s7.m4.o5 **A novel, fusion protein strategy for membrane protein crystallisation**. B. Byrne, M. Jormakka, J. Abramson, S. Iwata. *Department of Biochemistry, Uppsala University, Uppsala, Sweden*
Keywords: membrane protein crystallisation, cytochrome bo_3, fusion protein

To date, only a very small number of membrane protein structures have been solved to high resolution. This is not due to any lack of effort in this area but rather due to the difficulty involved in working with these proteins. The development of new methods to facilitate both the expression and crystallisation of membrane proteins is essential to increase our understanding of these important molecules.

It has been noted that large multi-subunit membrane proteins, e.g cytochrome bo_3 from *E. coli*, incorporate large solvent filled gaps within the crystal lattice structure. Within the cytochrome bo_3 crystals, subunit IV (3 transmembrane spanning domains, intracellular N-terminus and extracellular C-terminus) lies in close proximity to the large solvent filled gap. We have developed an E. coli expression system involving the fusion of the gene coding for a small (<100 kDa) integral membrane or membrane associated protein of unknown structure to the C-terminal end of subunit IV of cytochrome bo_3.

We have generated fusions with protein Z, a soluble protein, full-length human apolipropotein A-I (apo A-I) and the G-protein coupled receptor, the human cannabinoid 2 receptor as well as leader peptidase and ProW from *E. coli*. All of the fusion proteins express as assessed by Western blot and the function of the cytochrome bo_3 is maintained. These data demonstrate that it is possible to express a number of different proteins in this way. Crystals have been obtained for both the protein Z (diffract to 6 Å) and the full-length apo A-I (diffract to 5 Å) fusion constructs and these structures have been solved by molecular replacement. This is the first report of crystals for the full-length apo A-I demonstrating the application of this method to previously uncrystallisable proteins. Based on these results, the potential of this method for the crystallisation of membrane proteins will be discussed.

Notes