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# Generation of membrane tubules pulled from giant unilamellar vesicles (GUVs)

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

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**Keywords:** Generation of membrane tubules, giant unilamellar vesicles, ASAPCRN, giant unilamellar vesicles for fluorescence, giant unilamellar vesicle, lipid nanotube, generation of membrane tubule, membrane tubule, microscopy experiment, lipid, fluorescence

## Abstract

This protocol explains the methodology to generate lipid nanotubes pulled from giant unilamellar vesicles for fluorescence microscopy experiments.

## Attachments

DOCX

733-1844.docx

16KB

## Materials

**Materials**:

- Lipids:

1,2-dioleoyl-sn-glycero-3-phosphocholine (18:1, 18:1 PC) **Avanti Polar Lipids, Inc. Catalog #**850375P

12-dioleoyl-sn-glycero-3-phospho-L-serine (sodium salt) **Avanti Polar Lipids, Inc. Catalog #**840035

12-Dioleoyl-sn-glycero-3-phosphoethanolamine labeled with Atto 647N **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**42247

- Glass vials (2700 Supelco, Sigma-Aldrich).
- Silica Beads **Microspheres-Nanospheres Catalog #**140256-10
- Parafilm.
- Petri dish.
- Chloroform **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**650498
- Closed glass micropipettes prepared using a P-1000 micropipette puller (Sutter Instruments, USA).
- Bovine serum albumin 2 mg/ml **Thermo Fisher Scientific Catalog #**23209

**Solutions**:

- *Lipid films hydration buffer A*: 25 mM HEPES 7.4.
- *Lipid films hydration buffer B*: 1M Trehalose.
- *Working buffer*:

| | A |
|---|---|
| | 20mM HEPES 7.4 |
| | 150mM NaCl |
| | 2.5mM $MgCl_2$ |
| | 5% Glycerol |
| | 2mM DTT |

## Troubleshooting

## Protocol

3h 30m

1 Mix DOPC, DOPS and Atto 647N DOPE at 59.9:40:0.1 mol% respectively in a final volume of 200 µL with chloroform and 0.5 g/L lipid final concentration in a glass vial.

2 Dry the lipid mixture in the glass vials for 02:00:00 in a vacuum chamber forming the dried lipid films on the bottom of the glass vials. 2h

3 Add 200 µL of the lipid films hydration buffer A to the glass vial containing the dried lipid films.

4 Vortex the glass vials until visually seeing complete resuspension of the dried lipid films in the solution (seen by an increase in the turbidity of the lipid solution) forming the multilamellar vesicles (MLVs).

5 Mix 10 µL of MLVs with 2 µL of silica beads in an Eppendorf tube.

6 Deposit 6 drops of 2 µL each containing the mixture of MLVs and silica beads on a parafilm slide placed in the bottom of a petri dish.

7 Dry the drops for 01:00:00 in the vacuum chamber until the liquid is completely dried. 1h

8 Take one dried drop from the parafilm and insert it into a small plastic tip cutted at the thin end containing 6 µL of [M] 1 Molarity (M) trehalose solution until visually seeing how the dried beads get to the thin bottom.

9 Incubate the cutted plastic tip containing the drop and the trehalose for 00:15:00 at 60 °C attaching it to the cap of an Eppendorf with 500 µL destilled water inside by doing a small hole in the cap and inserting the cutted plastic tip. 15m

10 Passivate the microscopy chamber by adding 200 µL solution of 2 g/L BSA for 00:15:00 . 15m

11 Remove the cutted plastic tip from the Eppendorf and put the thin part of the cutted tip in contact with the microscopy chamber containing 200 µL of working buffer until

visually seeing how the beads are transferred from the tip to the observation chamber.

Note

**Note**: the microscopy chamber contains either [M] 20 nanomolar (nM) or [M] 0.5 micromolar (µM) of GFP-LRRK2 in the solution.

12 Gently stir the microscopy chamber to promote the detachment of the hydrated lipid films from the silica beads, leading to the formation of the GUVs.

13 Place a closed micropipette in the micro-positioning system (MP-285, Sutter Instrument, Novato, CA, USA), and use it to approach the pipette to the GUV membrane.

14 Touch the GUV membrane with the pipette and then move back the pipette until a lipid nanotube is pulled from the GUV.

15 Wait until protein coverage reaches the steady state in both the GUV and the pulled membrane nanotube.