**Generation of membrane tubules by lipid-covered silica beads rolling**

**Authors:** Javier Espadas1 and Aurelien Roux1

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

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

**Materials**:

Lipids:

* dioleoyil-phosphatidylcholine (DOPC) (850375 Avanti Polar Lipids).
* Dioleoyil-phosphatidylserine (DOPS) (840035 Avanti Polar Lipids).
* dioleoyl-phosphoethanolamine labeled with Atto 647N (Atto 647N DOPE) (42247 Sigma-Aldrich).

Glass vials (2700 Supelco, Sigma-Aldrich).

Silica beads (140256-10 Microspheres-Nanospheres, USA).

Parafilm.

Petri dish.

Chloroform (650498 Sigma-Aldrich).

Microfluidic chamber (80608 sticky-Slide VI 0.4, Ibidi).

Bovine serum albumin solution (2 g/L) (23209 ThermoFisher Scientific).

**Solutions**:

Lipid films hydration buffer A: 25 mM HEPES 7.4.

Working buffer: 20mM HEPES 7.4, 150mM NaCl, 2.5mM MgCl2, 5% Glycerol, 2mMDTT.

**Protocol:**

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 2 hours in a vacuum chamber forming the dried lipid films on the bottom of the glass vials.
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 1 hour in the vacuum chamber until the liquid is completely dried.
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 15 minutes.
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 500 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.