Last Update: April 10, 2023

VESICLE SYNTHESIS

CONTENTS

Apparatus Required

1. Lipids
    1. DMPC
    2. DPPC
    3. 14:0 PE, 16:0 PC, 14:0 Liss Rhod PE
2. Glass vials
3. Chloroform
4. Mineral Oil
5. Piranha glass coverslip
6. Adhesive sheet with slots (9 mm)
7. Magnetic Stir bars

SUV Synthesis

Lipid Vesicle Synthesis (Dehydration-rehydration method) – for SUV synthesis (~200nm)

- 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.

STEP 1

- Dissolve the lipid mixture (14:0 PE, 16:0 PC and 14:0 Liss Rhod PE) in chloroform.
- Chloroform can evaporate at room temperature conditions.
- Speed the experiment accordingly.
- Use glass vials for this preparation and not Eppendorf tubes.

STEP 2

- Dissolve the synthesized DNA-lipid in 1X PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, KH2PO4, PH 7.4).

STEP 3

- Mix the four components in a molar ratio of 50:50:1:1.
- This molar ratio is for 14 PE : 16 PC : 14 Liss Rhod PE : DNA-lipid
- Use the final concentration found from absorbance measurements on the prepared DNA-lipid conjugate.

STEP 4

- Dry the mixed solution in a vacuum chamber to form lipid bilayers.
- Use the vacuum desiccator for this step for 20 minutes or until the solution is dry.

Glass vial covered with parafilm

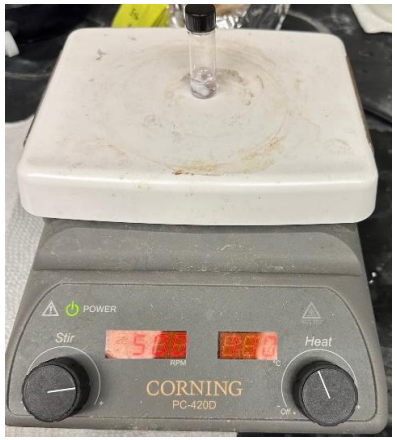
STEP 5

- 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.

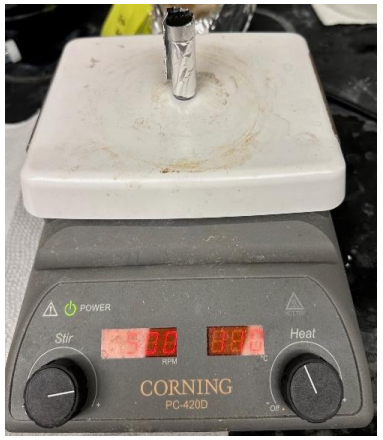
Magnetic stirrers of different sizes. Use the rightmost.

STEP 6

- Keep the mixture at 90 °C while stirring at 500 rpm for an hour in dark.
- Use the small magnetic stirrer and cover the glass vial with foil.



Glass vial on the heating plate



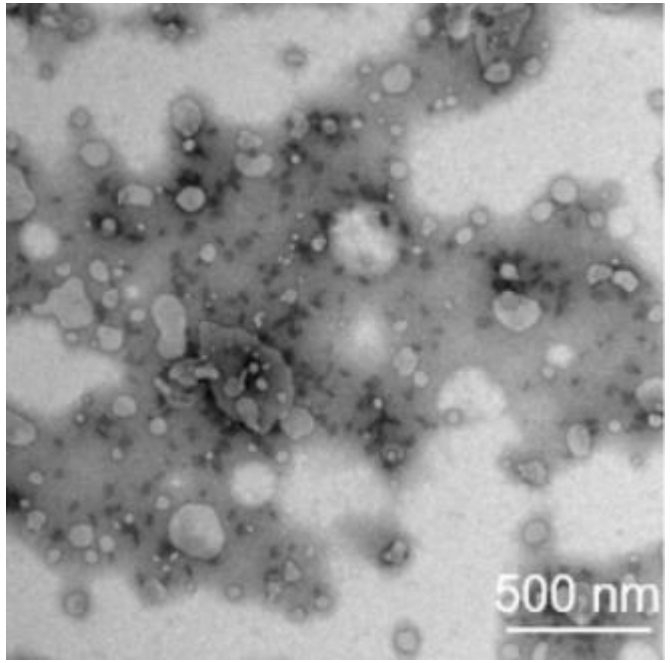
Glass vial on the heating plate (wrapped with foil)

STEP 7

- Perform purification with a 30 kDa molecular weight cut off (MWCO) spin column at 5,000 g for 5 minutes.
- This step involves centrifugation using filter tubes of MWCO 30kDA.

