Electronic Supplementary Information for the Communication

Soft Landing of Cell-Sized Vesicles on Solid Surfaces for Robust Vehicle Capture/Release

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Experimental Section.

Materials.

L-α-Phosphatidylcholine from egg yolk (Egg-PC), 1-Palmitoyl-2-oleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (POPG), lysozyme, tris(2-carboxyethyl)phosphine (TCEP), poly(ethylene glycol) methacrylate (average Mn~360), poly(ethylene glycol) methyl ether methacrylate (average Mn~300) and methyl methacrylate (MMA) were purchased from Sigma-Aldrich. Before the radical polymerization, the monomers were passed through a alkaline Al_2O_3 column to remove the inhibitors. HEPES buffer (pH=7.2~7.4, sterilized) was obtained from Solarbio. 3,3'-dioctadecyloxacarbocyanine perchlorate ($DiOC_{18}$) and 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate ($DiIC_{18}$) were purchased from Beyotime Institute of Biotechnology. The 5% polystyrene colloidal suspension (crosslinked) was purchased from Nano-MicroTech. Doxorubicin hydrochloride (DOX) was obtained from Melonepharma. 6-carboxyfluorescein was purchased from J&K Scientific. Ultrapure water was used in all experiments and was supplied by Milli-Q Advantage A10 (Millipore, USA).

The Formation of Lysozyme Layer on Solid Substrate.

The pristine glass substrate or the glass with a non-fouling comb copolymer coated could be used for lysozyme priming. Although most of data reported in this work were based on the glass substrate with the non-fouling comb copolymer coated unless otherwise noted, the other experiments (as shown in Figure 3, Figure S12-S15) have proved that the introduction of the non-fouling polymer layer is not the key factor to determine the capture and release of vesicles. The role of the non-fouling polymer layer was not specially correlated to the observed capture/release behavior, but potentially corresponding to a biocompatible platform with low non-specific adsorption of biomolecules. Such a substrate is beneficial for the future work on cell-inspired biomimetic research. A shortage of the non-fouling polymer substrate is that such kind of material easily adsorbs organic molecules, e.g. DOX. Consequently, when using this method to encapsulate DOX or 6-carboxyfluorescein, a glass substrate without such a non-fouling polymer layer was utilized (Figure 3).

The detailed synthesis procedures for the comb copolymer poly(methyl methacrylate) (PMMA)-b-poly[oligo(ethylene glycol) methyl ether methacrylate] (POEGMA) are referred elsewhere.[1] Generally, a cleaned substrate with or without the non-fouling polymer coating was immersed in a lysozyme phase transition buffer (pH 7.4) containing lysozyme, TCEP and

HEPES at determined concentration.[2] For lysozyme array, the phase transition buffer was spotted on a cleaned substrate by manually pipetting or automatic protein spot arrayer. After certain incubation time to induce the lysozyme phase transition,[2] the substrate was taken out and washed by Milli-Q water to remove unbound molecules and salts. The resultant surface with or without further drying (more stability was obtained with drying) could be directly utilized as a lysozyme-primed surface to provide a powerful platform for next vesicle immobilization.

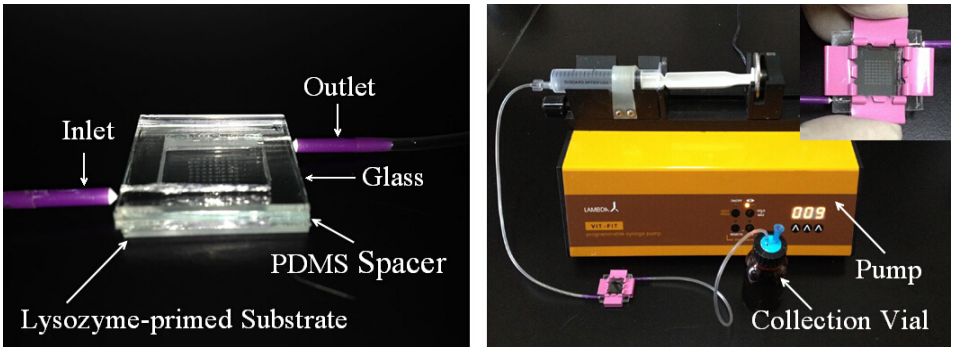
Vesicle Preparation.

Giant unilamellar vesicles (GUVs) were prepared by using a lipid mixture of Egg-PC and POPG. The electroformation method was utilized to grow GUVs.[3] Briefly, the lipids were dissolved in chloroform to form 2 mg/ml lipid solution. For the observation of the vesicles with fluorescent microscopy, the following dyes $DiIC_{18}$ (Ex, 549 nm; Em, 565 nm) or $DiOC_{18}$ (Ex, 484 nm; Em, 501 nm) were added to the lipid solution at a concentration as 0.1 mol %. Typically, A small drop (~ 20 μl) of lipid solution was placed onto a glass slide coated with indium tin oxide (ITO) and spread evenly on the surface. Two such coated ITO glasses were placed in a vacuum desiccator at room temperature for at least 2 hrs to evaporate the organic solvent. A closed chamber was assembled from the two ITO glasses (the slides with conductive coating were facing each other) and a 1 mm thick rectangular Teflon spacer with two holes as the solution inlet and outlet. The growing solution (100 mM sucrose with or without other functional materials added) was then introduced through the inlet to fill the chamber (c.a. 2 ml). The chamber was then connected to an AC field function generator for the electroformation of GUVs. The typical electroformation procedure started from the input of an AC voltage with (peak-to-peak) amplitude of 0.5 V and frequency of 10 Hz for 20 min. After that, the voltage increased gradually with a step as 0.5 V per 20 min to 2.5 V. The chamber was then kept at this stage (2.5 V, 10 Hz) for another 2 hrs to further grow GUVs. Then, the vesicles were detached from the ITO glass substrate by lowering the field frequency to 5 Hz and setting the voltage to 0.5 V for 20 min. The resultant vesicles were gently transferred from the electroformation chamber to a clean vial and stored at 4 ^{o}C.

Soft Landing of GUVs on Lipid Membrane-Deposited Lysozyme Layer.

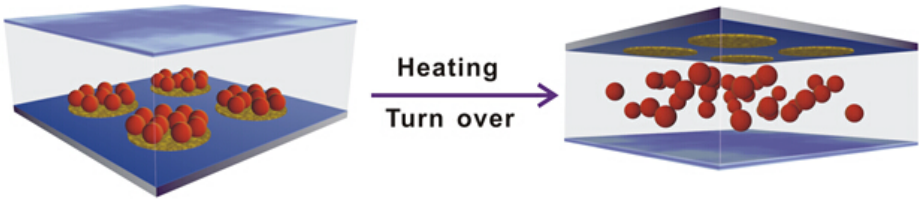
As shown in Scheme S1, a self-made chamber was firstly assembled from two glass coverslips (20*20 mm) and a 3 mm thick rectangular polydimethylsiloxane (PDMS) spacer with two holes as the solution inlet and outlet. The surface of the bottom coverslip was primed by the lysozyme phase transition product to provide the anchoring sites for vesicles. The as-prepared GUVs suspension was firstly diluted up to 2 times with isotonic glucose solution. The diluted GUVs suspension was then injected into the self-made chamber through the inlet by a polyvalent syringe pump. The chamber with GUVs suspension solution filled was allowed to stand for 1 hr. During this term, the glucose-sucrose asymmetry created a density contrast to accelerate the sedimentation of GUVs onto the bottom. The first settlement of GUVs on the lysozyme layer directly resulted in the rupture and fusion of GUVs with the surface (Movie S1, Figure S2), affording the lipid membranes on the lysozyme layer. Finally, the lysozyme layer was fully covered by the lipid membrane, which offered a soft bed for next safe soft landing of GUVs. After substantial sedimentation, the solution in the chamber was further slowly exchanged by a new isotonic glucose solution without vesicles contained. With an injection speed being 180 μl/min and the total injection volume being about 20 ml, the newly introduced solution was able to inoffensively purge the unbound vesicles out of the chamber.

