Last Update: 22 March 2023

<u>Lipid Vesicle Synthesis (Dehydration-rehydration method ~200 nm)</u>

All lipid reagents were purchased from Avanti Polar Lipids. The lipids used were 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine (14:0 PE), 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sufonyl) (ammonium salt) (or 14:0 Liss Rhod PE) and the synthesized DNA-DSPE.

- 1. Dissolve the lipid mixture (14:0 PE, 16:0 PC and 14:0 Liss Rhod PE) in chloroform
- 2. Dissolve the synthesized DNA-lipid in $1 \times$ PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, KH₂PO₄, PH 7.4).
- 3. Mix the four components a molar ratio of 50:50:1:1.
- 4. Dry the mixed solution in a vacuum chamber to form lipid bilayers.
- 5. Heat 1 ml PBS solution to 90 °C, which is above the phase transition temperature of the mixed lipid components and add to the dry bilayer.
- 6. Keep the mixture at 90 °C with stirring at 500 rpm for an hour in dark.
- 7. Perform purification with a 30 kDa molecular weight cut off (MWCO) spin column at 5,000 g for 5 minutes.
- 8. Repeat step 7 for 6 times and resuspend the vesicles in 500 μ l 1× PBS at a concentration of ~10¹³/ml.

<u>Lipid Vesicle Synthesis (Reverse emulsion method)</u>

- 1. Mix DNA-lipid with dipalmitoylphosphatidylcholine (DMPC) at a molar ratio of 1:1000 in a glass vial.
- 2. Dry the solution in vacuum for 30 min to evaporate the solvent.
- 3. Resuspend the mixture with 600 µL of liquid paraffin.
- 4. Sonicate the solution at 50 °C for 3 h.
- 5. Mix and add 10 μ L of 10 nM DNA origami pores and 5 μ L of Exo III particles and 5 μ L TAEM buffer to adjust the volume of the mixture to 20 μ L.
- 6. Add the mixture to the liquid paraffin containing lipids and vortexed for 25 s to form aqueous droplets. After vortexing, the vesicle solution becomes blurred.
- 7. Pour 600 μL of this vesicle solution to 300 μL of TAEM buffer and centrifuge for 15 min at 8000g.
- 8. Remove the supernatant. The giant vesicles including DNA strands, origami pores, and Exo III particles are in the precipitates.