How can the cell assemble complex structural motifs of integral membrane proteins?
Decoding different cotranslational mechanisms to insert charged residues and to place interfacial helices

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Membrane protein sequences must have evolved to fulfill the requirements for foldability, topogenesis, and functionality within the bilayer. But the molecular mechanisms and forces that explain how these requirements are achieved are not well understood.

To elucidate how complex structural motifs are assembled, we study the cotranslational insertion and folding of naturally occurring integral membrane proteins as well as designed sequences using *in vivo* force profile analysis (FPA). This method allows for monitoring vectorial insertion and assembly at the bilayer environment in which the protein evolved. This approach enables us to identify relevant steps during nascent chain insertion and elucidate the mechanisms cells evolved to assemble complex structural motifs, such as charged motifs and interfacial helices. Intramolecular interactions during insertion as well as the position of polar and hydrophobic residues, might play a role in the acquisition of the native functional conformation. To understand how this process takes place, we mutated polar residues involved in topogenesis and helix-helix interactions.

The results indicate that polar residues induce insertion pauses that could facilitate intramolecular interactions and that amphipathic helices undergo sequential conformational rearrangements before reaching the interfacial conformation.