Irina GUTSCHE is currently the leader of the Microscopic Imaging of Complex Assemblies group in the IBS Grenoble. In the last year of her PhD thesis on actin assembly in LEBS, Gif-Sur-Yvette, under the guidance of Marie-France CARLIER, she had a chance to work with Jean LEPAULT, one of the pioneers of cryo-electron microscopy. Fascinated by the beauty of the images, she then got a thorough training in cryo-electron microscopy and tomography in the lab of Professor BAUMEISTER in the Max Planck Institute of Biochemistry in Martinsried, where she worked on structure-function relationships of an archaeal chaperone system. As she returned to France, she combined her different areas of expertise and developed a research program focusing on structure, assembly and dynamics of flexible biological polymers and macromolecular complexes involved in particular in cellular stress response.

INTEGRATIVE STRUCTURAL BIOLOGY ANALYSIS OF AN ENTEROBACTERIAL STRESS RESPONSE PROTEIN TRIAD

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AAA+ ATPases are a diverse protein superfamily used by cells as motors to power mechanical work or to act as molecular switches or scaffolds, often as parts of macromolecular machines. A MoxR AAA+ ATPase termed RavA is part of an enterobacterial stress response system that includes also the acid stress-inducible lysine decarboxylase LdcI and the uncharacterized protein ViaA. In E. coli, five RavA hexamers bind two LdcI decamers to form a 3.3 MDa cage. Here we are using an integrated approach around cryo-EM and ranging from phenotypic analysis and molecular biology, through biochemistry, biophysics, mass spectrometry, X-ray crystallography and SAXS, to optical imaging, cellular electron microscopy and cryo-electron tomography. We present 3D structures of RavA alone and bound to LdcI, provide structural insights into the design principles of the RavA-LdcI assembly, discuss a molecular mechanism of RavA ATPase activity, characterise the RavA-ViaA interaction, assess the in cellulo distribution of the LdcI-RavA-ViaA triad and analyse its effects on cellular lipid homeostasis and membrane morphology. This results in a new model for the triad function and sheds first light on the mechanism of its involvement in sensitisation of enterobacteria to aminoglycosides, which is is essential because administration of smaller doses of antibiotics should reduce toxicity and allow safe treatment of a wider number of infections. In addition, this work opens up novel research directions and underscores the needs for further developments and creative combination of methods.