Lipid Preparation Protocol

1) Stock lipids are dissolved in organics such as Chloroform.

DOPC (25 mg/ml or 32 mM) - 1 ml = 25 mg or 32 umol
DOPG (25 mg/ml or 31 mM) - 1 ml = 25 mg or 31 umol
DOPS (10 mg/ml or 12.3 mM) - 1 ml = 10 mg or 12.3 umol
Brain PIP2 (1mg/ml or 0.9 mM)

2) Mix ~25mg (1ml) of lipids in a glass tube (~ 25mg lipid in 1 ml/tube)
   a) 670 ul DOPC (67 mol% = 21.4 umol) + 330 ul DOPG (33 mol % = 10.2 umol) = (100 mol % = 32 umol)
   b) 700 ul DOPC (70 mol % = 22.4 umol) + 752 ul DOPS (29 mol% = 9.2 umol) + 351 ul Brain PIP2 (1 mol% = 0.32 umol) = (100 mol % = 32 umol)
   c) 1ml DOPC Alone (100 mol % = 32 umol)
   d) 1ml E.coli Phospholipid Extract (25 ml ~ 32 umol)

* To visualize the Small Unilamellar Vesicles (SUVs), add 0.5 mol % of Rhodamine-PE (MW=1320, Ex560nm/Em583nm):
   1) Re-suspend 5mg of powder with 5ml CHCl3 (cf = 1mg/ml or 758uM).
   2) Add 211ul to achieve 0.5 mol % to the lipid mixes above.

3) Split volumes of each mixture into 2 glass tubes (16 umol/tube)

4) In fume hood, use dry N2 gas to dry lipid with rotation to make a thin film of dry lipid cake.

5) Speed vacuum to dry completely for 2hr @ 42°C for 1st hour.

6) Add 2.5ml degassed TK150 buffer to each tube in N2 box (Cf ~ 5mg/ml or 6.4 mM) and seal with parafilm.

7) Vortex for 1min, spin down, and hydrate o/n at RT in dark. Next day vortex for 1 min. and spin down.

8) Sonicate with Qsonica model #Q700A with #431C2 Cup Horn, #440 tube rack and #4900 Chiller.
   - Use of a chiller is highly recommended to accurately control temperature.
   - Maintain water level inside Cup just above the sample in each tube.
   - Use 1.5ml Polystyrene tubes (standard polypropylene tubes do not work efficiently).
   - Sonicate at 15% amplitude setting (70 watts) using pulse mode to help control temperature.
   - Sonicate 0.5ml/tube until translucent.

Sonication times:
   - 2 min (30s on, 10s off) for E.coli phospholipid extract
   - 5 min for 33% DOPE + 67% DOPC
   - Longer time period needed for DOPC alone (may need higher amplitude/wattage – 80W)
9) Spin down and syringe filter SUVs.

10) Make ~ 200ul aliquots, layer with N₂ gas, and store @ 4°C Teflon capped and parafilm sealed tubes.

* Aliquots are diluted to 0.5mg/ml with TK150 buffer and 5mM MgCl₂ before flowed into flowcell to make flat supported lipid bilayers.
* Lipid stocks are good for ~1 month after which they get cloudy as vesicles fuse over time.

<table>
<thead>
<tr>
<th>TK150 Buffer</th>
<th>[STOCK]</th>
<th>[1x]</th>
<th>1x (10ml)</th>
<th>[2x]</th>
<th>2x (30ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris-HCl (pH 7.4)</td>
<td>1 M</td>
<td>25 mM</td>
<td>0.25 ml</td>
<td>50 mM</td>
<td>1.5 ml</td>
</tr>
<tr>
<td>KCl</td>
<td>3 M</td>
<td>150 mM</td>
<td>0.5 ml</td>
<td>300 mM</td>
<td>3.0 ml</td>
</tr>
<tr>
<td>H₂O</td>
<td></td>
<td></td>
<td>9.25 ml</td>
<td></td>
<td>25.5 ml</td>
</tr>
</tbody>
</table>

Before Sonication

After Sonication