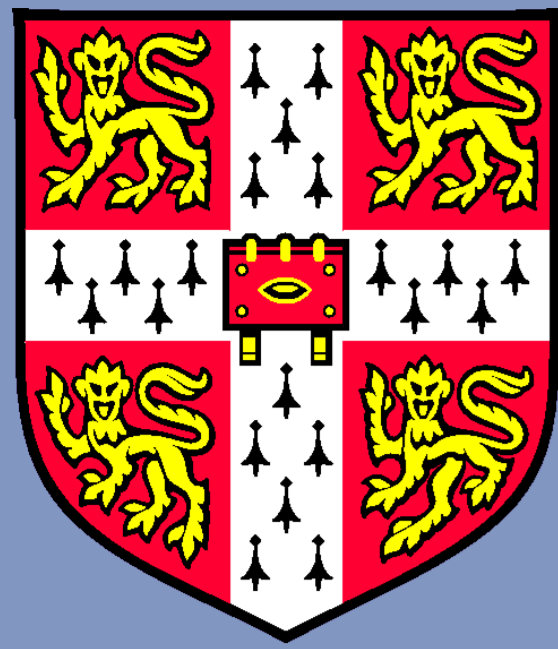


Studying the Structure of Biological Membranes on Titanium Implants using Neutron Reflection



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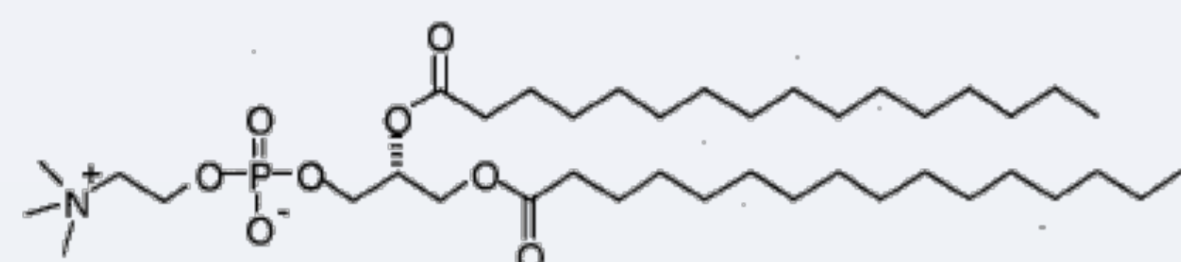
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INTRODUCTION

Titanium is an ideal substrate for use in biomedical applications due to its strength and non-toxicity. However very little is known of the interactions of biological material with titanium surfaces^{1,2}. Here we present the first structural characterisation of the purely zwitterionic phospholipid (DPPC) deposited onto a titanium surface.

A titanium surface for neutron reflection measurements was prepared by coating a silicon wafer with a 100Å layer of metal using electron beam evaporation. The substrate was then characterised with neutron and x-ray reflection and the DPPC bilayer deposited using a Langmuir Schaeffer technique.

