PROJECT PROPOSAL
for applicants for ITC fellowships (2018/19)

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project title: TARGETED CELL MEMBRANE DELIVERY OF SEMISYNTHETIC LIPOPROTEINS

PROJECT SUMMARY
Targeted protein transfer without transgenic methods represents huge biotechnological and medical potential. GPI-anchored proteins and related chimeras are suitable for that application because after isolation they can be reinserted into the membrane of recipient cells with the retention of the protein function. Cholesterol amphiphiles can mimic GPs, thus their incorporation as simplified GPI surrogates into proteins extends the scope of protein transfer methods. Bioorthogonal conjugations will be explored to prepare cholesteryl lipoproteins followed by the investigation of their host-guest complexes and exosome associates as protein transfer vectors. The usefulness of the method will be demonstrated by introducing cholesteryl interleukins into the plasma membrane of immune cells.

BACKGROUND
The exogenous introduction of semisynthetic lipoproteins into the plasma membrane offers control over the composition of the engineered recipient cell membrane. Thus, it is an accurate method for the investigation of membrane protein dynamics, for visualizing the cellular traffic of membrane proteins, and for improving the efficacy of biologically active proteins. Interleukins (IL) are secreted proteins, however, numerous engineered membrane-anchored cytokines have been found to be bioactive. Membrane anchoring of cytokines with GPI anchors or with simplified GPI surrogates followed by protein transfer are under investigation as an alternative approach to gene therapy. The main advantage of the method is that purified, pharmacologically active proteins are delivered directly to the plasma membrane of target cells. It was successfully applied for engineering T-cell or tumor cell membranes with IL-2, IL-4, IL-12, IL-15, B7-1 and GM-CSF.

CURRENT RESEARCH
Inspired by GPI-APs we have recently developed a method using cholesterol amphiphiles in order to deliver proteins into the plasma membrane under mild conditions (Figure 1). It was demonstrated that the cholesterol anchor and the attached protein had been incorporated into the membrane of live cells in stoichiometric ratio. Our anchor was found to be compatible with live cell applications, and the headgroup reporter made the direct imaging of the attached protein possible. Ongoing research is devoted to the general usefulness of the method that requires the extension of the protein-anchor conjugation chemistry and an optimal carrier system. For that, bioorthogonal functionalization of the C-terminus of proteins and the probed cholesterol anchor is investigated. Another aspect of the method development is to find an optimal carrier system for in vitro and in vivo applications. It is highly important because the amphiphilic cholesterol anchors form micellar associates in aqueous media, that exclude the
headgroup fluorescence-based protein imaging and also can perturb the plasma membranes of recipient cells.

Introduction of semisynthetic cholesteryl lipoproteins into the plasma membrane (Schäfer et al. 2013)

SPECIFIC AIMS

Beyond developing a general protein modification with cholesterol amphiphils, the method will be specifically applied for membrane anchoring of ILs to enhance their targeted delivery. Accordingly, the outlined research project requires the preparation of appropriate membrane anchors and bioorthogonally functionalized recombinant proteins, the optimization of protein cholesterol anchor conjugation, a flexible protein synthesis strategy to prepare ILs and finally in vitro experiments to evaluate the consequences of membrane anchoring on the structure and pharmacological effects of IL-15.

METHODS TO BE LEARNED / APPLIED

- Bacterial overexpression of proteins
- Chromatographic, spectroscopic and electrophoresis techniques for protein and small molecule analysis, purification
- Protein-small molecule conjugations, preparative organic chemistry
- Fluorescent confocal microscopy of live cells

SUGGESTED READINGS

Schäfer B, Ph.D., 2009-2013, “Preparation of semisynthetic cholesteryl lipoproteins”
Mórocz I, M.Sc., 2013-2014, “Exploring the application of methionyl aminopeptidase in the native chemical ligation-based preparation of polypeptides”
Göblyös D, M.Sc., 2011-2012, “Development of consecutive native chemical ligation to prepare synthetic proteins”