STEP 8

- Repeat step 7 for 6 times and resuspend the vesicles in 500 μL 1X PBS at a concentration of ~1013 /ml.



TEM images of synthetic vesicles. The negative stained vesicles appear in round shapes.

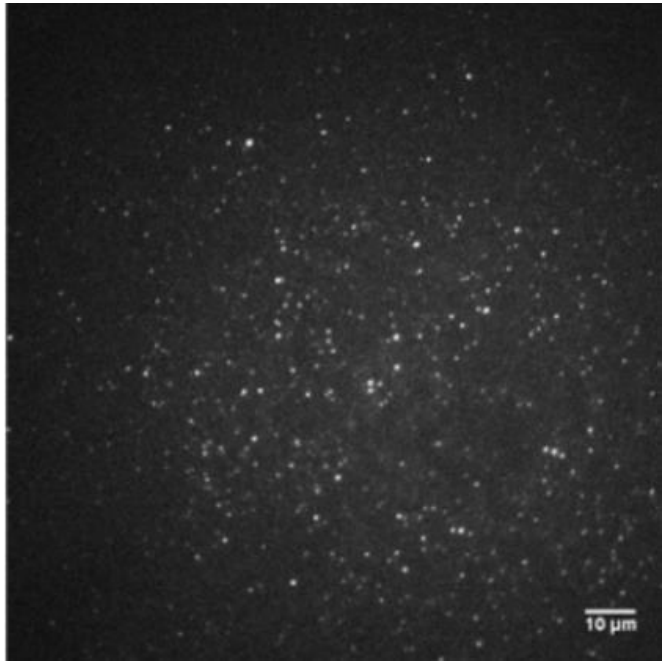
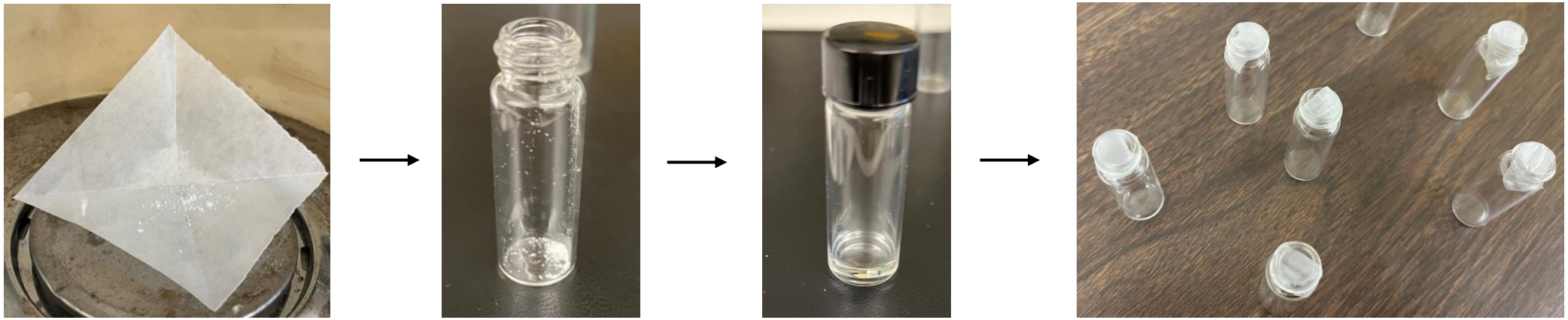


Image of fluorescent vesicles with complementary strands binding to the fuel surface

