Biomedical Applications Of Model Membranes

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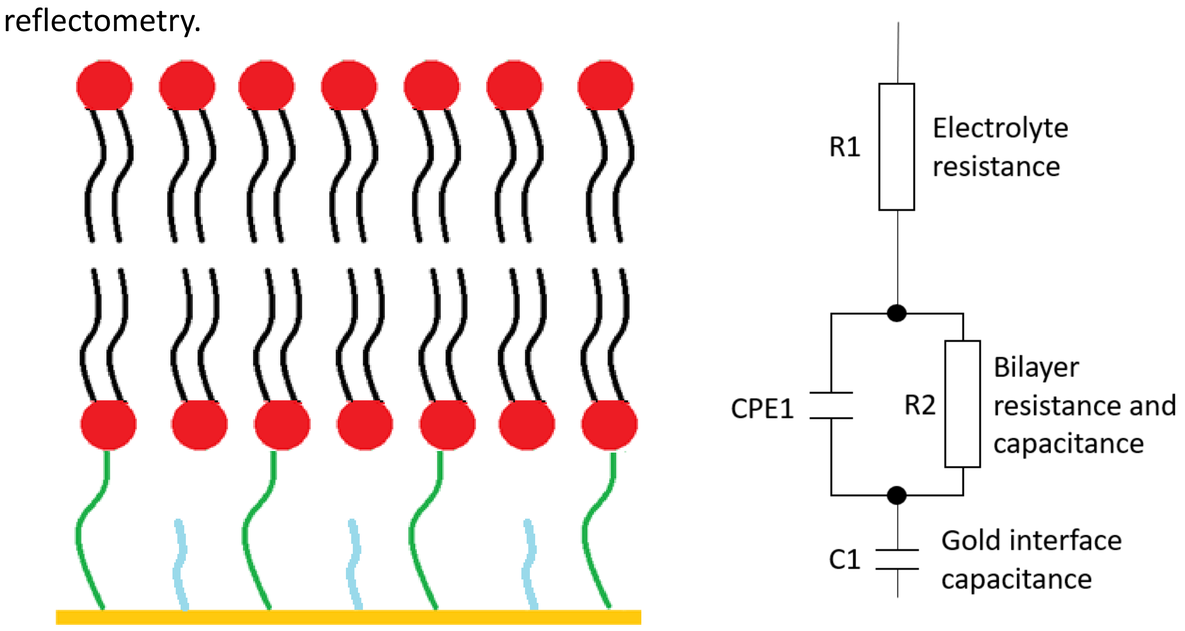
Tethered-Bilayer Lipid Membranes

All living organisms rely on biological membranes that act as a selectively permeable barrier controlling movement in and out of cells. Tethered-bilayer lipid membranes (tBLMs) are model systems designed to replicate the structure and function of key aspects of their biological counterparts.

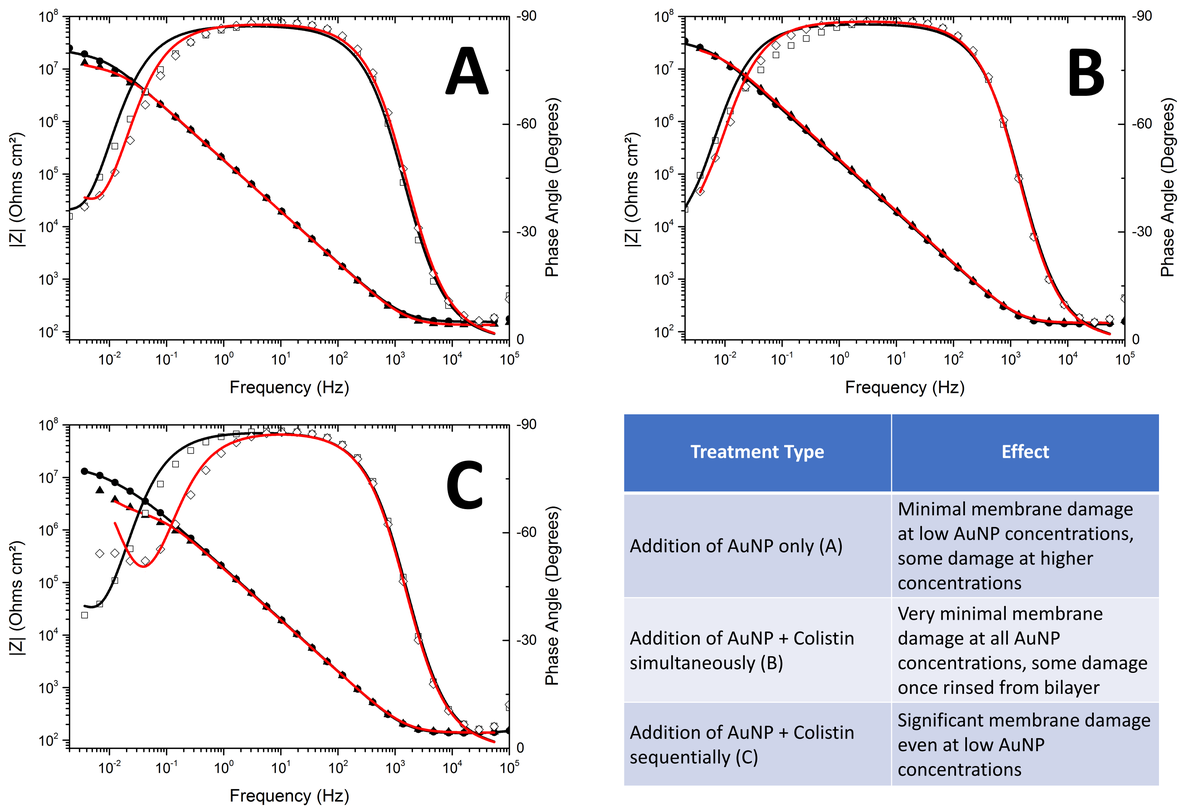
This project focuses on the use of tBLMs to determine and enhance the efficacy of various antimicrobial treatments in a controlled environment. This is achieved utilising two key techniques: electrochemical impedance spectroscopy (EIS) and neutron reflectometry.



