Supporting Information for

Tuning the Mechanical Properties of Recombinant Protein-Stabilized Gas Bubbles Using Triblock Copolymers

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EXPERIMENTAL SECTION

Materials. Protein surfactant, oleosin-30G, is prepared using the recombinant protein technique as described below. PEO-PPO-PEO triblock copolymer surfactants have been generously provided by BASF and are used as received. To prepare gas bubbles, oleosin-30G and PEO-PPO-PEO are dissolved in phosphate-buffered saline (PBS) solution (pH 7.2, Sigma-Aldrich). The concentration of purified oleosin-30G is measured using a Nano-Drop 1000 (Thermo Scientific) and adjusted to 1 mg/ml (≈ 66 μM). To investigate the effect of molecular structures of the triblock copolymer surfactants, we use four types of PEO-PPO-PEO: $(PEO)_{78}$-$(PPO)_{30}$-$(PEO)_{78}$ (Pluronic F68, M_w = 8,400 g/mol), PEO_{13}-PPO_{30}-PEO_{13} (Pluronic L64, M_w = 2,900 g/mol), PEO_{37}-PPO_{30}-PEO_{37} (Pluronic P105, M_w = 6,500 g/mol), PEO_{51}-PPO_{31}-PEO_{51} (Pluronic F77, M_w = 6,600 g/mol). Each PEO-PPO-PEO surfactant is mixed with oleosin-30G 1 mg/ml solution to the molar ratio of 1:18 (oleosin-30G:PEO-PPO-PEO).

Oleosin-30G Expression and Purification. The Oleosin-30G gene is incorporated in the expression vector pBamUK, a pET series derivative constructed by the van Duyne Laboratory (SOM, Penn). The pBamUK vector adds a 6-histidine tag to the C-terminus for purification purposes, and has kanamycin resistance. Oleosin-30G is expressed in the E.coli strain BL21 DE3, controlled by the lac promoter. Bacteria cultures are grown in lysogeny broth (LB) with 50 μg/ml kanamycin until OD600 ≈ 0.7. Expression of Oleosin-30G is induced by the addition of isopropyl β-D-thio-galactoside (IPTG) at a final concentration of 1.0 mM. Cells are collected by centrifugation at 15,000g and stored at -20 °C before purification. B-Per protein extraction agent (Fisher Scientific) is used for protein purification. Oleosn-30G is expressed in inclusion bodies and therefore requires a

modified protocol from the manufacture instructions. Cell pellet is suspended in B-PER (30 ml B-PER/L of cell culture), and DNAse is added to a final concentration of 0.7 μg/ml. The mixture is centrifuged at 15,000g for 15 min, and the supernatant is discarded. The pellet is then suspended again in denaturing buffer (8 M urea, 50 mM phosphate buffer, 300 mM NaCl), and centrifuged at 15,000g for 15 min. Next, the supernatant is added to an equilibrated Ni-NTA column (Fisher Scientific) for > 1 h, and washed 3 times with denaturing wash buffer (denaturing buffer with 20 mM imidazole). Protein is left to refold in refolding buffer (50 mM phosphate buffer, 300 mM NaCl, and 5 vol. % glycerol) at 4 °C for > 1 h. The column is washed extensively with wash buffer (50 mM phosphate buffer, 300 mM NaCl, and 20 mM imidazole) and eluted with elution buffer (50 mM phosphate buffer, 300 mM NaCl, 200 mM imidazole). The eluted protein solution is dialyzed against 1xPBS buffer (Fisher Scientific). Purified protein is stored at 4 °C.

Preparation of gas bubbles using hole array. Micro-post arrays with different diameter (30, 50, 100 μm) are fabricated using conventional photolithography. Briefly, negative photoresist SU-8 2025 is spin-coated onto a clean Si wafer to a thickness of 40 μm and photopatterned by UV light through a film photomask (CAD/Art Service) using ABM 3000HR Mask Aligner. The photoresist pattern is then chemically treated with hexamethyldisilazane for an hour. For soft lithography, prepolymer of poly(dimethylsiloxane) (PDMS, Sylgard 184, Dow Corning) mixed with curing agent with weight ratio of 10:1 is degassed thoroughly and poured on the photoresist pattern, and then cured for 5 hr at 65 ℃ in convection oven. The PDMS replica hole array is peeled off from the Si wafer and used for bubble generation. Approximately 30 – 100 μL

surfactant solution containing oleosin and PEO-PPO-PEO surfactants is placed on the PDMS hole array. Vacuum is applied to the sample in a chamber for 1-2 min until gas bubbles are formed.