GUV Synthesis

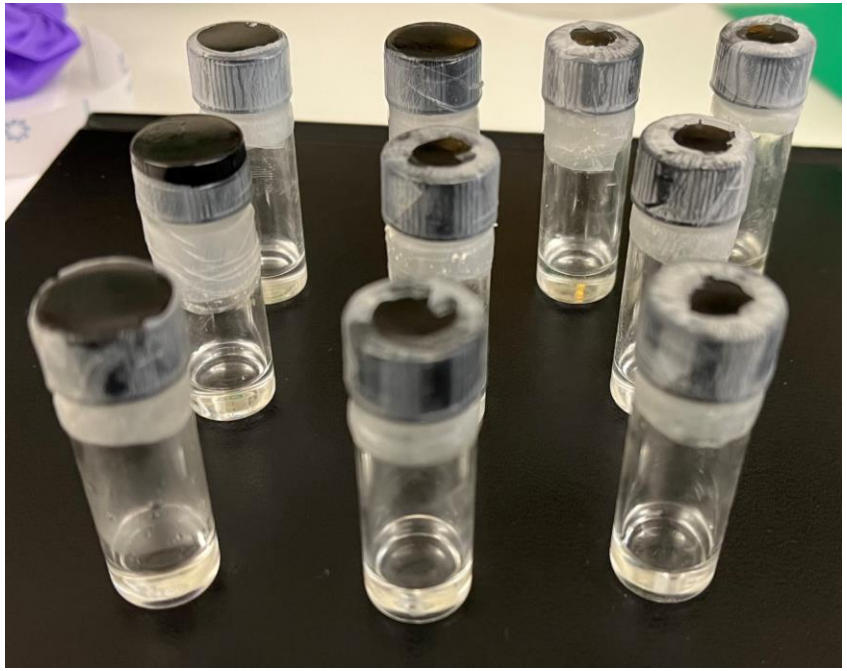
STEP 1:

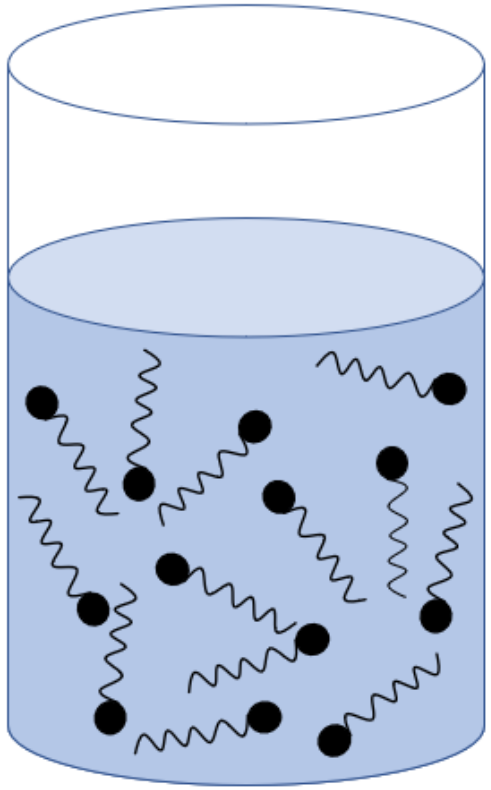
- Measure 4 mg of DMPC and introduce into a glass vial.
- Add 400 µL of Chloroform in the glass vial to make concentration of 10 mg/ml .
- Let the lipids be dissolved in chloroform and distribute into glass vials each containing 40 µL in each.
- Use paraffin tape to partially seal the glass vials
- Put all the glass vials in the vacuum chamber and let the chloroform evaporate in about 20 min



STEP 2:

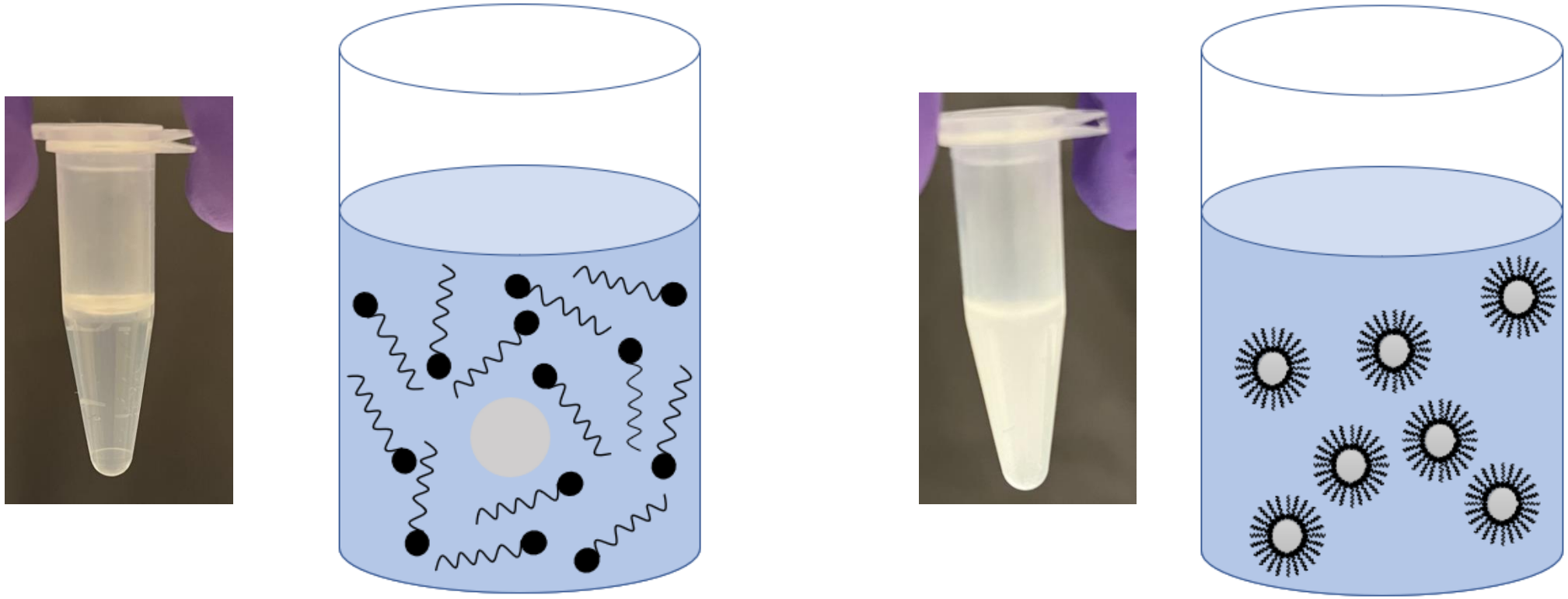
- Add 600 µL of mineral oil into each of the glass vial.
- Close the vial with lid and tight seal it with paraffin tape.
- Bath sonicate all the vials for 3 hr. at a temperature of 50^0 C.
- Now all the lipids that are adsorbed on the walls of glass vial are completely dispersed into the oil solution.





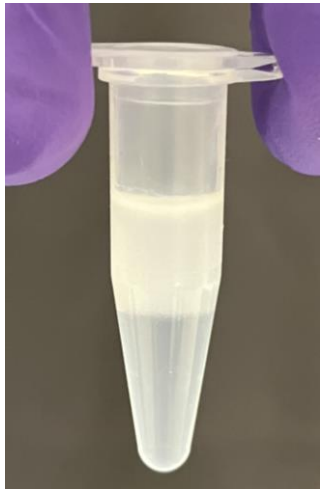
STEP 3:

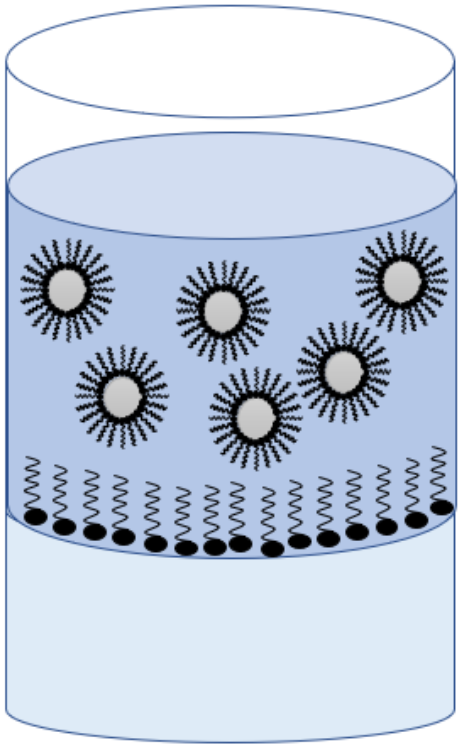
- Transfer the lipid dispersed oil into Eppendorf tube.
- Add the required aqueous solution/product you desire to trap into the GUV.
- Vortex the solution for 25 sec. This makes the giant aqueous bubble split into small microdroplets that will be immediately surrounded by the lipids in the solution because of hydrophilicity