Above: Structural components of a typical tBLM. A gold electrode forms the solid support, and gold-sulphur interactions allow for binding with thiol or disulphide terminating group of tethering lipids that form an initial monolayer. The rest of the bilayer is then be self-assembled on top. Additional spacer lipids are also used to provide a more realistic membrane environment. An electrical circuit model is used to approximate the properties of the membrane. Electrolyte resistance can be modelled by a singular resistive element, whilst the membrane itself can be modelled by a combined resistive-capacitive element, with an additional capacitive element often required to model the tethering region and gold interface.

Treatment of tBLMs with Gold Nanoparticles and Colistin

Gram-negative bacterial resistance is a critical issue for healthcare systems. An important factor in effective treatment is the susceptibility of bacteria to antibiotics. This has been studied by designing tBLMs using lipopolysaccharides (LPS) that mimic the outer membrane of gram-negative bacteria. Pre-treating these membranes with gold nanoparticles (AuNPs) was found to improve the efficacy of colistin sulphate, a cationic polypeptide and last-line antibiotic.



Treatment Type	Effect
Addition of AuNP only (A)	Minimal membrane damage at low AuNP concentrations, some damage at higher concentrations
Addition of AuNP + Colistin simultaneously (B)	Very minimal membrane damage at all AuNP concentrations, some damage once rinsed from bilayer
Addition of AuNP + Colistin sequentially (C)	Significant membrane damage even at low AuNP concentrations

Above: Bode plots showing impedance (closed shapes) and phase angle (open shapes) EIS measurements of LPS membranes before (black) and after (red) addition of different combinations of AuNPs and colistin.

Electrochemical Impedance Spectroscopy Analysis

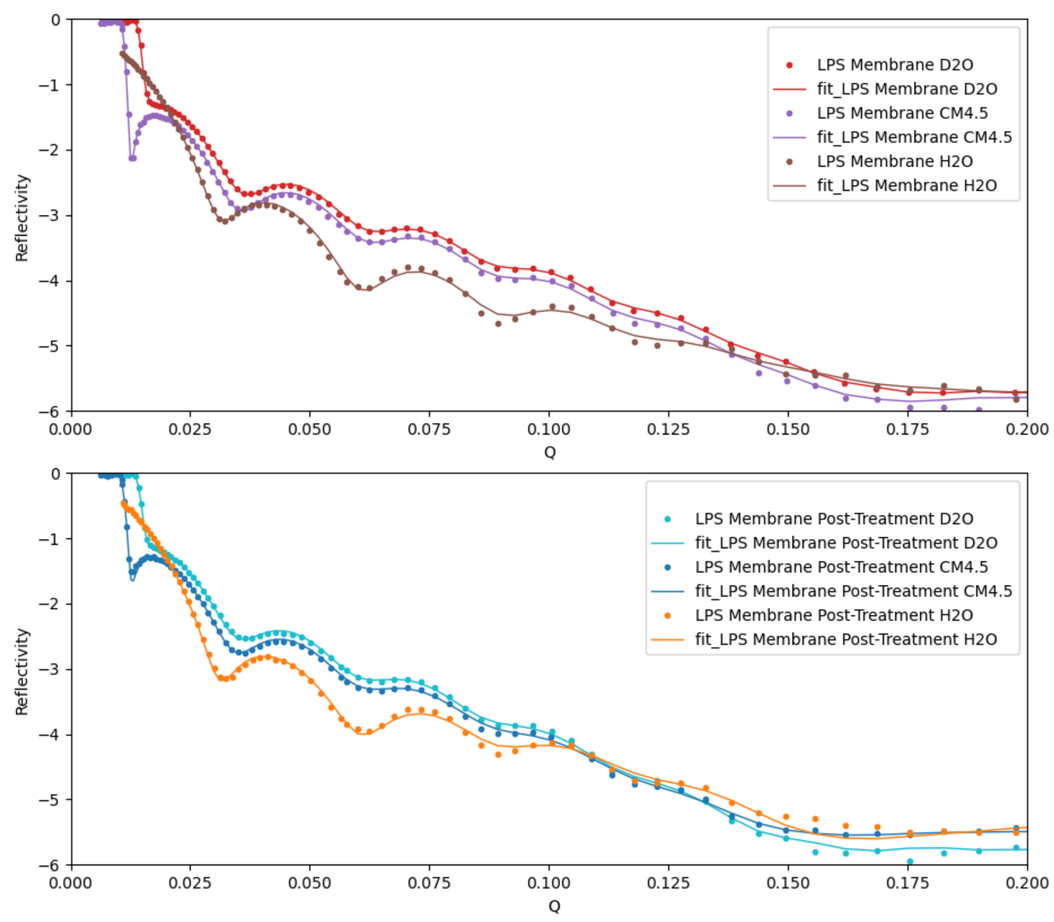
At frequencies where the model bilayer system is governed by resistive elements impedance does not change and phase angle tends towards 0 degrees, but at frequencies where the bilayer system is governed by capacitive elements the impedance increases and the phase angle tends towards 90 degrees. The resistance and capacitance of the bilayer can be modelled at the point where the impedance curve flattens out and the phase angle begins to dip from 90 degrees back down to zero. The EIS Bode plots are read and modelled from high frequency to low frequency.

