# PHD THESIS ABSTRACT – SOFIE FOGH HEDEGAARD

The need for highly efficient biopharmaceuticals for the treatment of many serious diseases is continuously increasing, and hence effort has been laid into the development of new strategies to optimize the efficiency of the biomolecular drug molecule itself, but also to improve the delivery of the drug molecule across biological barriers in the body. Covalent modification with hydrophobic moieties has proven to be advantageous in order to tailor the properties of therapeutic peptide/protein drugs and peptide excipients: Lipidation of therapeutic peptides/proteins can improve the effect of the drug in the body, and lipid modification of cell-penetrating peptides can potentially improve the membrane permeation ability. Fluorophore-labeling of cell-penetrating peptides enables high sensitivity detection in fluorescence-based techniques, however, tailoring such amino-acid-based molecules with a hydrophobic moiety like a fluorophore will change the physico-chemical properties of the molecules; a change that potentially can be accompanied with unforeseen outcomes. Therefore, upon modifying peptides/proteins, attention has to be drawn not only to the desired effect, but also to potential adverse consequences of the modification. The overall aim of this project was to explore and reveal the effects and role of peptide/protein-conjugated hydrophobic moieties on solid surface adsorption and fibrillation behavior, on the degree and mode of membrane interaction and membrane translocation ability, and moreover on the applicability as excipients for drug delivery purposes.

The impact that a lipid chain modification of insulin has on the protein's interaction with hydrophobic surfaces was addressed. Lipidation of insulin was observed to increase the extent of insulin adsorption, and change the subsequent mode of fibrillation on hydrophobic surfaces, under stressed experimental conditions. According to the complementary surface-sensitive techniques including QCM-D, AFM and NR, two layers of structurally different protein organization were formed on the surface for both insulin and the lipidated insulin analogue. However, the lipid chain attached to insulin noticeably affected the thickness of the two protein layers, the surface coverage, and the fibrillar morphology and structural arrangement.

Further, due to uncritical use of fluorophores for detection of short peptides in various *in vitro* bioassays, the effect of conjugating a range of different fluorescent molecules on biomembrane interaction was assessed, applying the 16-amino acid cell-

penetrating peptide penetratin as parent peptide. The conjugation of a rigid and bulky fluorophore moiety to penetratin induced noteworthy structural alterations in biomembranes upon exposure to the labeled peptides. The fluorophores mediated peptide insertion into the lipid core of a lipid bilayer, which induced lipid removal and membrane thinning. The most pronounced membrane disturbing effect was observed for penetratin labeled with the two most hydrophobic fluorophores; rhodamine B or 1-pyrene butyric acid.

Cell-penetrating peptides can facilitate the permeation of biopharmaceuticals across biological membrane barriers. The membrane interaction, binding and perturbation effect of four lipidated analogues of the 16-amino acid peptide penetramax (Syn1), a variant of penetratin, was assessed. The mode and degree of biomembrane interaction was highly dependent on the specific position of the conjugated C10 chain in the peptide sequence and a double-lipidated peptide analogue (Syn4) exhibited a more pronounced membrane disturbing effect compared to the single-lipidated analogues. Penetratin and the lipidated peptide analogues were moreover tested as epithelial membrane permeation enhances for insulin and lipidated insulin applying the Caco-2 cell culture model. Syn1 and the single-lipidated peptide analogues (Syn2, Syn3 and Syn5) significantly enhanced the permeation of insulin, an effect that was highly pH-dependent in the case of Syn2 and Syn5. The double-lipidated analogue Syn4 did not promote any insulin transepithelial permeation and no effect on cell viability was seen in the cells. In contrast, the rest of the tested peptides induced a decrease in cell viability, which was especially pronounced for the N-terminally lipidated analogue Syn5. The transepithelial permeation of lipidated insulin was not enhanced by the presence of the tested peptides.

Overall, tailoring amino acid-based molecules with hydrophobic moieties in order to achieve certain properties was shown to notably affect the surface interaction behavior of the molecules. Upon applying the pharmaceutically valuable tools; fatty acid chain conjugation and fluorophore-labeling, potential drawbacks and adverse effects of the modifications should be critically assessed.