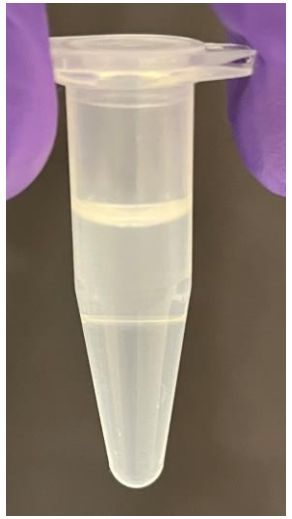


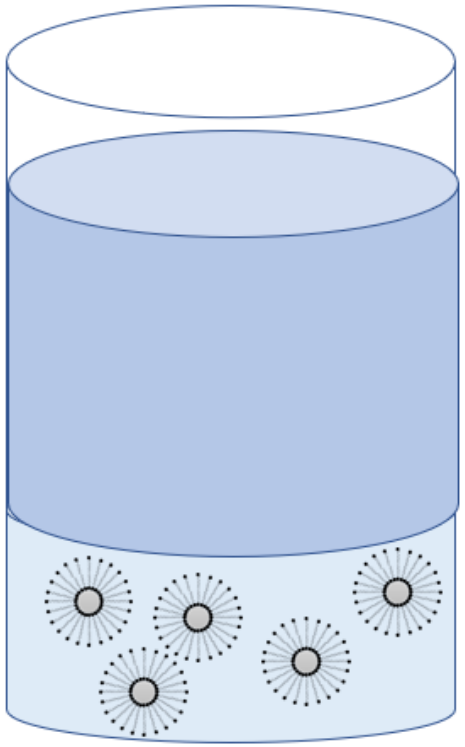
STEP 4:

- Take another Eppendorf and introduce 300 μL of buffer solution you want the GUV to be dispersed in.
- Take the vortexed solution and slowly flow it above the buffer to make a separation.
- Because of the hydrophilic interface free lipids in the solution form a layer at interface.
- Put the Eppendorf's in refrigerator for 5 min for better quality of GUV being synthesized
- Centrifuge the Eppendorf's at 8000g for 15 min. This make all the monolayer lipid vesicles pass through interface and become a bilayer GUV.



