Durable vesicles for stabilisation of membrane proteins in biotechnology

The Challenge

The Beales Laboratory, at The University of Leeds, are developing advanced vesicular materials as enhanced reconstitution systems for membrane proteins that improve their stability and functional lifetime.

Many challenges remain in understanding the mechanism of enhanced functional stability, optimising materials and protocols and exploring a wider range of membrane proteins in these systems.

The Research

The Beales laboratory use cytochrome bo3 as their model membrane protein for development of hybrid vesicle technology as it has quantitative functional assays that allow us to easily compare the efficacy of different material compositions and reconstitution methods for extended functional stability. Functional activity is measured by spectroscopically monitoring ubiquinol oxidation, and during this project we introduced an electrochemical method to characterise oxygen reduction, so we can monitor turnover of both substrates.

The functional output of proton transport is measured by a pH-sensitive fluorophore, a strategy we developin this project. We find that polymer and hybrid membranes have very low proton permeability (better than pure lipid membranes) but only small average pH shifts are observed due to random proton orientations pumping protons both into and out of the vesicle lumen.

Towards the development of hybrid vesicles as a robust and stable reconstitution system for membrane proteins, the Vacation Scholarhsip aimed to characterise proton pumping of cytochrome bo3 in these systems and investigate whether an alternative triblock copolymer (PMOXA-PDMS-PMOXA) could be substituted for our usual PBd-PEO diblock copolymer for successful functional reconstitution of the protein.

"CBMNet Vacation Scholarships are a great scheme for getting talented undergraduates into the lab to gain valuable research experience while making important contributions to ongoing projects."

Dr Paul Beales

"Overall this experience has been really enjoyable and has helped me confirm that I would like to continue studying past my undergraduate degree."

Ellen Moscrop

The Result

An alternative triblock copolymer was successfully developed for functional reconstitution of cytochrome bo3 in hybrid vesicles, expanding our current arameter space for these systems and giving greater flexibility for future material optimisation.

The project found that PBd-PEO vesicles and their hybrids with lipid POPC had much lower passive proton permeability than liposomes, however reconstituted proteins only showed small ensemble pH shifts inside these vesicles suggesting they were fairly randomly oriented after reconstitution. The enzyme showed good functional activity when econstituted using the new triblock copolymer, opening a further avenue for future investigation.

The student successfully reproduced previous work by functionally reconstituting cyt bo3 in hybrid vesicles and uantifying their functional activity by spectroscopically monitoring the oxidation of ubiquinol. Furthermore she used a new functional activity by electrochemically monitoring the reduction of oxygen to confirm enzyme activity by a second method.

The student measured the proton permeabilities of lipid, polymer and hybrid vesicles to protons, finding polymer and hybrid membranes had very low proton permeability. Proton pumping assays reported by HPTS fluorescence showed small (<0.1 pH unit) shifts inside vesicles in ensemble experiments, strongly suggestive of the proteins being fairly randomly oriented within the membranes.

The student successfully reconstituted cyt bo3 in vesicles using the alternative PMOXA-PDMS-PMOXA triblock copolymer, expanding our material parameter space for further study. (ii) We did not have time to investigate SMALP reconstitution methods.

As anticipated, this was ambitious within the time available and so F-ATPase was not investigated.

The Future

The Vacation Scholarship helped consolidate the Beales Lab newly established hybrid vesicles method and demonstrate feasibility of some new materials and methods within this framework. The data will contribute to preliminary data for future grant applications and may contribute to an invited submission to Methods journal on the hybrid vesicle technique.

In the immediate term this work will be carried forward by a first year PhD student funded through EPSRC SOFI CDT; the primary focus of this student will be characterising the physical properties of hybrid membranes and correlating this to the biochemical properties of enhanced membrane protein durability in these environments to better understand the stability mechanism and guide further optimisation.

We are also seeking to recruit a biochemistry PhD student through the BBSRC White Rose DTP, who would explore he properties of a wider range of membrane proteins reconstituted in hybrid vesicles and develop optimised methods for their reconstitution.

Finally, responsive mode grant applications to BBSRC/EPSRC are in the planning stages.