Scheme S1. The photograph of the experimental setup for the soft landing of GUVs.



Release of Immobilized GUVs after Soft Landing by Subtle Heating.
The chamber with immobilized GUVs was placed in a water bath with the constant temperature. Under mild shaking for certain duration, the captured vesicles gradually dissociated from the surface. It shoud be noted that the shaking is not necessary for the observed release after the capture, because in the absence of the shaking, when the heated chamber was turn over to make the upside down (Scheme S2), the vesicles located on the pattern could easily fall off from the surface. The released vesicles were then re-suspended in a new solution. The result reflected that the heating did induce a stimuli (heating)-responsive detachment of vesicles. The purpose of the shaking used in the heated chamber without the turnover was only to provide a turbulence field, so that the dissociated vesicles from the heated surface could move away from the original location without the turnover to result in a blurred pattern (Figure 2B).

Scheme S2. The schematic cartoon to show the release of GUVs by subtle heating.



Characterization.
Scanning electron microscopy (SEM) was performed on a FEI Quanta 200. Optical observations were carried out on a Nikon T*i*-U (Tokyo, Japan) equipped with bright field, Differential Interference Contrast (DIC) and fluorescent observation modes. Confocal laser microscopy was carried out by using a Olympus FV1200 microscope. The 3D projection was obtained from a series of images, and the image analysis was conducted by using Imaris 7.6.3. Automatic protein spotting was performed by Personal Arrayer 16 (CapitalBio Co, Beijing). AC field function waves was provided by Agilent 33500B 20 MHz function/arbitrary waveform generator. The solution injection and exchanging in the chamber was performed by a polyvalent syringe pump (Lambda Vit-fit, the Czech Republic). Surface Zeta potential measurement was performed by SurPASS electrokinetic analyzer (Anton Paar GmbH, Austria). During the test, an electrolyte solution containing 1 mM KCl, 50 mM glucose, 50 mM sucrose was used, and an adjustable slit sample cell was equipped for the measurement. The flow rate of the electrolyte and the slip gap were setted as 90 ml/min and 100 μm. The measurement could be started until the overlapping extent of the two flow check lines was good enough. The pressure during the test was 300 mbar.

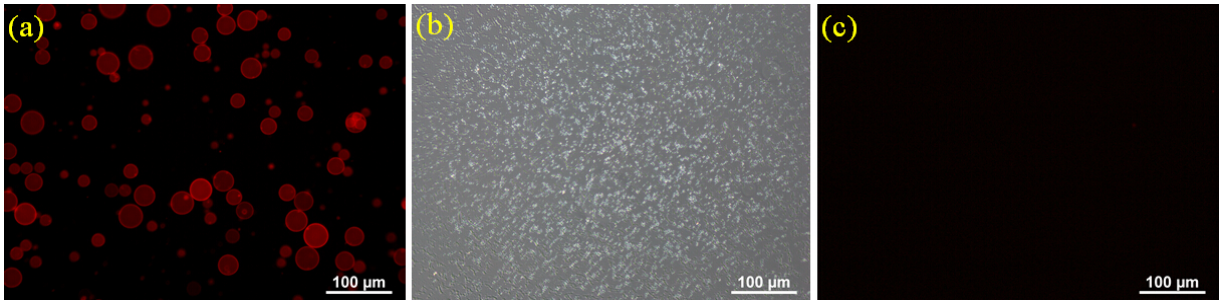


Figure S1. The interaction of zwitterionic Egg-PC GUVs on the lysozyme layer. (a) The fluorescent image for the gravimetric sedimentation of GUVs on the lysozyme layer; (b) and (c) presented the DIC (b) and fluorescent (c) images for the surface shown in (a) after the flushing by a solution flow. There was no obvious capture of GUVs observed in (b) and (c). Lysozyme, 2 mg/ml; POPG/Egg-PC=0:100 (w/w) with 0.1 mol% $DiIC_{18}$ added.

