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The dynamics of a protein during its insertion into a membrane

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Most membrane proteins are inserted co-translationally by the Sec-translocase or the YidC/Oxa1/Alb3 insertases. The folding of these proteins occurs within the membrane during the interaction with the insertases. We have purified and reconstituted YidC, the membrane insertase of *Escherichia coli*. The protein spans the membrane 6 times, and the recently solved structure shows a hydrophilic cavity and a greasy slide between the transmembrane segments TM3 and TM5. Hydrophobic residues of TM3 and TM5 interact with the substrate, with a prospective transmembrane segment of an inserting membrane protein as documented by disulfide crosslinking experiments. The membrane insertion process can be studied with the reconstituted vesicle system. The purified substrate proteins are solubilized in 10% isopropanol or kept unfolded with urea or GuHCl. When the substrate proteins are added to the proteoliposomes by dilution 1:100, they rapidly bind to YidC and become membrane inserted within 2 msec. FRET-based kinetic measurements show that the substrate proteins approach YidC to a close distance during the insertion event. Time-resolved fluorescence anisotropy shows that the periplasmic domain of YidC moves when a substrate protein was added. This suggests that both the insertase and the substrate protein undergo conformational motions.



Figure1: YidC-mediated insertion of the Pf3 coat protein

Biography

Andreas Kuhn has his expertise in protein folding of membrane proteins. Studies include reconstituted systems with bacterial translocases and insertase, as well *in vivo* studies with *Escherichia coli*. For biophysical experiments, the membrane proteins are purified and their folding is monitored spectroscopically in real time after their addition to liposomes. Andreas Kuhn obtained his PhD from the Universities Basel and Freiburg im Breisgau 1982. After a Postdoc at UCLA with Bill Wickner he continued at the Biozentrum Basel from 1986 to 1989 and accepted a professorship at the University Karlsruhe. Since 1996 he is at the University of Hohenheim in Stuttgart.

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