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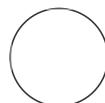
Protocol status: Working
We use this protocol and it's working

Vesicle Fusion on SiO₂ Substrates V.2

Nicole Voce¹, Paul Stevenson¹

¹Northeastern University

Stevenson group



Nicole Voce
Northeastern University

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ABSTRACT

This protocol outlines the steps to produce large area, uniform supported lipid bilayers on SiO₂ substrates with or without patterned features.

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Keywords: supported lipid bilayer, membranes, lipid bilayer

Substrate cleaning

- 1
 - Clean plain SiO₂ or TiO₂-patterned substrates by sonicating them in a 1:1 IPA:Acetone mixture for ~2 minutes
 - Dry with a steady stream of N₂
- 2 Oxygen plasma clean the substrates for 2 minutes to remove any residual surface contamination

Note

For our Anatech SP-100 system, the RF power is set to 120 watts, the O₂ flow rate to 15 ccm, and the pressure to 0.35-0.40 torr

Vesicle prep

- 3 Prepare a 1.2 mg/mL DLPC 0.5mol% Rho-PE solution (suitable for FRAP measurements and general fluorescence imaging) or a 0.3mg/mL DLPC 0.0025mol% Rho-PE solution (suitable for FCS measurements)

3.1 To prepare 1.2 (0.3) mg/mL DLPC 0.5 (0.0025) mol% Rho-PE solutions:

- Measure out appropriate amount of DLPC (stored as powder) into a glass vial

Note

6 mg of DLPC for FRAP concentration; 1.55 mg of DLPC for FCS concentration

- Add appropriate amount of Rho-PE (stored as 1 mg/mL in chloroform) to DLPC

Note

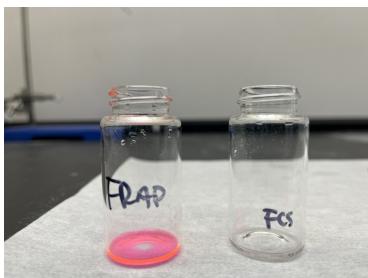
60.6 μL of 1 mg/mL Rho-PE for FRAP concentration; 0.0781 μL of 1 mg/mL Rho-PE for FCS concentration

mol % = mols Rho-PE/(mols Rho-PE + mols DLPC)

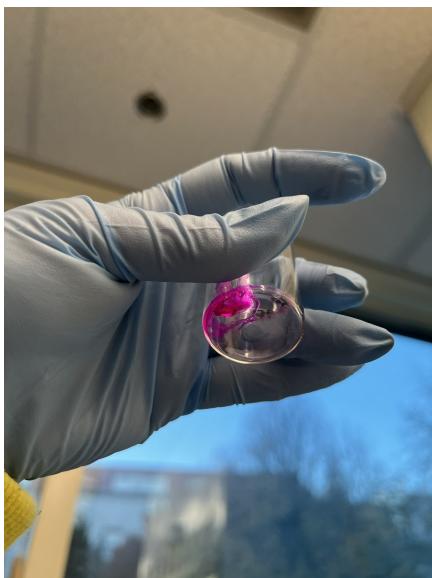
For 1.2 mg/mL DLPC 0.5mol%:

$$\blacksquare 0.005 = x/(x + 0.006\text{g}/621.85\text{g/mol})$$

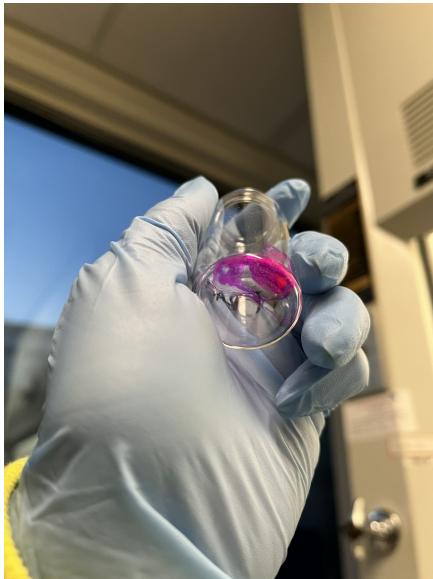
- Add 100-200 μL chloroform to vial



- Dry with N_2 so the solution doesn't look glassy-- it should have a dull appearance



Glassy: FRAP solution before N_2 dry



Past glassy: FRAP solution after N₂ dry

- Leave in vacuum desiccator overnight to fully remove residual chloroform.