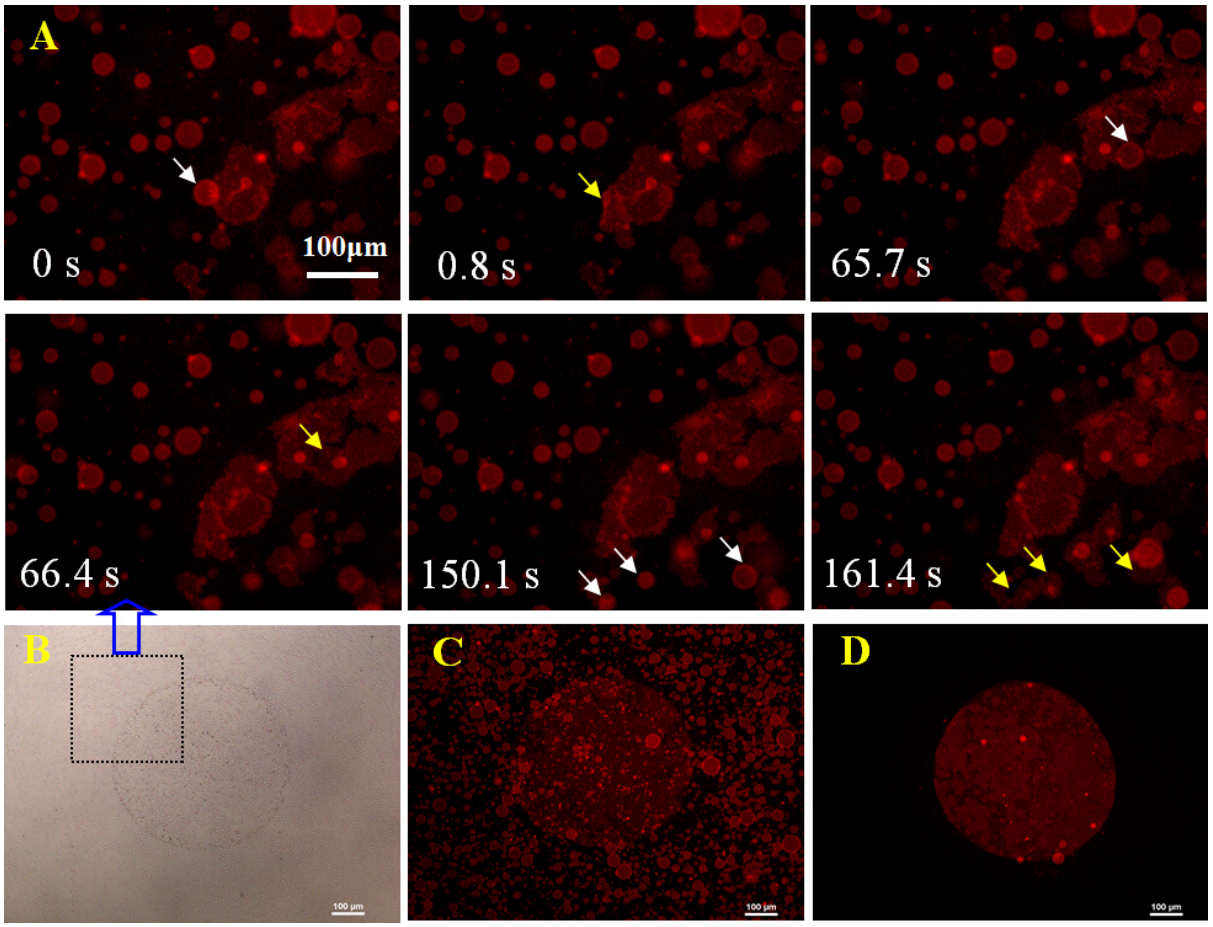


Figure S2. The formation process of a lipid membrane on the lysozyme layer by the fusion of GUVs with the surface. (A) The time-resolved fluorescent snapshots (abstracted from the Movie S1) of the observation region as indicated by a square in (B); (B) the bright field image of one typically prepared lysozyme spot; (C) the fluorescent image of the one typically resultant lipid membrane-coated lysozyme spot after the sedimentation of GUVs for 0.5 hr; (D) the fluorescent image of the one lipid membrane-coated lysozyme spot after deliberately flushing away the vesicles outside the spot. As shown in (A), a sharp contrast on the fate of vesicles could be easily found between the inside and outside of lysozyme spot. In the frames (0-0.8 s), (65.7-66.4 s) and (150.1-161.4 s), the vesicles (pointed by the white arrows) quickly ruptured (in c.a. 1 s) upon the contact with the underlying lysozyme layer (pointed by the yellow arrows), while the vesicles outside the lysozyme spot kept intact. Lysozyme, 5 mg/ml; POPG/Egg-PC=5:95 (w/w) with 0.1 mol% $DiIC_{18}$ added.

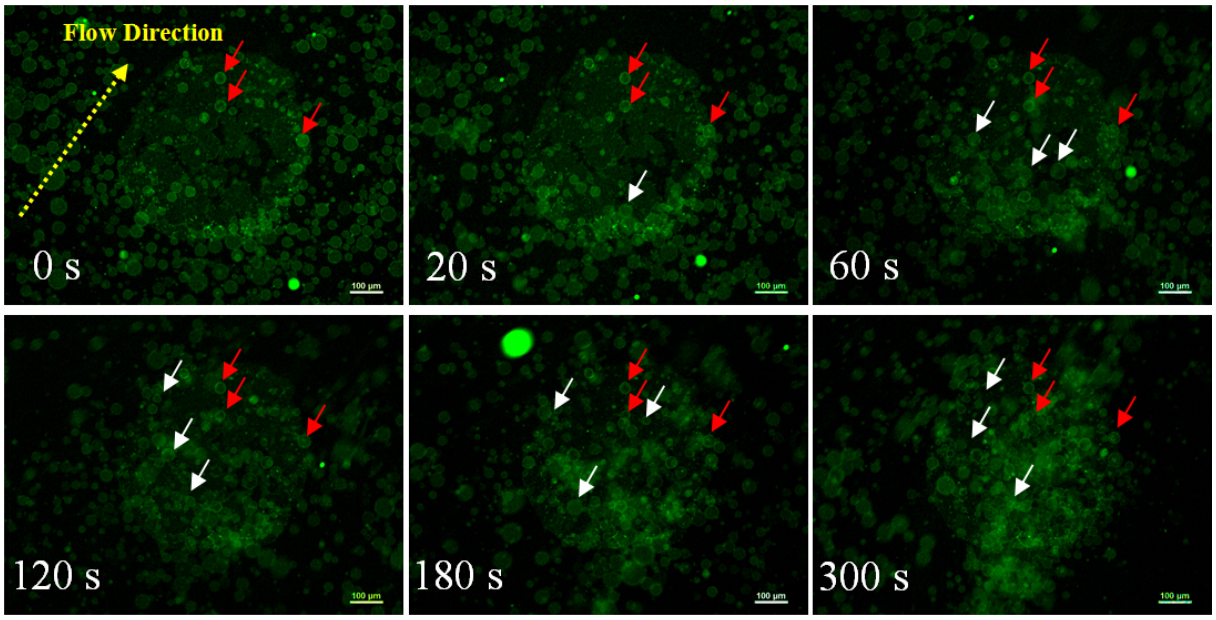


Figure S3. The stable tether of GUVs on the lysozyme layer during the solution exchange. The time-resolved fluorescent snapshots were abstracted from the Movie S2. The red arrow indicated the vesicles originally subsided on the lysozyme layer, and the white arrow illustrated the immigrated vesicles from the exterior space of the lysozyme layer and subsequent capture by the lysozyme array. Lysozyme, 2 mg/ml; POPG/Egg-PC=5:95 (w/w) with 0.1 mol% $DiOC_{18}$ added.

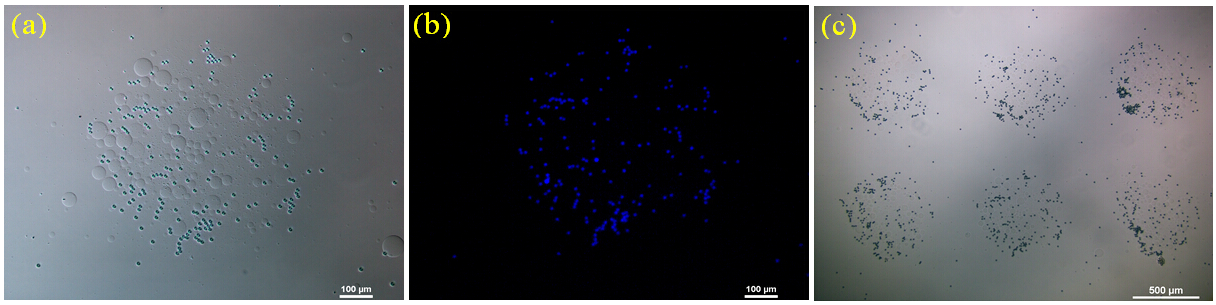


Figure S4. The co-adsorption of the negatively charged polystyrene colloids and anionic GUVs on the lysozyme layer by the soft landing. The 5% colloidal suspension was diluted 100 times by 100 mM glucose solution, and then mixed with the undiluted GUVs population at the volume ratio as 1:1. The mixture was then injected into the chamber and settled down based on the same method as used in the case for the capture and release of GUVs. (a), (c) the DIC images for the co-adsorption; (b) the fluorescent image for the co-adsorption (Ex, 365 nm). The fluorescence of colloids might be from the additives in the particles. Lysozyme, 2 mg/ml; POPG/Egg-PC=5:95 (w/w).

