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The University Of Sheffield.

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Optimising the disulphide load in the periplasm of *E. coli* cell factories

The Challenge

E. coli-based production of therapeutic proteins often requires that the products are exported to the more oxidising environment of the periplasm in order to acquire the disulphide bonds that are necessary for proper function. One example of this is the production of antibody fragments which have wide ranging applications in diagnostics, human therapeutics and as fundamental research tools.

A major problem with industrial production of antibody fragments arises when disulphide bonds are not incorporated properly, leading to diminished quality and value of the end product. To address this issue, this project aimed to exploit the *E. coli* ABC transporter CydDC (Figure 1), which exports low molecular weight thiols such as cysteine and glutathione to the *E. coli* periplasm where they can participate in disulphide exchange and impact upon the formation of disulphide bonds.

The Research

Dr Mark Shepherd is a Senior Lecturer in Microbial Biochemistry at the University of Kent. The research in his laboratory focusses mainly on bacterial stress tolerance, respiratory metabolism, haem biosynthesis, disulphide folding, and biofuel production.

Fujifilm Diosynth Biotechnologies are a world leading cGMP Contract Development and Manufacturing Organization (CDMO) supporting the biopharmaceutical industry with the development and production of their therapeutic candidates.

Dr Shepherd applied for a CBMNet Business Interaction Voucher with Fujifilm Diosynth Biotechnologies to develop a collaborative partnership and produce preliminary data that could be built on in future funded projects.

The project aimed to measure the quality of antibody fragments produced in strains of *E. coli* with varied CydDC levels. The yield and quality of D1.3 Fab (Figure 2), a model antibody fragment used routinely by the industrial partner, was measured using a state-of-the-art Waters Synapt G2-Si electrospray mass spectrometer.

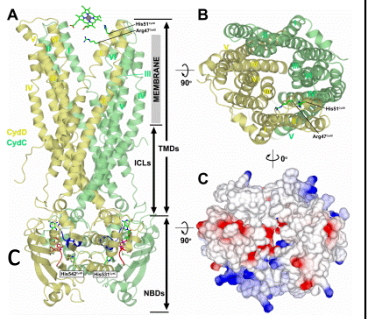

Figure 1. The CydDC ABC transporter of *E. coli.*

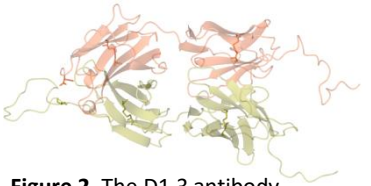

Figure 2. The D1.3 antibody fragment. Cysteine sidechains are shown to highlight S-S bonds.

CBMNet Business Interaction Voucher

The Result

The main focus of this work became the development of a quantitative technique to measure disulphide folding in antibody Fab fragments. The University of Kent recently acquired a state-of-the-art Waters Synapt G2-Si electrospray mass spectrometer that was used to directly measure the incorporation of disulphide bonds into the D1.3 fragment.

D1.3 tryptic-digest peptide fragments were analysed from *E. coli* strains expressing various levels of the CydDC transporter. The extent to which D1.3 was disulphide bonded was quantified for each strain, and the data revealed that loss of CydDC expression elicited a 25 % increase in antibody quality (in terms of disulphide incorporation).

The Future

Diminished CydDC expression was shown to enhance disulphide incorporation in D1.3 Fab, hence improving protein quality. This approach will be extended to other disulphide-containing protein targets of biotechnological importance in future.

The collaboration and data that has come out of this project will lead to further collaboration between Dr Shepherd and Diosynth Biotechnologies, e.g. CBMNet Proof of Concept funding, Innovate UK funding and possibly Knowledge Transfer Partnership funding. This collaboration has recently been supported by funding from the 'Metals in Biology NIBB', and the project partners are currently planning antibody experiments using the approaches described herein.

"This work is of value to our research as it resulted in method development in terms of proteomics approaches to measure disulphide incorporation and quantification of low molecular weight thiols. These approaches will certainly be exploited for other projects involving disulphide folding"

Dr Mark Shepherd
University of Kent

"This could be of major importance to Diosynth if this approach could be extended to other Fab fragments that are expressed in high abundance when CydDC is co-expressed"

Dr Chris Lennon
Fujifilm Diosynth Biotechnologies

This project was funded through the Crossing Biological Membranes Network (CBMNet) by the Biotechnology and Biological Sciences Research Council (BBSRC)

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