

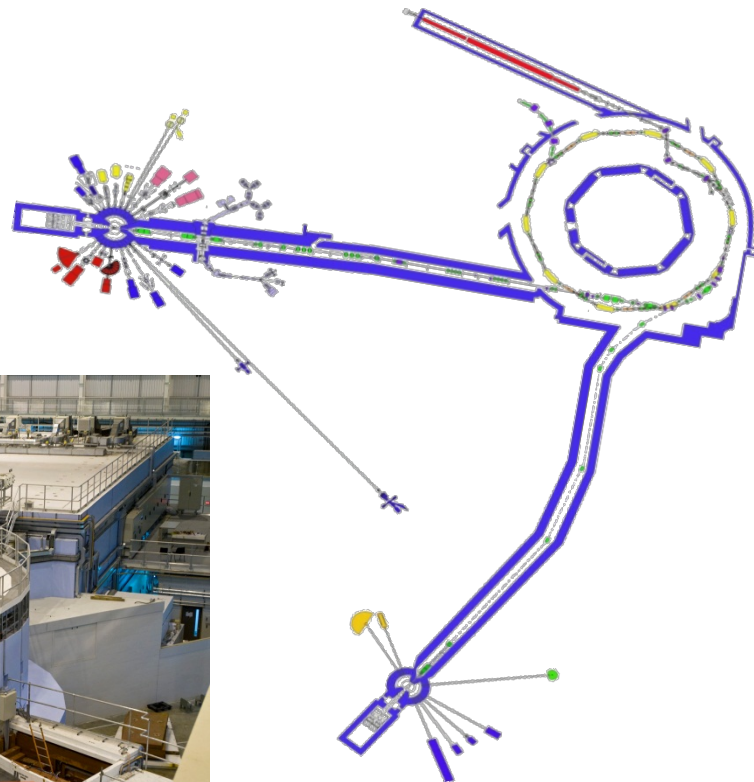
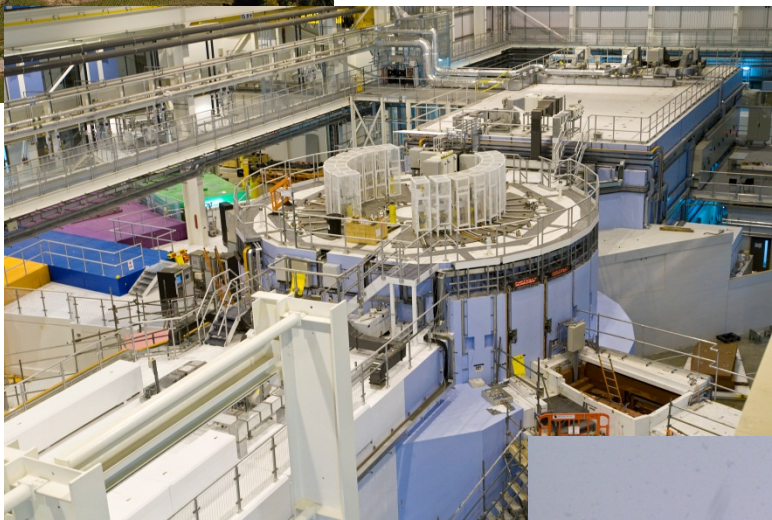
The Interaction of Thionins with Model Pathogen Membranes

Luke Clifton

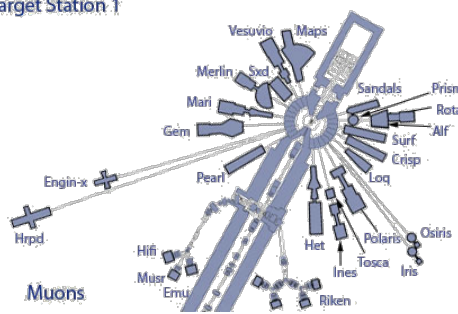
(luke.clifton@stfc.ac.uk)



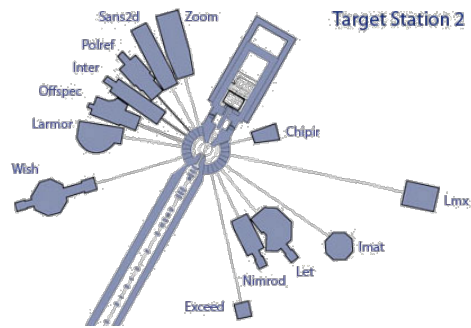
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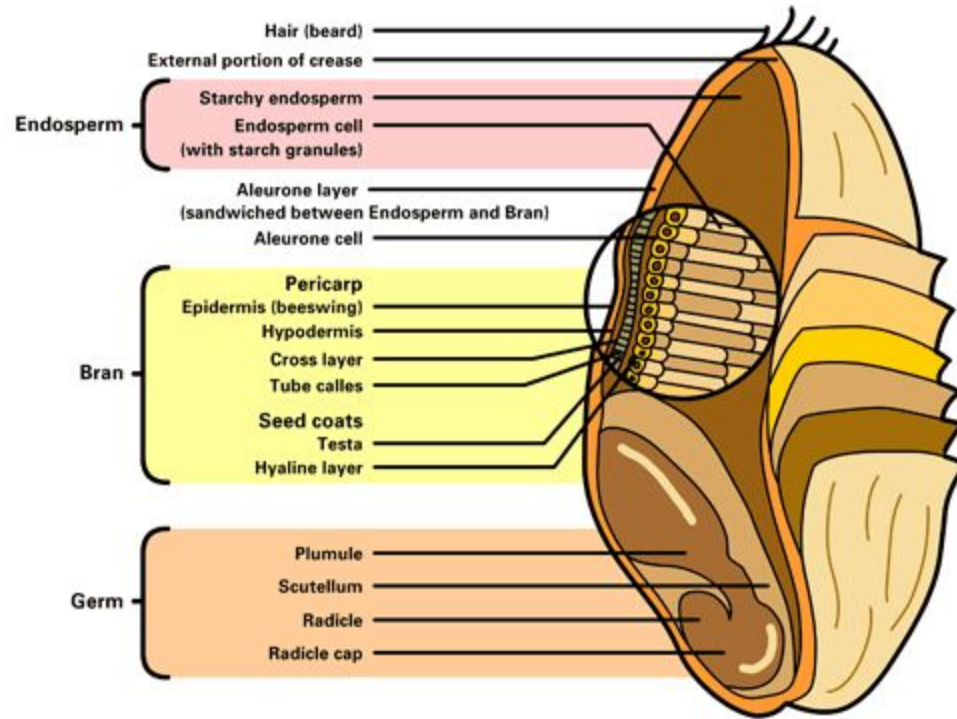


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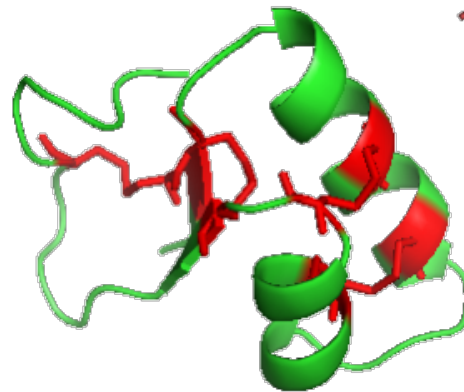
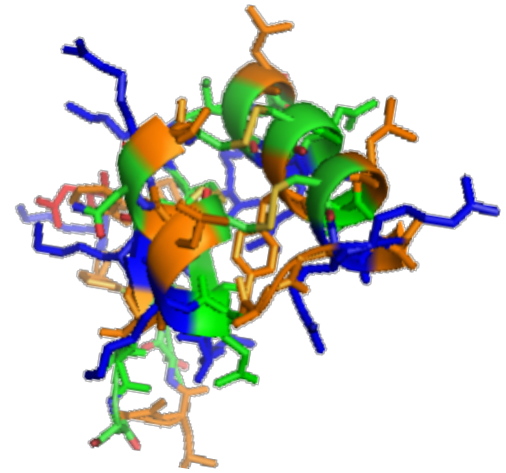
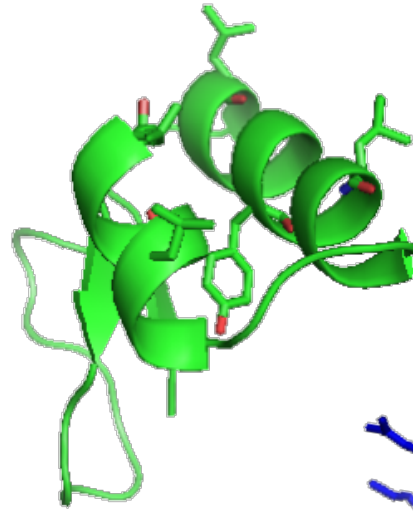
Target Station 2





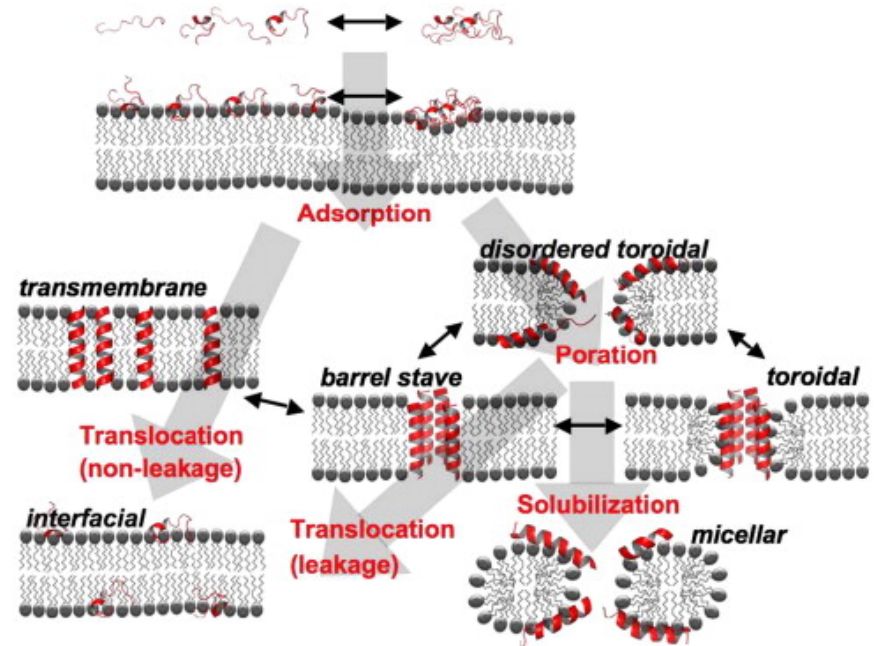
Thionins

- Thionins are small (<5KDa) proteins produced as part of plants innate immune response system.
- All members are highly cationic (pI 9-11) which enables interactions with the anionic bacterial and fungal membranes.
- Most are also amphiphilic allowing partition into the hydrophobic core of the membrane.
- Thionins are cysteine rich, they contain between 8 and 10 cysteine residues forming 4-5 disulphide bonds per protein. Making these proteins structurally stable.