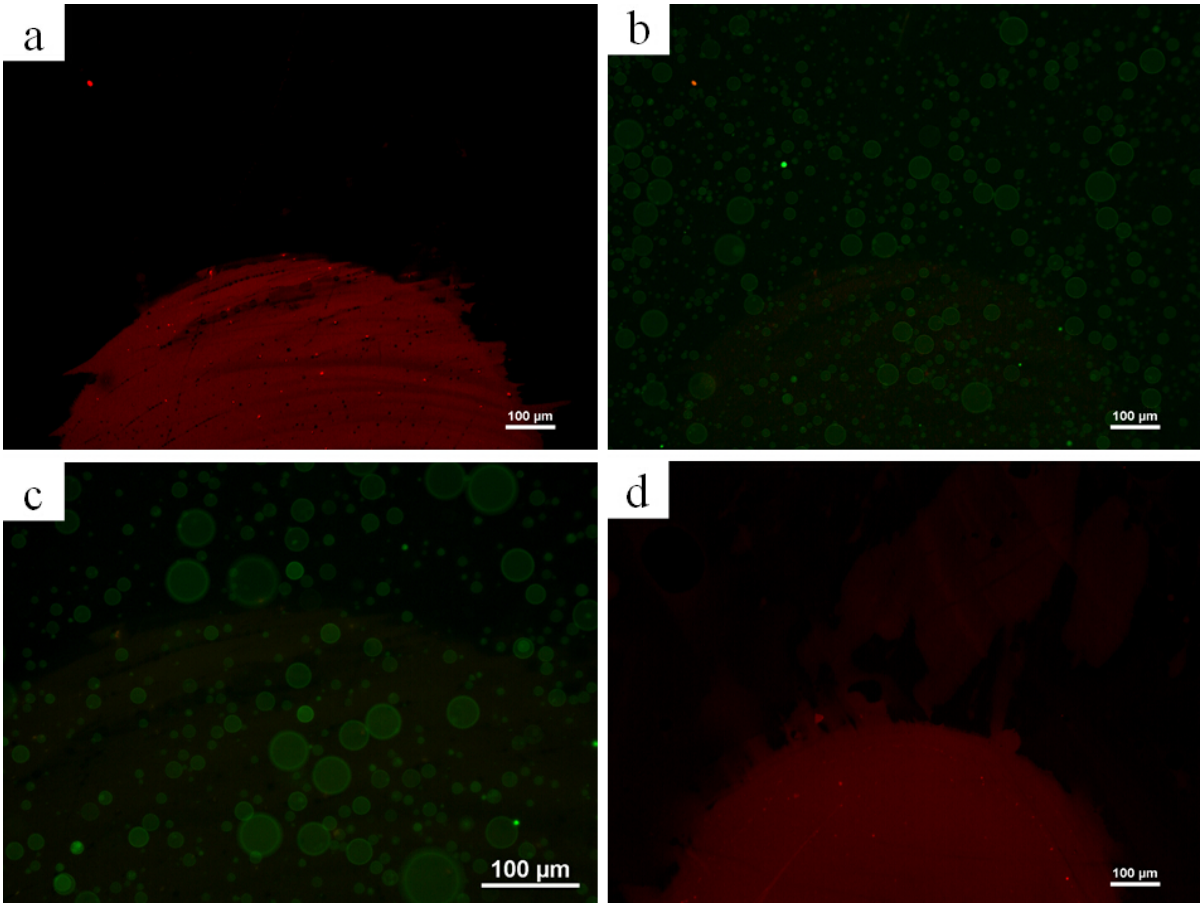


Figure S5. The interaction of GUVs with a dry lipid film. (a) The fluorescent image for the lipid film prepared by direct deposition and evaporation of lipid solution in chloroform on the lysozyme layer through a capillary; (b)-(c) the fluorescent images to show the sedimentation of GUVs on the lipid film for 1 hr. After flushing by a solution flow, the sedimented GUVs in (b) and (c) were easily detached from the lysozyme film surface, and there was no immobilized GUVs observed on the lysozyme film (d). From (d), it could be also seen that some lipid film species were flushed away from the spot. Lysozyme, 2 mg/ml; the formula for the lipid film preparation, POPG/Egg-PC=5:95 (w/w) with 0.1 mol% $DiIC_{18}$ added; the formula for the GUVs preparation, POPG/Egg-PC=5:95 (w/w) with 0.1 mol% $DiOC_{18}$ added.

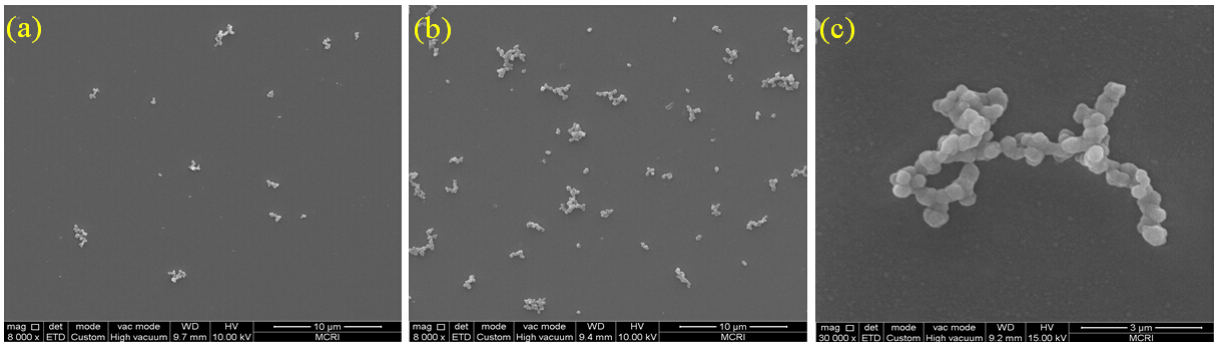


Figure S6. The SEM images for the glass substrate primed by the lysozyme layer at different concentrations. (a) 0.5 mg/ml, (b) 2.0 mg/ml. A typical phase-transited lysozyme product was shown in (c). In this experiment, the non-fouling comb copolymer coating was not used.

