Cotranslational folding of an integral membrane protein GlpG into an artificial protein WRAP

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Most membrane proteins are cotranslationally integrated into the membrane with the assistance of translocons such as bacterial SecYEG and YidC. During biogenesis of a membrane protein both cotranslational insertion of transmembrane domains and local contacts contribute to topogenesis and folding. These events can be hard to separate when studying the folding *in vivo*, as they often occur simultaneously and are influenced by multiple chaperones and translocon complexes. In contrast, *in vitro* experiments can provide a more controlled and simplified environment, which alongside in vivo experiments allows us to discover mechanistic insights and paint a more complete picture of the mechanisms underlying membrane protein biogenesis.

Design proteins such as water-soluble RFdiffused amphipathic proteins (WRAP) can be customized for the solubilization of a given membrane protein, facilitating the study of membrane proteins in solution. The design protein WRAP-GlpG, a fusion between WRAP and GlpG, was shown to preserve the GlpG fold, maintain functional integrity, and enhance thermostability [1].

The *E. coli* protease GlpG is widely used for membrane protein folding studies to dissect the mechanisms of insertion and folding within the membrane. *In vivo* force profile analyses (FPA) of GlpG, identified the different insertion and folding steps during biogenesis in the membrane [2].

In this work, we aimed to study the folding of the WRAP-GlpG protein, and the interaction between the transmembrane domain (TMD) of GlpG and the hydrophobic region of WRAP *in vitro*, comparing this behavior with co-translational folding in the membrane.

We achieved by FPA using arrested peptides (AP), as sensors. We employed this methodology by expressing this WRAP-GlpG design protein *in vitro* using AP sensors of different strength, *E. coli* (Ec) SecM AP and a hybrid between the *E. coli* and the AP from Mannheimia succiniproducens SecM (EcMs).

Preliminary data indicate an interaction between the TMD1 of GlpG and the hydrophobic region of WRAP.

References:

[1] L. Mihaljević *et al.*, 'Solubilization of Membrane Proteins using designed protein WRAPS', 2025, *Biochemistry*. doi: 10.1101/2025.02.04.636539.

[2] F. Nicolaus et al., 'Residue-by-residue analysis of cotranslational membrane protein integration *in vivo'*, 2021, eLife. doi: 10.7554/eLife.64302.