Layer-by-Layer Modelling of tBLMs

One of the most effective methods for in-depth analysis of tBLM structure is neutron reflectometry. This technique involves the specular reflection of incident neutrons from the bilayer surface at different angles for each part of the system. A model is then produced using the detected scattering that can approximate the thickness, scattering length density (SLD), roughness and hydration of each layer.

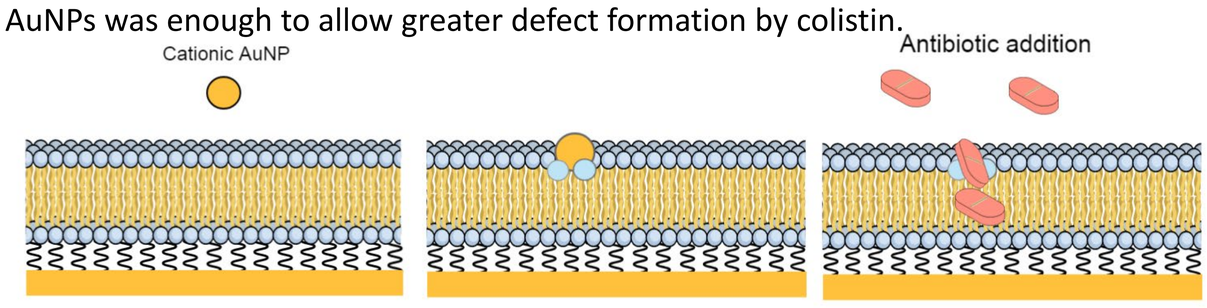
Layer	Typical SLD	Typical Thickness (Å)
Silicon fronting	2.07	Inf
Silicon oxide	3.47	10-30
Chromium	3.01-3.03	20-80
Gold	4.1-4.5	100-300
Tethering region	0.5-2	12-18
Inner leaflet	-0.4-0	12-18
Outer leaflet	-0.4-0	12-18
Outer head groups	1-3.5	6-16
Binding region	Solvent dependent	1-20
Backing solvent	Solvent dependent	N/A

Left: Table showing the different regions that a bilayer can be split into for neutron reflectometry modelling. **Below:** Neutron reflectometry data showing the reflectivity at different Q values for LPS membranes before and after sequential treatment with AuNPs and colistin. Q is the momentum transfer vector that changes depending on the angle of incidence of the neutron beam. Three different sets of data are shown with a different solvent for each – deuterium oxide (D_2O), H_2O and CM4.5 (a combination of D_2O and H_2O that is designed to match the SLD of the gold substrate and nanoparticles).



Neutron Reflectometry Analysis

By modelling the bilayers with layer-by-layer fitting values, changes in the outer leaflet of the membrane were determined. Simultaneous addition of AuNPs and colistin was shown to decrease hydration in the outer head group layer and increase roughness of the outer hydrocarbons to beyond the thickness of the outer head groups, suggesting defects in the outer leaflet of the membrane. Addition of AuNPs by themselves had minimal effect unless at high concentration, however sequential addition of colistin afterwards showed the same change to outer hydrocarbon roughness and a dramatic decrease in outer head group hydration. This suggests that the pre-treatment with AuNPs was enough to allow greater defect formation by colistin.



Above: Schematic of the effect that AuNPs are proposed to have on the lipid bilayer in order to increase the membrane damaging potential of colistin.

Further Work

Our research group has been working on multiple other biomedical applications of tBLMs beyond what is presented here. This includes studying the stability of these bilayers when dried out and rehydrated – useful for understanding their water retentive properties and potential for use as biosensors in a variety of different environments, as well as using LPS-based tBLMs as screening mechanisms for testing the efficacy of a library of synthetic antimicrobial polymers as an alternative to live bacterial cultures.