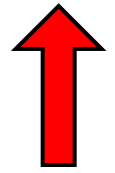
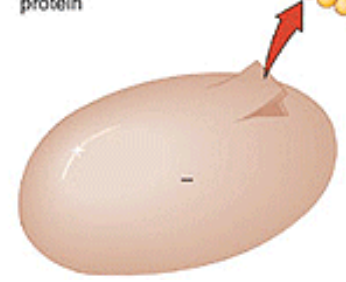
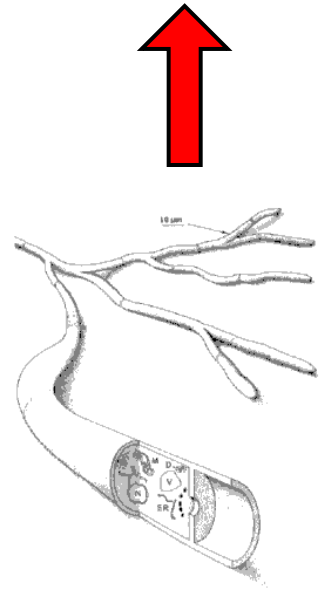
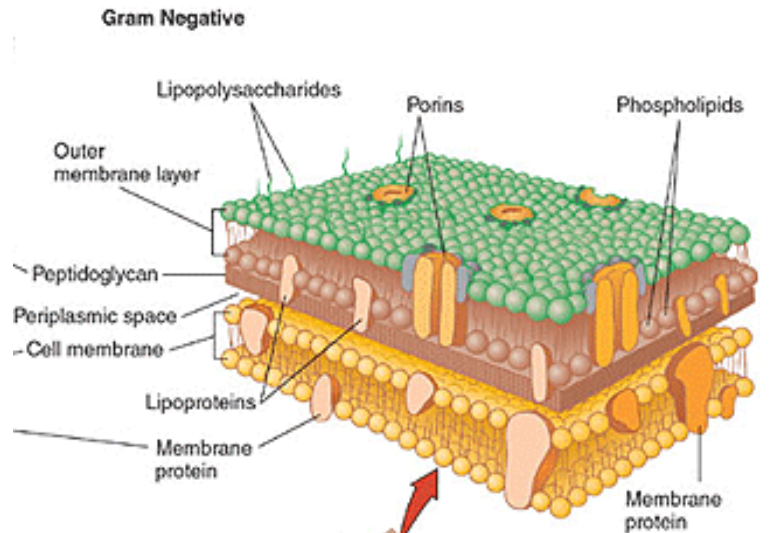
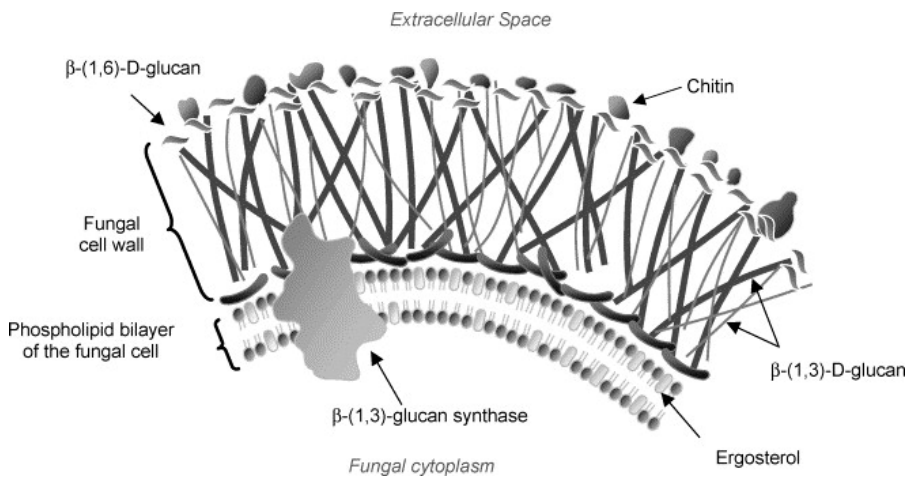


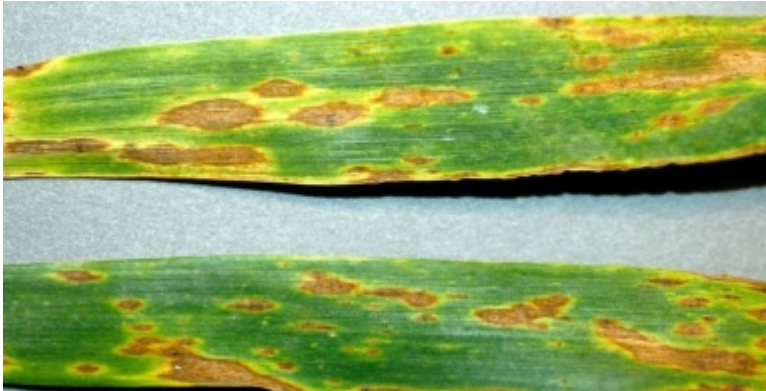
Antimicrobial Proteins which act at the membrane level.

- *Charge.*
- *Hydrophobic and hydrophilic domains (amphiphilicity).*
- *Hydrophobicity.*
- *Stable molecular conformation.*
- *Size.*
- The balance of these attributes modulates binding selectivity (prokaryotic/eukaryotic) and activity.



Fungal and bacterial membranes





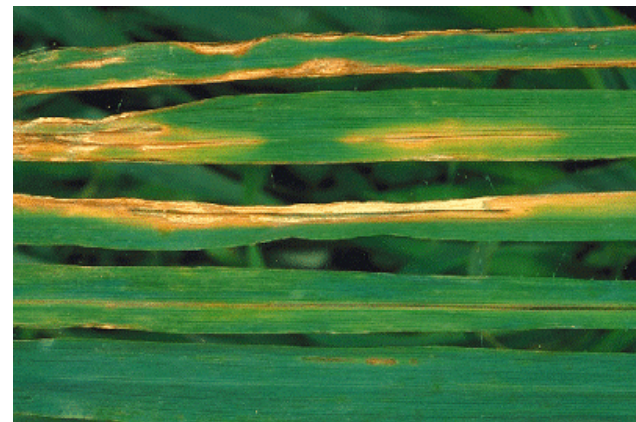
Tan spot (*Pyrenophora tritici-repentis*)



Glume Blotch (*Stagonospora nodorum*)



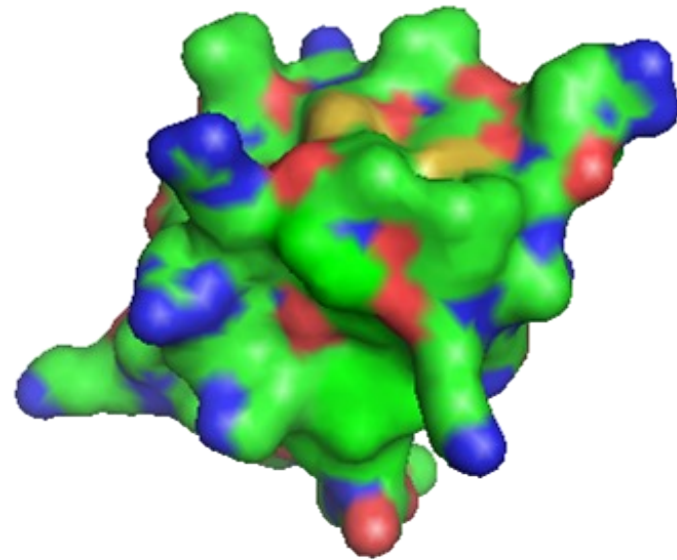
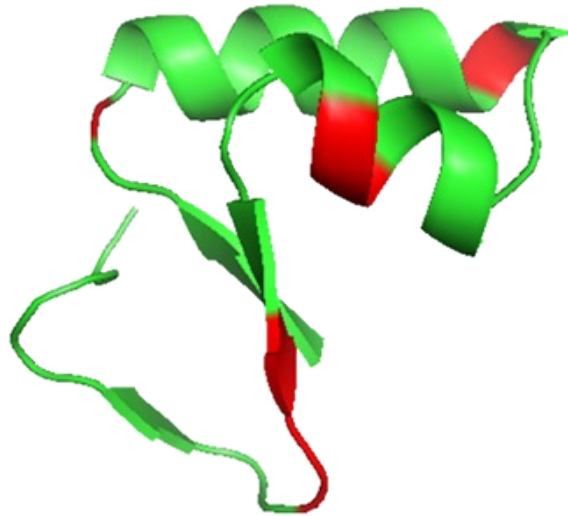
Common Smudge (*Cochliobolus sativus*)



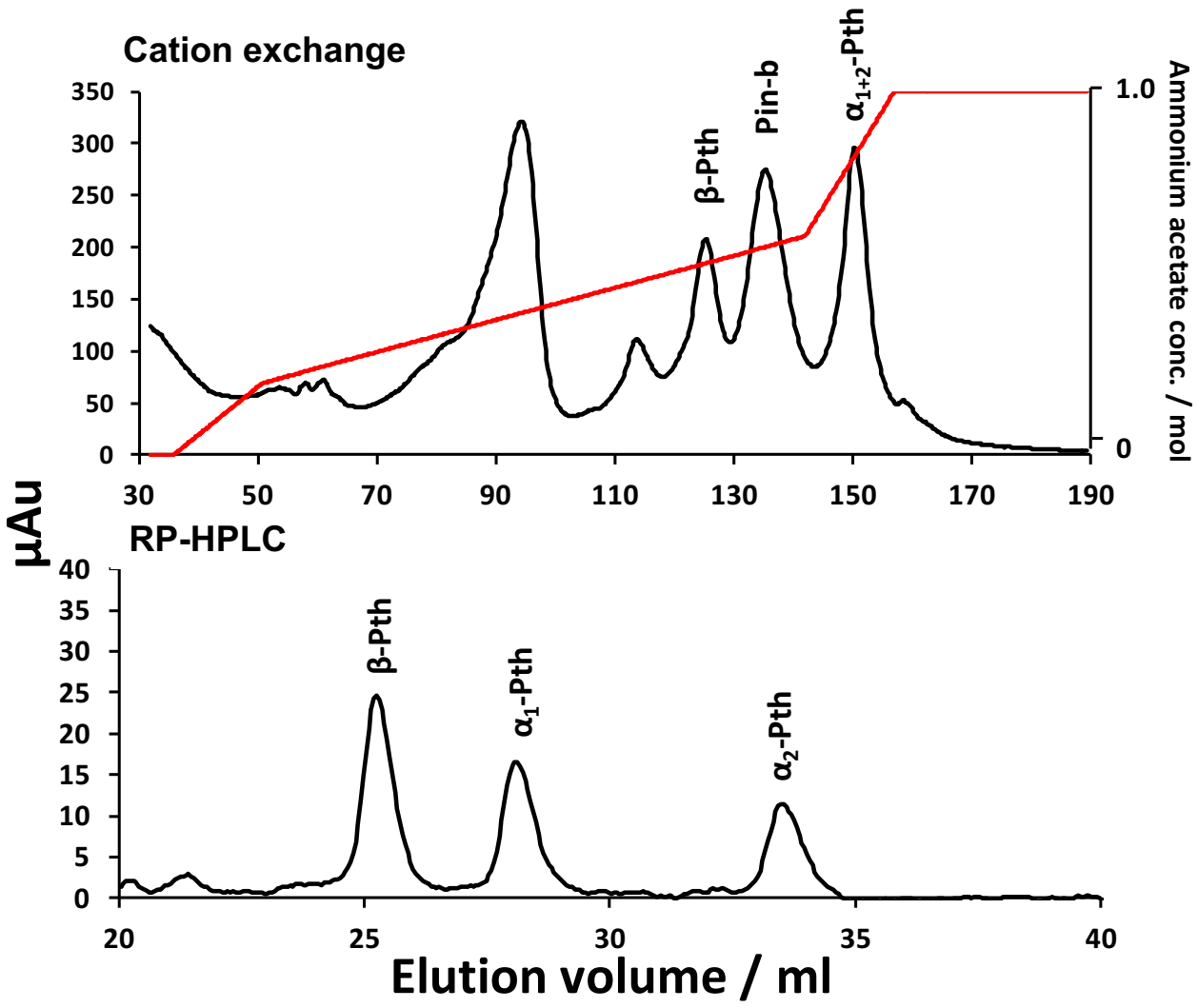
Stripe blight (*Pseudomonas syringie*)