3.2 After drying, re-hydrate solution with phosphate buffered saline to achieve desired concentrations of 1.2 mg/mL or 0.3 mg/mL

Note

After adding phosphate buffered saline to the solution, shake or sonicate the glass vial(s) for ~30 seconds or until the dried DLPC/Rho-PE have been fully dissolved



Re-hydrated FRAP solution



Re-hydrated FCS solution

- 4 Extrude 1.2 (0.3) mg/mL DLPC 0.5 (0.0025) mol% Rho-PE solution 21 times

Note

Equipment

Mini Extruder

NAME

Avanti Polar Lipids

BRAND

610000-1EA

SKU

<https://avantilipids.com/product/610000>

LINK

Equipment

19mm Nuclepore™ Polycarbonate Track-Etched Membranes

Whatman

NAME

10419504

BRAND

<https://scientificfilters.com/whatman/membranes-track-etched-polycarbonate-800310>

SKU

LINK

Equipment

10mm Filter Supports

Whatman

NAME

610014-1EA

BRAND

<https://avantilipids.com/product/610014>

SKU

LINK

To prepare the Mini Extruder, place filter supports in the middle of the rubber o-rings on the plastic cylinders. Deposit ~15 µL phosphate buffered saline on each support and use the pipette tip to move buffer around so each support is fully wet. Place the 100nm filter membrane on one of the supports, making sure it's secure with the phosphate buffered saline. Place the cylinder with the filter 3/4 of the way into the metal cylinder, place the other cylinder on top, sandwiching the membrane filter between the filter supports. Allow the cylinders to rest fully in the metal cylinder, then screw on the metal nut. Use one of the extruder syringes to measure out at least 300 µL of 1.2 (0.3) mg/mL DLPC 0.5 (0.0025) mol% Rho-PE solution and stick the syringe into one end of the just-assembled filter apparatus. Stick the other extruder syringe into the other end of the filter apparatus. Push the DLPC solution back and forth 21 times, ending in the syringe that was empty to begin with. Note that some solution will be lost during the extrusion process.



FRAP solution before extrusion



FRAP solution after extrusion



FCS solution before extrusion



FCS solution after extrusion

- 5 After extrusion, aliquot the solution into Eppendorf tubes and store at -20°C until ready to use

Bilayer formation

6

Equipment

Imaging Chamber

NAME

CoverWell

BRAND

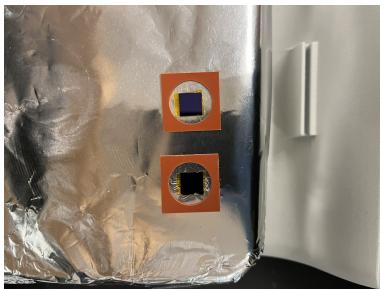
635011

SKU

<https://gracebio.com/product/coverwell-imaging-chambers-635011/>

LINK

- Set hotplate to 30°C
- Use double-sided kapton tape to adhere a substrate to the bottom of an imaging chamber



Substrates adhered to imaging chambers

- 7
 - Place the substrate on the hotplate and deposit 30 μL of 1.2 (0.3) mg/mL DLPC 0.5 (0.0025) mol% Rho-PE solution and 90 μL phosphate buffered saline onto the substrate
 - Cover the substrate with a pipette box top or something else to protect from dust
 - Let the substrate incubate for 10 minutes



TiO₂-patterned substrates with DLPC solution and phosphate buffered saline

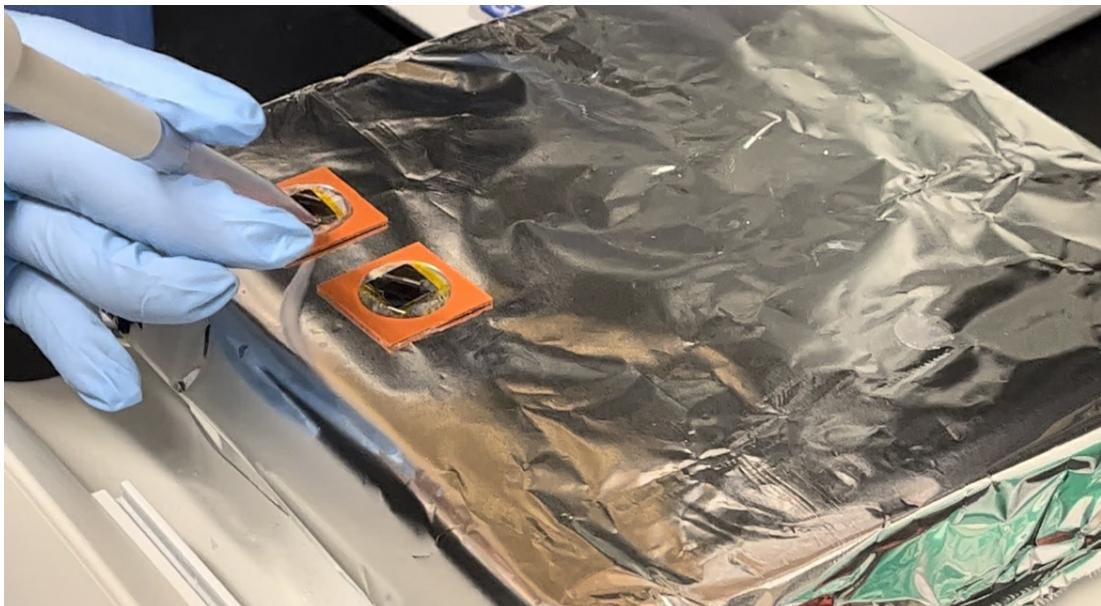
- 8 After 10 minutes of incubation, deposit 700 μL of phosphate buffered saline onto the substrate, flooding the imaging chamber



