

Aug 26, 2023

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

DOI

dx.doi.org/10.17504/protocols.io.j8nlkw7p5l5r/v1

Javier Espadas¹, Aurelien Roux¹

¹Department of Biochemistry, University of Geneva, Geneva, 1211, Switzerland



xinbo.wang

OPEN  ACCESS



DOI: dx.doi.org/10.17504/protocols.io.j8nlkw7p5l5r/v1

Protocol Citation: Javier Espadas, Aurelien Roux 2023. Generation of membrane tubules pulled from giant unilamellar vesicles (GUVs). **protocols.io** <https://dx.doi.org/10.17504/protocols.io.j8nlkw7p5l5r/v1>

License: This is an open access protocol distributed under the terms of the **[Creative Commons Attribution License](#)**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: June 02, 2023

Last Modified: May 31, 2024

Protocol Integer ID: 82801

Keywords: Generation of membrane tubules, giant unilamellar vesicles, ASAPCRN

Abstract

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

Attachments



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

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 distilled 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 (μ M) 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.