How transmembrane proteins insert and fold into cellular membranes is not fully understood. One of the problems is the lack of experimental methods allowing investigating the process of polypeptides insertion and folding into lipid bilayers at physiological conditions. We introduced a method to observe the assisted insertion and folding of individual transmembrane proteins. As a model system, we employ a polytopic α-helical membrane protein, the lactose permease (LacY). Here we study the insertion and folding of single LacY assisted by (i) the SecYEG translocon, (ii) the YidC insertase, and (iii) the combined action of SecYEG translocon and the YidC insertase.

In our experiments, we first mechanically unfold and extract a single LacY from a phospholipid membrane, transport the fully unfolded polypeptide to another membrane and characterize the insertion and folding of LacY into the lipid bilayer. We observe insertion and folding of LacY only in the presence of translocases or insertases. Both SecYEG and YidC insert LacY along multiple co-existing pathways to complete folding of the native LacY structure. We will present our methodological development and discuss our results revealing fundamentally different mechanisms by which YidC and SecYEG individually and collectively insert and fold α-helical membrane proteins.