Experimental report

23/04/2022

Proposal: 8-02-960 **Council:** 4/2021

Title: Potential-dependent interactions of a redox protein with lipid bilayers tethered to electrode surfaces.

Research area: Chemistry

This proposal is a new proposal

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Samples: DMPC
Cytochrome C
Ethanol
DMPG
Gold coated silicon substrates
MPA

Instrument	Requested days	Allocated days	From	To
D17	3	3	03/09/2021	06/09/2021

Abstract:

This proposal builds on previous work that successfully characterised behaviour of a redox protein at the electrode surface under electrochemical control, as well as initial data suggesting possible different mechanisms of interaction with deposited bilayers formed from two different lipids. Here, we wish to improve the applicability of the model by using an improved deposition technique and tether molecule to prevent denaturation of the redox protein, and hence better model the electron-transfer mechanisms occurring between biofilm cell membrane proteins and electrode surfaces.

Experimental report: 8-02-960: Lipid bilayers/redox protein interactions

Background

In bioelectronic devices, biological species such as bacteria or extracted electroactive membranes (such as thylakoids) are interfaced with electrode surfaces in order to tap into energy naturally harvested e.g. photosynthetically from the sun (biophotovoltaics) or by digestion of wastewater products (microbial fuel cells). In such devices, the limiting factor is the electron-transfer step between the cells/membrane and the electrode, the mechanism of which is generally poorly understood. In this experiment, we aimed to construct model lipid membranes using DMPC and DMPG (neutral and negatively-charged respectively) and use neutron reflectometry (NR) to determine structural changes as a result of applied potential. By using deuterated lipids we also aimed to determine the position of a redox protein (cytochrome c) with the membrane during the electron-transfer process.

Results

We are still in the process of fitting the data to obtain the final structural models, but we are able to make some initial qualitative observations. Our experimental protocol involved firstly characterising the gold films in D-buffer and H-buffer before introducing solutions of peptide-belts (vesicles of DMPC and DMPG with a peptide introduced in order to reduce the liposome diameter and ensure complete fusion). From QCM-D (quartz crystal microbalance) measurements obtained

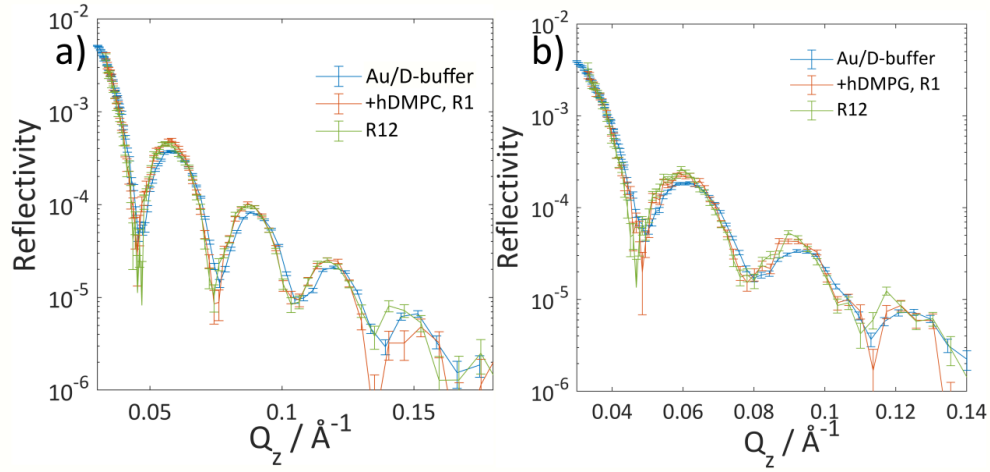

Figure 1: Reduced data for a) the Au/hDMPC system and b) Au/hDMPG, where 'R1' and 'R12' denote the first and final runs at 3.0°, immediately after injection of the peptide belts into the cell and an hour later.

prior to the NR experiment, we were expecting to see initial adsorption of whole liposomes before fusion occurred to form the bilayer (timescale of around 1 hour). We therefore sat at the second angle (where most changes were expected) and ran 12 x 5 minute scans as soon as the peptide-belt solution was injected into the cell, before finally fully characterising the resultant layer. Interestingly, we didn't see the same trend as expected from the QCM—as shown in Figure 1, the changes were fairly immediate for hDMPC with little further change over the course of the hour, whereas progressive changes were seen for hDMPG in this time period.

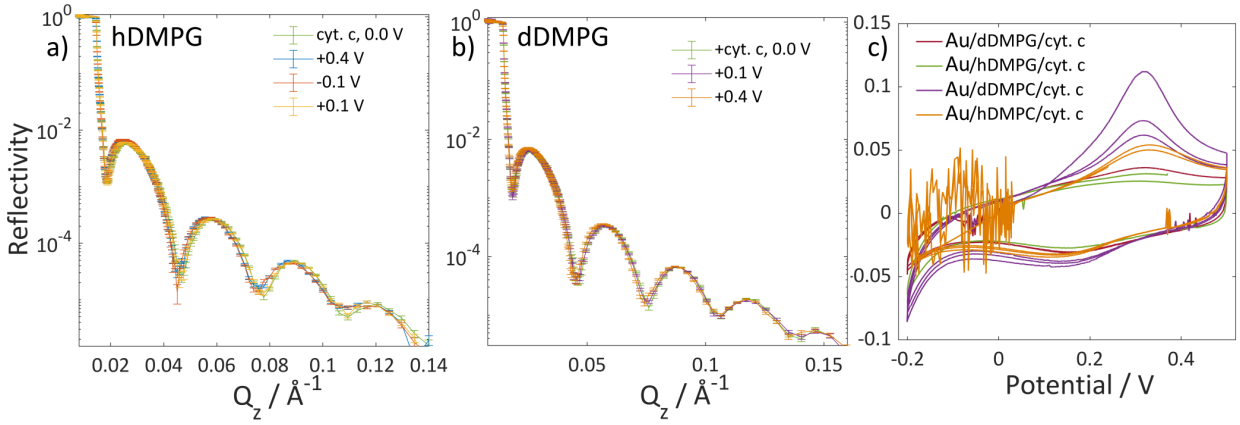

Figure 2: Reduced data for a) the Au/hDMPG/cyt. c system and b) Au/dDMPG/cyt. c, showing semi-reversible and irreversible changes upon changing applied potential. c) CVs for all samples run at the end of the experiment.

As our main interest was to observe the interactions of the redox protein cytochrome c with

these lipid bilayers, we subsequently injected solutions of the protein into the cell, characterised at OCV (open circuit voltage) before cycling stepwise through a range of applied potentials. By using protonated and deuterated lipids, we hoped to monitor changes in the structures of the lipids and added protein respectively. In all cases, we saw shifts in the fringe spacing when cycling through the applied potentials. In the case of the protonated lipids, this was partially reversible—we expect this to arise from the reversible tilting of the lipid bilayer (Figure 2a). However, when using the deuterated lipids, and therefore focusing in solely on the cytochrome c, we saw irreversible changes, suggesting the protein may be adsorbing and then deforming irreversibly at a given onset potential (Figure 2b).

Finally, we ran CVs with each sample *in situ* after all NR data had been collected. Interestingly, we saw that the redox peaks for cytochrome c were at higher intensity when interacting with the deuterated lipids compared with their protonated counterparts (Figure 2c). We are still uncertain as to the underlying cause for this observation (and intend to repeat this part of the experiment in the lab to ensure it wasn't merely a coincidence), but it may suggest that the deuterated and protonated lipid systems are not entirely exchangeable (as previously suggested by other researchers), which will be a key question to address in the final analysis.