# Electrodes to study membrane proteins

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## Introduction

Membrane proteins, which are estimated to account for a third of the human genome, perform a myriad of functions in biology. They perform key roles in signal processing and bioenergetics. Our lab develops new techniques to study membranes and membrane proteins and we focus on ion channels and redox enzymes. Ion channels have key roles in signal transduction, while redox enzymes catalyse redox reactions in many vital processes, including photosynthesis and metabolism. We aim to link membranes and membrane proteins to electrode surfaces, which allows us to probe electron consumption/production (redox enzymes) or transmembrane charge transport (ion channels) using electrochemical methods.

## Cholesterol tethers to 'wire' membranes

have prepared electrode surfaces which enables the characterisation of redox-active membrane enzymes and ion-channels in a native-like environment. For this, we have used two approaches. In the first approach, tethered bilayer lipid membranes (tBLMs) are prepared, in which lipid bilayers are attached to the electrode surface via special chemical anchors that are bound to the surface on one side and insert into a bilayer leaflet at the other (Figure 1). Cholesterol derivatives have been synthesised, which, via a hydrophilic linker, are connected to a thiol group that form self-assembled monolayers (SAMs) on gold electrodes. These cholesterol-lipids have been mixed with small thiols to provide space for transmembrane proteins. In the second approach, lipid vesicles are attached intact on solid surfaces. By appropriate modification of the gold-electrode surface, the vesicles do not loose their integrity when adsorbed, as indicated by their ability to retain encapsulated fluorescent dyes.

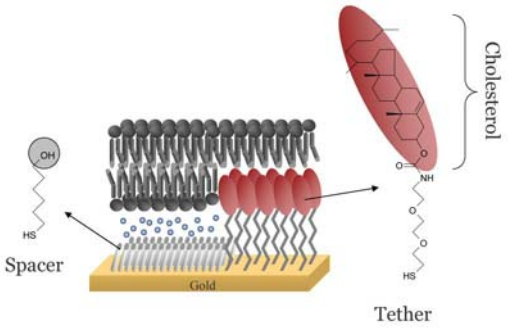


**Figure 1:** chemical structures of 6-mercaptohexanol (spacer) and the cholesterol tether molecule used to form tBLMs.

## Ion channels

Studies were performed to provide a proof-of-principle that our tBLM system can be used to characterise ligand gated ion-channels. For this purpose, tBLMs were formed on either (a) pure tether lipid or on (b) mixed self-assembled monolayers (SAMs) of tether and spacer molecules (see Figure 1). While the tether lipid is required to form a tBLM with high resistivity, the spacer molecule dilutes the cholesterol content in the lower leaflet of the bilayer forming "ionic reservoirs" required for the sub-membrane hydration. By using simple ion-channels (gramicidin) and ionophores (valinomycin) we have shown that these ionic reservoirs are required for ion transport through the membranes. This is most likely due to the thermodynamic requirements of ions to be solvated once transported through the membrane. Unexpectedly, electrochemical impedance spectroscopy (EIS) shows an increase of capacitance upon addition of gramicidin, while valinomycin addition decreases the membrane resistance in the presence of $K^+$ ions. We hypothesise that this is due to previously reported phase separation of tether-lipid and spacer molecule on the surface.

**Proton-pumping enzymes**

Surface adsorbed vesicles were used to study a proton-pumping enzyme, cytochrome $bo_3$, which is a terminal oxidase and proton-pumping enzyme from *Escherichia coli*, Cytochrome $bo_3$ was reconstituted in lipid vesicles, which were subsequently 'loaded' with a pH-responsive fluorescent dye. Cytochrome $bo_3$ oxidises lipophylic ubiquinol to ubiquinone and reduces molecular oxygen to water. Ubiquinone included in the vesicles can be electrochemical reduced to ubiquinol and this property was exploited to drive the formation of a proton gradient in the adsorbed lipid vesicles as schematically indicated in Figure 2. In nature, transmembrane proton gradients are formed using energy supplied by light or chemical reactions, but in our biomimetic or hybrid organic–inorganic systems we have for the first time shown that surface-applied electrochemical potentials can also be used as an energy source.

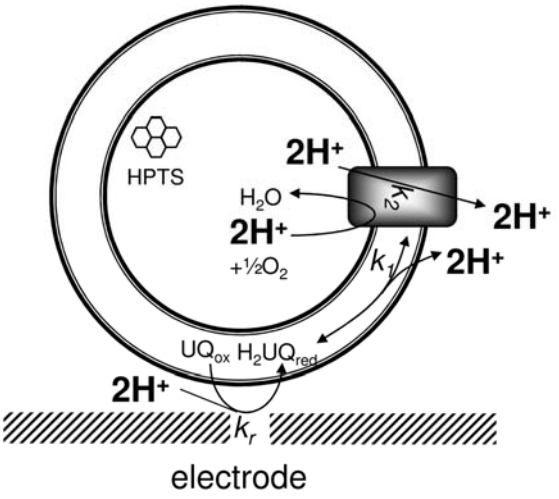


**Figure 2:** illustration of the experimental setup used and the four protons removed from the intravesicular compartment per 2 electrons required to reduce ubiquinone to ubiquinol. This illustration assumes a single orientation of the enzyme in the

**Future directions**

We aim to continue to the development of the proton-pumping system with the ultimate aim to use this system to study the enzymes on the single molecule level. Other quinone enzymes, the NapC and CymA are also being studied with the particular focus on properties that made UQ a special substrate when compared to aqueous solutes. The proteins CymA and NapC both have a single α-helix that binds them to the The work with ion channels will be continued by testing more complex ligand-gated ion-channels. Finally, EU funding has been obtained to study if the tBLM platform can be used to study the interaction between nanoparticles and lipid membranes, with the aim to characterise potential toxicological effects of nanoparticles.

**Publications**

Kendall, J., Johnson, B., Symonds, P., Imperato, G., Bushby, R., Gwyer, J., van Berkel, C., Evans, S. & Jeuken, L. (2010) Effect of the structure of cholesterol-based tethered bilayer lipid membranes on ionophore activity, *Chem. Phys. Chem.*, **11**:2191-2198**.**

Weiss, S., Bushby, R., Evans, S. & Jeuken, L. (2010) A study of cytochrome bo3 in a tethered bilayer lipid membrane. *Biochim. Biophys. Acta* **1797**:1917-1923.

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