Co-translational protein folding in lipid membranes

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Summary:

Classic *in vitro* folding studies of membrane protein folding use overexpressed protein which has been purified and unfolded using chemical denaturants. This however cannot replicate folding in the cell, which occurs unidirectionally and co-translationally as the polypeptide emerges from the ribosome. To assess co-translational folding, membrane proteins can be expressed using a cell-free *in vitro* transcription/translation (IVTT) system with a supplied lipid bilayer. Folding is measured while synthesis occurs. A sucrose flotation gradient is used to separate inserted from aggregated protein, and the amount of successfully inserted protein can be quantified by counting incorporated [35S]Methionine. Altering the lipid composition can give insight into how bilayer properties can aid or inhibit protein insertion and folding in the bilayer. The protein can also be labelled at Cysteine residues to assess the topology, and function can be measured using a fluorescent assay.

Advantages:

Different cell-free IVTT kits are available commercially to suit the protein being studied, or can be made for low cost in-house

Can be used to measure co-translational folding yield, the topology of inserted helices, and structure formation can be measured as it occurs

Easily tuneable by changing the membrane mimetic (lipids, detergent, nanodiscs), fluorescent labels, radioactive or heavy labelled amino acids, or unnatural amino acids

Difficult-to-express or toxic proteins can be studied

Folding and insertion can be very efficient when unaided - but folding chaperones and insertion apparatus can be added or omitted as desired – for example the *E.coli* translocon SecYEG

High yield of expression relative to reaction size

Early stages of folding and insertion can be accessed without the need for purification

Challenges:

Very small reaction sizes mean that experiments with commercial kits can become prohibitively expensive – but this can be alleviated by making an IVTT extract in-house

Purifying the protein of interest from the IVTT components can be challenging, leading to problems for structure and function measurements

Measuring the function of protein can be difficult if there is not a sufficiently sensitive functional assay

Significant empirical optimisation can be necessary for successful protein expression - this can involve altering the reaction temperature, the supplied mimetic, or addition of insertion apparatus such as the translocon

Acknowledgements:

We acknowledge funding from the European Research Council, ERC Advanced grant 294342 to PJB

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