This is a research project aimed at the design of a protein for its application as a cryoprotectant in bacterial stocks, as an alternative to glycerol, whose long term cytotoxicity has been demonstrated.

**BACKGROUND AND SIGNIFICANCE**

**ANTIFREEZE PROTEINS**

**PROJECT PURPOSE**

- Ice recrystallization is the main cause of cell damage during cryopreservation.
- Ice recrystallization inhibition activity prevents this process, while thermal hysteresis tends to generate an ice crystal with sharp ends.
- Cell preservation studies have been performed with soluble AFSs, which can't prevent intracellular ice formation.

**METHODOLOGY**

**PRIMARY SEQUENCE DESIGN**

After analysing 11 AFP, the main candidates were:

- Soluble (phage display)
- Small size (phage display)
- Defined ice binding site
- Low stability at 25 and 0ºC

**MEMBRANE BINDING SITE DESIGN**

The membrane targeting sequence, MTS (from the protein MinD) has shown to be a transpaltable lipid-binding motif.

**FINAL DESIGN ANALYSIS**

- **In silico testing**
  - Secondary structure prediction (Quick2D)
  - Topology prediction (Membrane Protein Explorer)
  - Aggregation (Aggscan)
  - Stability prediction (FoldX)

- **In vitro testing**
  - Western Blot
  - Liposome

**WORKING PLAN**

**BUDGET AND STAFF**

**BIBLIOGRAPHY**