Micropipette Aspiration. Micropipettes are prepared using a micro-puller (David Kopf Instruments) and a microforge (Technical Products International INC.). The inner radius of micropipette is around 10 μm. Gas bubbles stored in the surfactant solution are placed on a cover glass and dispersed in a chamber. Negative pressure of approximately 80-100 cmH_2O is usually needed to grab a bubble, and the captured bubble is moved into the middle of the chamber for aspiration. We perform two aspirations with each bubble and 3-5 different bubbles are characterized for each set of conditions.

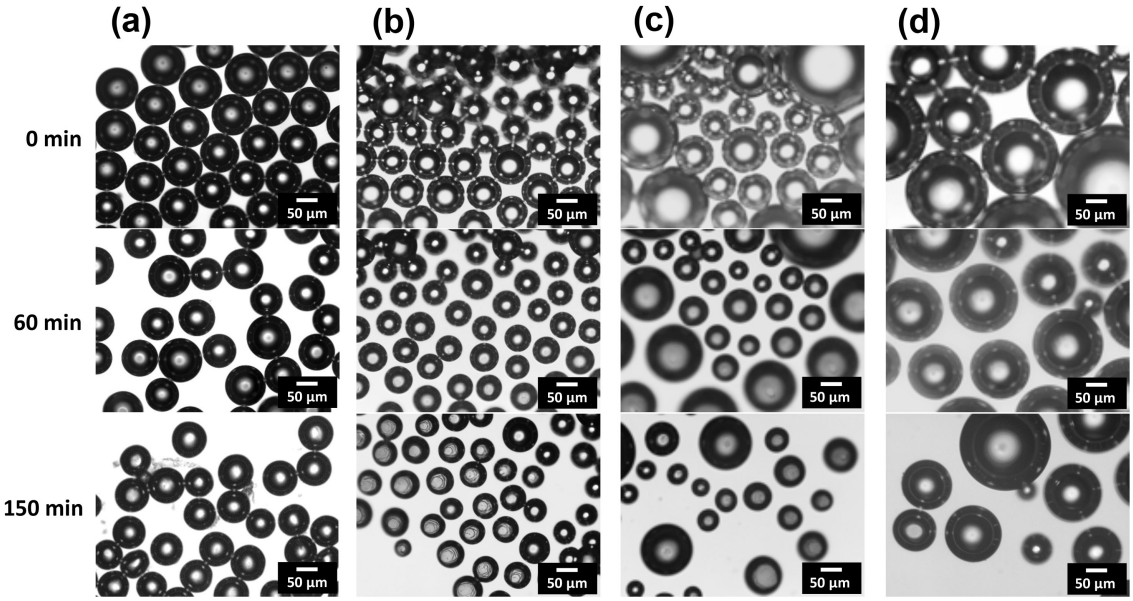

Figure S1. The effect of blend ratio of oleosin-30G and $(PEO)_{78}$-$(PPO)_{30}$-$(PEO)_{78}$ on the bubble stability (Oleosin-30G (mg/ml): $(PEO)_{78}$-$(PPO)_{30}$-$(PEO)_{78}$ (mg/ml) = (a) 1:0, (b) 0.7:0.3, (c) 0.5:0.5 and (d) 0.3:0.7)).

