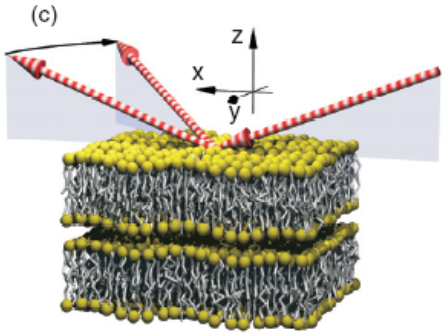
Biomembranes Developments at ISIS

Arwel Hughes

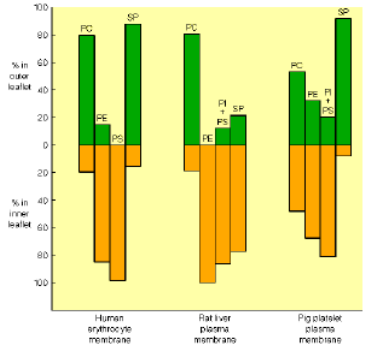
Basic Requirements of Model Bilayer for Reflectivity

Large uniform area of bilayer on a substrate

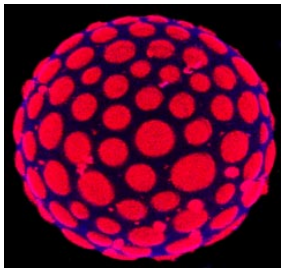


Wide range of lipid compositions

- *Saturated + unsaturated lipids;*
- *Charged headgroups;*
- *Sterols;*
- *Proteins;*

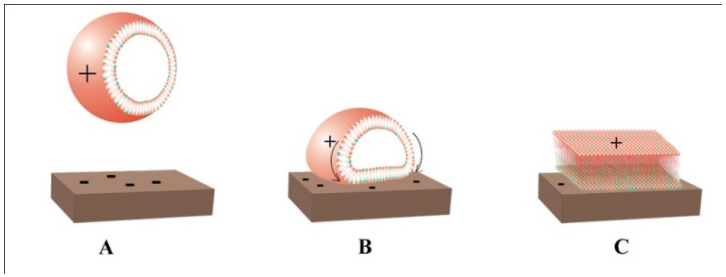


Unconstrained Lateral Diffusion of components..

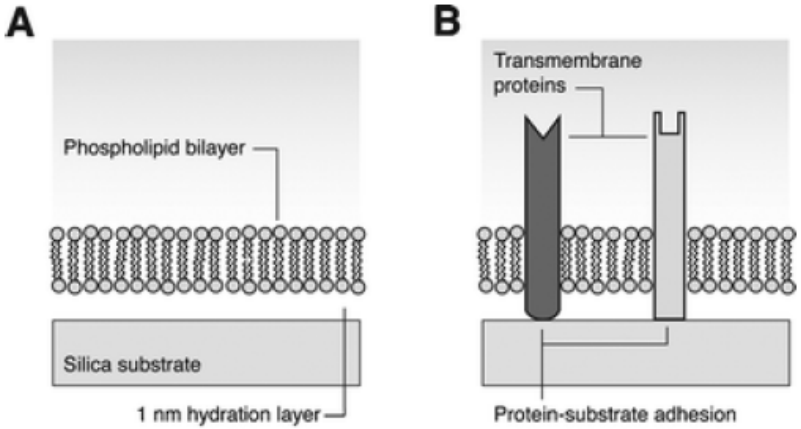


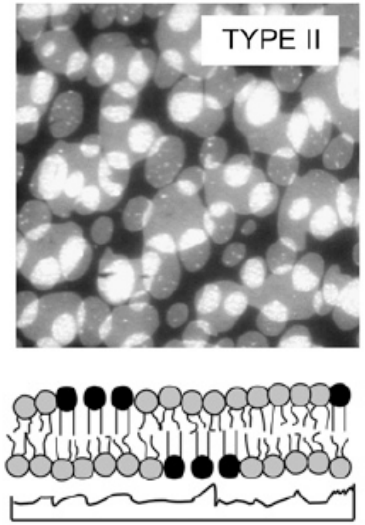
Easiest models are simple supported bilayers…

Easy to make – Vesicle fusion (or LB)..



