PREPARATION OF SUPPORTED LIPID BILAYERS

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BACKGROUND

- This protocol details how supported lipid bilayer (SLB) is prepared via small unilamellar vesicle fusion.
- SLB is lipid bilayer formed on planar surfaces, which are required to be hydrophilic and smooth, such as glass, quartz and mica. It is a simplified experimental model for studying biomembrane properties and functions, as it retains a number of the structural and dynamic properties of cellular membranes.
- The adsorption and fusion of small unilamellar vesicles (SUVs) is one of the most convenient methods for preparation of SLBs. Spreading of SUVs on solid supports may be affected by numerous factors, such as structure of the lipids forming the vesicles, vesicle size, pH and ionic strength of the solution, the properties of the substrate surface, etc.
- Lipid vesicles are formed when thin lipid films or lipid cakes are hydrated and stacks of liquid crystalline bilayers become fluid and swell. The hydrated lipid sheets detach during agitation and self-close to form large multilamellar vesicles (LMV). Small unilamellar vesicles (SUV) can then be formed by reducing the size of LMV, usually through energy input in the form of sonic energy (sonication) or mechanical energy (extrusion).
- Success in SLB formation can be observed by fluorescence microscopy, if a fluorescent dye is used to dope the SLB. Fluorescence recovery after photobleaching (FRAP) is the common technique to characterize the fluidity of the SLB both qualitatively and quantitatively.

A. MATERIALS

- Lipid solution in chloroform or other solvent
- Lipid labeled with fluorescent dye (optional, for fluorescence microscopy only)
- Milli-Q grade water
- 1X Phosphate buffer saline (1X PBS)
- Clean and smooth substrate (glass cover slip, SiO_2 wafer, mica, etc.)
- Glass syringes
- Vortex mixer
- Vacuum oven or desiccator
- Extrusion set
- Pipettes
- Incubator

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B. PROTOCOL

Small unilamellar lipid vesicle solution preparation

1. Calculate the amount of lipid chloroform solution and the amount of 1X PBS buffer needed to obtain the desired lipid bilayer compositions. (The stock lipid vesicle solution used is typically 1mM.)
2. Use glass syringes to transfer appropriate amount of lipid chloroform solution for each lipid component into a small glass vial that has been cleaned appropriately (e.g. chloroform washes or piranha solution).
3. Evaporate the chloroform in the mixture very gently in the glass vial, by rotating the vial under nitrogen flow. To get a gentle nitrogen flow, a Pasteur pipette can be used to connect to the Nitrogen line. The lipid cakes are formed on the walls of the glass vial.
4. Leave the lipid cakes under vacuum (vacuum oven or desiccator) for at least 2 hours (usually overnight) for complete solvent removal.
5. Rehydrate the dry lipid cakes with the required amount of 1X PBS, and vortex until the lipid films are completely resuspended, forming a cloudy solution containing LMV of various sizes. **Note: The solution should be kept above the phase transition temperature of the lipid during hydration.**
6. Filter the LMV solution through a 0.45 µm porous polyethersulfone filtering membrane to reduce the vesicle size. Filtration is done by pushing the solution through the filter with a plastic syringe.
7. Extrude the solution ten times through a 100 nm porous polycarbonate filtering membrane at a temperature above the highest transition temperature of the lipids in the mixture. The resulting solution would contain SUVs of approximate 100 nm in diameter. **Note: After extrusion, the solution should become clear. If not, you need to check if the filter membrane is in place, and redo the extrusion.**
    a. When higher temperature is needed, heat the extruder stand on a hot plate. Insert a thermometer into the well in the stand, and allow the temperature of the extruder stand to reach the desired value. **Note: The temperature should not go above 80°C, or the syringes will be damaged.**
    b. Assemble the extruder apparatus. Put down the filter support on each of the Teflon block, use Mili-Q water to wet them and keep them in place. Place the polycarbonate membrane on one of the Teflon block. Make sure it is centered, and the inner part of the membrane is intact. Seal the two Teflon blocks in the hex nut. The edges of the hex nuts should be aligned.
    c. Insert the fully assembled extruder apparatus into the extruder stand. Insert the hex nut so that the apex of the hex nut points down. Check the temperature of the heating block before placing the assembled extruder apparatus into the heating block.
    d. The lipid suspension should be heated above its lipid phase transition temperature as well. Load the warm solution into one of the gas-tight syringes and carefully place into one end of the extruder.

e. Place the empty syringe into the other end of the extruder. Make sure its plunger is set to zero. Allow the temperature of the lipid suspension to equilibrate with the temperature of the heating block (approximately 5-10 minutes).
f. Gently push the plunger of the filled syringe until the lipid solution is completely transferred to the alternate syringe. Gently push the plunger of the alternate syringe to transfer the solution back to the original syringe. Repeat this for a minimum of 5 times (total of 10 passes through membrane). The final extrusion should fill the alternate syringe, in order to reduce the chances of contamination with larger particles.
g. After extrusion, remove the mini-extruder from the heating block. Remove the filled syringe from the extruder and inject the lipid solution into a clean glass vial. **Note: the syringes should be pulled straight out, as removing at angles may cause cracking of the glass syringe.**
h. Store the vesicle solution at 4°C when not in use. The solution is stable for one week.
i. Clean the apparatus thoroughly before using it for another lipid solution.

Supported lipid bilayer formation

8. Dilute the 1mM lipid vesicle solution with 1X PBS to 0.2 mM or other desired concentration. This is to prevent too many vesicles from binding to the SLB surfaces.
9. Clean the substrates. For glass cover slips or SiO_2 wafer, perform piranha cleaning and air plasma cleaning. For mica, cleave with tape to have a fresh layer exposed.
10. Put the substrates in a petri dish, and cover the substrate surface with diluted SUV solutions. If the substrate is small or light, it is suggested to affix it to the bottom of the petri dish with glue or double sided tape.
11. Leave the sample in a moisture box, and incubate at a temperature that is above the lipid transition temperature for at least half an hour. (For example, DOPC can be incubated at room temperature, and DSPC is suggested to be incubated at 60 °C, as its transition temperature is 55 °C.)

Washing the sample

12. After incubation, wash the SLB in petri dishes with 1X PBS at least 3 times to remove the vesicles in excess. This is done by creating liquid flow in the solution (pipetting the solution up and down 3 times) and substitute with fresh 1X PBS after each wash (removing part of the solution and adding fresh solution in for 3 times). More washes may be required if the vesicles are hard to remove.

    Note: The substrate surface should never be completely dried, as SLB structure would be destroyed.

