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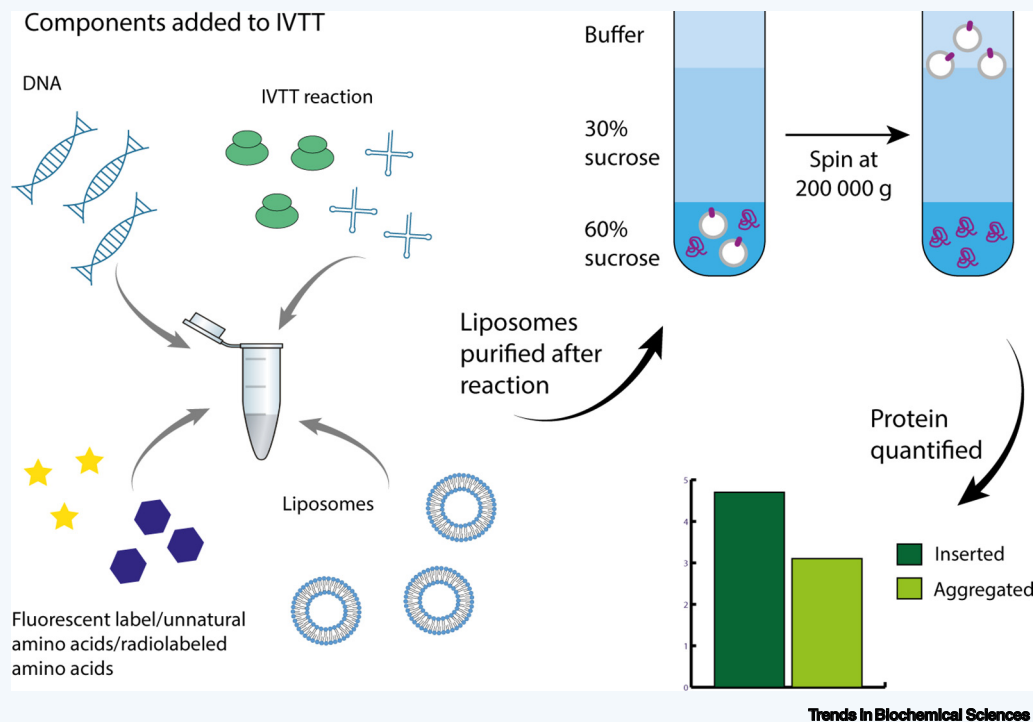
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Co-Translational Protein Folding in Lipid Membranes

Nicola J. Harris¹ and Paula J. Booth^{1,*}

¹King's College London, Department of Chemistry, Britannia House, 7 Trinity Street, London, SE1 1DB, UK



ADVANTAGES:

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

IVTT kits can be used to measure co-translational folding yield and the topology of inserted helices; the structure formation can be measured as it occurs.

IVTT kits are easily tuneable by changing the membrane mimetic (lipids, detergent, or nanodiscs), fluorescent labels, radioactive or heavy labeled amino acids, or unnatural amino acids.

Difficult-to-express or toxic proteins can be studied.

Folding and insertion can be efficient when unaided, but folding chaperones and insertion apparatus can be added or omitted as desired, with for example the inclusion of the *Escherichia coli* translocon SecYEG.

Classic *in vitro* folding studies of membrane protein folding use overexpressed protein that has been purified and unfolded using chemical denaturants. However, this 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.

There is a high yield of expression relative to reaction size.

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

CHALLENGES:

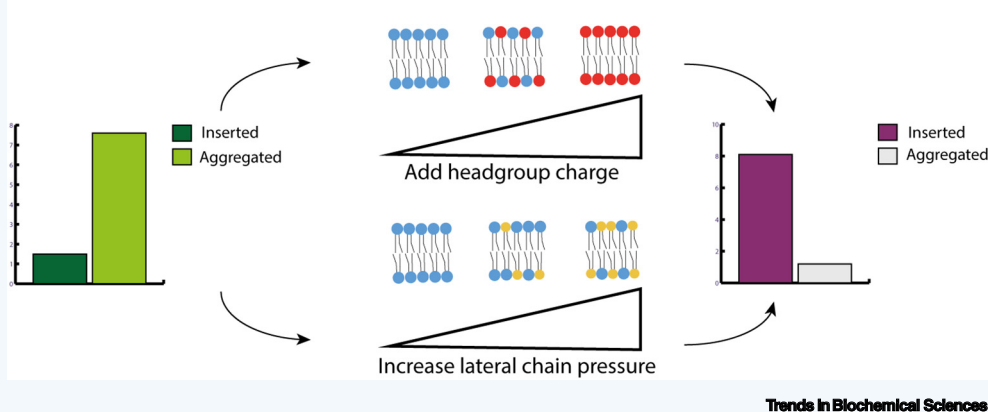
Very small reaction sizes mean that experiments with commercial kits can become prohibitively expensive, but this can be alleviated by making an IVTT extract in-house.

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 the addition of insertion apparatus, such as the translocon.

Altering headgroup charge and lateral chain pressure of liposomes can optimise the yield of inserted, folded protein



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.

*Correspondence: paula.booth@kcl.ac.uk (P.J. Booth).

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