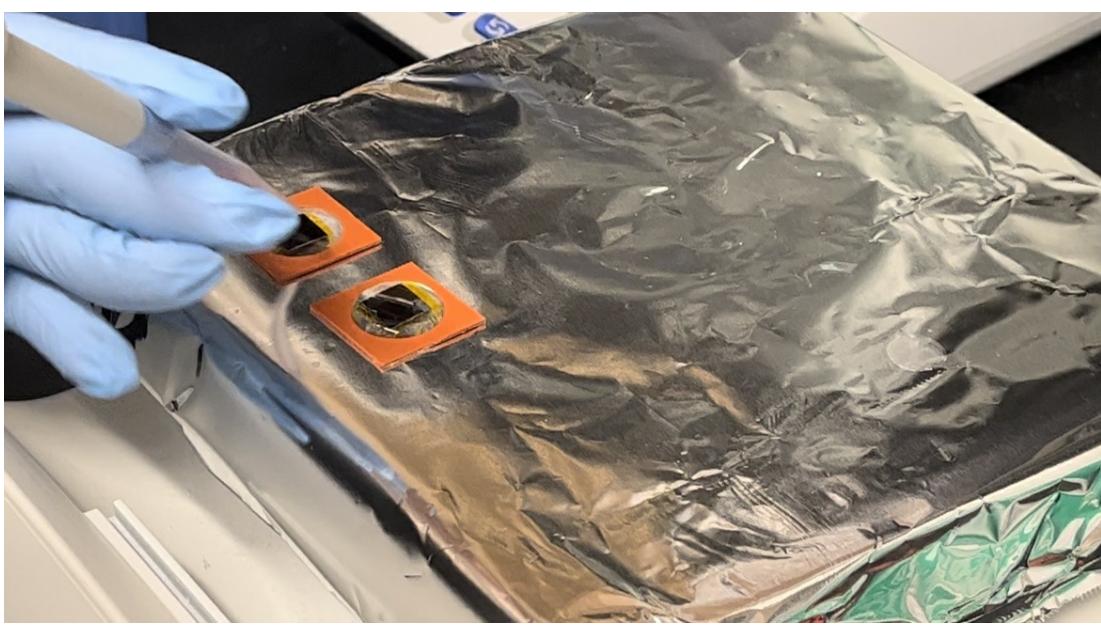
TiO₂-patterned substrates after flooding the imaging chamber

- 9
- Wash the substrate by pipetting 500 μL off and then depositing it back on, moving around the substrate
 - Repeat 15 times

