Project A2: Membrane active polypeptides and liposomal complexes for nucleic acid delivery

Principal investigators: B. Bechinger (Strasbourg) / R. Süss (Freiburg)

Current state of the research. Membrane-active peptides and liposomal systems have been developed for nonviral transfection of eukaryotic cells with nucleic acids for research and with the goal to develop medical treatments including gene therapy. Little is known about the molecular events during transfection and the underlying mechanisms. Therefore, there is a need to investigate the formation of transfection complexes and the transfection activities on a molecular, supramolecular and cellular level.

Contributions of the principal investigators. The cationic amphipathic histidine-rich peptide LAH4-L1 was developed by the Bechinger group and already proven to be membrane active and to deliver nucleic acids very efficiently. In addition to their high transfection efficiency, LAH4-L1/siRNA complexes are characterized by low toxicity, good biodegradation and ease of preparation. The Süss group is experienced in developing carriers for nucleic acids (DNA, siRNA) and has recently shown that Lipid-Protamine-siRNA systems are highly effective.

Research project and collaboration. The Bechinger and Süss groups have already started to collaborate on LAH4 peptides and first data are very promising, as it was shown that LAH4-L1/siRNA complexes are highly active in biological assays.

Work plan. This PhD project involves the following tasks: LAH4-L1/DNA or siRNA and LPR complexes will be prepared and characterized in both laboratories and freely exchanged between partners.

The Süss group will then focus on: *i*) Biological studies: cellular interaction, cellular toxicity, cellular uptake, cellular processing and evaluation of biological efficacy in terms of protein production or gene-silencing of targeted protein (flow cytometry). *ii*) Cellular location of polypeptide, lipid and DNA or siRNA.

The Bechinger group will focus on: *i*) Biophysical studies of the supramolecular morphology of lipids, of intermolecular distances between NA and peptides and the peptide topology within the complexes (NA or membranes) by solid-state NMR. *ii*) Secondary structure of LAH4 and RGD peptides in transfection complexes using MAS solid-state NMR and optical spectroscopies. *iii*) Fusion and pore-forming activities of the complexes using optical spectroscopies. *iv*) Thermodynamics of complex formation using ITC and other biophysical approaches.

Although effective transport of polypeptide- as well as LPR-nucleic acid-complexes into the cell has already been reported, it has not yet been thoroughly studied by which mechanism these complexes interact with membranes and what is the fate of the complexes after cellular uptake. This knowledge will be essential for the understanding and further development of transmembrane delivery systems of nucleic acids. Our transnational collaboration enables comparative studies between LAH4/DNA or siRNA and liposome-protamine-DNA or siRNA (LPR) complexes with highly complementary methods. The results will constitute the foundations for a deeper understanding of how these transfection complexes and their individual components interact with each other and with membranes. The cell-biological and *in vivo* aspects in the study of LAH4 as well as adding biophysical-structural information to the LPR studies will provide a new quality of research and development for these systems and will only be possible through this collaboration.