Determining the Location of Ubiquinone in a Biomimetic Membrane using Neutron Reflectometry

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In situ neutron reflectometry was used to study the location of ubiquinone (coenzyme Q_{10} in biomimetic lipid bilayers as a function of potential and temperature. Ubiquinone is vital to biological electron transport, however, the location of ubiquinone in the membrane is unknown. Through its role in the NADH respiratory chain, ubiquinone changes oxidation states. One of the goals of this work is to study the location and conformation changes of ubiquinone as a function of redox potential as well as how the applied electric field affects the ubiquinone-membrane structure.

Neutrons are non-charged particles that have extremely weak interactions with matter. Neutrons are deeply penetrating, making them ideal probes for buried interfaces in soft matter systems. With the use of isotopic substitution the contrast is varied between different regions of the system, highlighting the scattering of each component of the system. Neutrons are a particularly powerful probe for the study of biological systems which contain hydrogen atoms and need to be studied in controlled systems of precise pH, temperature and electric field conditions. Neutron reflectometry provides a unique tool for determining the structure of the lipid membrane and the location of small biologically relevant molecules.

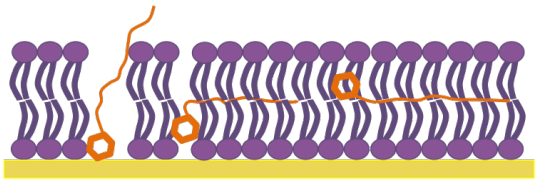

Figure 1: Cartoon representation of some possible locations of coenzymeQ_{10} in a biomimetic membrane. The orange molecules (○) represent the CoQ_{10} molecules and the purple (○) the lipid molecules

To probe the location of ubiquinone, a phospholipid bilayer was prepared on a gold coated solid substrate using a combination of Langmuir-Blodgett and vesicle fusion techniques. The combination of these two methods allowed for the composition of the inner and outer membrane leaflets to be varied. The inner leaflet is composed of 100% DMPC and the outer leaflet 90 % DMPC and 10% ubiquinone.

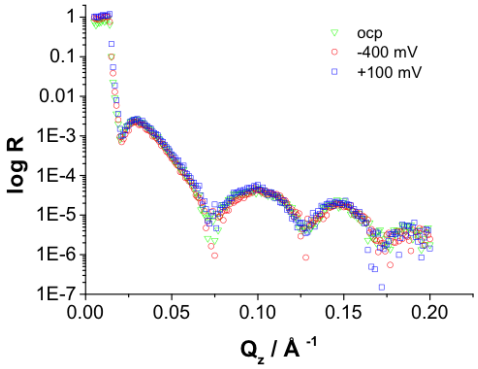

Figure 2: The neutron reflectivity curves do not vary with potential potential changes from ocp (△), -400 mV(○) or +100 mV (□)

In neutron reflectometry experiments, neutron scattering spectra are collected and a model is built to fit the data using the variable parameters scattering length density (SLD), thickness and roughness. For our system the expected result of the membrane structure had the UQ located in the outer leaflet and the DMPC close to gold surface. The actual result result was much different suggesting the UQ moves close to gold surface.

Coenzyme Q_{10} undergoes a structural change from ubiquinone to ubiquinol with change of oxidation state. Using a system with a supported model membrane on a conductive metal surface, the applied potential can be controlled and the oxidation sate of the molecule changed. Figure 2 shows the result with potential control at three different potentials: open circuit potential (ocp), -400 mV and +100 mV. It was expected the reflectometry curve would change with potential control if the location of the coenzyme Q_{10} changed, but no shift in the curve was seen.

A second series of measurements changing the bilayer environment were conducted decreasing the tem-

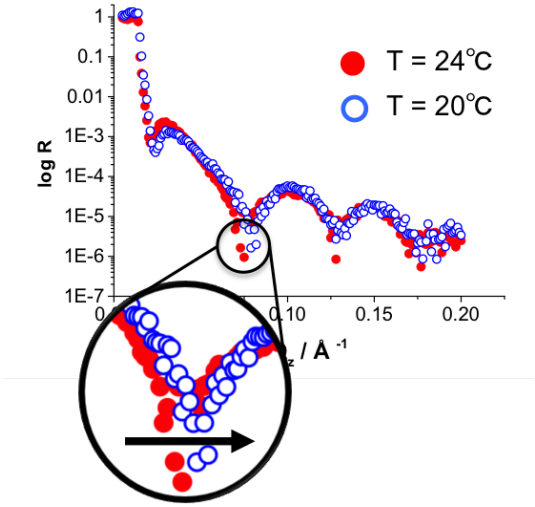

Figure 3: Neutron reflectivity curves at 24°C (●) and 20°C (○)

perature from 24°C to 20°, below the lipid transition temperature, and back to 24°C. Shown in Figure 3, there is a shift in the reflectometry curves. Simultaneous electrochemical experiments conducted revealed a loss of the redox peak from coenzyme Q_{10} at low temperatures and a recovery of the peak when the temperature was raised back to 24°C. More neutron experiments are needed to say with certainty the effect temperature on the location of Coenzyme Q_{10} in the phospholipid membrane.

Neutron reflectometry experiments allowed us to probe the location of coenzyme Q_{10} in a biomimetic membrane. Using this technique changes in the lipid structure is observed and preliminary results show sensitivity to the location of a small biologically relevant molecule. With more extensive lipid membrane preparation protocols it will be possible to evaluate more extensively the membrane structure with the incorporation of coenzyme Q_{10}. Future work includes changing to the lipid system (changing the lipid composition or increasing fluidity with the addition of cholesterol to the system), and improving neutron scattering data by employing contrast matching.