α -purothioins

α_1 -Pth	KSCCR <u>S</u> TLGR NCYNLCR <u>A</u> RG AQKLC <u>A</u> GVCR CK <u>I</u> SGLSCP KGFPK
α_2 -Pth	KSCCR <u>I</u> TLGR NCYNLCR <u>S</u> RG AQKLC <u>S</u> TVCR CK <u>L</u> TSGLSCP KGFPK



α_1 and α_2 -purothionin – same charge, differing hydrophobicity

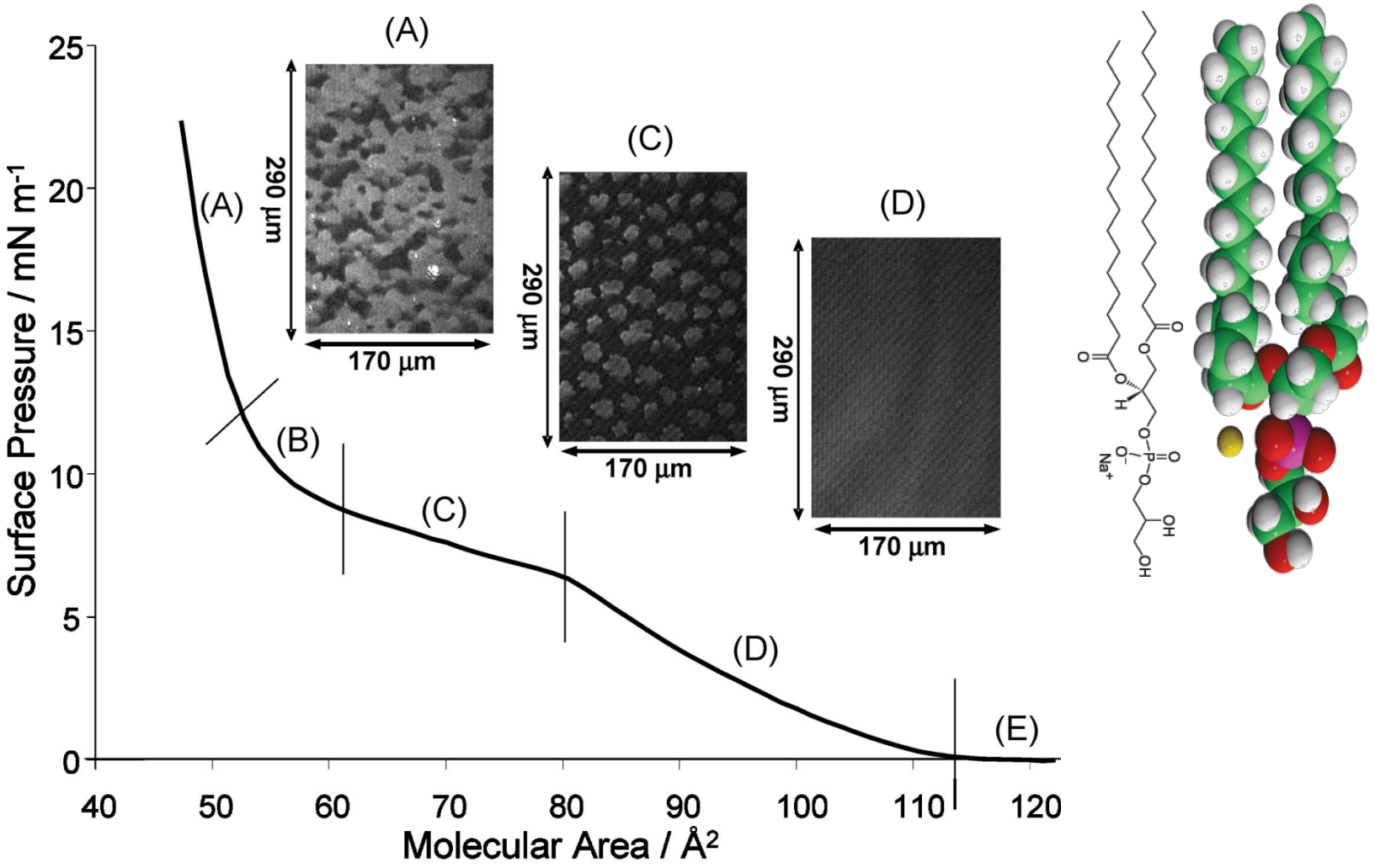


Aim

To determine the method by which thionins exert their seed defence activity, and determine how a difference in antimicrobial protein hydrophobicity effects protein membrane disruptive effects.



Comparing defense protein interactions with condense phase anionic phospholipid monolayers.



Brewster Angle Microscopy vs. time imaging

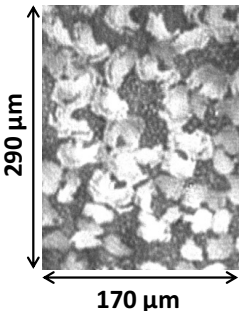
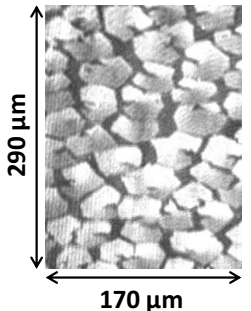
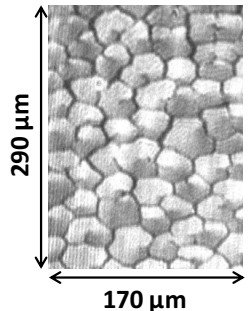
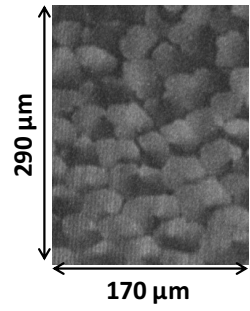
$\alpha 1$ -Pth

22 mN m⁻¹ before protein injection.

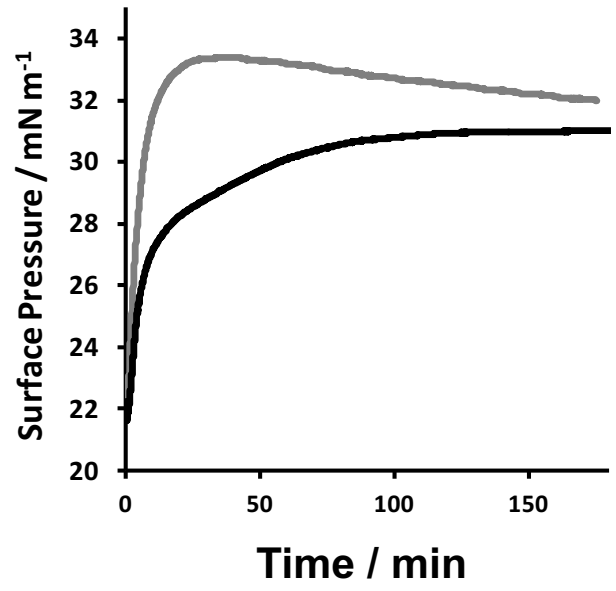
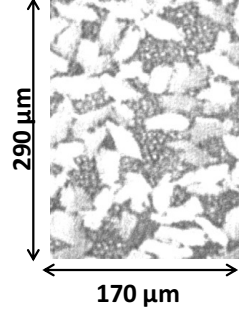
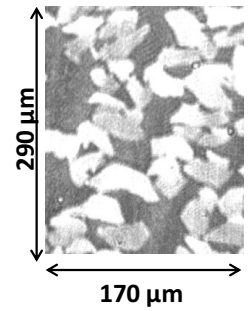
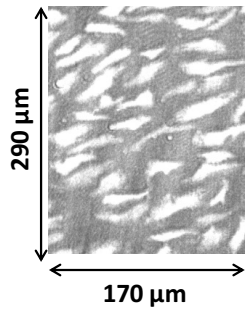
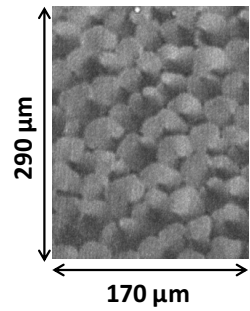
20 minutes after protein injection