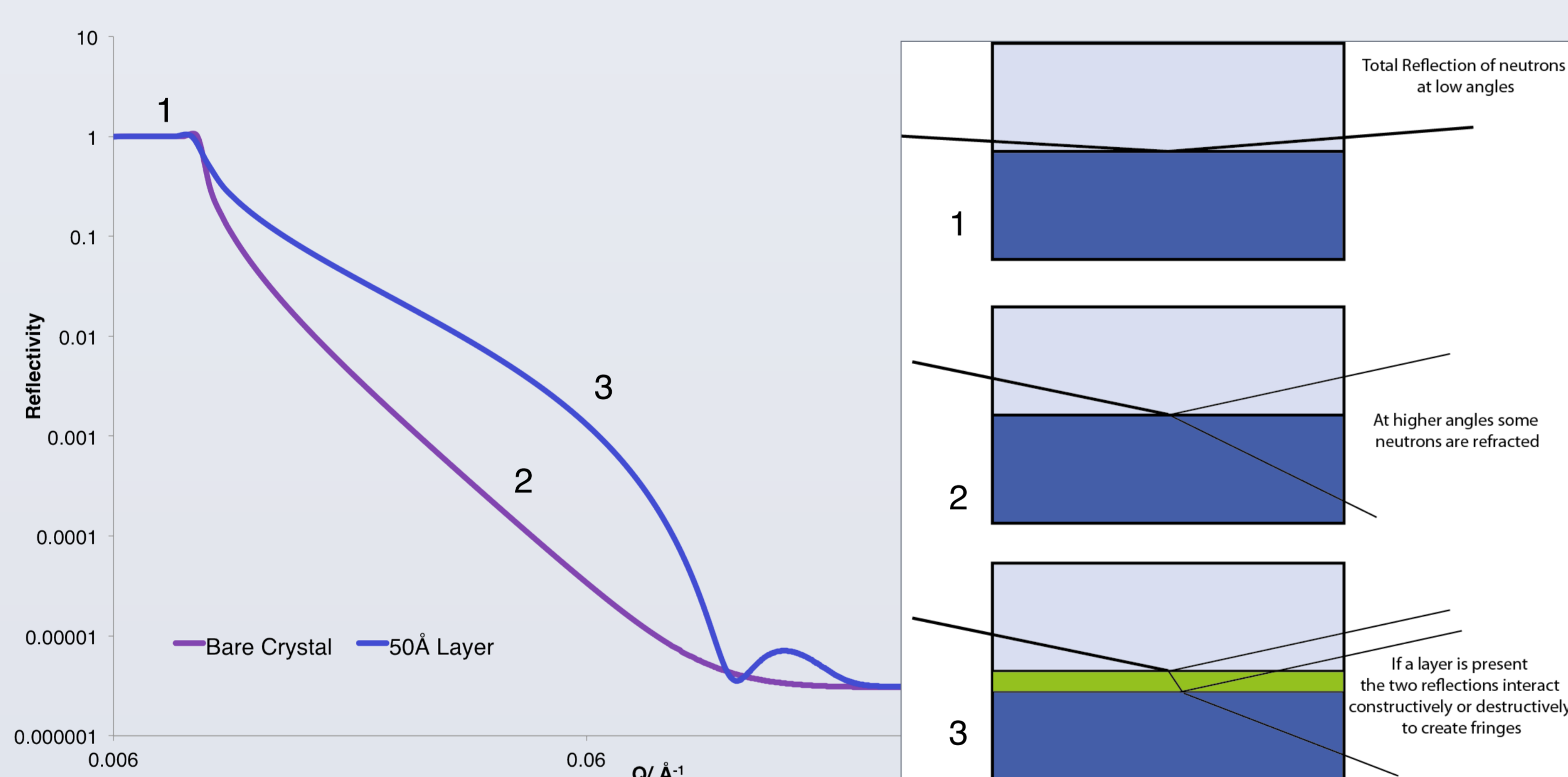


Zwitterionic dipalmitoylphosphatidylcholine (DPPC) molecule which forms a simplified biological membrane at interfaces.

Results from neutron reflection data suggest the formation of a defect-free bilayer at the titania water interface with a calculated area-per-molecule of 51Å². Upon addition of 20mmol CaCl₂ the area per molecule increases to 70Å² suggesting significant membrane disruption.

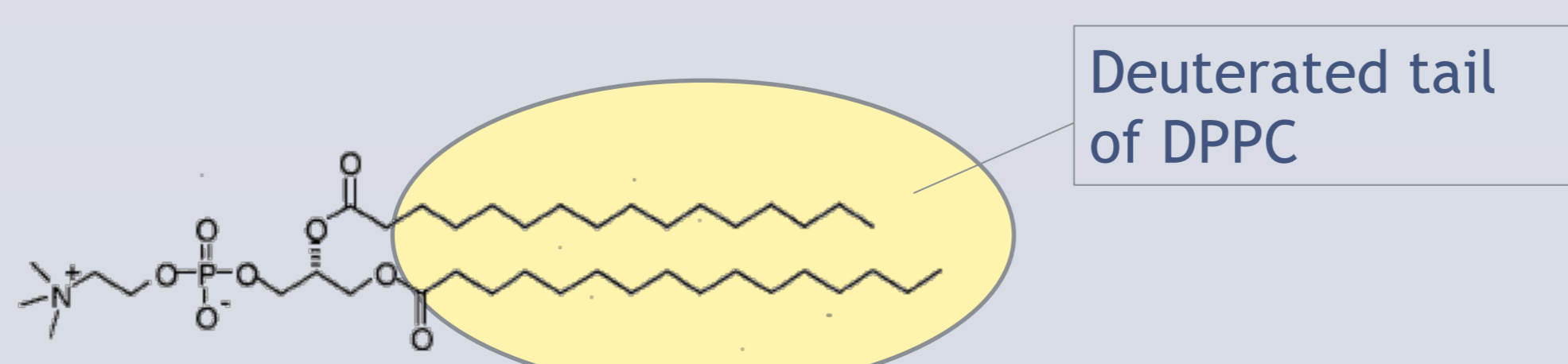
NEUTRON REFLECTION

- Neutron Reflection is a technique to study flat buried interfaces in a non-destructive manner.
- The reflectivity of a neutron beam impinging on a surface at grazing angles is measured as a function of the angle of incidence. As the angle is increased fewer neutrons are reflected and more are refracted (2).
- A layer at the interface causes a phase lag between the two reflections leading to interference fringes which are directly proportional to the thickness of the layer (3).