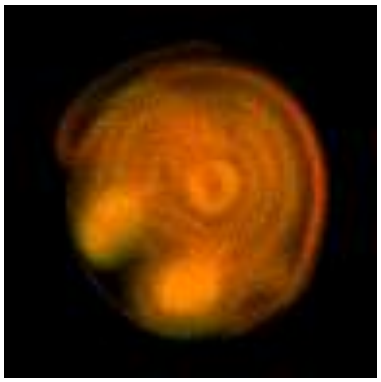
But, substrate effects….

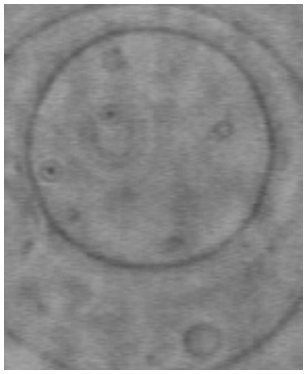




How can we make supported bilayers that are not affected by the proximity of the substrates????

Reducing Substrate Influence – 'Floating' Bilayers..





Multilamellar Vesicles made of 'concentric shells of lipid bilayers.

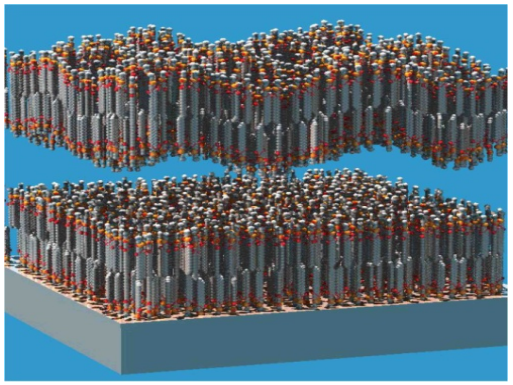
Bilayers remain widely separated due to balance of forces between them

Van der Waals interaction energy

$$P(a,T) = -\frac{H}{6\pi}\left(\frac{1}{a^3} - \frac{2}{(a+b)^3} + \frac{1}{(a+2b)^3}\right) + P_h e^{-a/\lambda_h} + \left(\frac{k_B T}{2\pi}\right)^2 \frac{1}{K_C}\frac{A_{fl}}{\lambda_{fl}} e^{-a/\lambda_{fl}}$$

Hydration energy

'Helfrich' fluctuation potential



We mimic the MLV structure on a substrate to give an unconstrained upper bilayer….

First example created at the ILL…

EUROPHYSICS LETTERS 1 January 2001

Europhys. Lett., **53** (1), pp. 100–106 (2001)