90 minutes after protein injection

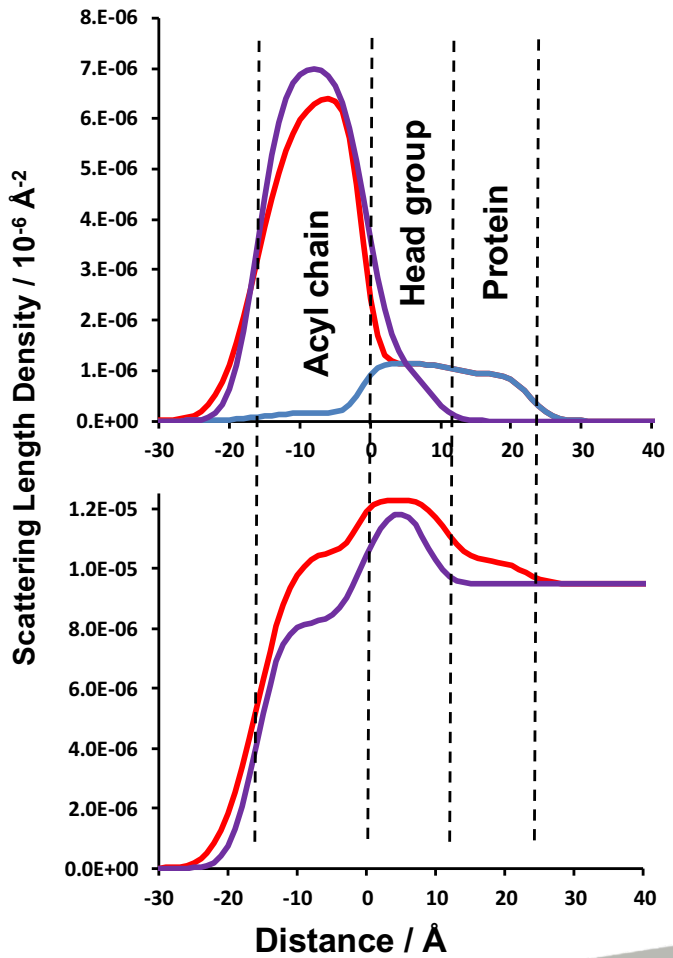
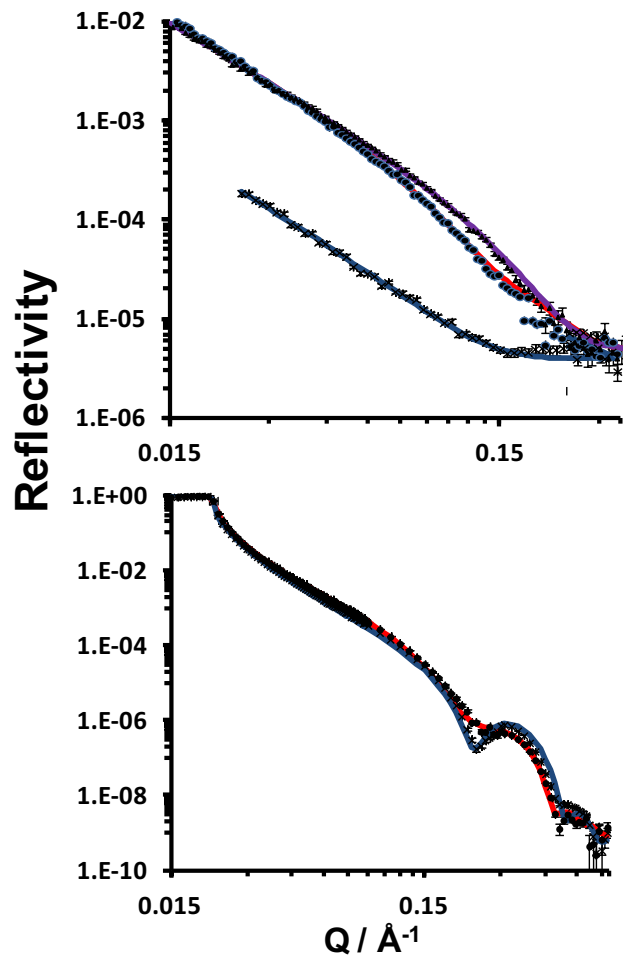
180 minutes after protein injection



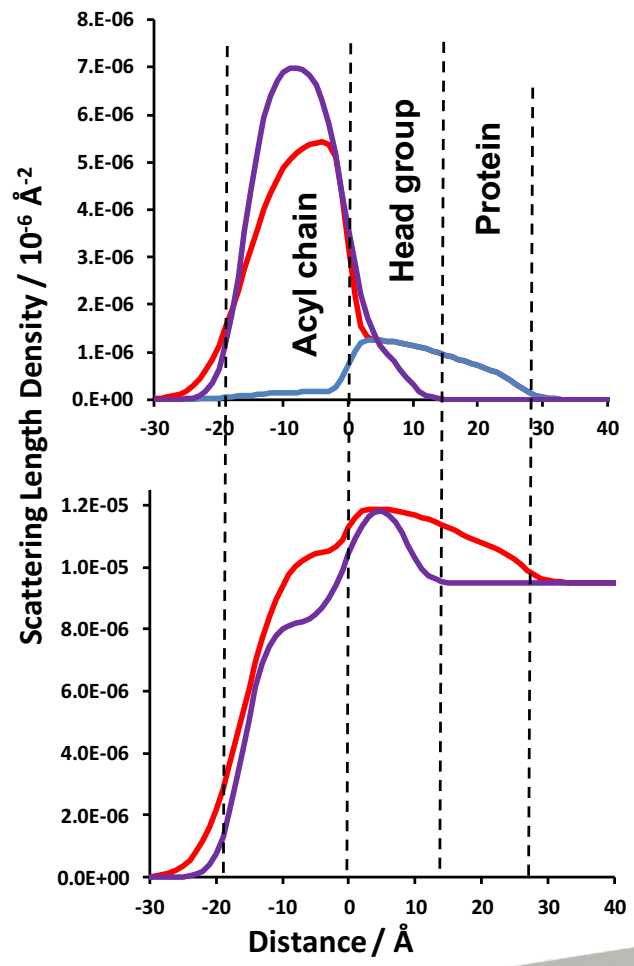
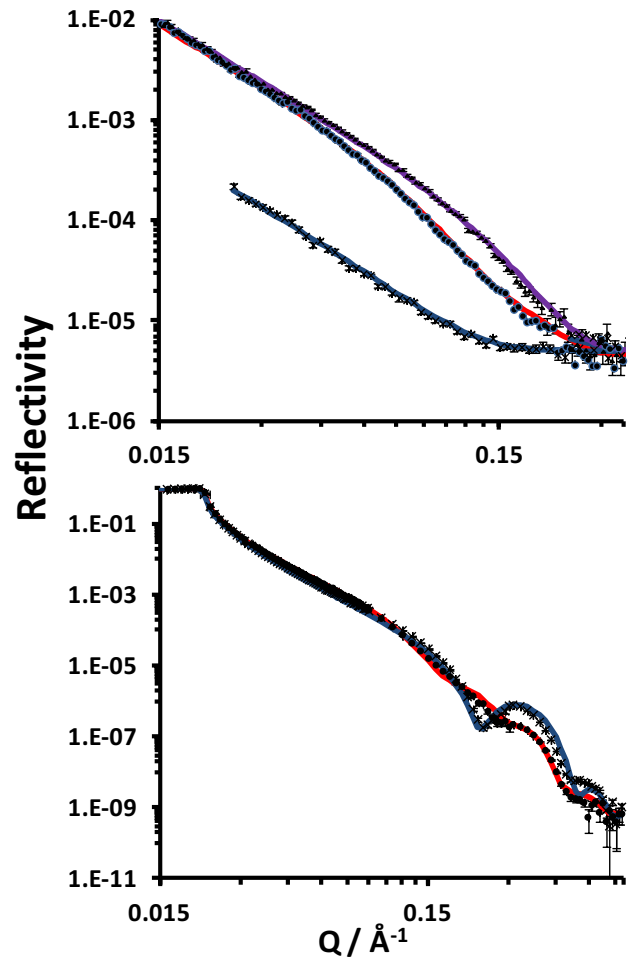
$\alpha 2$ -Pth



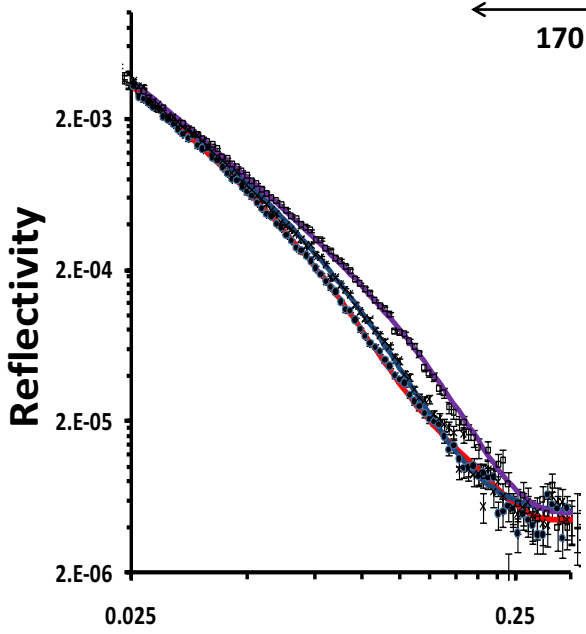
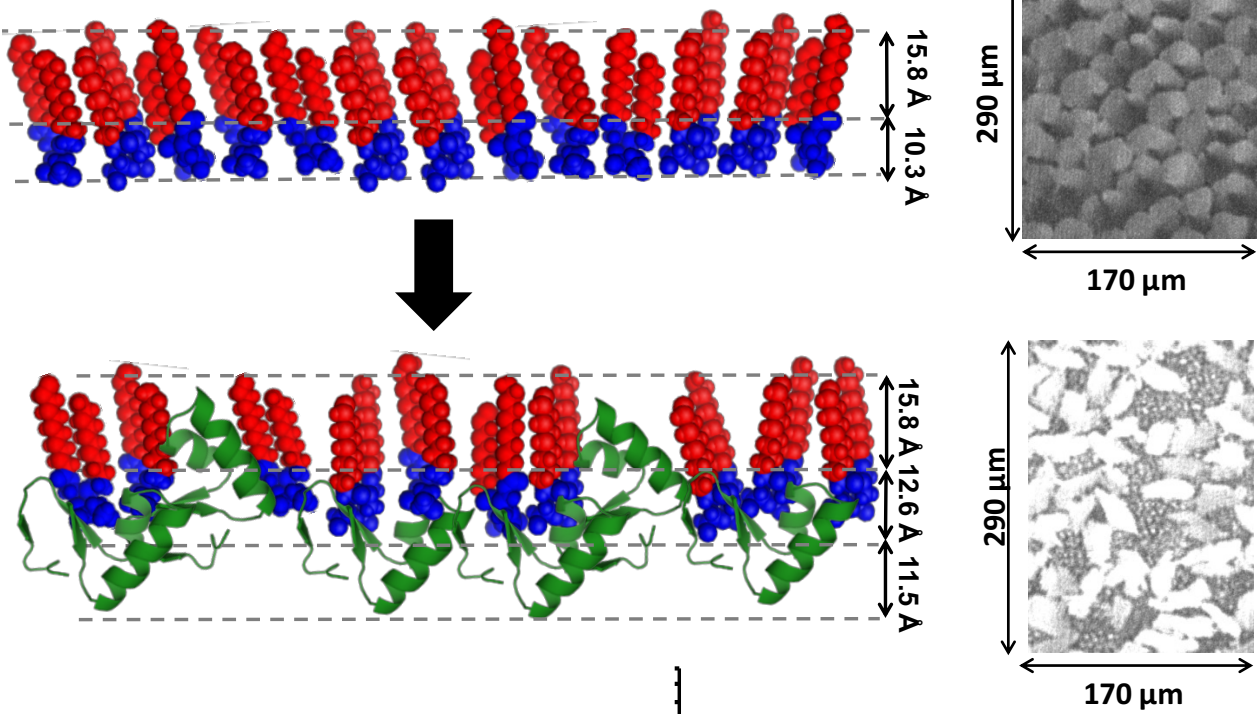
Equilibrium $\alpha 1$ -Pth adsorbed DPPG monolayer



