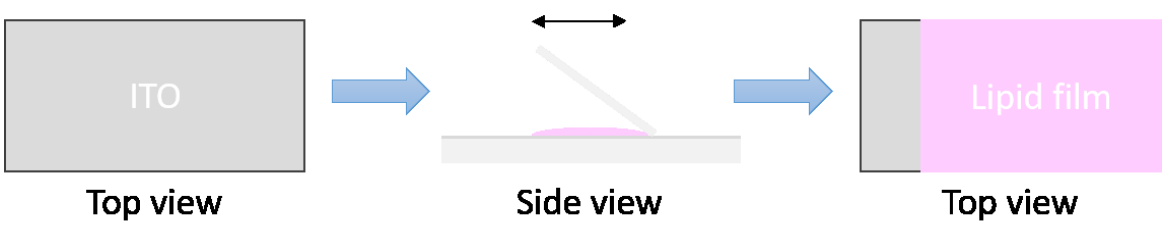
Electroformation of Giant Unilamellar Vesicles (GUVs)

Materials & Reagents

- Indium-tin-oxide slides
- Lipid mixture (DOPC + TexasRed-DHPE at 0.8% molar ratio) prepared mixing (with solvent safe tips) in a clean glass (amber coloured) vial
    - 171 µL of DOPC lipids (from a stock at 25 mg/mL)
    - 20 µL of TexasRed-DHPE lipids (from a stock at 3 mg/mL)
    - 892.75 µL of chloroform
- Sucrose solution (300 mM)
- PDMS Spacer (~1 mm thick)
- Glass coverslips (24×24mm)
- Voltimeter
- Solvent safe tips
- Gel-loading tips
- Silica dessicator
- Function generator
- Crocodile clips
- Paper clips

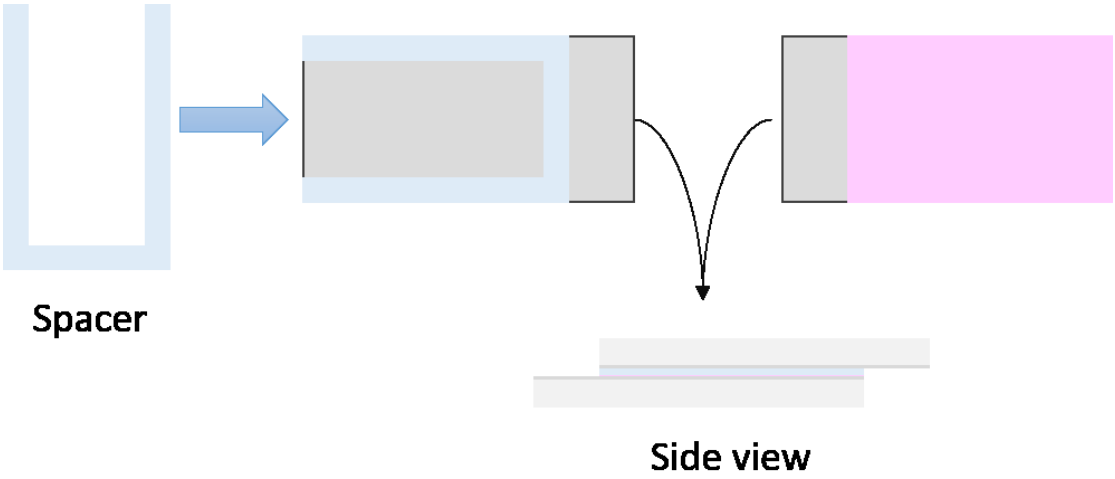
a. Cleaning ITO slides
    1. Sonicate in Hellmanex III solution (2%) for 15 min
    2. Wash twice with MilliQ water
    3. Sonicate in isopropanol for 15 min
    4. Wash twice with MilliQ water
    5. Sonicate in MilliQ water for 15 min
    6. Dry slides using Nitrogen flow
    7. Determine the conductive side with a voltimeter

b. Lipid film spreading
    1. Bring the lipid mixture to room temperature (without opening the vial)
    2. Vortex thoroughly the lipid mixture
    3. Pipette 45 µL of the lipid mixture on the conductive side of the ITO slide
    4. Using a glass coverslip (24mmx24mm) at an angle of ~45°, spread a lipid film on the conductive side of the ITO slide covering almost the entire area of the slide (as shown in the schematic). Do so multiple times without lifting the coverslip until the solvent has evaporated.

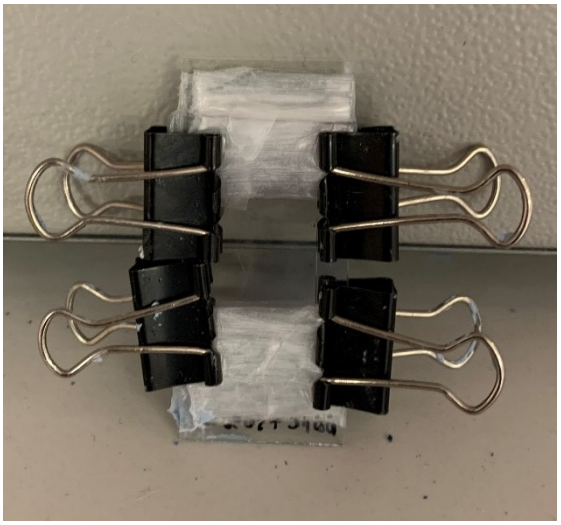


5. Place the slide in the silica dessicator, and apply vaccum with a pump for >20 seconds, then close the valve and leave under vacuum for an hour (in a dark place).

c. Electroformation chamber assembly and GUV preparation
   1. Stick the U-shaped PDMS spacer to the non-lipid ITO slide. Make the U-end match the edge of the slide.
   2. Press on the spacer to make sure its sticking properly to the slide.
   3. Stick to the other lipid-covered ITO slide sandwiching the spacer, as shown in the schematic. Leave two ends on opposite sides (big enough to attach the crocodile clips).



   4. Put paper clips on the sides to make sure the chamber is firmly sealed.
   5. Degas around 600 µL of sucrose buffer. (You can use a syringe for this – grab the volume, create vacuum with your finger and pulling the plunger, and hitting the syringe with a pen or something similar to start creating bubbles. Then, release your finger. Doing this three or four times gets rid of most of the gas).
   6. Fill the chamber with degassed buffer, making sure you do not leave any bubbles inside. Put parafilm on both ends to help prevent leakage and evaporation. From this point onwards, make sure the chamber is always kept in an upright position. (See photo)



7. Connect the crocodile clips to the free ends of the chamber.
8. Apply sinusoidal function, 2V peak-to-peak amplitude: 10 Hz for 2 hours (growth stage) and 2 Hz for 1 hour (detachment)

d. Retrieval
   1. Right after turning the generator off, use gel-loading pipette tips to gently retrieve the volume inside the chamber. Be careful not to create any bubbles when pipetting.
   2. Store in an Eppendorf tube (room temperature is fine, in a dark place). Use before 7 days.