

Redox active proteins in the hunt for biotech tools

Translating research into useful biotechnological tools, such as biosensors and devices that can be used for a broad range of medical diagnostic applications, is the ultimate aim of Dr Lisandra Martin and her team at the School of Chemistry, Monash University, Victoria, Australia.

With a background in synthetic inorganic chemistry and application of physicochemical techniques to characterize the electronic properties of these molecules including magnetochemistry and electrochemistry supplemented by further research into metalloproteins and enzymes, specifically, nitrogen fixing, nitrogenase, Lisandra Martin, has turned her interests to the understanding of structure, reactivity and function of redox active proteins. This work on biomolecules is aimed at underpinning the development of biosensors based on immobilized enzymes. "These biomolecules can detect (bind) substrates at nanomolar concentrations which when coupled to a transducer can lead to a direct measurement of the concentration," says Lisandra Martin.

Moving forward with such research requires new tools and approaches and led to the recognition that a suitable 'material' is essential to provide an interface for the investigation of biophysical properties of proteins and other biomolecules. Included in those tools is the use of QCM-D combined with electrochemistry to study the interfacial properties of proteins immobilized to a (electrode) surface.

"Our recent research has focused on artificial membranes as the biocompatible material on which biomolecules can reveal mechanistic and functional data. We have made several discoveries; firstly showing that polyelectrolyte capsules can be sealed using lipid coating and this prevents passage of drug-like molecules. Secondly, we have used supported membranes to investigate and reveal mechanism(s) of action for a range of peptides that disrupt or traverse membranes to intracellular targets and thirdly, we have looked at the assembly of membrane bound proteins associated with steroid synthesis on a membrane and demonstrated that

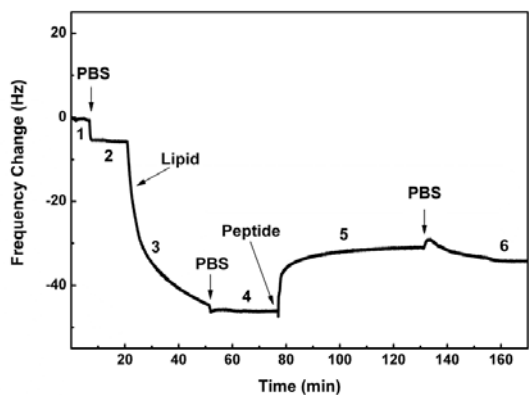


Figure 1 Typical Δf - t curve from a QCM-D experiment showing lipid membrane deposition step followed by the maculatin peptide ($10 \mu\text{M}$) addition. Points of interest on trace are: 1 - initial baseline (water) 2, 4, 6 - baselines in phosphate buffer 3 - lipid deposition curve 5 - peptide effect

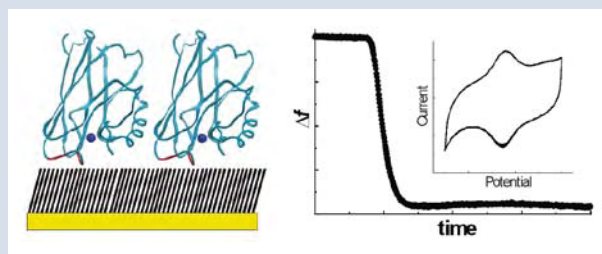


Figure 2 Simplified model of azurin adsorbed in idealized orientation onto an alkanethiol-modified gold electrode. After adsorption of azurin, a cyclic voltammogram was measured *in situ* at the scan rate of $0.02 \text{ V}\cdot\text{s}^{-1}$ by using electrochemical QCM-D module.

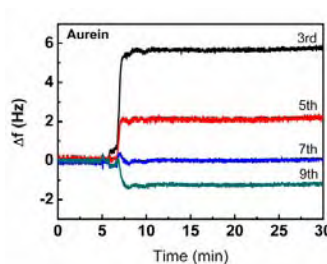
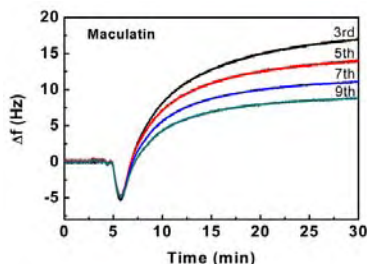
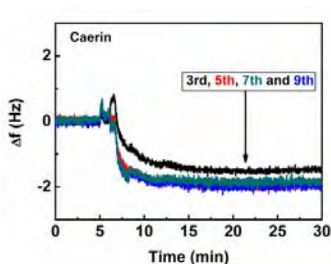


Fig. 3 Overtone effect for three antimicrobial peptides at $7 \mu\text{M}$ on DMPC lipid membrane.