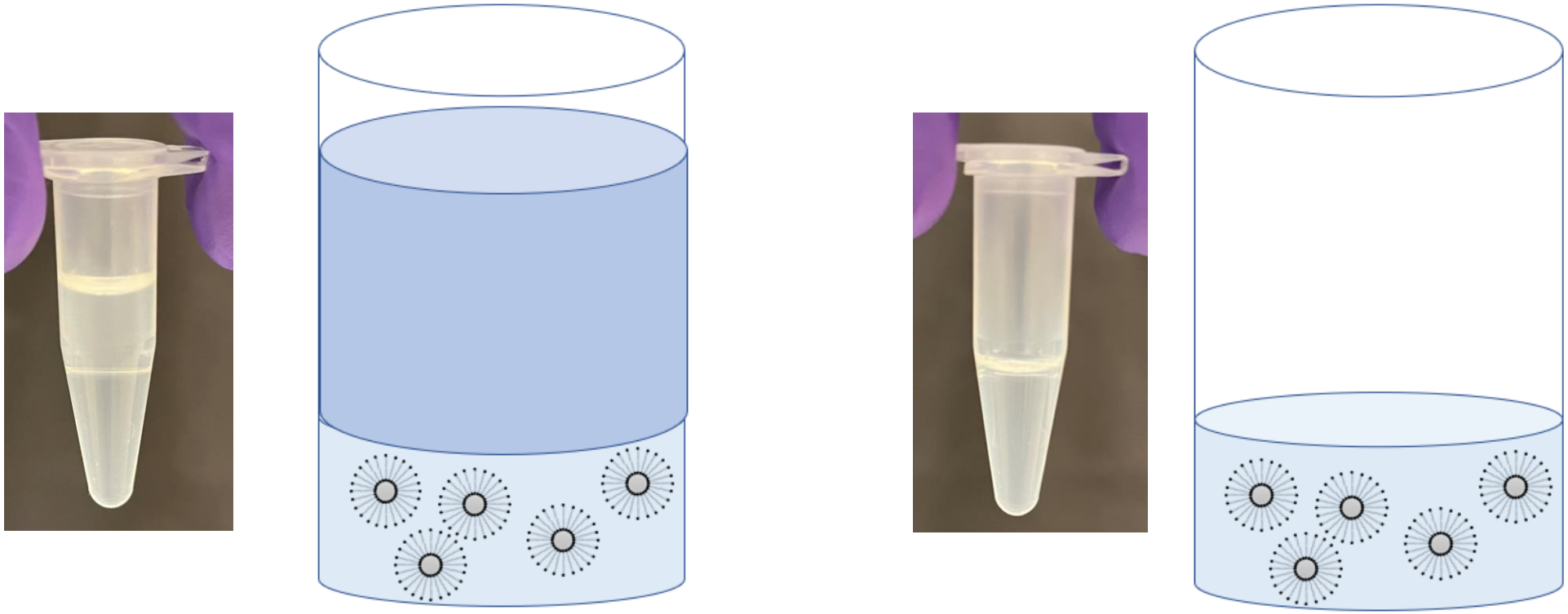






STEP 5:

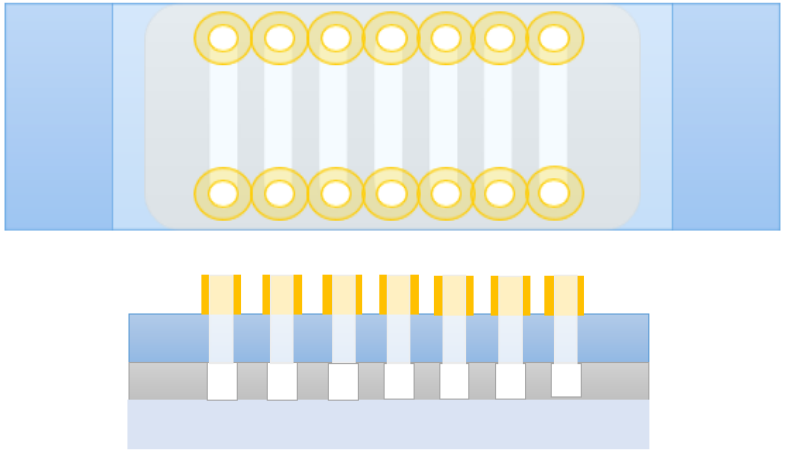
- Remove the supernatant and the buffer containing GUV with entrapped materials are dispersed at bottom.
- On average vesicles of size 5-20 μm are synthesized.

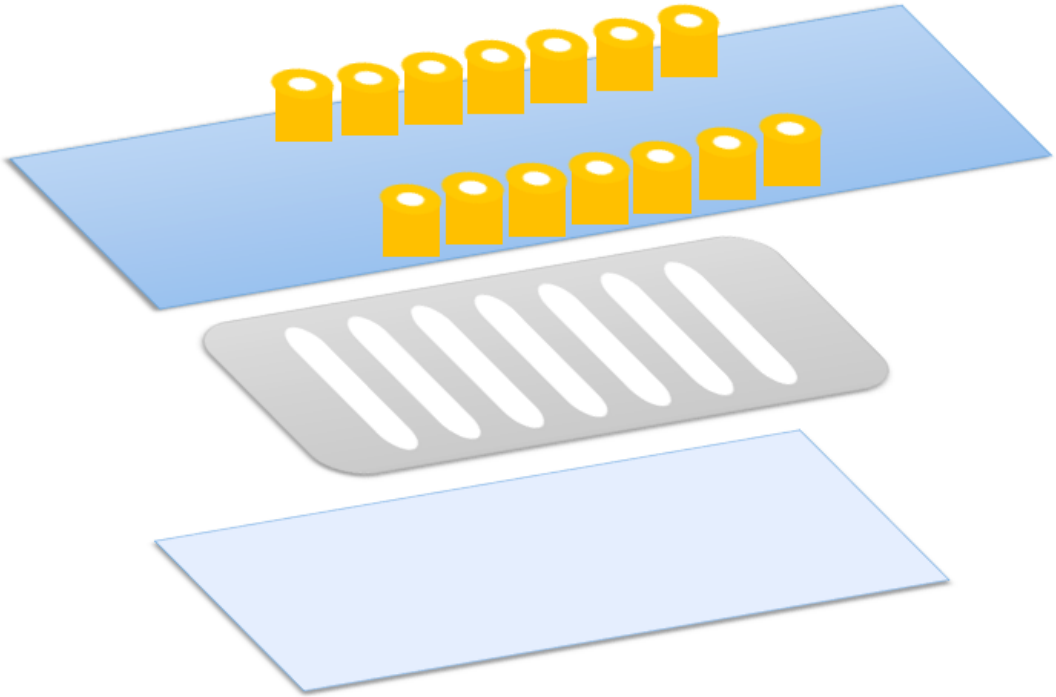


Visualization in Microchannel

Microchannel Sandwich Assembly:

