

B235

On Demand Control of Lipid Composition in Individual Bilayers

John S.H. Danial, Brid Cronin, Chandini Mallick and Mark I. Wallace
University of Oxford, Physical and Theoretical Chemistry Laboratory, Oxford, OX1 3PS, U.K.

wallace.chem.ox.ac.uk

INTRODUCTION

• Reorganize transmembrane cell trafficking

• It has been proposed that specific lipid compositions in phase diagrams can be mapped out

• The formation of Giant Unilamellar Vesicles (GUVs) as a model system with which to understand phenomena. By creating GUVs with a range of lipid compositions a phase diagram can be mapped out

• The equilibration of lipid mixtures has been achieved as a potential pitfall of such experiments, leading to a heterogeneous population of GUVs

AIMS

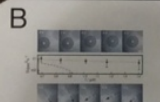
• The aim of the project is to produce a versatile and easy tool whereby the composition of single bilayer can be precisely determined by the *in situ* titration of calculated amounts of lipids.

• We use Droplet Interface Bilayers (DIBs) formed by the contact of a lipid coated water droplet and a thin hydrogel spun coat on a coverslip immersed in a solution of lipids in oil.

• Access to the oil phase enables us to vary the lipid composition of a single bilayer, and control phase separation.

PHASE SEPARATED BILAYERS

SEPARATED DIBs



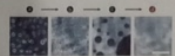
Massive viscosity $\approx 2 \times 10^7$ Pa.s. Surrounding bulk viscosity $\approx 1 \times 10^2$ Pa.s. Aggregating well with values reported in GUVs¹ (Scale bar = 1 μ m).

Different lipid compositions of DIBs: DPPC + Cholesterol (Chol) separating the phase diagram: (1) 1:1, (2) 1:2, (3) 1:3, (4) 1:4, (5) 1:5, (6) 1:6, (7) and (8) lie in the single phase region. (Scale bar = 1 μ m).

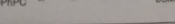


IN SITU CONTROL OF BILAYER COMPOSITION BY LIPID TITRATION

A One way titration of cholesterol: (a) lies in the bulk ordered - Lipid disordered Phase coexisting region. (b) and (c) lie in the two phase - Lipid ordered - Lipid disordered coexisting region. (d) lies in the single phase - Lipid disordered region. Initial molar ratio (a) is 1:1.6. Final molar ratio (d) is 1:1.8. (Scale bar = 17 μ m).



Reversible titration of lipids reveal domains switching. Three titrations are performed: (1) to (3), (3) to (2) and (2) to (3), hence the term reversible. Initial lipid molar concentration: (1), 1:1.5:1.6. Final lipid molar concentration: (3), 1:1.5:1.8. Phase boundaries have been determined from ref (9) for DPPC:Chol.



DROPLET INTERFACE BILAYERS

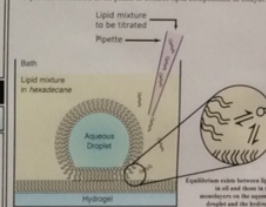
• Simple, robust approach to making stable *in vitro* lipid bilayers.

• Optical imaging by Total Internal Reflection Fluorescence (TIRF).

• Fluid substrate supported bilayers.

• Bilayers can be formed from binary mixtures of lipids.

• Lipid can be titrated in oil phase to control lipid composition in bilayer.



FUTURE WORK

• Study the effects of Lipid Phase Separation on the function of membrane proteins using Single Channel Recording and SM Fluorescence techniques.

• By titrating oil amounts of lipid to immediately control the composition of a DIB, we aim to study the effect of domain switching on the insertion dynamics of membrane proteins.

REFERENCES

1. J. S. H. Danial, B. Cronin, C. Mallick, M. I. Wallace, *Soft Matter*, 2007, 3, 1000.
2. P. S. C. and the challenge of lipid bilayers, *Soft Matter*, 2007, 3, 1000.
3. M. I. Wallace, B. Cronin, C. Mallick, *Soft Matter*, 2007, 3, 1000.
4. M. I. Wallace, B. Cronin, C. Mallick, *Soft Matter*, 2007, 3, 1000.
5. M. I. Wallace, B. Cronin, C. Mallick, *Soft Matter*, 2007, 3, 1000.
6. M. I. Wallace, B. Cronin, C. Mallick, *Soft Matter*, 2007, 3, 1000.
7. M. I. Wallace, B. Cronin, C. Mallick, *Soft Matter*, 2007, 3, 1000.
8. M. I. Wallace, B. Cronin, C. Mallick, *Soft Matter*, 2007, 3, 1000.
9. M. I. Wallace, B. Cronin, C. Mallick, *Soft Matter*, 2007, 3, 1000.