A fluid floating bilayer

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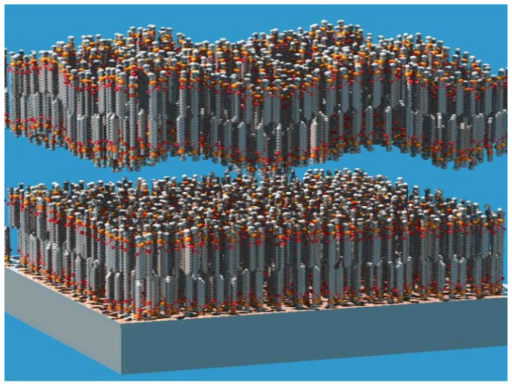
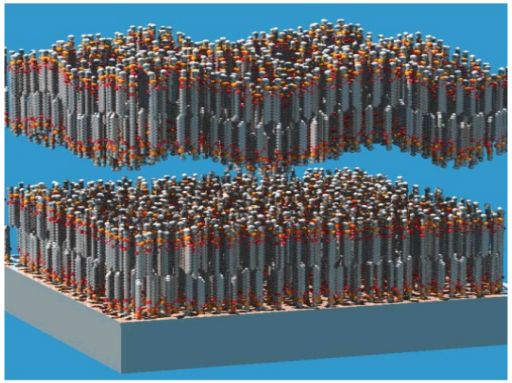
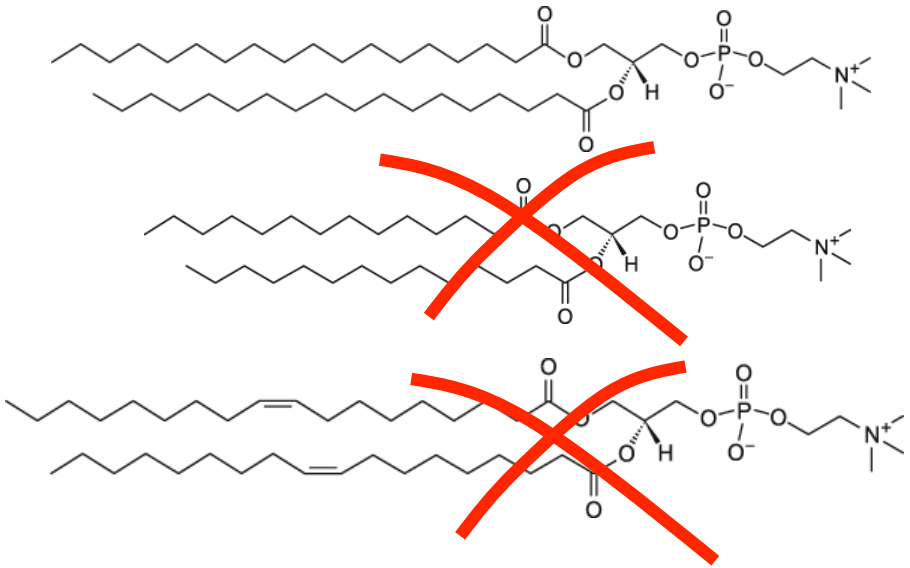