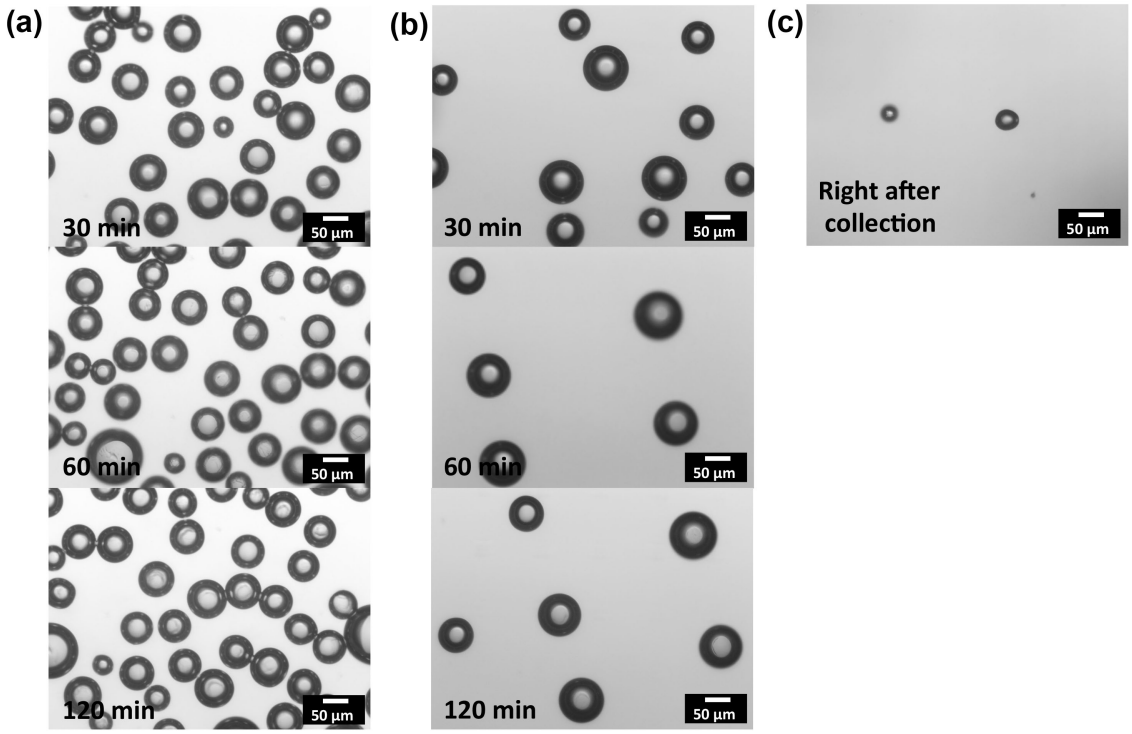

Figure S2. The effect of concentration of oleosin-30G ((a) 1 mg/ml ≈ 66.5 µM, (b) 0.75 mg/ml ≈ 50 µM and (c) 0.15 mg/ml ≈ 10 µM) on the bubble stability..

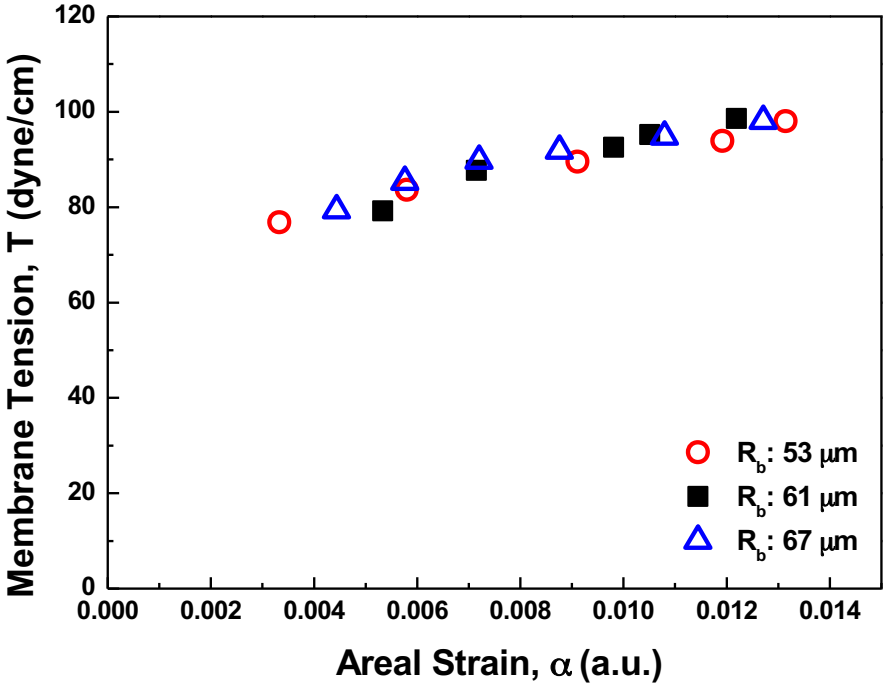

Figure S3. The effect of bubble sizes on the tension-strain curve. The bubbles with different radius (R_b = ○: 53 μm, ■: 61 μm, △: 67 μm) were aspirated with a micropipette (R_p = 10.5 μm).