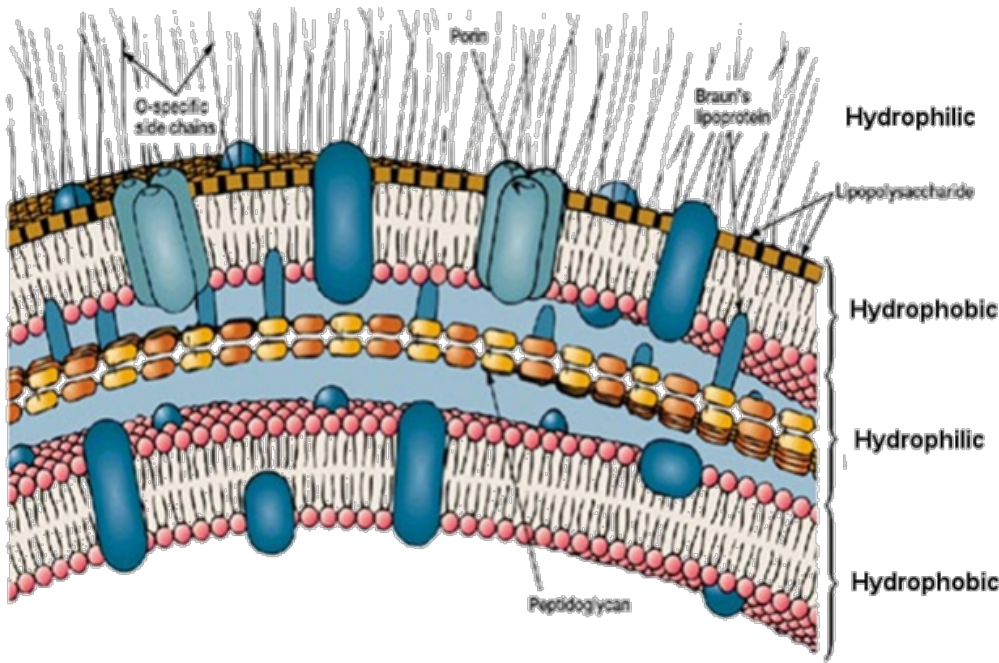
Equilibrium $\alpha 2$ -Pth adsorbed DPPG monolayer



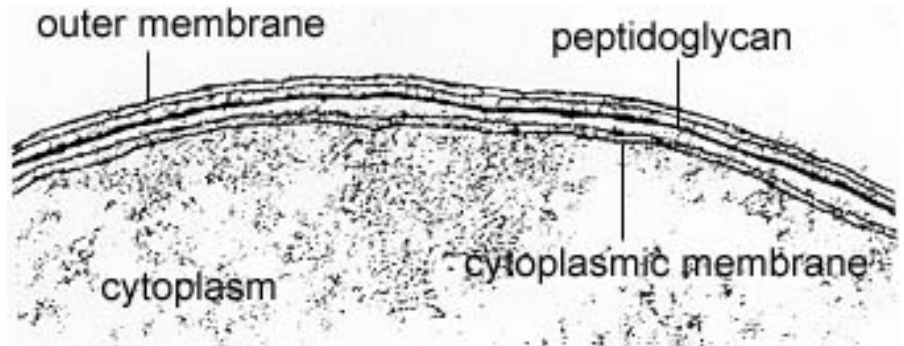
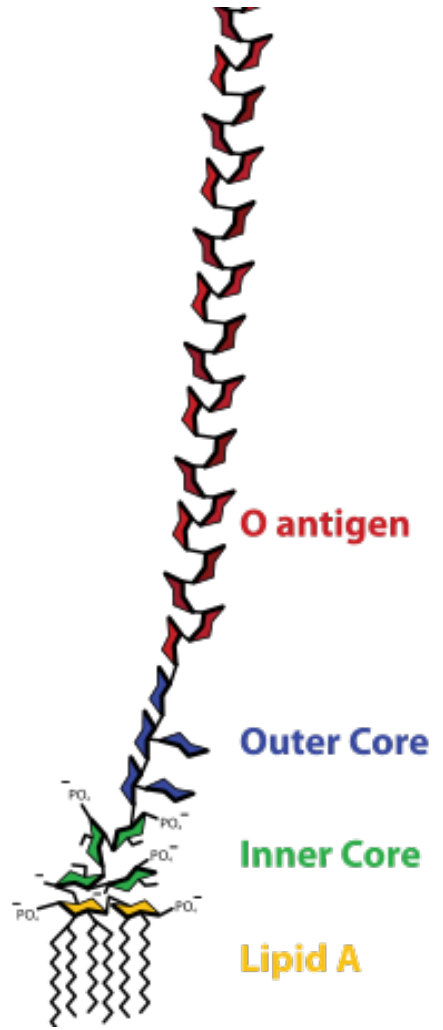
Monolayer results summary



Bilayer models of the Gram negative bacterial outer membrane

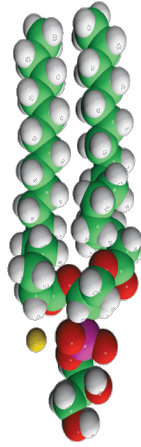


Hydrophilic
 Hydrophobic
 Hydrophilic
 Hydrophobic

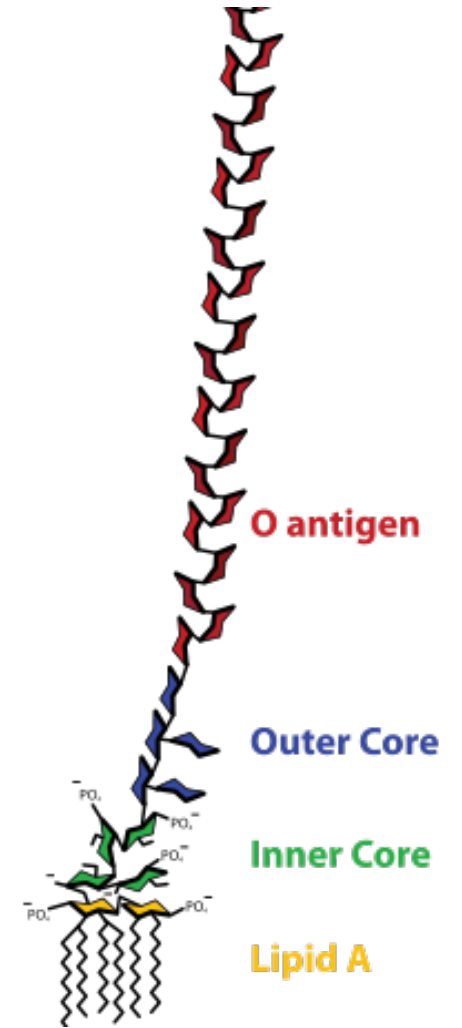
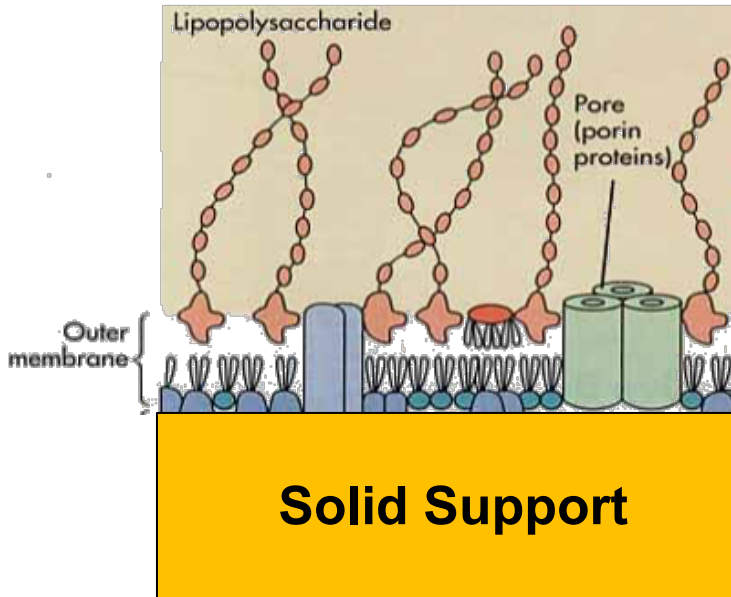
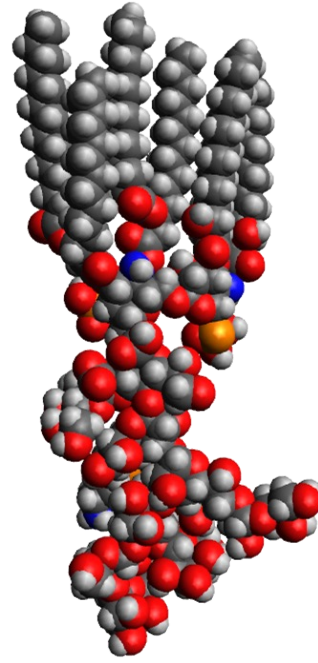


outer membrane
 peptidoglycan
 cytoplasm
 cytoplasmic membrane

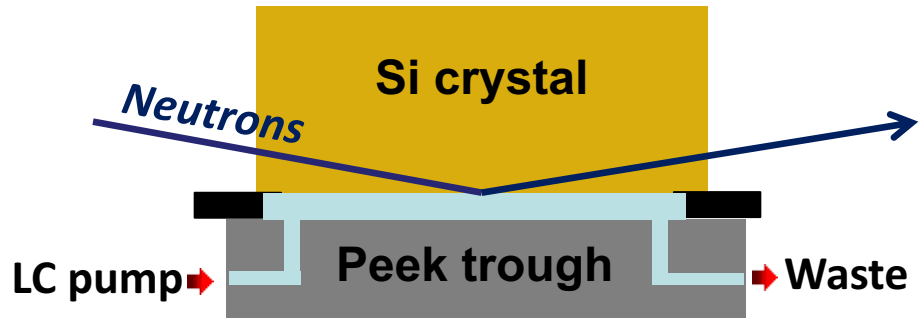
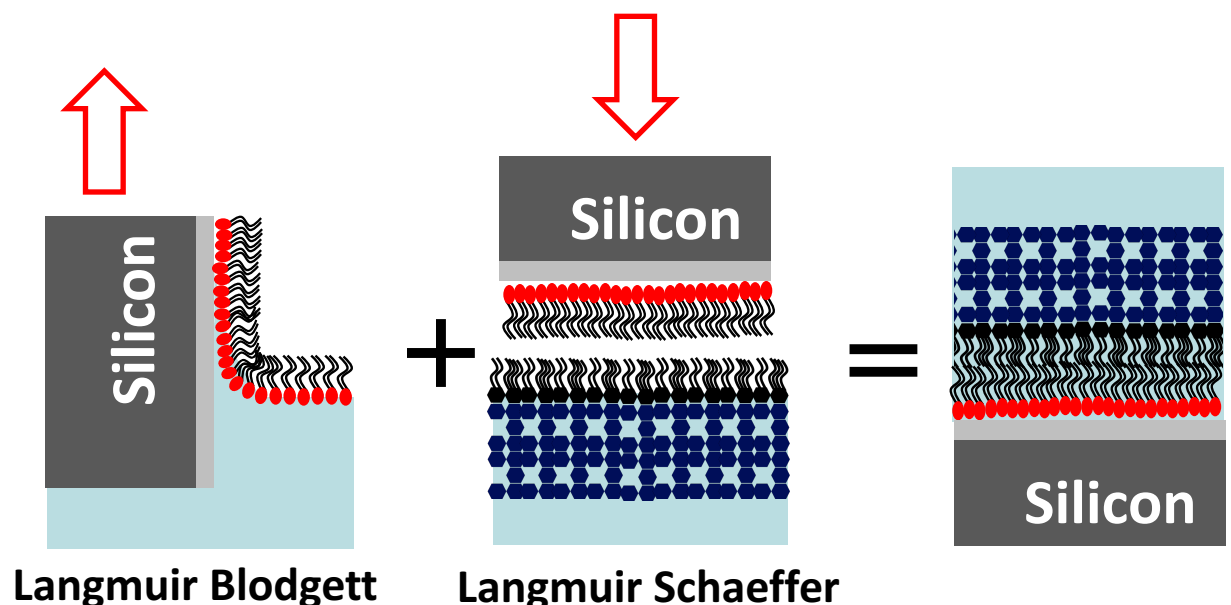
DPPC