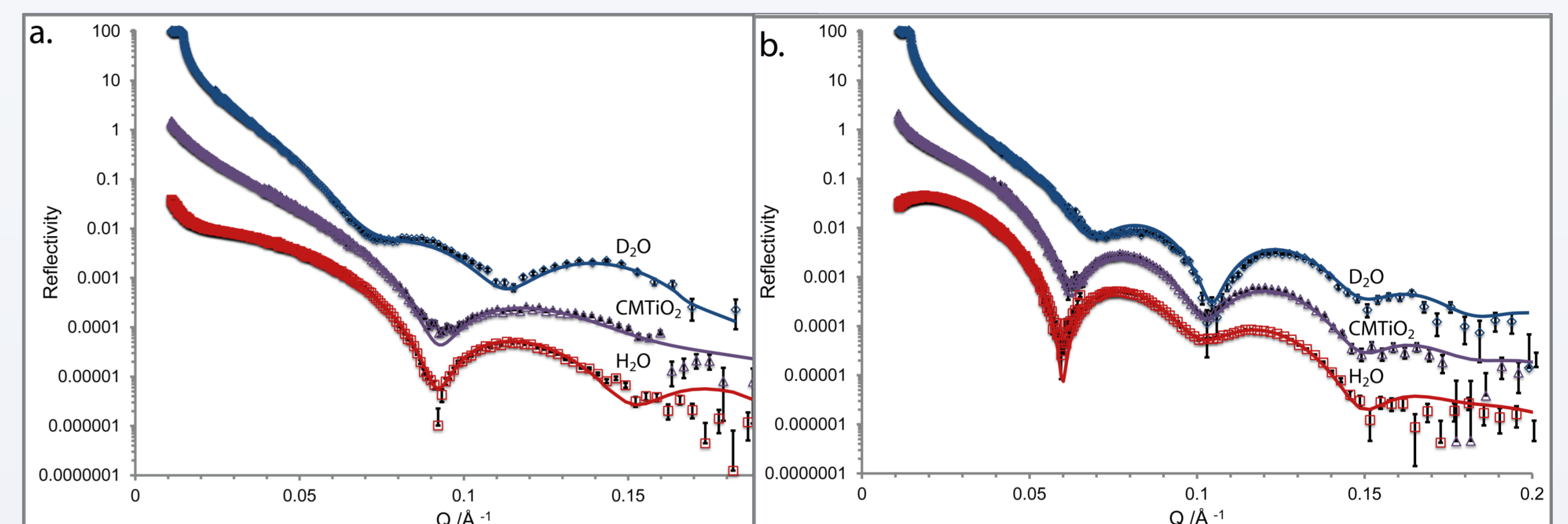


CONTRAST VARIATION

- One of the most powerful aspects of neutron research is the vastly different interaction of neutrons with different elements and isotopes. This parameter is quantified by a scattering length density (SLD) and allows structural characterisation of highly complicated systems.
- Most useful is the difference in SLD of hydrogen and deuterium. Through variation of the water subphase different reflectivity profile can be recorded for chemically identical systems.
- In this experiment 3 water contrasts were used, H₂O (SLD = $-0.56 \times 10^{-6} \text{Å}^{-2}$), D₂O (SLD = $6.35 \times 10^{-6} \text{Å}^{-2}$) and a mixture that matched the scattering length density of the titanium oxide (SLD = $2.9 \times 10^{-6} \text{Å}^{-2}$). Allowing a unique structure to be found.
- The DPPC molecule was also selectively deuterated at the hydrophobic tail region so that the head and tail groups of the molecule can be distinguished.



