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Generation of membrane tubules by lipid-covered silica beads rolling

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

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Abstract

This protocol explains the high throughput methodology to generate membrane tubules from lipid-covered silica beads.

Attachments

DOCX

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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
- sticky-Slide VI 0.4 **Ibidi Catalog #**80608
- Bovine serum albumin 2 mg/ml **Thermo Fisher Scientific Catalog #**23209

Solutions:

- Lipid films hydration buffer A: 25 mM HEPES 7.4.
- Working buffer:

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

Protocol

3h 15m

1 Mix of 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.

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 Stick a microfluidic device on a 1.5 borosilicate coverslip.

9 Passivate the microfluidic channels by adding 200 µL solution of 2 g/L BSA for 00:15:00 . 15m

10 Clean the BSA solution by passing 200 µL working buffer solution in each channel 5 times.

11 Add 200 µL of working buffer to each channel with a final concentration of GFP-LRRK2 of [M] 500 nanomolar (nM) .

12 Pick one of the dried drops and add it to the inlet of the microfluidic device.

13 Gently tilt the chamber towards you 60 degrees with the inlet in the upper part and the outlet in the lower part, and wait until visually seeing the lipid-covered silica beads moving from the inlet to the outlet openings of the microfluidic device.

14 Wait until reaching the steady state of protein coverage on the lipid tubules.