'Double Bilayer'



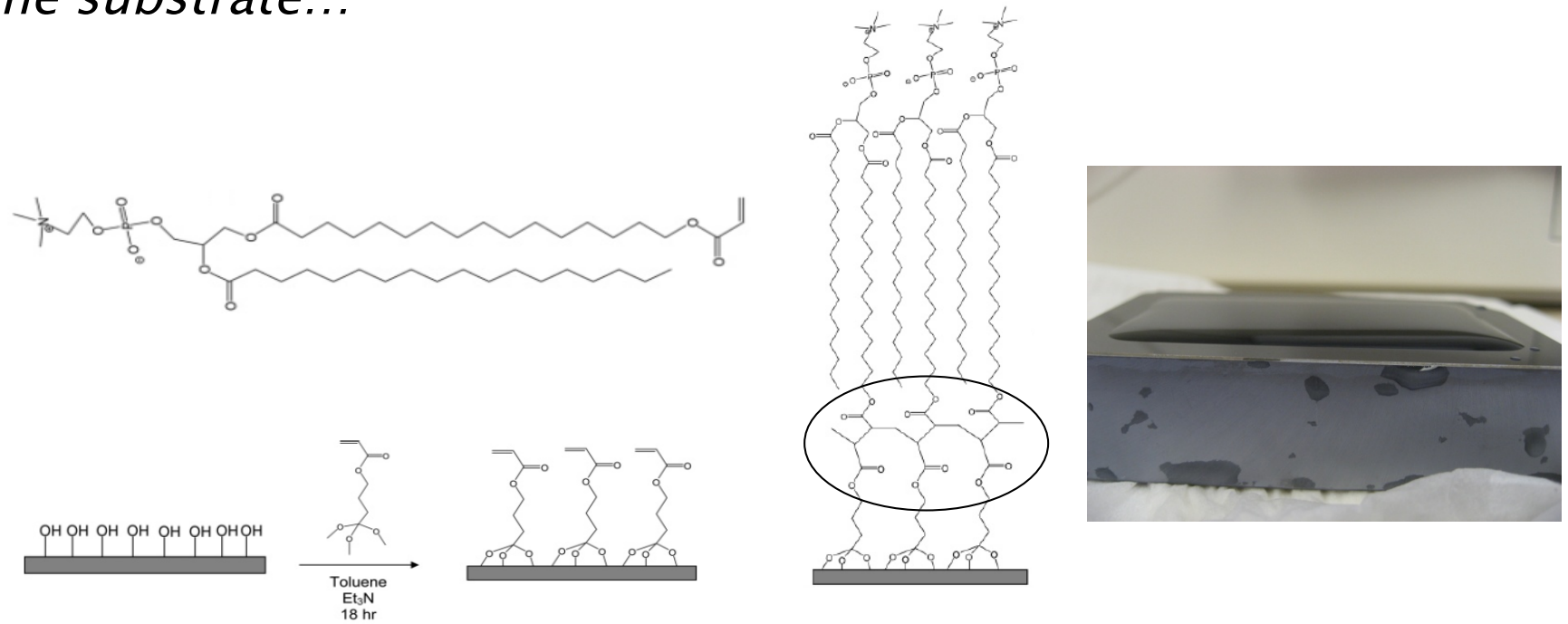
Double bilayer works – Unconstrained bilayer, large area of uniform coverage, correct phase behaviour. But..