RESULTS



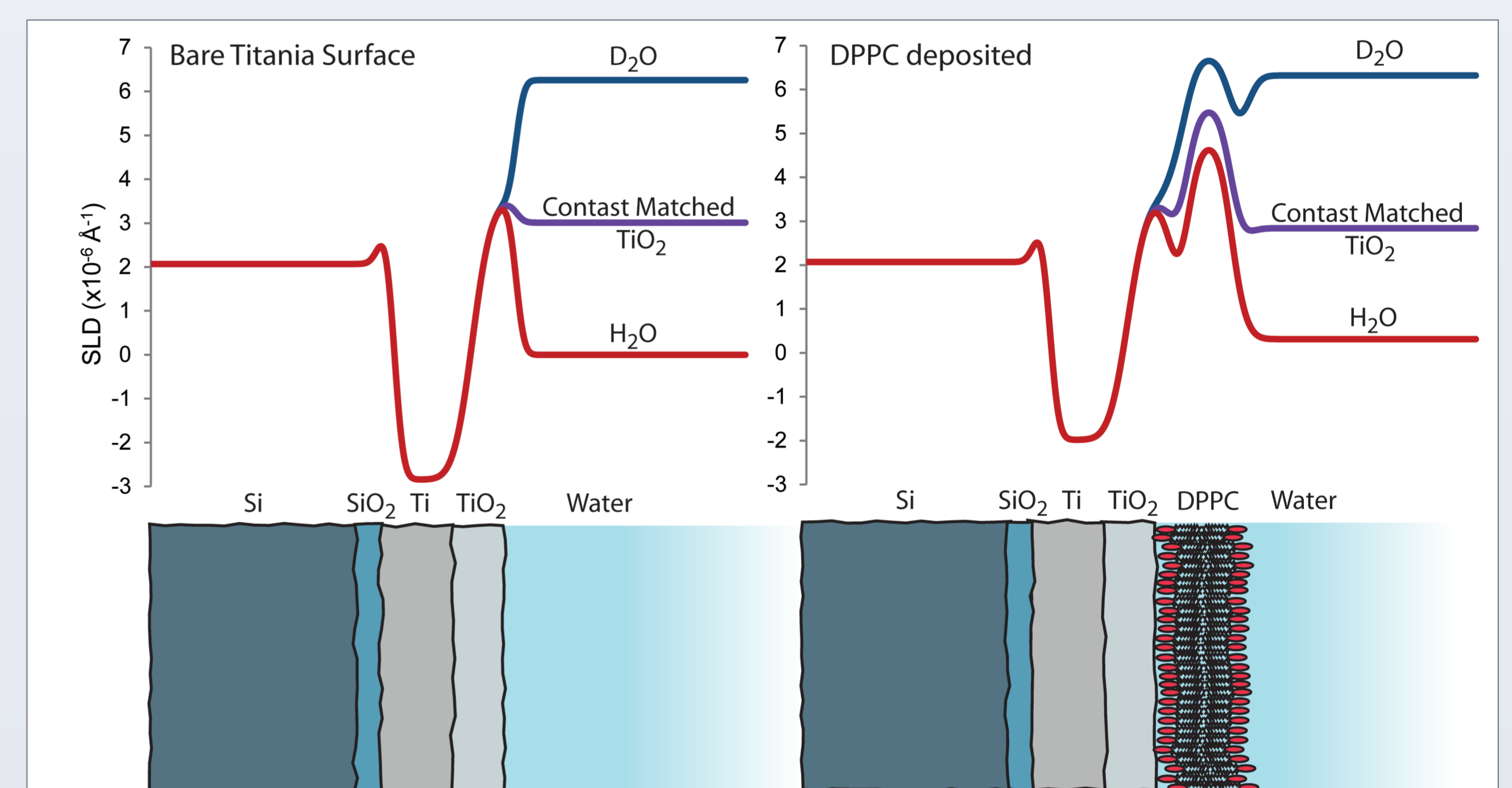
The above figure (left) shows the reflectivity profiles obtained for three contrasts of the bare titanium substrate. Data obtained is shown as discrete points with the best calculated fit overlaid as a continuous line. Upon deposition of the lipid bilayer the reflectivity changes drastically as shown in the figure above right.

Material	SLD (10^{-6}Å^{-2})	Thickness (Å)
Silicon	2.07	-
Silicon Oxide	3.47	11 ± 1
Titanium	-2.87 (-1.99 ^a)	68 ± 1
Titanium Oxide	2.9	38 ± 1

^aSLD of titanium after deposition of the lipid membrane
Fitting parameters used to characterise substrate

It is clear that a stable bilayer has been deposited.

