Protocol for Preparation of Supported Lipid Bilayers: DB 01/07/13

Glass Prep

- Sonicate Glassware in 3M KOH for 30 minutes covered in parafilm.
    - Rinse 5 times in ddH2O
- Sonicate glassware in 100% EtOH for 30 minutes covered in parafilm.
    - Rinse 5 times in ddH2O
- Sonicate in ddH2O for 5-10 minutes.
    - Rinse 5 times in ddH2O
- Blow dry glass with argon or nitrogen until all water is gone.
- Place flat in plastic petri dish (usually fits ~9 coverslips).
- Plasma clean using AC Glow Discharge procedure for ~10 minutes.
- Using UV glue, attach cut PCR tubes to coverslips with UV light for ~5 minutes.

Lipid Prep (Performed Simultaneously)

- Pre rinse glass syringes with chloroform at least 5 times.
- Deposit ~50 µL chloroform into a capped tube in which the lipids will be mixed.
- Mix lipids to desired molar percentage using a glass syringe. I start with PC, move to PI, then finish with PI(4,5)P2.
- Immediately cover lipid stocks with nitrogen or argon, wrap tubes in parafilm.
- Blow solvent off of lipid mixture using a very light stream of gas. Avoid huffing chloroform.
- Place in vacuum for at least 1 hour.
- Resuspend lipid mixture in supported lipid bilayer buffer pre-heated at 37^{o} C. Wrap top of tube tightly with parafilm. Sonicate for ~2 minutes to resuspend. Place at 37^{o} C in water bath for ~10-30 minutes (while plasma cleaning glass). When ready, sonicate for 2-5 minutes. Lipid suspension should begin to clear. Place at 37^{o} for another 5-10 minutes. Remove and sonicate until lipid suspension is clear. Dilute lipids in SLB buffer to 1 mg/mL.

Supported Lipid Bilayer Preparation

- Apply 50 µL of lipid prep to well on coverslip.
- Add 1uL of 100mM CaCl2 immediately afterword.
- Let fusion occur for 30 minutes at 37^{o} on hot plate.
- Wash each well 6 times with 150 µL SLB buffer at room temperature.
- Store hydrated lipids at 4^{o}C when not in use.
- Wash lipids with 150 µL of reaction buffer immediately before use. Remove reaction buffer, add septins.

Supported Lipid Bilayer Buffer

- 20 mM Tris pH 8.0
- 300 mM KCl
- 1mM $MgCl_2$