**Liposome preparation**

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**Abstract:** This protocol details methods for the preparation of 100% PS liposome and GC/PS lipid nanotubes used for LRRK2 binding, tubulation assays.

**Materials:** Brain PS (Avant, 840032); Galactosylceramide (GC) (Avant, 860546P); Rhod-PE (Avanti, 810150); Cy5-PE (Avanti, 810345).

Solutions to prepare:

Liposome buffer: 20 mM HEPES (pH 7.4), 100 mM KCl, 0.5 mM TCEP

**Protocol:**

1, Dissolve lipid mixtures with chloroform in glass vials in moles percent as follows:

PS liposomes: 99.5% brain PS:0.5% Rhod-PE.

GC/PS nanotubes: 39.5% Galactosylceramide:60% brian PS:0.5% Cy5-PE.

2, Chloroform was evaporated under a stream of nitrogen gas to produce a lipid film on the glass surface.

3, Lipid film was further dried in a vacuum oven for 1 hour.

4, Dried lipid films were rehydrated in liposome buffer at a final concentration of 1 mg/ml (~1.2 mM)

5-1, For PS mixtures, liposomes were formed by three freeze (liquid N2)–thaw (37°C water bath) cycles.

5-2, For GC/PS mixtures, lipid nanotubes were formed by a brief vortexing instead of freeze-thaw cycles.

6, Large aggregates were removed by a brief centrifugation (500xg for 5min) and stored in the dark at 4 ̊C to avoid photooxidation.