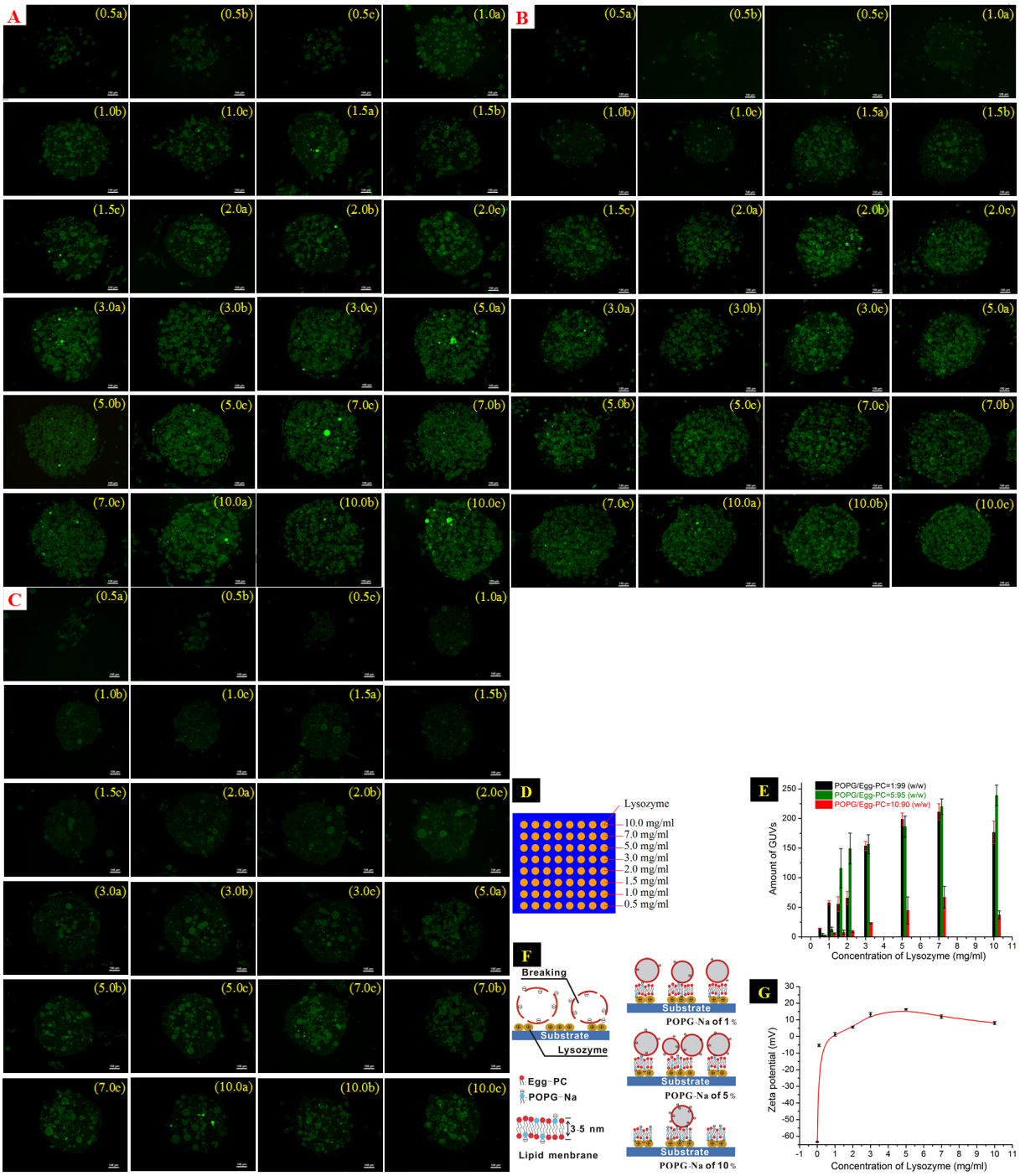


Figure S7. The effects of lysozyme concentration and POPG proportion on the immobilization of GUVs. In (A)-(C), the proportion of POPG/Egg-PC was 1:99, 5:95 and 10:90 respectively. The systematical study on the lysozyme concentration was performed by arraying a series of lysozyme spots at different concentrations on one slide (D) and the assay for GUVs immobilization on such slide was evaluated by the fluorescent images (A)-(C). For each combined image, three parallel trials (a-c) at different lysozyme concentration varying from 0.5 to 10.0 mg/ml were provided. The counted amount was quantitatively depicted in (E). In (F), a cartoon to show the schematic process for the differentiated adsorption of GUVs on the substrate with different POPG percentage was given. In (G), the zeta potential curve on the lysozyme-primed glass substrate surface as a function of the lysozyme concentration was presented. The lipids with 0.1 mol% $DiOC_{18}$ added were used. The scale bar is 100 μm.

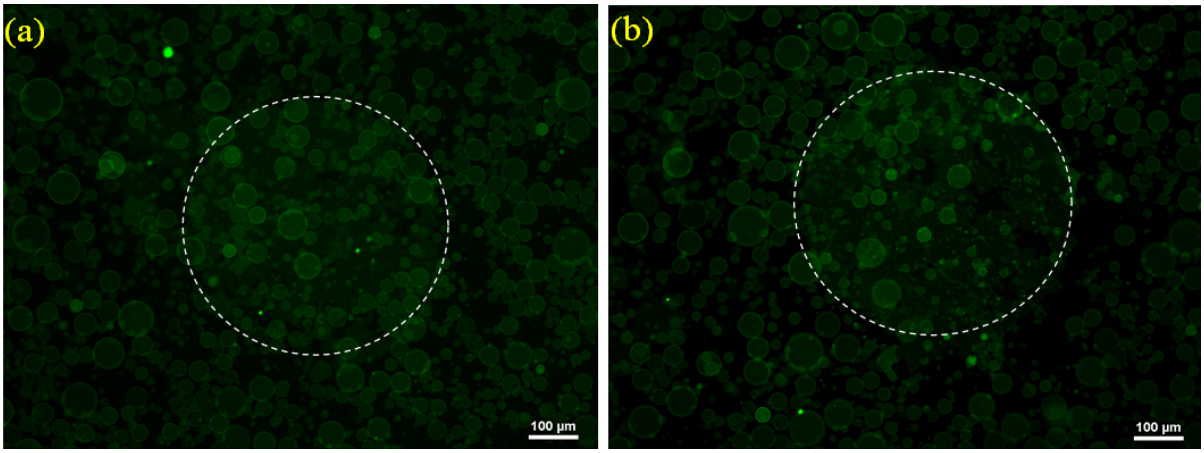


Figure S8. The sedimentation of GUVs on the lysozyme spot (as indicated by the white circle) for 1 hr at the lysozyme concentration being 2.0 (a) and 10.0 (b) mg/ml respectively. The amount of GUVs captured at 2.0 mg/ml was higher than that at 10.0 mg/ml. POPG/Egg-PC=5:95 (w/w) with 0.1 mol% $DiOC_{18}$ added. The images were taken under the fluorescent microscope.