Only works for the simplest lipids – lower layers peel off to easily.
Need to hold lower 'support' more firmly.

Solution – Grafted phospholipid Self Assembled Monolayers

Developed at ISIS. Attach the lower layer of lipid chemically to the substrate…



Having a grafted SAM for the lowest layer prevents 'peeling off' during deposition.
Allows fabrication of membranes from more complex mixtures.

First example was DSPC bilayer….

Langmuir **2008,** *24,* 1989−1999

Floating Lipid Bilayers Deposited on Chemically Grafted Phosphatidylcholine Surfaces

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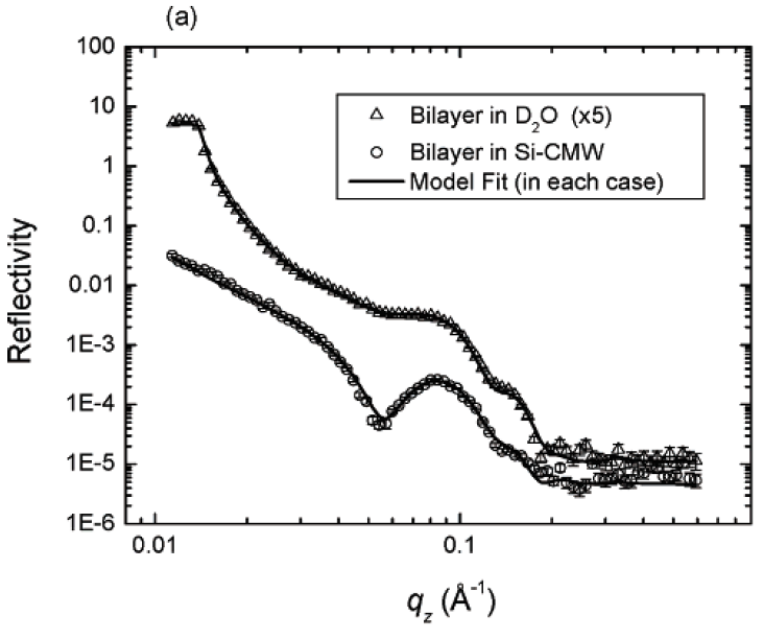
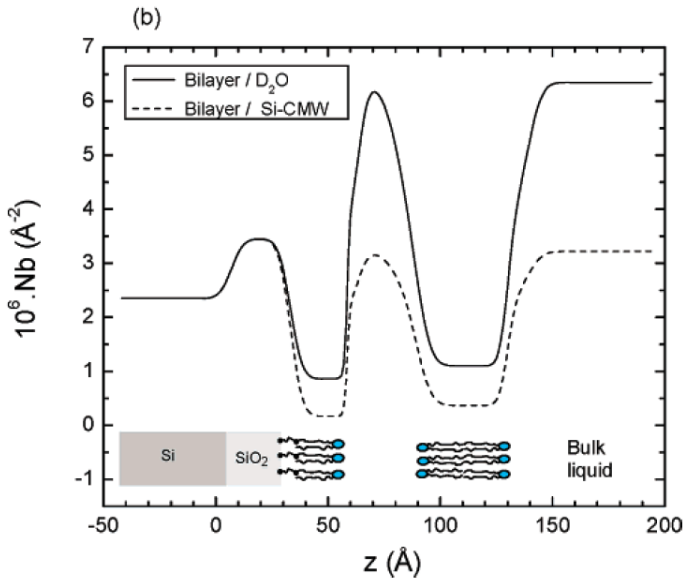
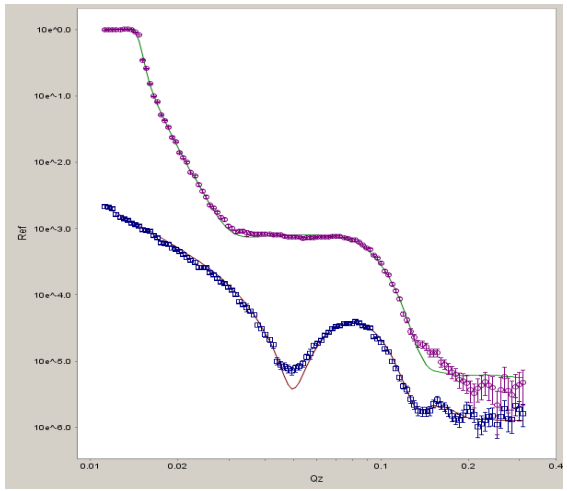
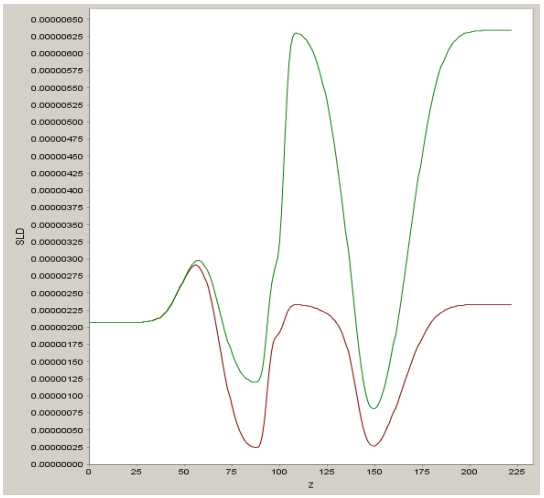




Grafted SAM works for unsaturated and charged lipids…

e.g. DOPC bilayer at a coverage of 75%…..





PL-SAM support means we can make floating bilayers of a wide range of compositions, including….

