Life in the Cold
- Permanently low temperature habitats have been successfully colonised by a wide variety of psychrophilic organisms which not only survive, but thrive, in this environment.
- Life at low temperatures requires a multitude of adaptations, both structural and functional, at all levels within the cells.
- Enzymes produced by cold-adapted organisms have successfully overcome the low temperature challenge and maintain efficient catalytic rates at low temperatures with, in addition, a reduced stability also being commonly reported.
- Currently it is believed that this low temperature adaptation is brought about by an increase in the protein flexibility which can also lead to the observed reduced stability.
- The proposed increased flexibility is a difficult parameter to demonstrate and yet unequivocally direct experimental evidence of this is lacking.
- Presently 23 3D-structures of cold-adapted enzymes are known and all have been obtained by X-ray crystallography.

Objectives
- To determine the solution structure of a cold adapted enzyme.
- To carry out a comparative biochemical and structural characterisation of homologous cold adapted and mesophilic enzymes.
- To obtain a better understanding of the molecular basis of cold adaptation.

This is the first report of an NMR structure for a cold-adapted enzyme and should open up a new dimension in the study of cold adaptation. The potential power of NMR to monitor both local and global motions over a large range of time scales should allow for a better understanding of the role of dynamics in the protein adaptation to temperature.

The model protein for the study: a cold adapted DsbA (PshDsbAp)
DsbA: Thiol-Disulphide Oxidoreductase (EC 1.8.4.-)
DsbA catalyses the extracytoplasmic formation of disulphide bonds in newly synthesised proteins. Catalyses a third disulphide exchange reaction during which substrate is oxidised and DsbA is itself reduced. Reduced DsbAs are then re-oxidised by the enzyme DsbB.
Cold adapted DsbA isolated from the Antarctic marine bacterium Pseudoalteromonas haloplanktis TAC125
PshDsbAp UniProtKB/TREMBL Accession code: Q31L4N
187 amino acid / 20804 Da protein.

Overproduction and Purification
- Recombinant PshDsbAp production at 18°C using the pET22b(+)/E. coli BL21(DE3) expression system.
- Protein purification: periplasmic extraction hydrophobic exchange (Phenyl Sepharose) anxious exchange (DEAE-Sepharose PF) and filtration (Superdex 75).

Thermal Unfolding
- The mesophilic homolog (VcDsbAm) from Vibrio cholerae was produced and purified as previously described.
- Purified DsbAs were reduced with 100-fold excess of DTT or oxidised with 1.5 mM copper phenanthroline, these agents were subsequently removed by gel filtration.

Irreversible Thermal Inactivation
- Higher rate of thermal inactivation for cold-adapted DsbA as compared to its mesophilic homolog.
- No thermal inactivation observed for Red. or Ox. mesophilic DsbA at temperatures investigated.

NMR Structure Determination of Reduced PshDsbAp

Conclusions
- We report here the first NMR structure of a cold adapted enzyme.
- The cold-adapted DsbA is characterised by a reduced thermal stability as compared to its mesophilic homolog.
- PshDsbAp is a two-domain protein with an overall architecture and fold very similar to previously described DsbAs.
- Short 3 to 4 amino acid insertions in two critical inter-domain regions may play central roles in adaptation to low temperatures in thiol-disulphide oxidoreductases.

Future Studies
- In-depth structural comparison with its mesophilic homologs, in particular comparing the number and strength of stabilising interactions.
- Development of an assembly assay to evaluate and compare the thermodynamic stability of this enzyme with that of its mesophilic homolog.
- Comparative dynamics using NMR.

References