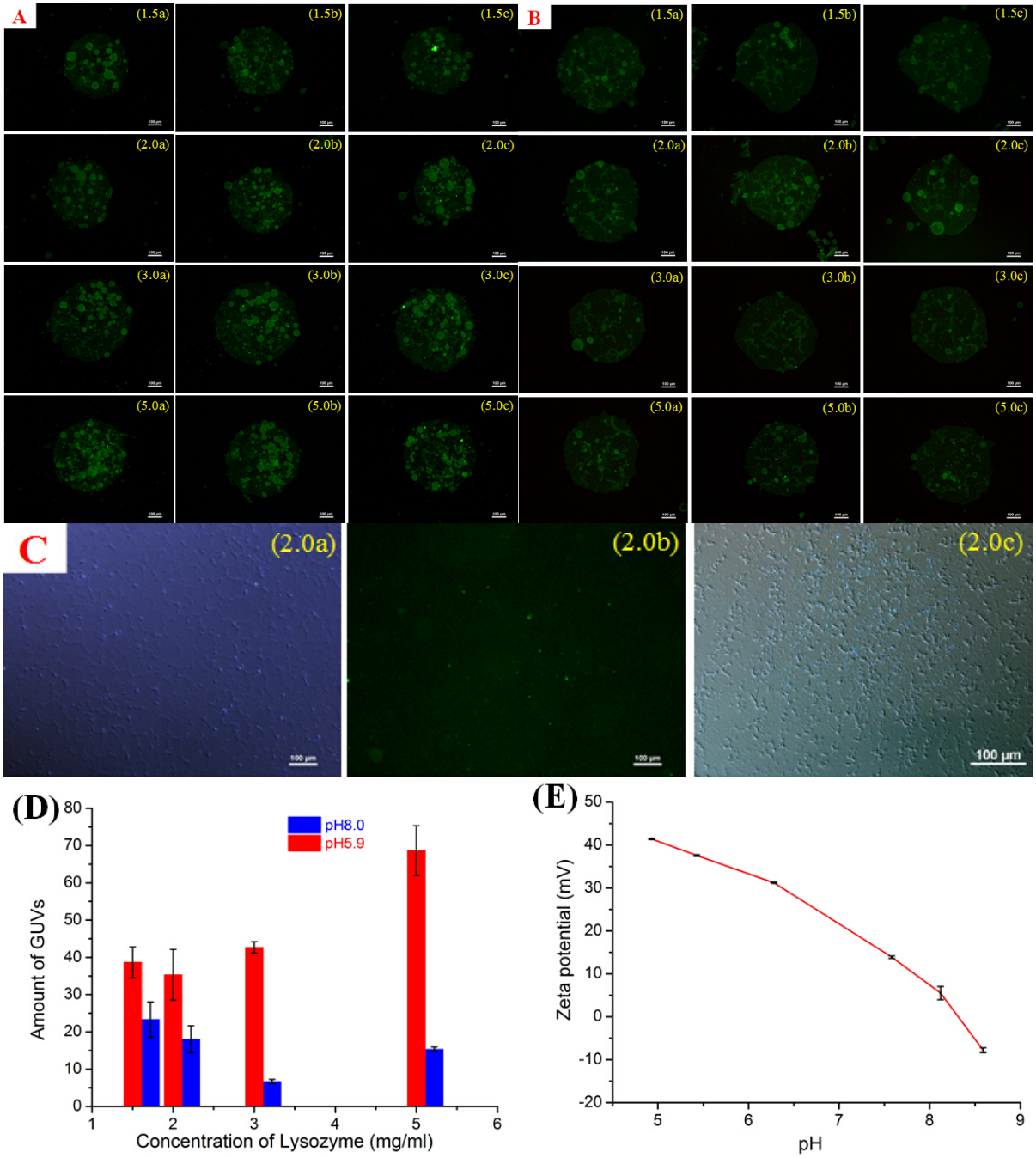


Figure S9. The effect of pH on the capture amount of GUVs, as obtained by counting vesicles on the fluorescent snapshots. (A-C) The optical snapshots for the immobilized GUVs obtained at different pH; (D) the quantitative amount of the captured GUVs obtained at different pH; (E) the effect of the solution pH on the surface zeta potential of the lysozyme layer (5 mg/ml) coated on a glass substrate. (A-B) The fluorescent snapshots for pH at 5.9 (the normal electroformation condition) (A) and 8.0 (mediated by NaOH) (B). In (A-B), the lysozyme concentrations were tried at 1.5, 2.0, 3.0 and 5.0 mg/ml respectively and three repeats for each concentration (a~c) were given. In (C) for pH at 4.0 (mediated by HCl) with the lysozyme concentration being 2.0 mg/ml, the fluorescent signal from $DiOC_{18}$ was largely quenched by low pH so that the introduced GUVs as revealed under DIC mode (C-2.0a) showed weak fluorescence (C-2.0b); the DIC image (C-2.0c) further revealed a large amount of lipid fragments were formed upon the contact of GUVs with the lysozyme layer, which indicated that the GUVs after sedimentation for 1 hr were broken at such condition. POPG/Egg-PC=5:95 (w/w) with 0.1 mol% $DiOC_{18}$ added. The scale bar is 100 μm.

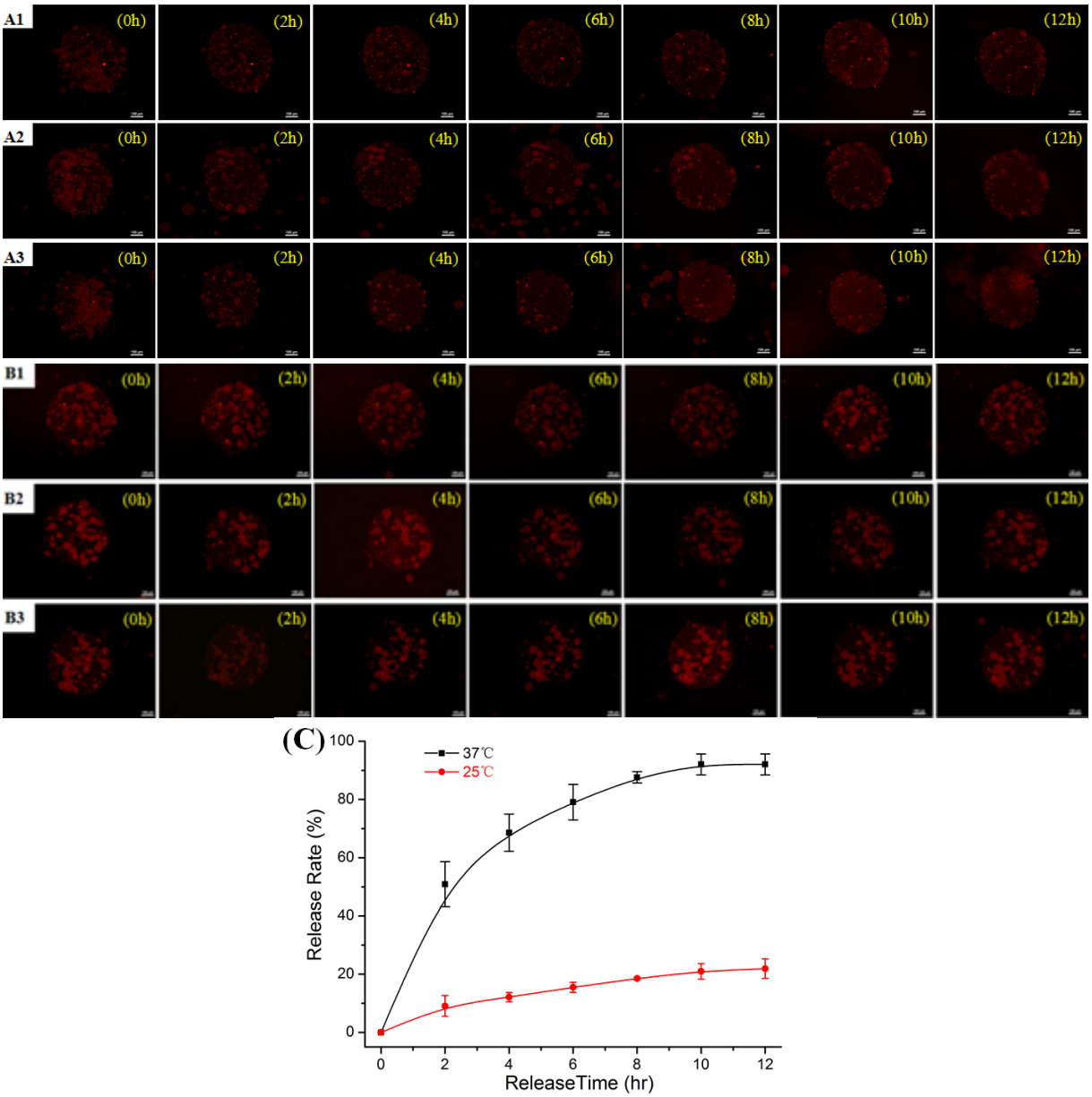


Figure S10. The release of GUVs from the surface by gentle heating. (A1-A3) and (B1-B3) the repeated measurements for typical fluorescent snapshots at different incubation time (hr) to describe the release process of GUVs from one lysozyme spot with the heating operated at 37 °C and 25 °C, respectively. The quantitative calculation on the release rate was shown in (C), which was obtained based on the equation $(N_0\text{-}N_t/N_0)\times100\%$ where N symbolized the number of GUVs immobilized on the lysozyme spot at the time t (before starting release, t=0). Lysozyme, 2 mg/ml; POPG/Egg-PC=5:95 (w/w) with 0.1 mol% $DiIC_{18}$ added. The scale bar is 100 μm.

