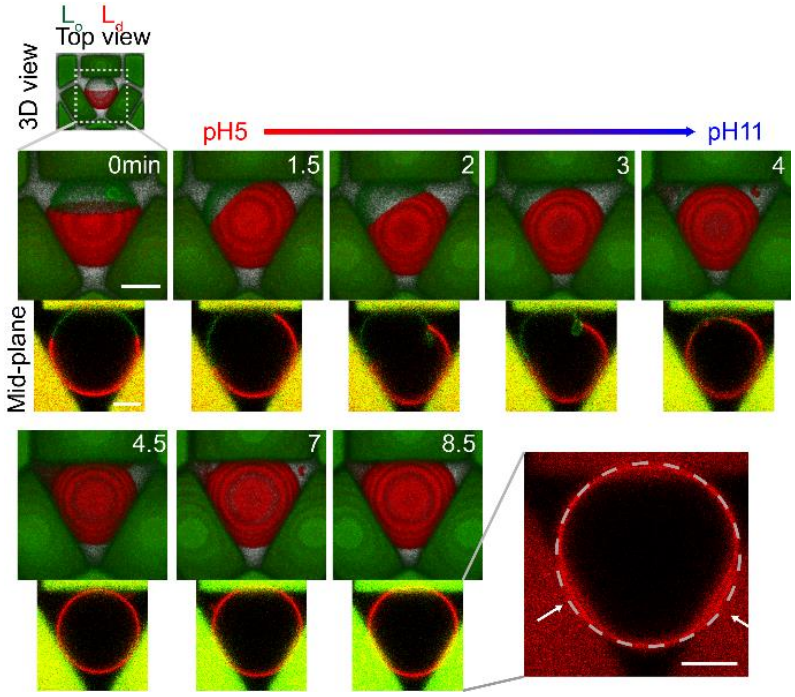


Figure S6. Dynamic lipid domain fusion and reorganization adapting to the space-induced membrane deformation, Scale bar, 10μm. Top: confocal imagines of middle plane of the vesicle. Bottom: top view of the 3D z-stack reconstitution. The numbers show the domain numbers of L_o and L_d phase. GUVs were produced from DOPC:SM:cholesterol (2:2:1). GUV



in the images was labelled with Atto655-DOPE (blue).

Figure S7. Dynamic membrane domain reorganization under pH-induced compression in the hydrogel chambers, scale bar 5μm. GUVs were produced from DOPC:SM:cholesterol (2:2:1) and labelled with NBD-DSPE(green) and Atto655-DOPE (red). The top views of 3D images were compiled from Z-stack confocal images with ZEN software.

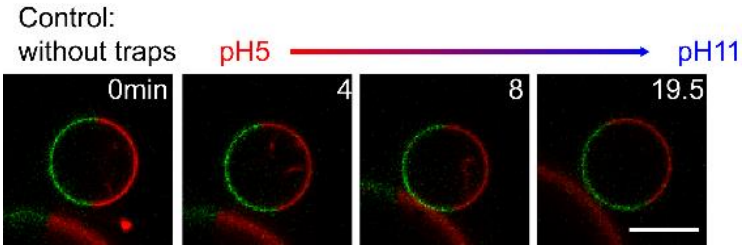


Figure S8. Free standing phase-separated GUVs upon pH stimuli, scale bar, 10μm. GUV in the images was labelled NBD-DSPE (green) and Atto655-DOPE (red).

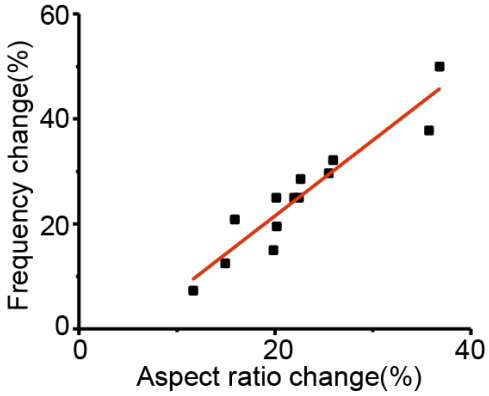


Figure S9. Pulsing oscillation acceleration during vesicles compression (1.5μM MinD, 1.5μM eGFP-MinD, 3μM MinE, 5mM ATP). Pulsing frequency change versus aspect ratio (W/H) change of vesicles that were before- and after- compressed.

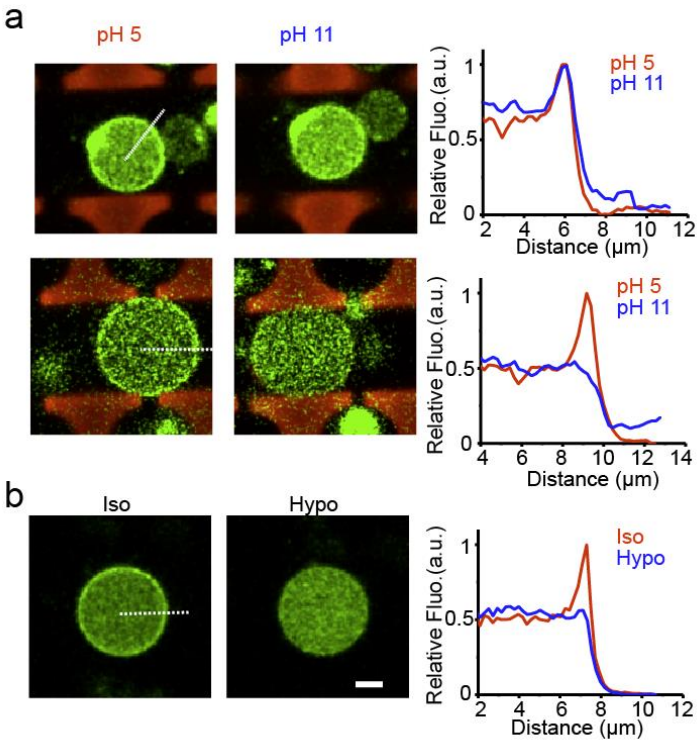


Figure S10. a.) Vesicle compression could oppose Min protein binding (1.5μM MinD). b.) MinD-membrane interaction under hypotonic and isotonic condition. To generate the hypotonic shock, the outer solution osmolality was increased about 20mOsm by adding water in the surrounding buffer. Scale bar, 5μm. The white dash lines show the position for radial plot profiles.

Supplement Movies

Supplement Movie S1. pH responsive swelling of group trap, when pH was shift from 5 to 11.

Supplement Movie S2. Dynamic membrane phase separation and reorganization in pH-stimuli 3D hydrogel chamber (top view)

Supplement Movie S3. Dynamic membrane phase separation and reorganization in pH-stimuli 3D hydrogel chamber (orthogonal view)

Supplement Movie S4. Membrane budding driven by the space compression of the pH-stimuli 3D hydrogel chambers (top view)

Supplement Movie S5. Pulsing oscillation acceleration during vesicles compression

Supplement Movie S6. Min oscillation modes transition from pole-to-pole to circling.

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