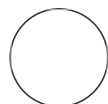




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Generation of membrane tubules pulled from giant unilamellar vesicles (GUVs)

Javier

Espadas¹, Aurelien Roux¹¹Department of Biochemistry, University of Geneva, Geneva, 1211, Switzerland

xinbo.wang

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ABSTRACT

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

ATTACHMENTS

[733-1844.docx](#)

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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


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

- 



1 Mix DOPC, DOPS and Atto 647N DOPE at 59.9:40:0.1 mol% respectively in a final volume of $200\ \mu\text{L}$ with chloroform and $0.5\ \text{g/L}$ lipid final concentration in a glass vial.
- 2h

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.
- 

3 Add $200\ \mu\text{L}$ 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 $10\ \mu\text{L}$ of MLVs with $2\ \mu\text{L}$ of silica beads in an Eppendorf tube.
- 6 Deposit 6 drops of $2\ \mu\text{L}$ each containing the mixture of MLVs and silica beads on a parafilm slide placed in the bottom of a petri dish.
- 1h

7 Dry the drops for $01:00:00$ in the vacuum chamber until the liquid is completely dried.
- 8 Take one dried drop from the parafilm and insert it into a small plastic tip cutted at the thin end containing $6\ \mu\text{L}$ of $1\ \text{M}$ 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 **20 nanomolar (nM)** or **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.