Caerin: there is no significant difference between the traces, suggesting transmembrane insertion.

Maculatin: there are slight differences in the disruption phase.

Aurein: the difference between the overtones reveals a surface dominated process.



Monash University: was established in 1961 and is the largest university in Australia. The School of Chemistry is a leading chemistry department and attracts high quality international and local students. The School of Chemistry has research strengths in biological chemistry, analytical chemistry and synthetic organic and organometallic chemistry. In particular, electroanalytical chemistry has two Federation Fellows (A.M. Bond and D. McFarlane) – the highest research positions available in the Australian system.

Lisandra Martin's group collaborates with a number of other research groups. International collaboration includes working with Professor Alan Conley at UC Davis, USA on cytochrome P450 protein structure

and function, and with Professor Ralf Hoffmann, at the University of Leipzig in Germany for Antimicrobial peptides derived from insect species. In Australia, there is close collaboration with Professors John Bowie (University of Adelaide) and Frances Separovic (University of Melbourne) on peptide-membrane interactions, and with Monash colleagues Professor Alan Bond on electroanalytical chemistry of new semi-conducting materials based on TCNQ and Andrea Robinson on synthetic peptides. Collaborations with a number of biomedical colleagues Professors Raymond Rodgers, John Wallace, and Briony Forbes from the University of Adelaide have recently stimulated the study of peptide activated insulin receptor examining conformational and structural changes linked to signalling and function.

they have functional activity, catalyzing substrate to product," Lisandra Martin points out.

Lisandra Martin's research team consists of post-doctoral fellows, PhD and research assistants, including Dr's Barry Fleming, Adam Mechler, Victor Qu and Ms's Slavica Praporski, Stefania Piantavigna and Mr George McCubbin. Their current research focuses on using biomimetic membranes to create a stable interface. This biocompatible material is being used to assemble proteins and also to examine the activity of Antimicrobial peptides. The latter area has been rewarding in the establishment of biomimetic surfaces that reflect the composition of mammalian and bacterial membranes.

In addition, the group has developed a synthetic molecule that binds histidine-tagged proteins without the problems of leakage that occurs in Ni-NTA approaches. This molecule allows the protein to be tethered in such a way that the reactivity, for example redox processes can be examined while allowing conformation freedom in both redox states.

Revealing material properties with QCM-D

"We have started to use biomimetic membranes and SAM (self-assembled monolayer) materials on electrodes to examine the redox properties of some electron transfer proteins," says Lisandra Martin. "The QCM-D instrument was an obvious choice to assess the wet mass and composition of the lipid that we were using to create the membrane."

Lisandra Martin cites three examples where QCM-D has provided significant advances to her group's knowledge of material or biomolecule properties. These are:

I. Binding of an electron transfer protein, Azurin, to a SAM of octane thiol revealed that the amount of protein binding was concentration independent, correlated well with the current

measured by electrochemistry immediately following binding to the chip, and finally that there was zero change in dissipation. This indicates that the group could demonstrate the presence of a tightly coupled protein-SAM in which there was no covalent attachment.

II. The activity of peptides (AMP) that disrupt bacterial membranes was distinguished using the harmonics from the change in frequency response. In this case, the two predominant mechanisms were surface or detergent-like action or transmembrane pore formation. The differential response of the harmonics (3rd – 9th) for the surface active peptide interaction was contrary to the transmembrane pore forming peptide in which all the harmonics responded with the same frequency change.

III. The structure and homogeneity of lipid bilayers was examined following deposition of vesicles onto a carboxylate modified gold QCM-D sensor. This study included "data mining" from 170 independent experiments and we were able to classify membrane formation as monolayer, multilayer or vesicular composites. By estimations of the layer thickness and shear modulus, the surface structure was characterized in Q-Tools modeling software.

As regards the usefulness of QCM-D, Lisandra Martin highlights the wide range of harmonics to probe the surface of the material under study. "We are continually amazed at the value of this ancillary information to our understanding of structural and interfacial properties of the species under interrogation," she notes. "For our studies we have increasingly found that the measurement of dissipation per unit mass is valuable data for the analysis of the surface interaction of biomolecules."

The group's latest result is available in the paper Electrochemical Quartz Crystal Microbalance Study of Azurin Adsorption onto an Alkanethiol Self-Assembled Monolayer on Gold (Langmuir 2008,24,323-327).