Note



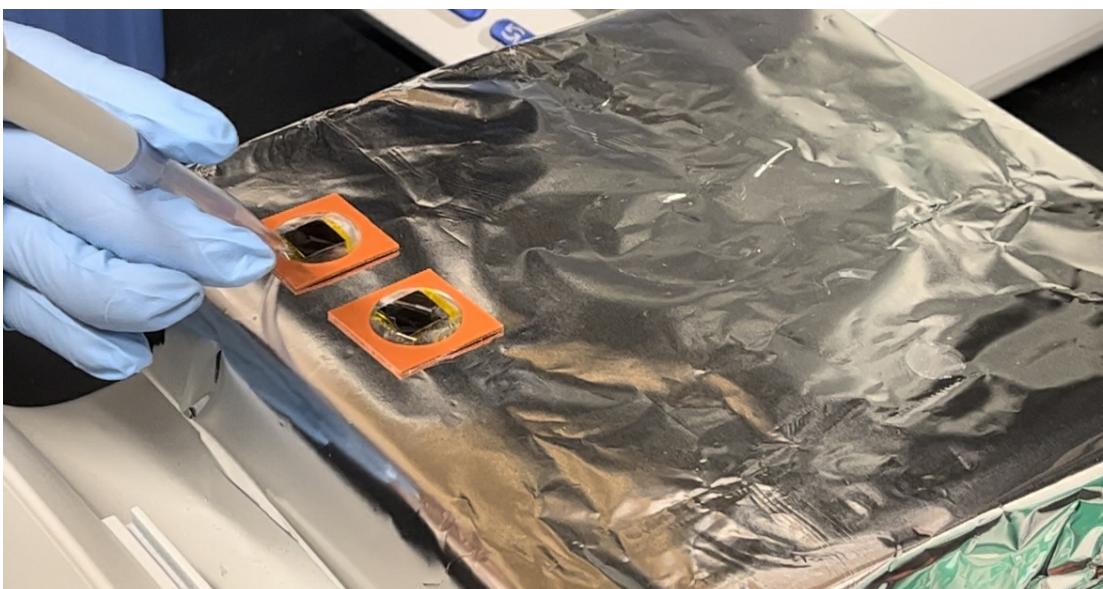
Take 500 μL of the solution off



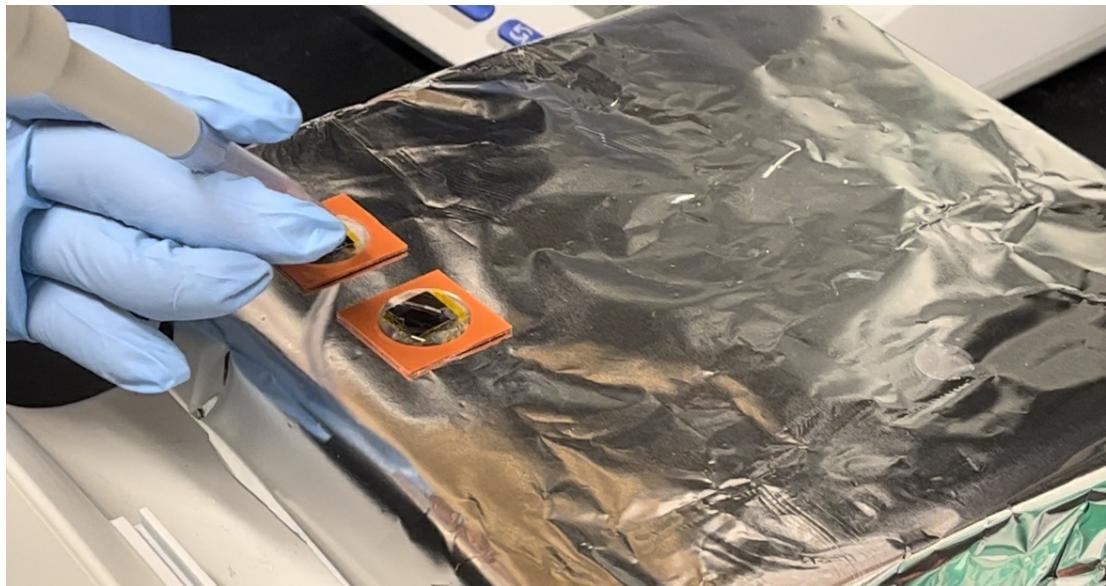
Move to a different side of the substrate



Deposit the 500 µL of solution back into the imaging chamber



Move to a different side of the substrate and take 500 µL of solution off



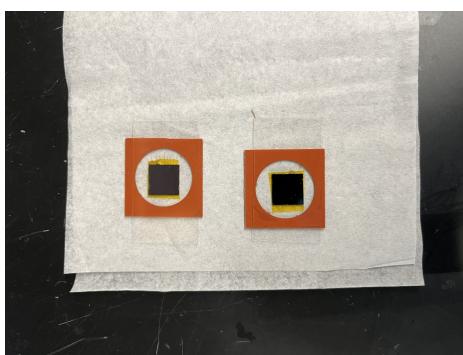
Move to a different side of the substrate and deposit the 500 µL of solution back into the imaging chamber

10 After 15 washing steps, take 500 µL off and depose of it

11 Roll a coverslip over the imaging chamber

Note

For FCS measurements, coat the sides of the coverslip with clear nail polish to seal it to the imaging chamber



Substrates with coverslip

- 12 Image the samples to confirm bilayer formation. A representative image is shown below: black circles are TiO₂ features deposited on the surface to inhibit bilayer formation (scale bar is 100um)

