Translating bacterial ribosomes can form structurally ordered polysomes. The three-dimensional (3D) organization of such polysomes has been described in solution using cryoelectron tomography (CET) and template matching [1]. However, little is known about how polysomes are organized in space when translating membrane proteins, and many questions related to the ribosome association to the membrane remain open. We propose to examine the 3D arrangement of membrane associated ribosomes in situ, for an optimal preservation of polysomes architecture; and in vitro, for a better control of translation conditions. We used the mannitol permease (MtlA) as a substrate for translation-coupled translocation through the membrane. In order to form densely packed polysomes, we inserted a SecM sequence at the end of the MtlA messenger. Thus, we stalled ribosomes in situ and in vitro, while the ribosome nascent chain complexes are still attached to the membrane. In situ this transmembrane protein construct is expressed in slow- and fast-growing BL21 E. coli cells. Fast-growing cells are thinned using a Focused Ion Beam technology [2]. In vitro, a transcription-translation reaction is supplemented with inverted vesicles isolated from E. coli inner membrane. First results show that the described approaches are adequate for revealing how membrane-associated ribosomes are spatially related in the context of polysomes. Furthermore the analysis of cytosolic ribosomes reveals a fraction of particles in similar 3D organization of dense polysomes previously described in vitro [1].

Reference:

Acknowledgement:
Prof. Dr. Wolfgang Baumeister
Prof. Dr. F. Ulrich Hartl
Dr. Friedrich Förster
Dr. Julio Ortiz
Elitenetzwerk Bayern: Protein Dynamics in Health and Disease
Fondation Fourmentin-Guilbert
Fig. 1: Tomogram from induced E. coli cells expressing MtlA385-SecM-Stop construct. (A,C) Slices from whole cell grown in minimal media (slow growing); (B,D) Slices of FIB-milled cell grown in rich media (fast growing). In the image ribosomes are visible as highly contrasted particles. - (A,B) XY-slices; (C,D) XZ-slices. Bar 100 nm.