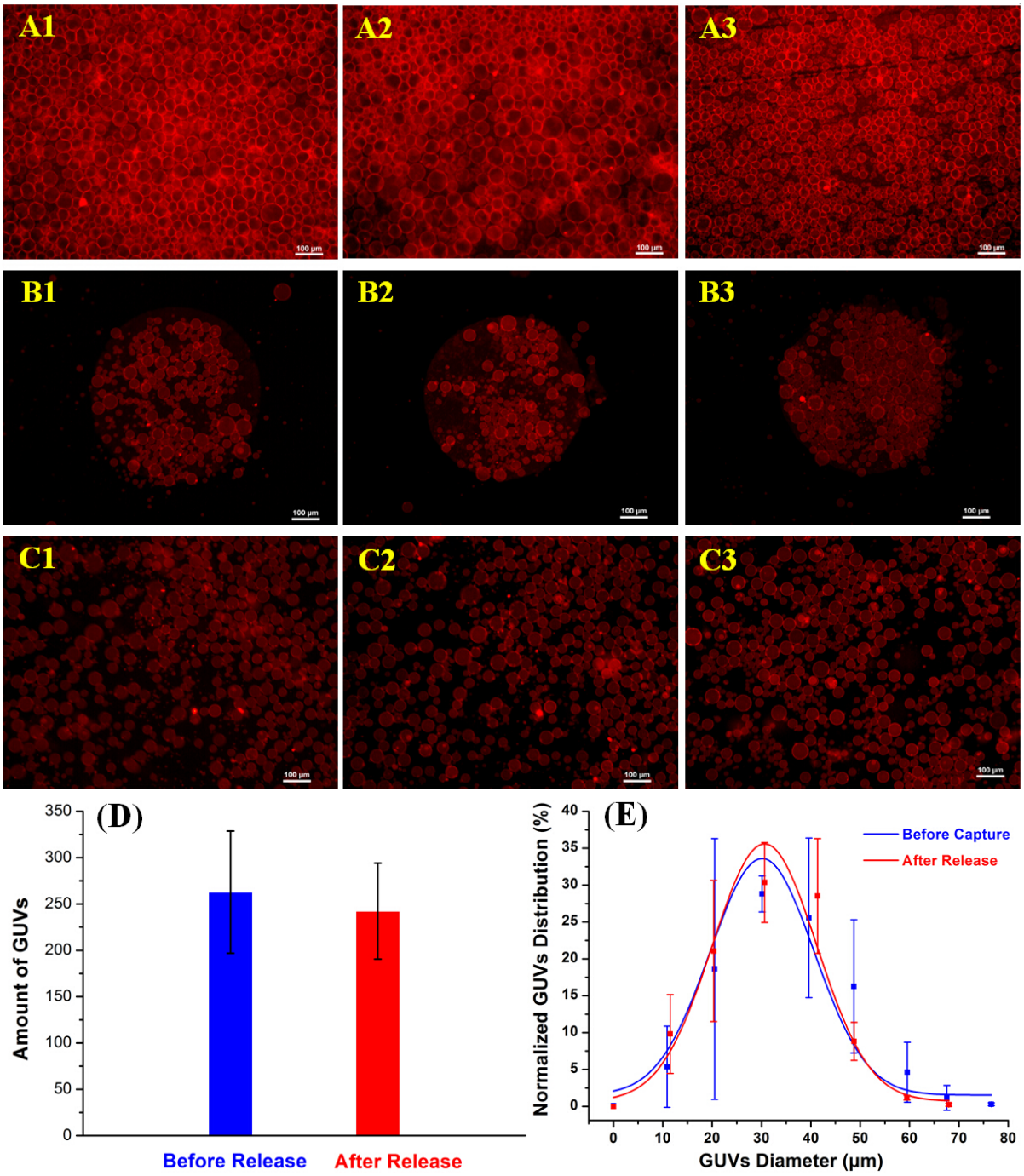


Figure S11. The variation of the amount and size distribution of GUVs before and after the capture-release cycle. (A1-A3) The as-prepared GUVs by the electroformation; (B1-B3) the captured GUVs on the lysozyme spot; (C1-C3) the GUVs after the capture-release cycle to be re-suspended in a new solution. In each panel, three repeats for fluorescent microscopic images were provided. The comparison on the quantified amount of GUVs before and after release (D) indicated the recovery ratio of GUVs was around 92%. The size distribution variation shown in (E) presented the scatter diagram and fitting curves for Gaussian distributions of GUV diameter before capture (blue) and after release (red). The sampling site for the statistics was selected randomly and the sampling number was over 400 for each preparation. Lysozyme, 2 mg/ml; POPG/Egg-PC=5:95 (w/w) with 0.1 mol% $DiIC_{18}$ added.

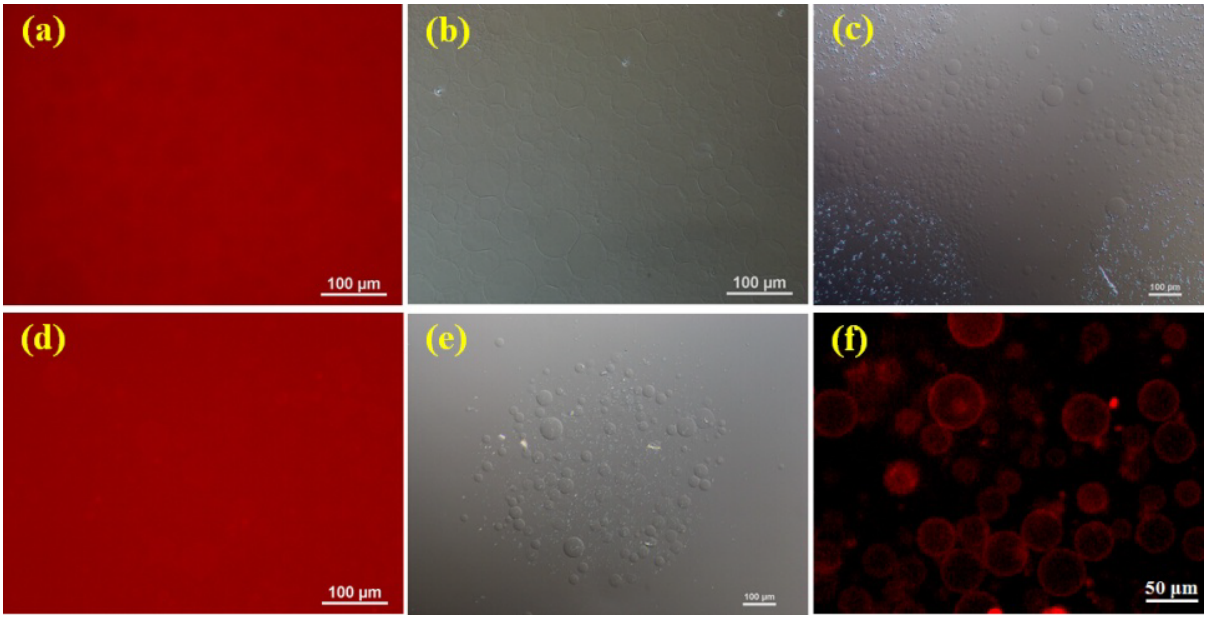


Figure S12. The encapsulation of DOX in GUVs by the capture/release of GUVs from the lysozyme-primed substrate. (a) and (b) represented the fluorescent (a) and DIC (b) images for the GUVs grown in a sucrose solution of DOX (25 µg/ml); (c) and (d) presented the DIC (c) and fluorescent (d) images for the sedimentation of the prepared GUVs on the lysozyme spots for 1 hr; (e) showed the DIC image for a typical capture of GUVs on a lysozyme spot; (f) presented the fluorescent image for the GUVs with DOX encapsulated inside after the capture-release cycle, and the red fluorescence emitted from DOX was only observed in the interior space of GUVs. Lysozyme, 5 mg/ml; POPG/Egg-PC=10:90 (w/w). No fluorescent dyes ($DiIC_{18}$ or $DiOC_{18}$) were added, and the glass substrate without the comb copolymer coating was used.

