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Preparation of unilamellar liposomes

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Ayan Adhikari[1,2], Suzanne R Pfeffer[1,2]

[1]Stanford University School of Medicine;
[2]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network

Ayan Adhikari
Stanford University

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We use this protocol and it's working

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Abstract

We present here a protocol for preparing liposomes that can be used to monitor binding of proteins to vesicles of various membrane curvatures. We used this to study the association of the PPM1H phosphatase with highly curved membranes due to its N-terminal amphipathic helix.

Materials

1.(18:0-20:4)PC (1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphocholine)
[Avanti #850469]

2.(18:0-20:4)PI (1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphoinositol)
[Avanti #850144]

3.(18:0-18:2)PS (1-stearoyl-2-linoleoyl-sn-glycero-3-phospho-L-serine)
[Avanti #840063]

4.(18:1)PI(4)P {1,2-dioleoyl-sn-glycero-3-phospho-(1'-myo-inositol-4'-phosphate)}
[Avanti #850151]

5. Cholesterol sulfate [Avanti #700016]

6. 0.4 µm pore size polycarbonate filters [Avanti #610007]

7. 0.1 µm pore size polycarbonate filters [Avanti #610005]

8. 0.05 µm pore size polycarbonate filters [Avanti #610003]

9.Chloroform [Fischer Chemical #C298-500]

10.Hand held mini extruder with heating block [Avanti # 610000)

Make lipid stocks

1 All lipids were purchased from Avanti Polar Lipids. PC and PS were obtained as 10mg/ml chloroform solutions. PI, PI(4)P and cholesterol sulfate were obtained as powders. Dissolve in chloroform to prepare 2mg/ml, 1mg/ml and 25mg/ml stock solutions respectively.

Prepare the lipid mixture

2 Prepare unilamellar vesicles by mixing:

940 µL of (18:0-20:4)PC
470 µL of (18:0-20:4)PI
60 µL of (18:0-18:2)PS
140 µL of (18:1)PI(4)P
26 µL of cholesterol sulfate

from the respective stock solutions in a glass vial. The lipid composition of the liposomes is in the ratio (mol %) 78:7:5:1:9 to represent the mammalian cell Golgi composition (Fasimoye et al., 2023).

3 Dry the above-mentioned lipid mixture in chloroform under nitrogen flow by pointing a Pasteur pipette into the glass vial. This vial is subsequently incubated under house vacuum for at least 01:00:00 . 1h

4 Resuspend the dried lipids by pipetting up and down in 1ml of resuspension buffer (50mM HEPES pH 7.5, 120mM KCl)

Liposome preparation

5 Sonicate the liposome suspension by two brief 00:00:05 cycles of bath sonication followed by sequential extrusion through 0.4, 0.1 and 0.05 µm pore size polycarbonate filters for 21 times using a hand extruder. 5s

Liposome storage

6 The final lipid concentration of the liposome suspension will be 15mM; store at 4 °C for a maximum of 2 weeks. Small liposomes are best used close to the time of their preparation.

Protocol references

1. R. Fasimoye et al., Golgi-IP, a tool for multimodal analysis of Golgi molecular content. Proc Natl Acad Sci U S A **120**, e2219953120 (2023)