Protocol: supported lipid bilayer experiments

Lipid film stocks

Lipid mixtures containing sphingomyelin (SM), dioleoylphosphatidylcholine (DOPC), dioleoylphosphatidylserine (DOPS), and cholesterol (Chl) in a ratio of 44:24:12:20 (SM:DOPC:DOPS:Chl) is prepared for subsequent use to make supported lipid bilayer and liposomes. This composition is inspired from Chatterjee and colleagues [1].

The dye Atto655 is included in some of the lipid mixtures to enable detection of the lipid bilayers and liposome by fluorescence microscopy. Biotin was also included in some lipid mixtures to allow for attachment of biotin-containing liposomes to microscopy slides covered with NeutrAvidin.

Lipid	**MW (g/mol)**
DOPC	786.113
DOPS	810.025
SM	760.2
Chl	386.7
Atto655	1366.0
Biotin DSPE PEG (2000)	3016.0

Lipids solubilized in chloroform and methanol should be handled in a fume hood and pipetted using a Hamilton syringe.

Materials

- 25 mg/mL stocks of each lipid* (referred to as “stock 1” in notebook)
- Lipid dye Atto655, 0.5 mg/mL (referred to as “stock 1” in notebook)
- Biotin, PEG, 0.5 mg/mL (referred to as “stock 1” in notebook)
- Chloroform and methanol**

*Lipids: SM, DOPC, DOPS and Chl.

**A mix of chloroform and methanol is used for DOPC and SM solutions and chloroform alone for the rest of the lipid solutions.

Procedure

Dilute 25 mg/mL lipid stocks to new stocks with concentrations of 1 µg/µL (referred to as “stock 2” in notebook).

Dilute biotin and dye to stocks of 0.1 µg/µL and 0.1 µg/µL (referred to as “stock 2” in notebook).

Make lipid mastermixes 1, 2 and 3 from the stocks 2s by adding the following.

Lipid	**Mastermix 1**	**Mastermix 2**	**Mastermix 3**
DOPC (1 µg/µL)	75 µL	75 µL	75 µL
DOPS (1 µg/µL)	39 µL	39 µL	39 µL
SM (1 µg/µL)	133 µL	134 µL	134 µL
Chl (1 µg/µL)	31 µL	31 µL	31 µL
Atto655 (0.01 µg/µL)	27 µL	27 µL	-
Biotin DSPE PEG (2000) (0.1 µg/µL)	60 µL	-	-

Aliquot mastermixes into 2 µmol portions (10 aliquots). Use brown light-blocking vials for the mixtures containing dye.

Dehydrate lipids by using a nitrogen flow or a ScanVac evaporator for vacuum concentration.

Store lipid film stocks at -20°C.

Liposomes

Materials

- Lipid film stocks
- LB media

Procedure

Rehydrate lipid stocks and extrude liposomes as described in the Liposome preparation protocol step 6 to 16. Use LB media to rehydrate lipids.

Store at -20°C if not used immediately.

Confocal microscopy

Materials

- Liposomes made from lipid mastermix no. 2
- LB media
- Bacteria (SIEC and SIECΔp1 transformed with GFP gene)

Procedure

Grow bacteria in media for 2.5 hours while inducing expression, 37 oC at 160 rpm*.

Prepare a supported lipid bilayer on a microscopy slide as described in the Moran-Mirabel protocol step 8 to 12. Use LB media to wash the slide.

Add 100 µL bacteria culture to each slide.

Incubate at 37 °C for 3 hours.

Prepare the confocal fluorescence microscope. Use *fluorescence recovery after photobleaching* (FRAP) to to investigate if the membrane is intact.

Take pictures before and after washing the slide with 100 µL LB media several times. Use 488 nm laser (for GFP) and 635 nm (for Atto655).

*Incubation time was inspired by Ruano-Gallego *et al.* 2015 [2].

References

[1] Chatterjee, A., Caballero-Franco, C., Bakker, D., Totten, S., Jardim, A. (2015) Pore-forming Activity of the Escherichia coli Type III Secretion System Protein EspD. J Biol Chem. 290 (42) pp. 25579-25594.

[2] Ruano-Gallego, D., Álvarez, B., Fernández, L. A. (2015) Engineering the Controlled Assembly of FIlamentous Injectisomes in E. Coli K-12 for Protein Translocation into Mammalian Cells. ACS Synth. Biol. 4, pp 1030-1041.