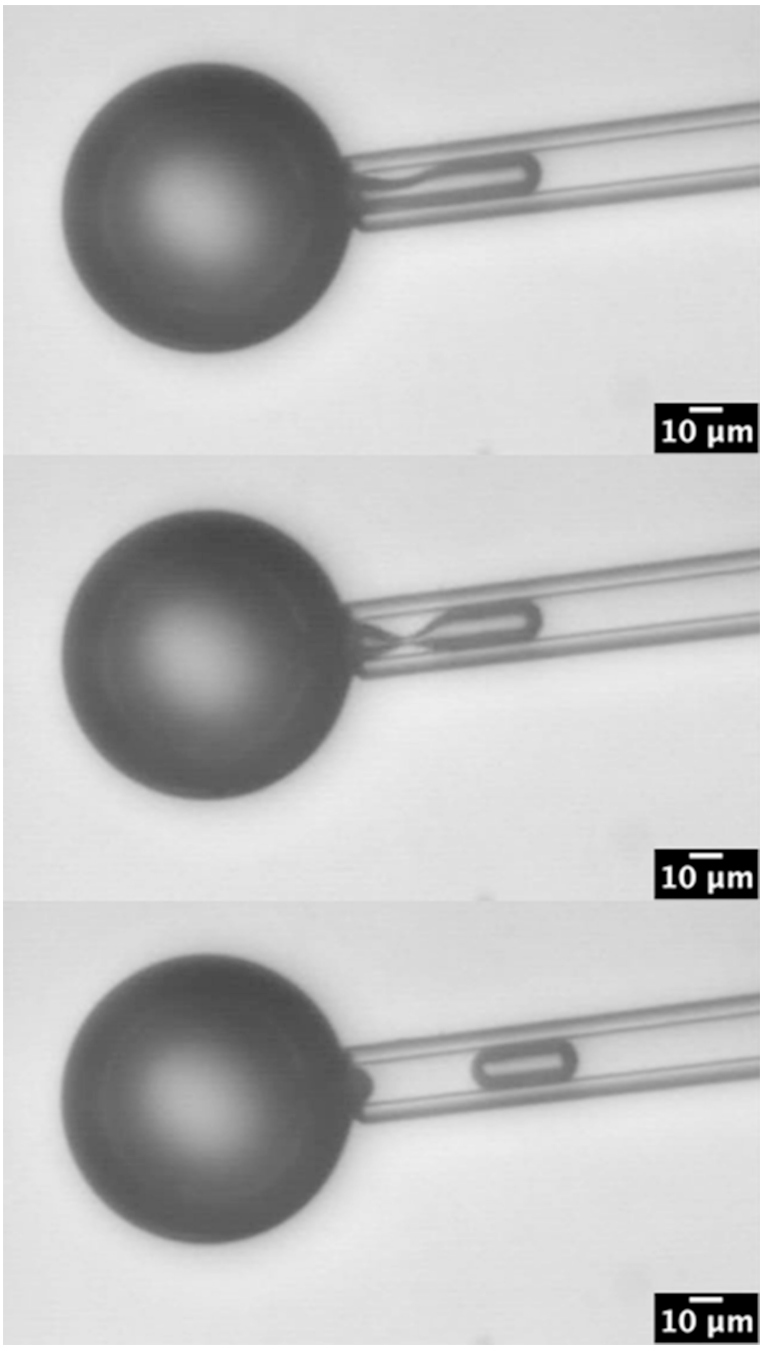

Figure S4. Optical images of budding behavior of a oleosin-30G-stabilized gas bubble during micropipette aspiration with a narrow micropipette (R_p < 7.5 µm) due to high bending stress. Therefore, we used a micropipette with radius around 10 µm in this study.

Table S1. Molecular weight and structure of PEO-PPO-PEO triblock copolymers used in present study, and the corresponding mechanical properties of oleosin-30G-stabilized gas bubbles blended with PEO-PPO-PEO.

Surfactant Types	Molecular Weight (g/mol)	Number of Both End Hydrophilic Units (x)	Number of Central Hydrophobic Units (y)	Mechanical Properties (dyne/cm)
Oleosin-30G	**15,022**	**42 (N-terminal), 63 (C-terminal)**	**30**	**1.8×10^3**
Blended with Different $(PEO)_x$-$(PPO)_y$-$(PEO)_x$ Triblock Copolymers	8,400	78, 78	30	3.0×10^3
	6,600	51, 51	31	1.4×10^3
	6,500	37, 37	56	1.0×10^3
	2,900	13, 13	30	0.8×10^3