Lipid Vesicle Synthesis (Dehydration-rehydration method ~200 nm)

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-phosphocholine (16:0 PC), 1,2-dimyristoyl-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× PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, KH_2PO_4, 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 μl 1× PBS at a concentration of ~10^{13} /ml.

Lipid Vesicle Synthesis (Reverse emulsion method)

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 μL of 10 nM DNA origami pores and 5 μL of Exo III particles and 5 μL TAEM buffer to adjust the volume of the mixture to 20 μL.

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.