Model-membrane electrodes to study membrane-protein function and interaction with nanoparticles

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Abstract

Novel electrodes have been applied to the study of physical properties of lipid membranes and membrane proteins. By tuning the electrode-surface chemistry, biomembranes are immobilised on the electrodes in a planar geometry or as intact liposomes (see Figure). We have used these model-membrane electrodes in biosensor applications or to study the catalytic mechanism of redox-active membrane proteins. We will present two applications of our model-membrane electrodes.

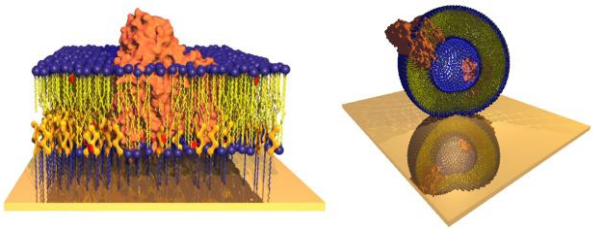

Figure: Representation of (left) a planer model-membrane electrode and a (right) liposome model-membrane electrode.

In the first application, the system has been used to study the enzyme activity of bacterial respiratory enzymes. Bacteria have highly diverse and highly branched respiratory chains, which consist of a range of enzymes that transfer electrons from many different substrates into a common pool of lipid soluble electron carriers, known collectively as quinones. Most biochemical studies of quinone-converting enzymes have used water-soluble quinone analogues. However, our model-membrane systems are able to electrochemically reduce and oxide the lipophilic quinones in the membrane and this property has been used to study the interaction of a range of enzymes with their native-like quinone substrates. Some examples will be presented on how the interaction of the lipophilic quinones deviate from literature in which assays are used that use water-soluble quinone analogues.

In the second application, the system has been used to study the interactions of silica nanoparticles (Ludox SM-30) with membranes as part of a nanotoxicity study. The interactions and toxicity were studied using the model-membrane electrodes, but also cultured cells. The mean size of silica particles was 15 nm in water but particles were found to aggregate to a mean size of 500 nm in culture medium (DMEM). Cytotoxicity was observed at ≥10 µg/ml, while significant DNA damage was seen at ≥1 µg/ml silica in all cells. Transmission Electron Microscopy showed particles inside cytoplasm, but not the nucleus. There was no evidence of particles inside endosomes and when treatment was carried out at 4^{o}C for 30 minutes particles were observed inside the cells. Surpricingly, model-membrane electrodes did not indicate structural damage to the lipid membrane by the nanoparticles, although the nanoparticles exhibit a strong affinity for the lipid membranes. This suggests that the particles can enter the cells by a process that does not require endocytoses, nor damages the integrity of the membrane.