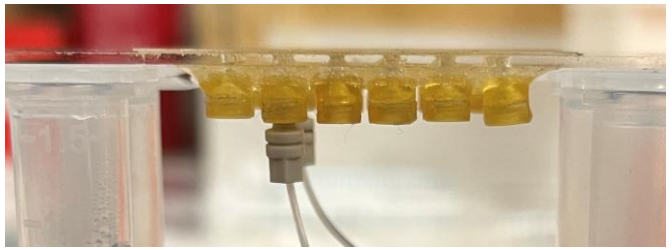
- A thick glass slide with inlet and outlet channels has been made prior.
- Take an adhesive sheet (9mm) which has been cut slots to make a channel when stick to the glass coverslip.
- Paste it onto the glass channel holder so that all the inlet and outlet holes align with the slots on sheet.
- Place the piranha washed glass coverslip onto it and make a sandwich of the 3 components.
- Make sure the boundaries of slot are well stick to the glass coverslip.

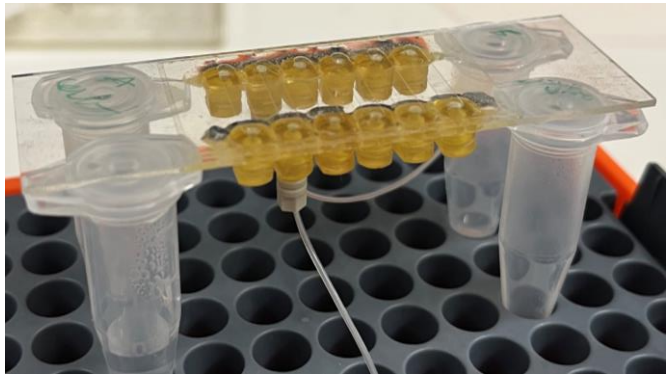


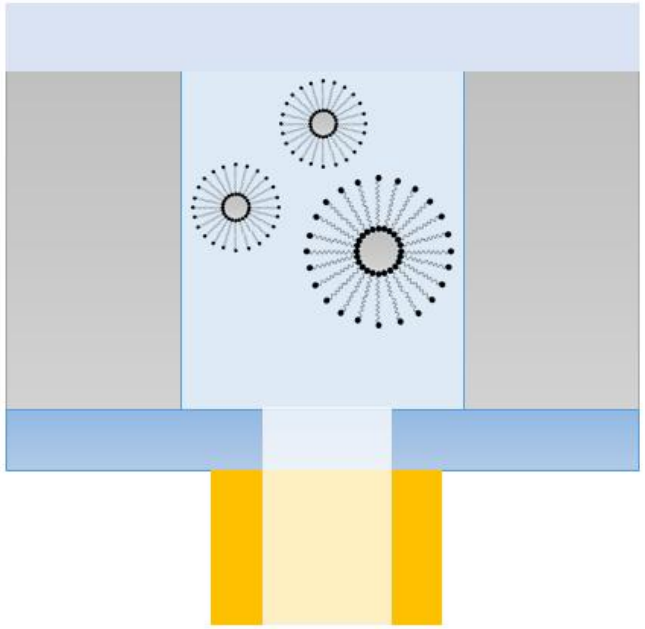


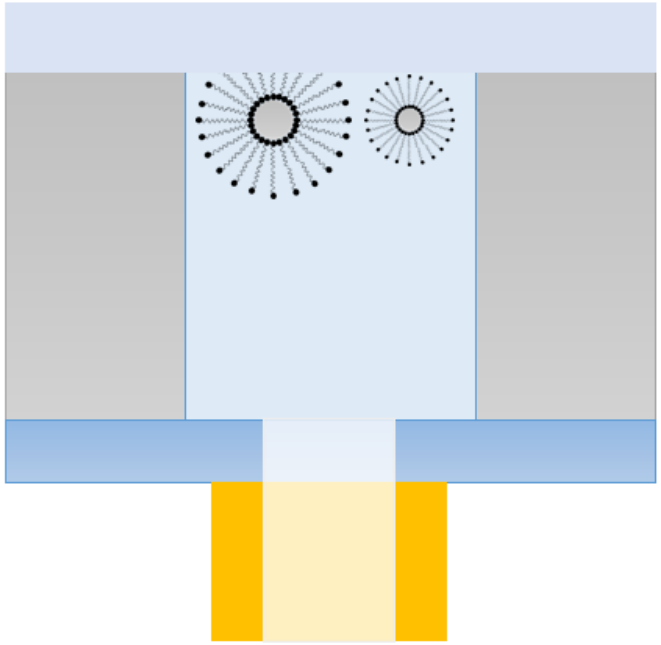
Flowing GUV solution into Microchannel:

- Use the microtubes to connect both ends of channel. One goes to the syringe, and one ends to the Eppendorf containing GUV solution. Use the syringe to suck the generate pressure for the flow.
- Before flowing GUV solution it is recommended to wash the channel by flowing buffer first.
