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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.

ATTACHMENTS

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

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MATERIALS

Brain PS **Avanti Polar Lipids, Inc. Catalog # 840032**

Galactosylceramide (GC) **Avanti Polar Lipids, Inc. Catalog #860546P**

Rhod-PE **Avanti Polar Lipids, Inc. Catalog #810150**

Cy5-PE **Avanti Polar Lipids, Inc. Catalog #810345**

Solutions to prepare:

Liposome buffer:

	A	B
	HEPES (7.4)	20 mM
	KCl	100 mM
	TCEP	0.5 mM

Liposome preparation

1h 5m

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 Evaporate chloroform under a stream of nitrogen gas to produce a lipid film on the glass surface.

3 Dry the lipid film in a vacuum oven for 01:00:00. 1h

4 Rehydrate the dried lipid films in liposome buffer at a final concentration of [M] 1 mg/mL (~

[M] 1.2 millimolar (mM)).

5 For PS mixtures, form the liposomes by three freeze (liquid N2)–thaw (37 °C water bath) cycles.

5.1 For GC/PS mixtures, form the lipid nanotubes by a brief vortexing instead of freeze-thaw cycles.

6 Remove large aggregates by a brief centrifugation (500 x g for 00:05:00) and store in the dark at 4 °C to avoid photooxidation. 5m