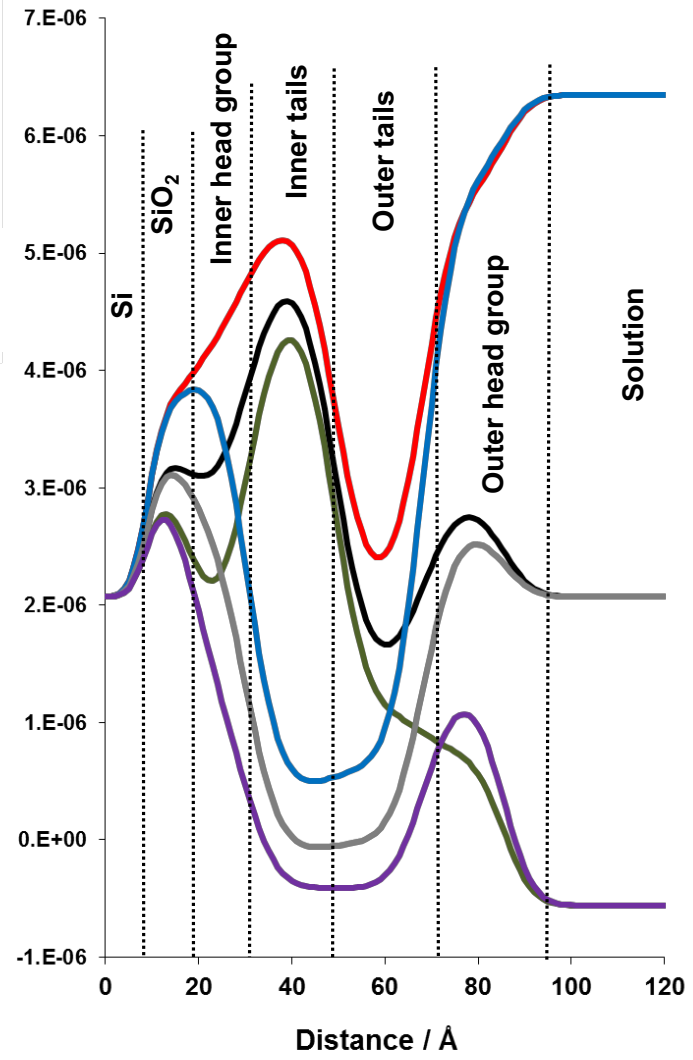
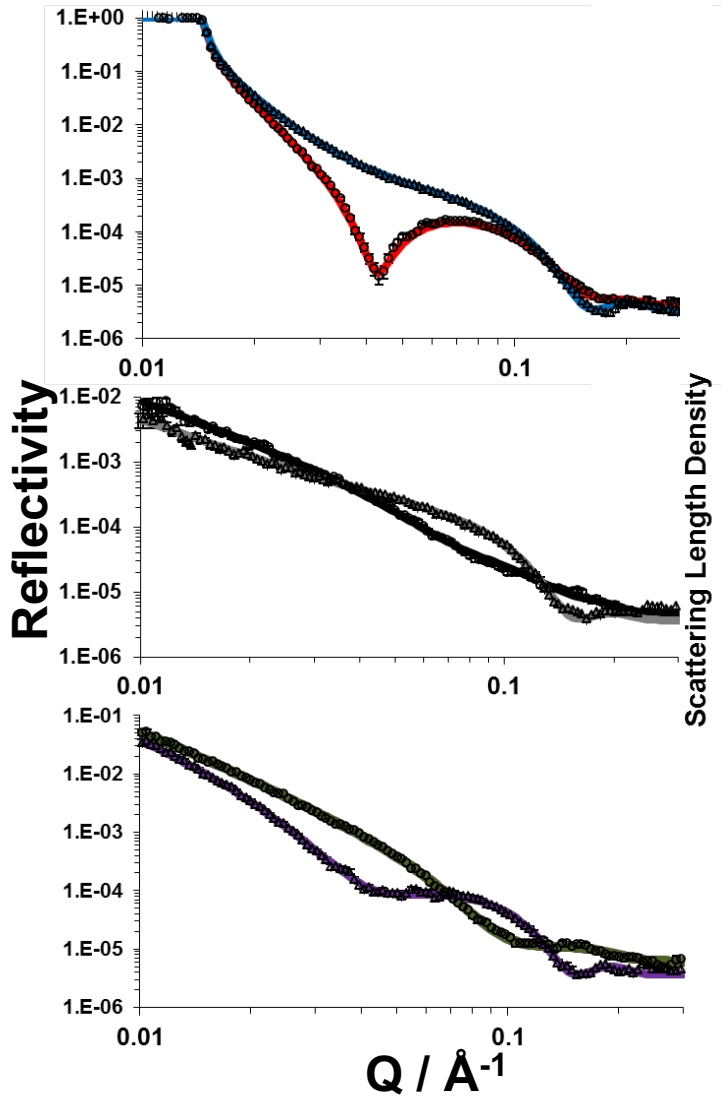
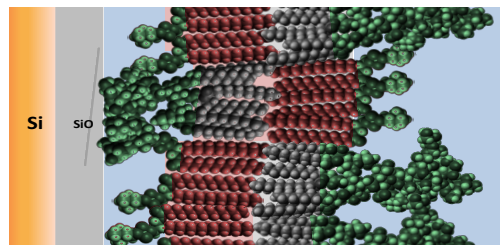
Rough mutant-LPS



Fabrication of asymmetric phospholipid : LPS membranes on silicon supports

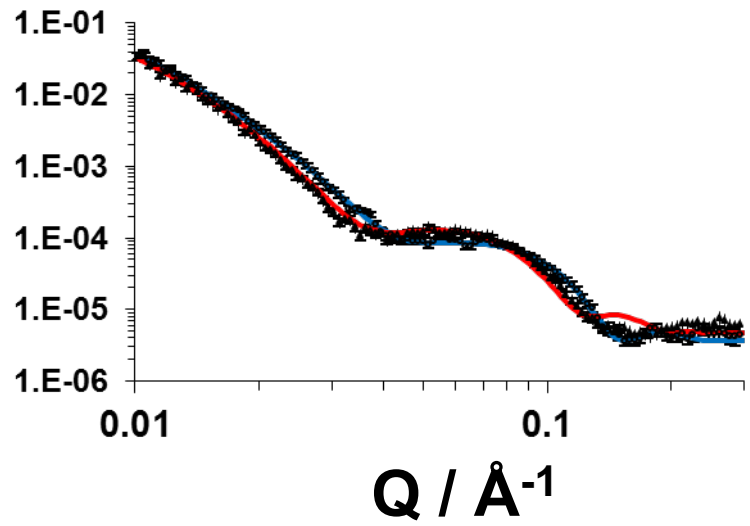
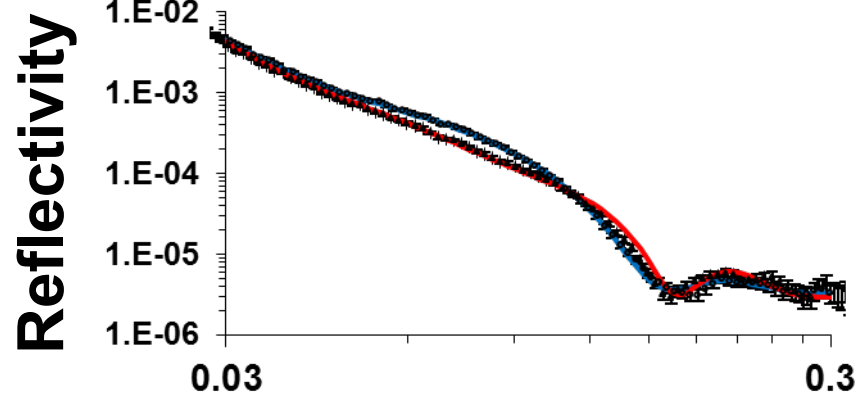
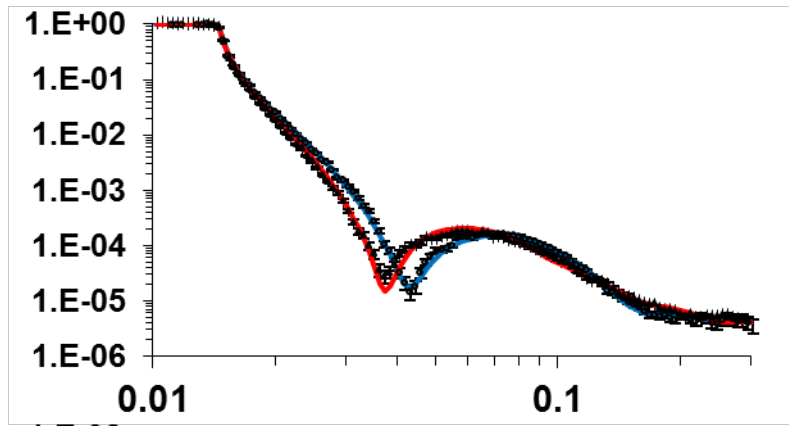


