CBMNet
CROSSING BIOLOGICAL MEMBRANES NETWORK, A BBSRC NIBB

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Designing a new coat for *E. coli*

The Challenge

E.coli and other Gram negative bacteria coat themselves in remarkably stable protein fibres, polymers of around a 100 copies of a single protein, on their cell surface. These fibres are assembled via the well-recognised chaperone-usher (CU) pathway.

These highly stable polymers represent an appealing scaffold structure. Replacing a small fragment on this protein, potentially permits display of countless copies of such foreign peptides allowing changes in properties of the cell surface.

One limitation to manipulation of this bacterial coat is that each CU assembly system has evolved to export a specific substrate.

The Research

Dr Sheila MacIntyre is an Associate Professor of Microbiology at the University of Reading. The research in her laboratory focuses on the cell surfaces of Gram negative bacteria and Secretion via the chaperone: usher pathway.

Dr MacIntyre applied for a CBMNet Vacation Scholarship, which provided funding for an undergraduate student to exploit knowledge of secretion and surface assembly of proteins from Gram negative bacteria to design optimum export of modified fibres.

A molecular model of desired constructs within the export machinery was designed. Modified fibre subunit and export machinery were assessed *in silico* and then optimum constructs tested in *E. coli* for surface assembly.

CBMNet Vacation Scholarship

The Result

Two successful constructs of interest were prepared, one of which is predicted to change surface properties and a second, a possible vaccine candidate.

Modified polymers showed only a small decrease in thermostability. Thus, the study has demonstrated flexibility of this pathway for surface display of different peptides.

It also highlights the value of the modelled assembly complex to design constructs permissive to export with the native usher and to manipulate the usher sequence to replace specific interfering residues.

The Future

With the CBMNet vacation scholarship funding, Dr MacIntyre now has substantial evidence for potential exploitation of this surface display approach.

The study produced some very interesting results showing potential for development of different applications to modify the bacterial surface. The potential of inducing flocculation as required is currently being optimised and assessed with the intention of sourcing industrial interest and support for further study.

"The summer studentship has helped me to gain an insight into future career opportunities in the research environment as well as invaluable laboratory experience.. I have thoroughly enjoyed learning new skills - computer programmes for protein structure and DNA analysis, and laboratory procedures relating to mutagenesis and phenotype determination. "

Ash Griffin, Undergraduate Student

"This CBMNet Scholarship has funded an excellent student giving him the opportunity to experience research and provided us with the basis to explore industrial interest for our approach to adapting the surface of bacterial cell factories."

Dr Sheila MacIntyre

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