- While flowing GUV solution the glass coverslip should face up, so that GUV floats because of less density and binds non-specifically to it. Leave the setup undisturbed for 30 min.
- Then, proceed to the microscope for imaging.



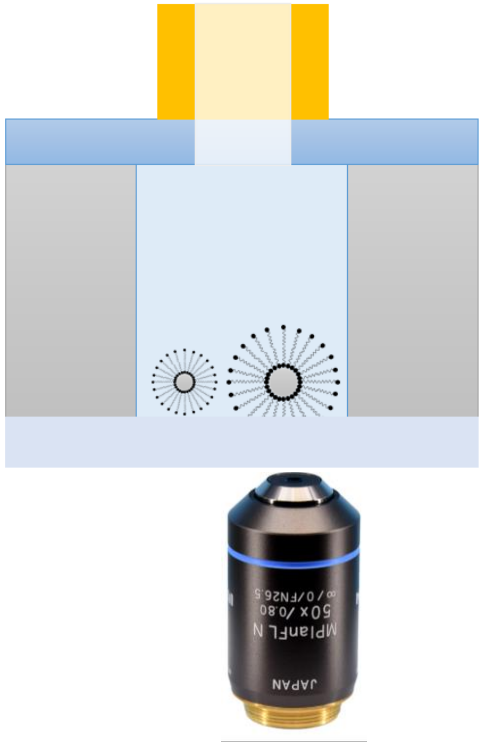


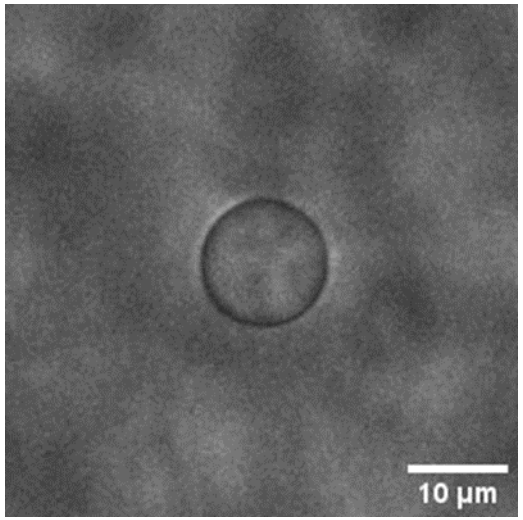




Observing under Microscope:

- When using the inverted microscope set the CCD temperature to -90, andor gain to 40 and let it stabilize.
- By changing objective height try to focus onto the boundary of channel and then move into channel.
- Once you feel you are focusing in the channel, search for GUV and capture their image.





Notes

References:

1) Jing Pan, Yancheng Du, Hengming Qiu, Luke R. Upton, Feiran Li, and Jong Hyun Choi, Nano Letters 2019 19 (12), 9138-9144, DOI: 10.1021/acs.nanolett.9b04428

2) Hengming Qiu, Feiran Li, Yancheng Du, Ruixin Li, Ji Yeon Hyun, Sei Young Lee, and Jong Hyun Choi, ACS Synthetic Biology 2021 10 (6), 1268-1276, DOI: 10.1021/acssynbio.0c00550