DPPC : Rc-LPS bilayer

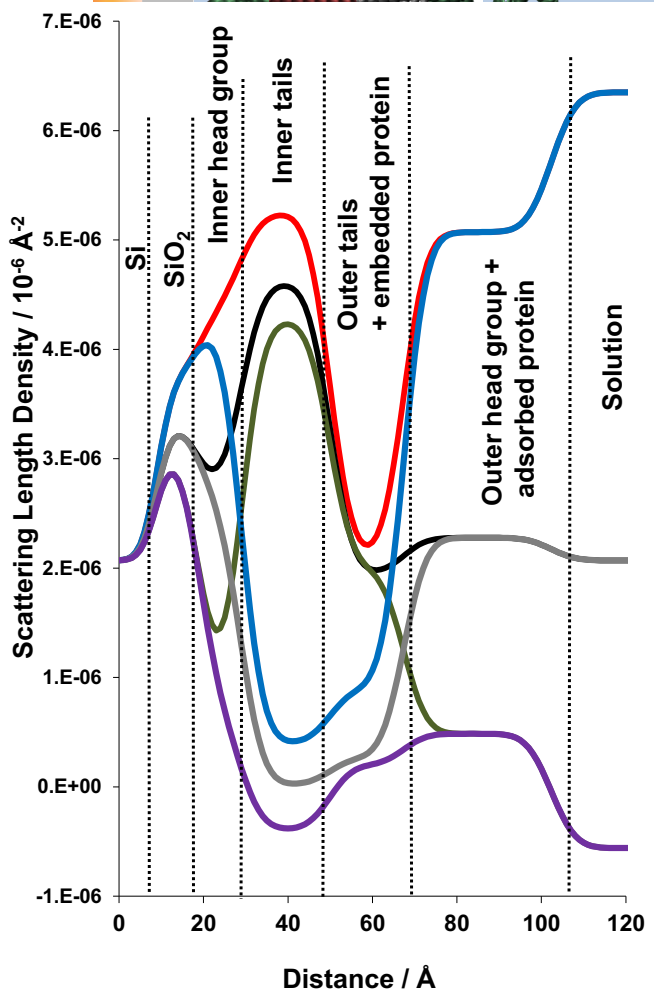
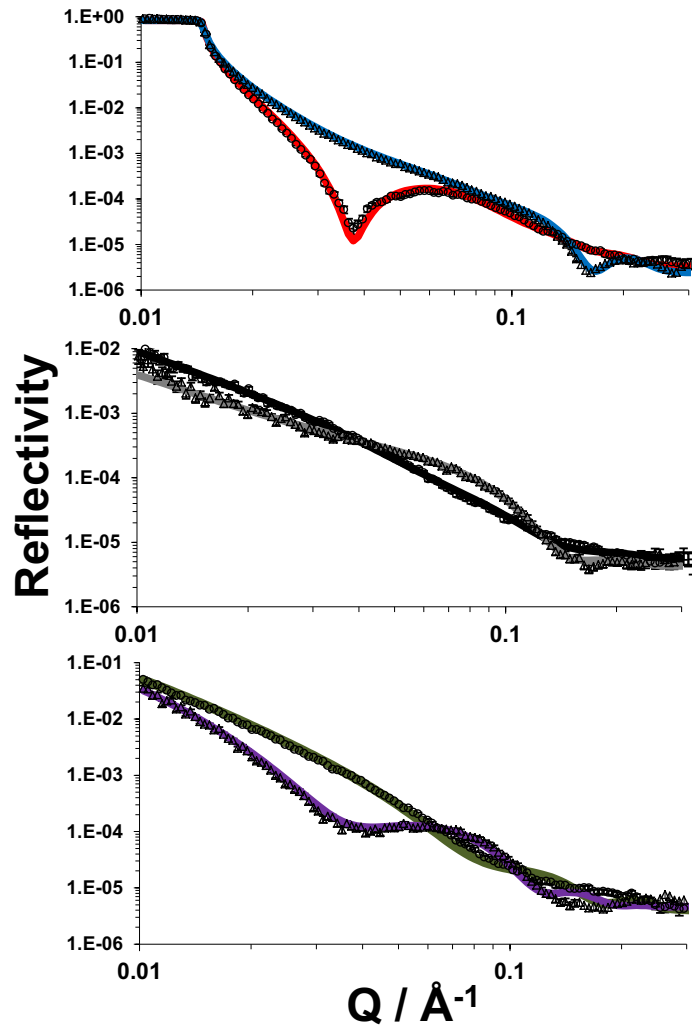
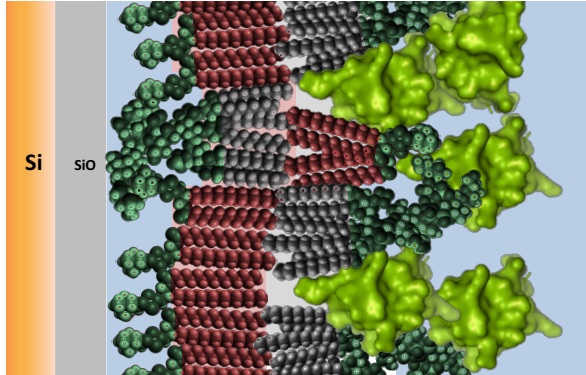


Changes Upon addition of $\alpha 1$ -Pth

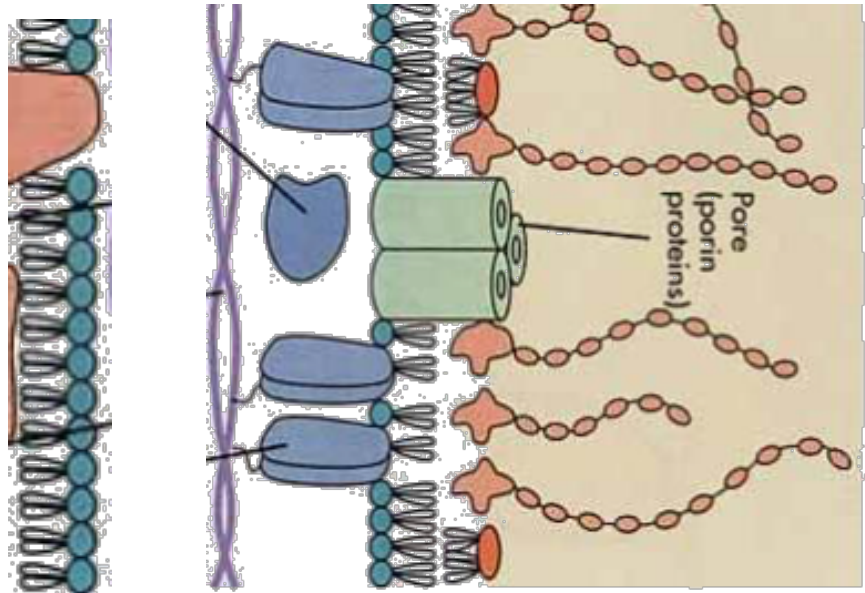
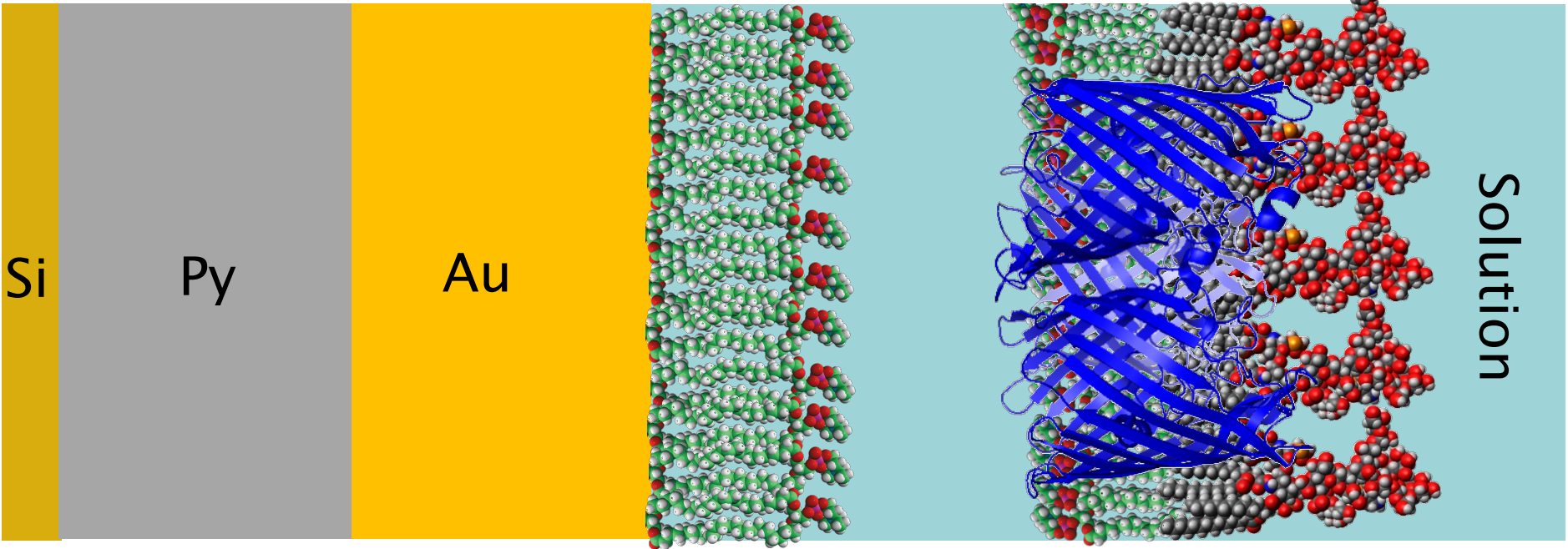
— = before protein addition
— = after 0.01 mg / ml injected into cell



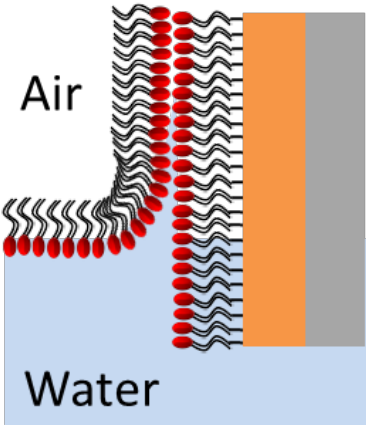
DPPC : Rc-LPS bilayer + α 1-Pth



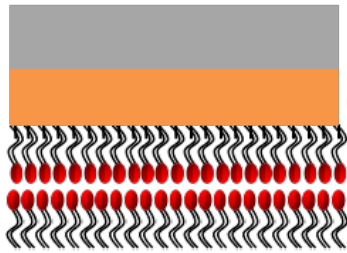
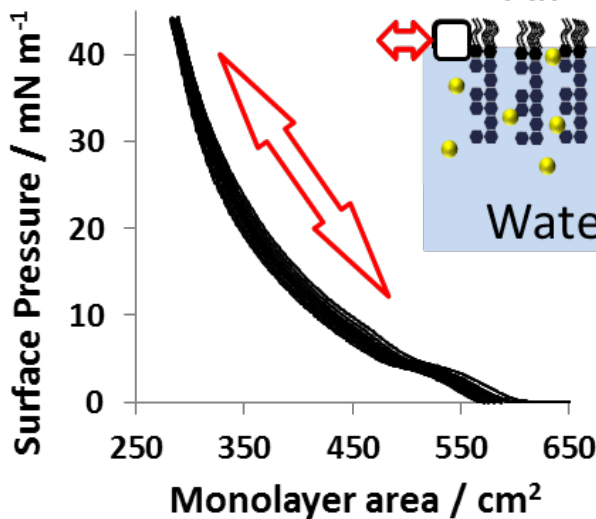
Accurate Model of Bacterial Membranes for Structural Studies



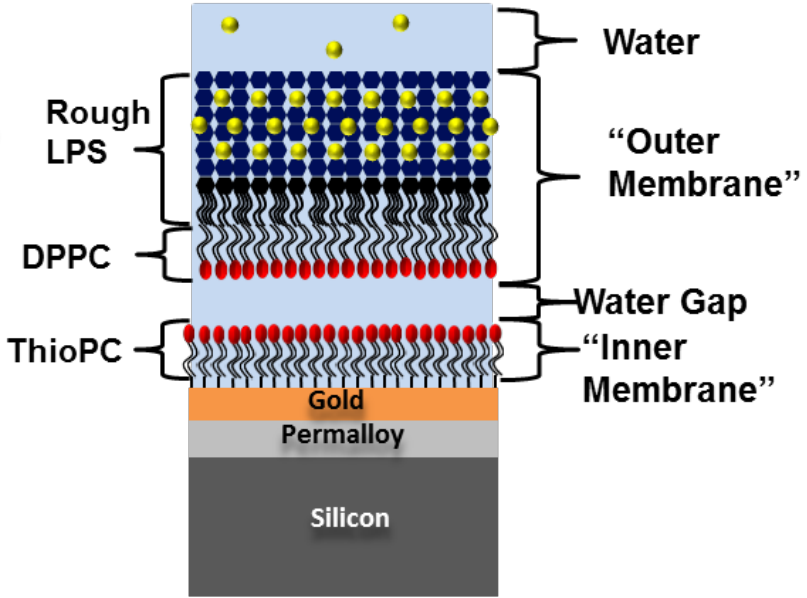
Floating Model Gram Negative Bacterial Membranes : Fabrication