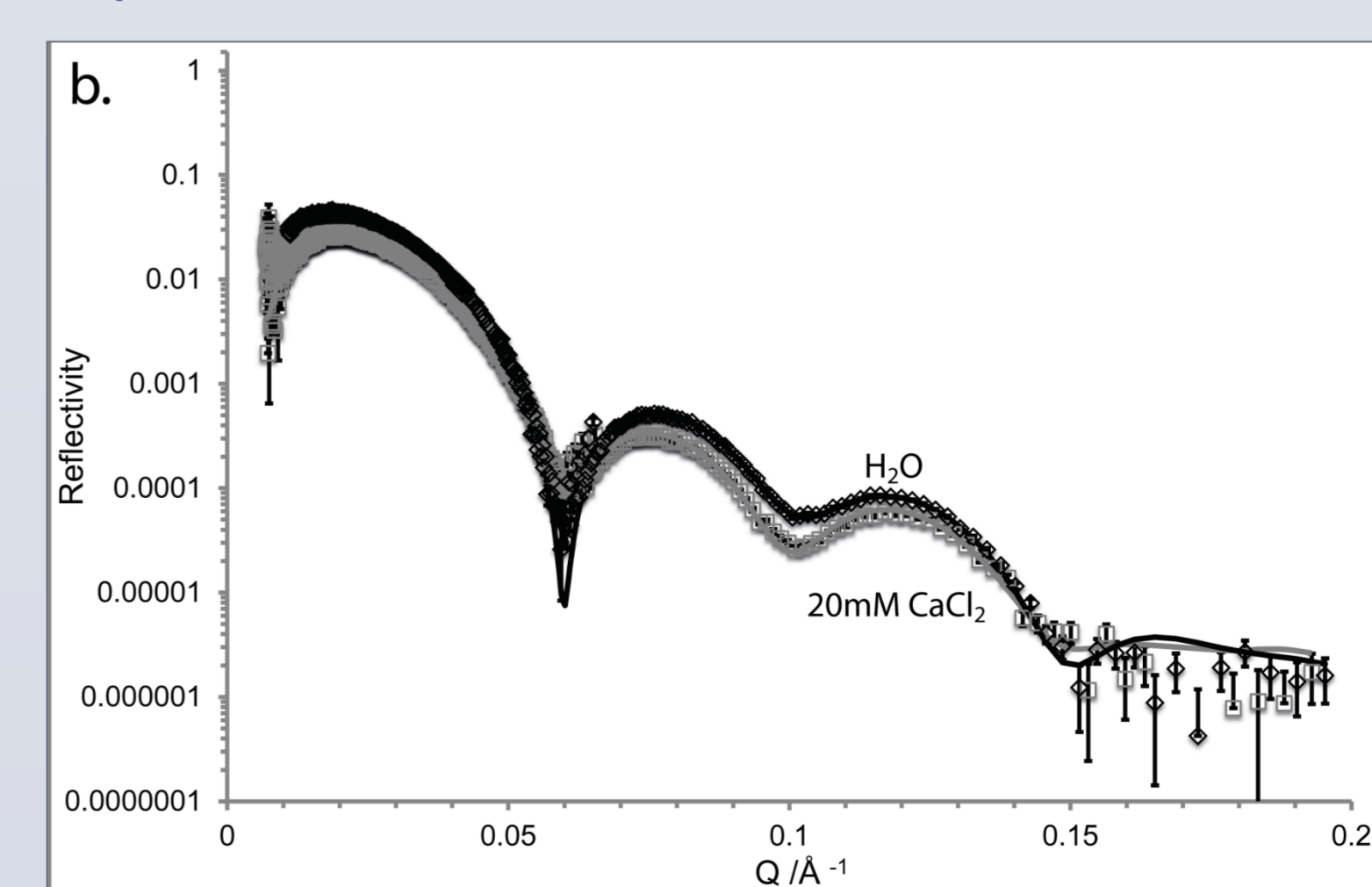
From calculations of the fit an area per DPPC molecule was found to be 51Å² in good agreement with expectation for a defect-free bilayer.



The SLD profiles above show how the calculated scattering length density changes as distance perpendicular to the interface changes. On addition of the DPPC bilayer the deuterated tails can be seen clearly as a rise in SLD at the interface. To aid the visualisation a cartoon of the of the system lies below the SLD profile.

DISCUSSION AND CONCLUSIONS

- The structure of the bilayer differs from that deposited on more traditional surfaces like quartz and silicon. Normally the bilayer sits on a 20Å cushion of water and counter-ions^{3,4}. On titanium the bilayer is in direct contact with the surface due to the lower charge density at physiological pHs.
- On addition of biological concentrations of calcium (20mmol) the bilayer is irreversibly disrupted by the loss of molecules from the surface and the area per molecule increases to 70Å².
- Upon heating the bilayer does not form a highly disordered ripple phase as seen with neutron reflection on silicon substrates. Molecules are again lost from the surface as the area per molecule increases.



Neutron reflectivity profiles of the DPPC membrane deposited on titanium in water (black) and 20mmol CaCl₂ (grey)