Mechanisms and applications of disulfide bond formation in the cytoplasm

The research undertaken by the Ruddock group covers both i) mechanistic understanding of fundamental cellular processes and ii) the application of the knowledge gained to study other cellular systems and associated disease states. Specifically, mechanistic understanding of the natural pathways for disulfide bond formation has allowed for the development of efficient artificial systems for disulfide bond formation by the application of synthetic biology. Our systems under development combine the advantages of prokaryote cell factories (e.g. *E.coli*) with those of eukaryotic cell factories (e.g. PTMs and secretion) and will have significant advantages over currently available protein production systems.

Studies with our system, known as CyDisCo (cytoplasmic disulfide bond formation in *E.coli*), have shown that the system is very successful, that knockout of the reducing pathways in the cytoplasm is not essential for disulfide bond formation and that high yields of active, correctly folded, eukaryotic proteins can be obtained. CyDisCo can be combined with other technologies, such as systems that secrete folded proteins from the cytoplasm and N-glycosylation in the cytoplasm to generate Gen2Co, 2nd generation *E.coli* cell factories.

Current versions of the CyDisCo system can easily be transferred between **any** *E.coli* strain, used in **any** media and allow the production of homogeneously folded human proteins with multiple disulfide bonds in *E.coli* grown in shake flasks with yields of up to 250 mg/litre culture along with multi g/L yields from fermentation. This system has been patented by the University and negotiations for commercial development are ongoing, with three companies already having licensed its use.

These two Pro Gradu positions are to use the CyDisCo system to generate proteins which naturally fold in the ER, whose study will give new insights into their mechanisms of action and associated disease states. Both are aimed at structure/function studies *in vitro* with parallel local collaboration for *in vivo* studies.

1) Angiopoietins play a key role in vascular development. There are no structures available for the N-terminal half of any angiopoietin and only limited structural studies on the C-terminal fibronectin-like domain which contains three disulfide bonds, including an unusual CXC disulfide. For Dr Lauri Eklund we have generated both N- and C-terminal domains for human Ang2 in yields of up to 25mg/L and have protein produced in good yields for full length mature protein Ang4 as well as its individual domains. Ang4 has no prior structural studies. This Pro Gradu will take forward structural and functional characterization of Ang2 and Ang4 constructs. In addition, the student will initiate studies on angiopoietin-like proteins which have a similar domain organization, a range of physiological functions and associated disease states and no structural data.

2) Perlecan is a key player in extra-cellular matrix organization. It is a 467kDa protein and less than 7% of the structure is solved. It is made up of a large number of repeating domains including many Ig-like and EGF-like domains. Since CyDisCo has successfully made both Ig- and EGF-like domain containing proteins previously, this project will systematically test production of multiple perlecan fragments for structural & functional studies. This will tie in with *in vivo* studies by Dr Raija Soininen. If successful we plan to extend these studies to other extra-cellular matrix proteins. Synthetic codon optimized gene fragments of perlecan will be used.

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