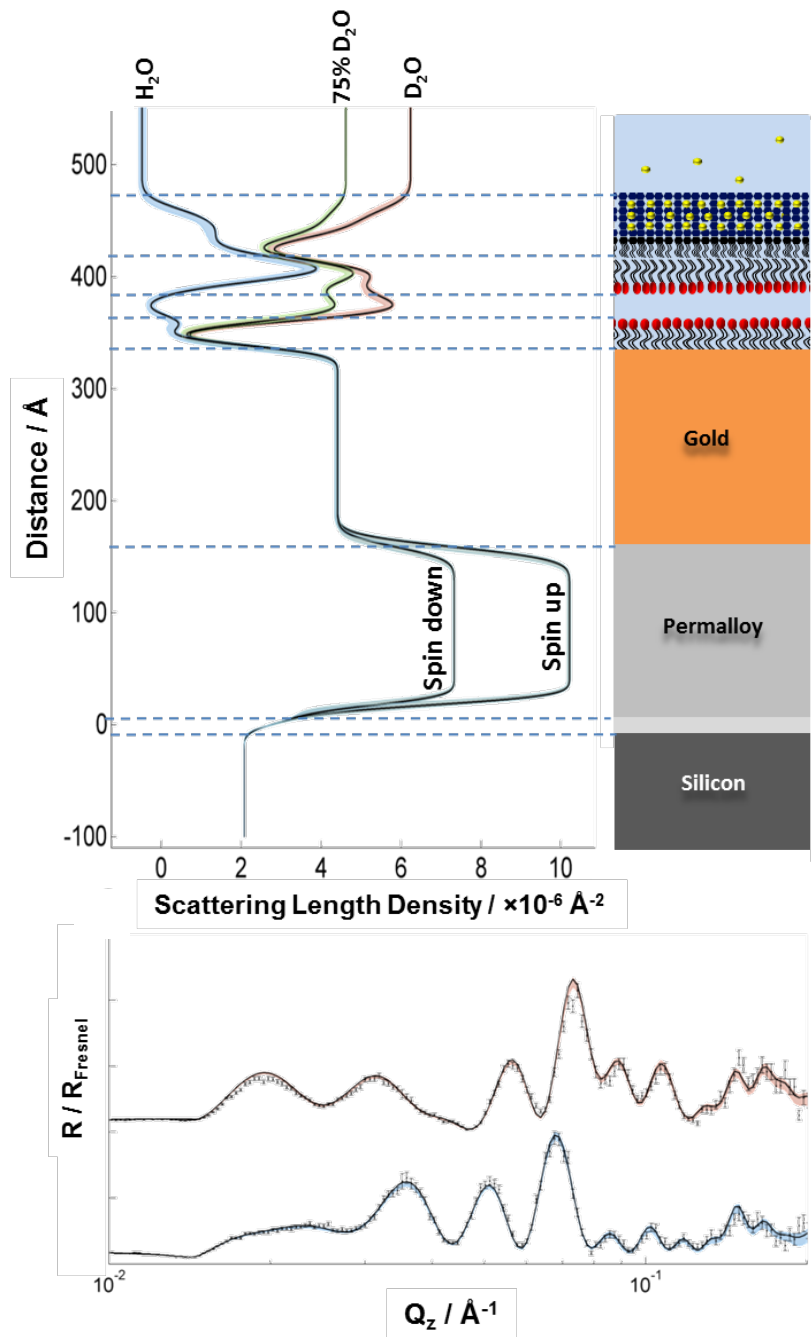
Langmuir Blodgett



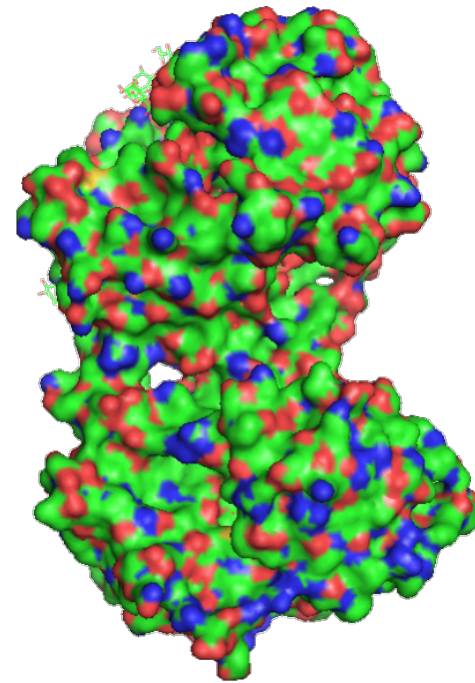
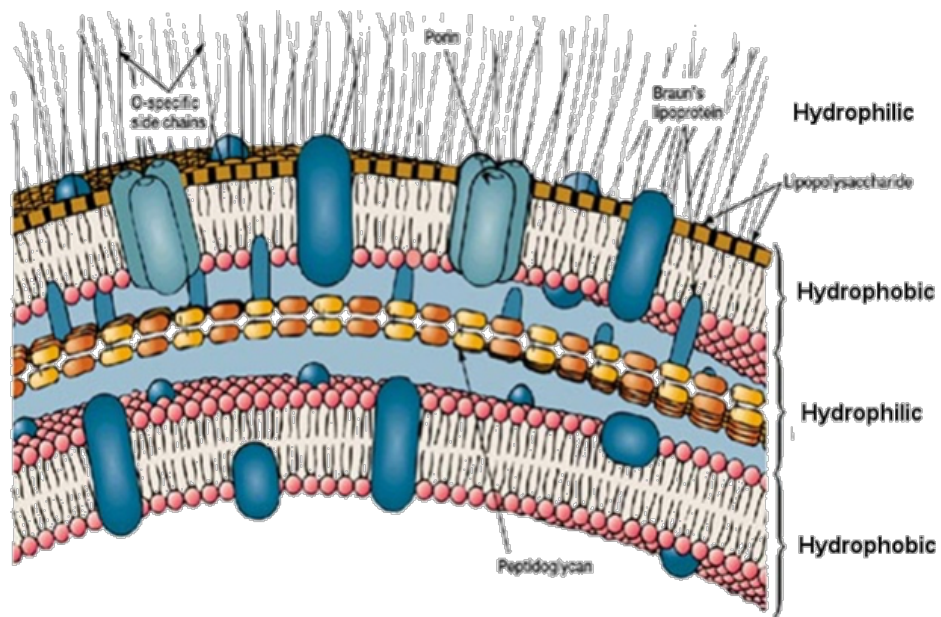
Langmuir Schaeffer



Floating Model Gram Negative Bacterial Membranes



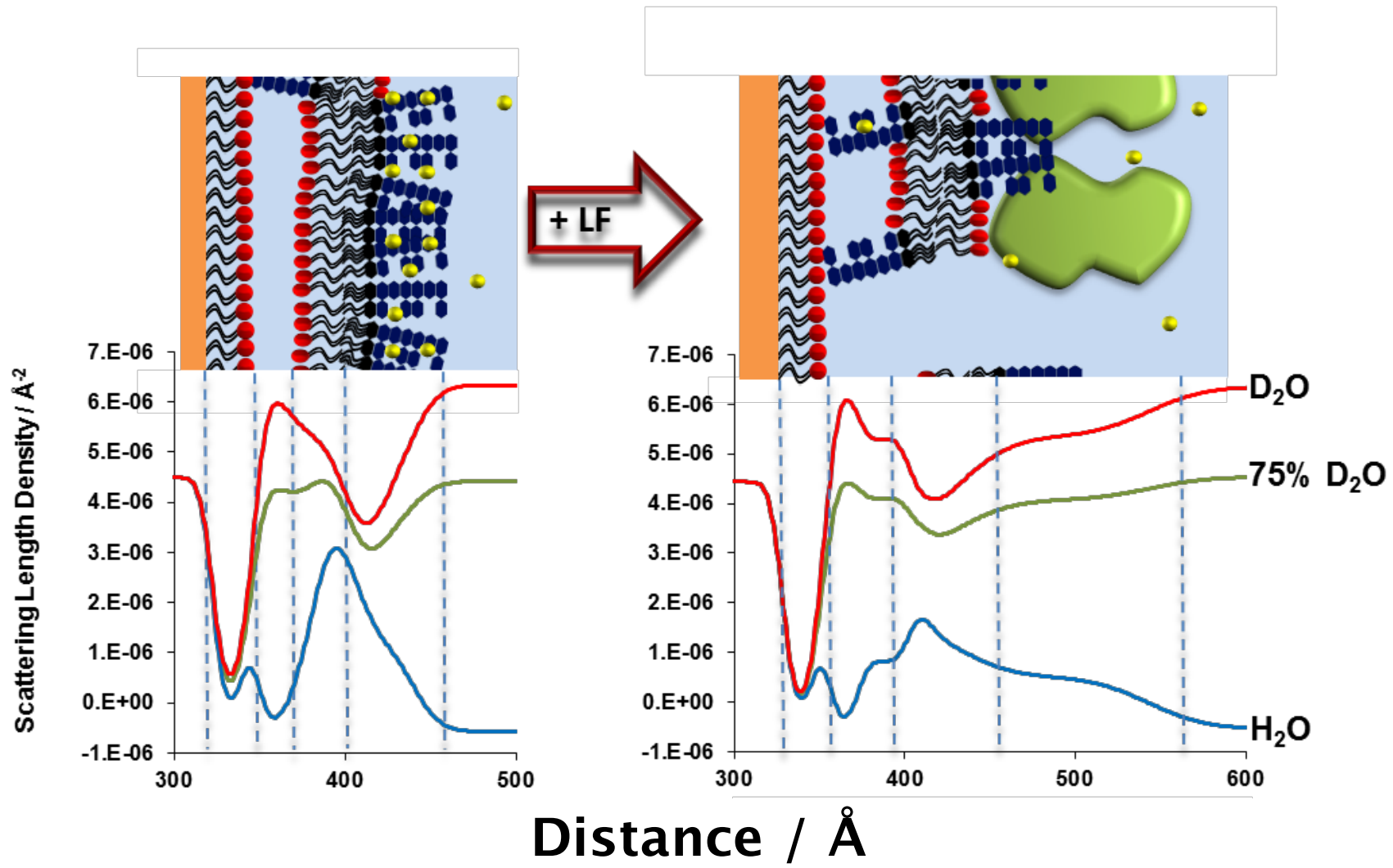
Antimicrobial protein interactions with the OM



Lactoferrin
pI 8.7

Floating Model Gram Negative Bacterial Membranes : Interaction Studies

Lactoferrin



Conclusions



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Facilities Council

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Nicoló Paracini

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Max Skoda

LSS group

ANSTO

Stephen Holt

Diamond Light Source

Tom Arnold



Science & Technology Facilities Council

ISIS



diamond