Unsaturated lipids ✓

Sterols ✓

Mixed bilayers ✓

Asymmetric Bilayers ✓

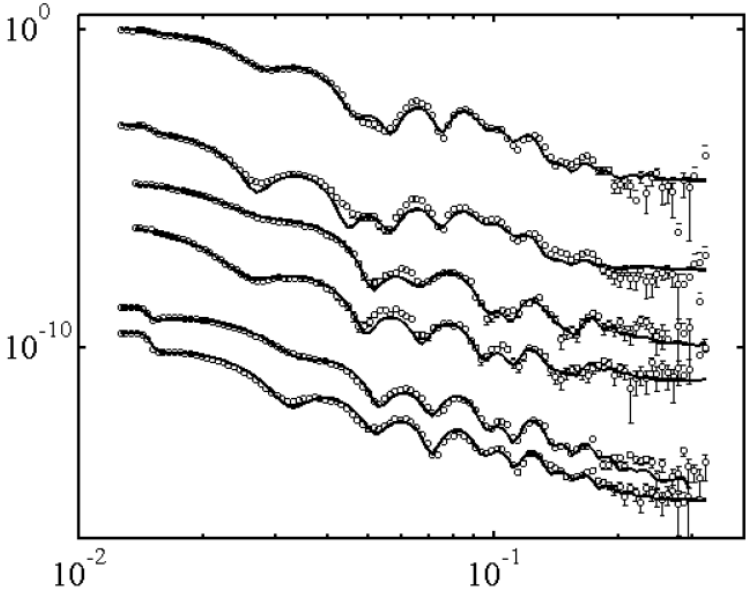
Re-usable SAM means quick, repeatable sample prep (~ 1 hr per sample)

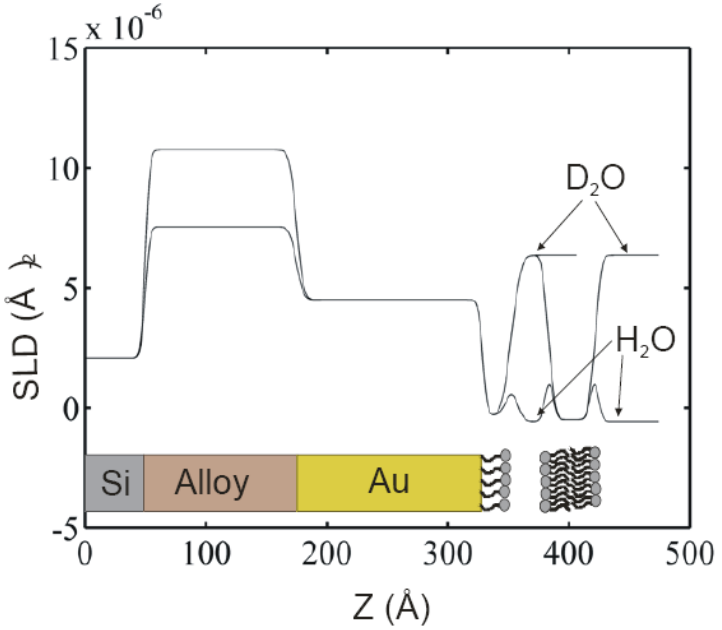
Also, development of new technology to aid membrane fabrication….

Latest developments – Gold/Thiolipids

Coverage of Silane SAM never exceeds about 80%. Found that coverage of subsequent membrane usually matches the SAM coverage.

Latest improvement is to use a thiolipid on gold. This gives full coverage and subsequent improvement in membrane quality





A.V. Hughes, S. A. Holt, A. Soliakov, T. R. Charlton and J. H. Lakey, *Submitted to Langmuir.*.

Progress so far….

•‘Floating bilayers’ developed over last 10 years at ILL / ISIS as realistic unconstrained membranes.

• Use of Self Assembled Monolayers as supports means able to use more useful lipids – e.g. unsaturation, charge etc.

• Silane based SAM’s allow membranes of 70 – 80 % coverage. Thiolipid SAM’s on gold allow membranes approaching 100% coverage.

• Developed novel LB trough technology for accurate positioning of substrates. Quick and repeatable sample preparation.

In this JRA….

- JRA contribution of €125k will allow recruitment of PDRA at ISIS for 2 years.
- Advertisement about to 'go live', with interviews scheduled for end of January 2013.

– ISIS PDRA will….

1. Continue development of floating bilayer technology. Specific aims are more realistic membrane compositions, particularly 'raft' containing membranes, and bacterial membrane mimics containing lipopolysacharides.
2. Investigate potential methods of incorporating membrane proteins into floating bilayers
3. Develop new methods of data analysis, particularly involving molecular modelling techniques to analyse neutron data.