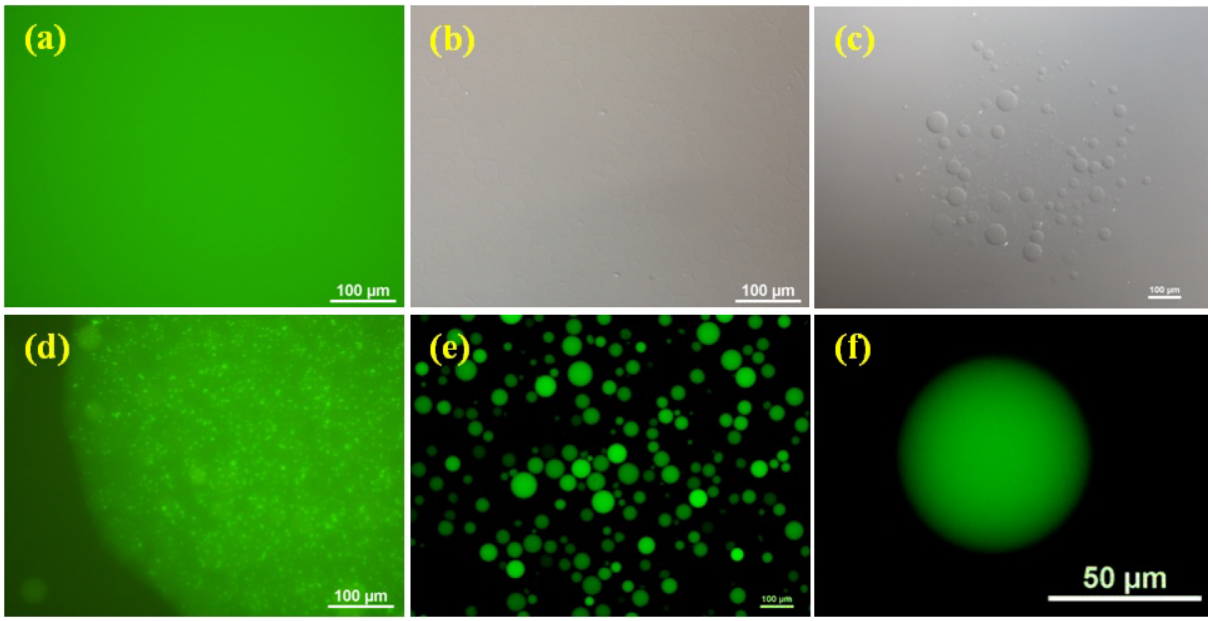


Figure S13. The encapsulation of 6-carboxyfluorescein in GUVs by the capture/release of GUVs from the lysozyme-primed substrate. (a) and (b) represented the fluorescent (a) and DIC (b) images for the GUVs grown in a sucrose solution of 6-carboxyfluorescein (10 μg/ml); (c) and (d) presented the DIC (c) and fluorescent (d) images for a typical capture of GUVs on a lysozyme spot, and in (d), due to the adsorption of 6-carboxyfluorescein on the lysozyme layer, the GUVs could not be told clearly; (e) and (f) showed the fluorescent image for the GUVs with 6-carboxyfluorescein encapsulated inside after the capture-release cycle, and the green fluorescence emitted from 6-carboxyfluorescein was only observed in the interior space of GUVs. Lysozyme, 5 mg/ml; POPG/Egg-PC=10:90 (w/w). No fluorescent dyes ($DiIC_{18}$ or $DiOC_{18}$) were added, and the glass substrate without the comb copolymer coating was used.

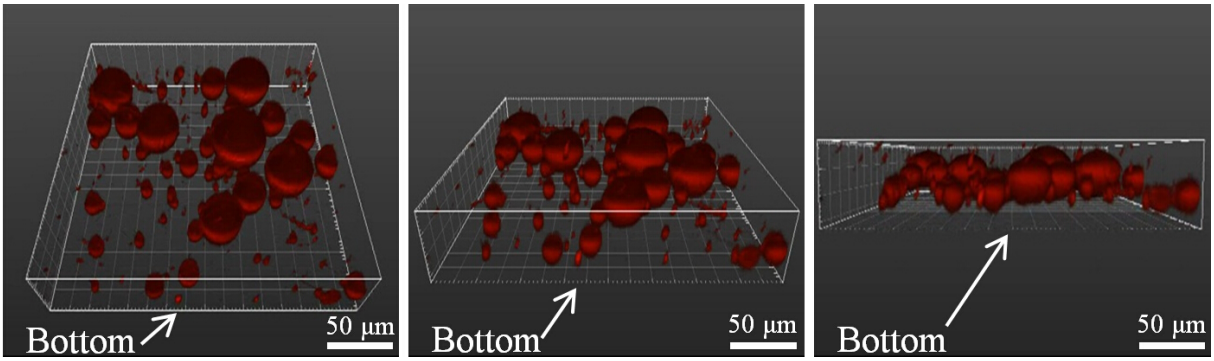


Figure S14. The z-stack confocal images of the GUVs. The GUVs in these images were the captured vesicles on the lysozyme spot after the solution exchange.

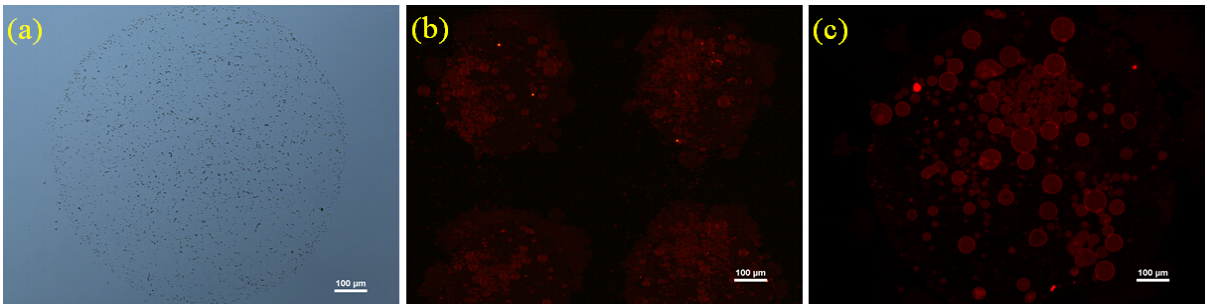


Figure S15. The capture of GUVs on the lysozyme-primed glass substrate without the non-fouling comb copolymer coating. (a) A typical optical image for the lysozyme spot; (b) and (c) represented the fluorescent images for the GUVs capture on the corresponding lysozyme spot. Lysozyme, 2 mg/ml; POPG/Egg-PC=5:95 (w/w) with 0.1 mol% $DiIC_{18}$ added.

References

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