**Investigation of the transport and metabolic targeting of bile salts**

**by human ileal bile acid-binding protein using an integrated biophysical approach**

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The main goal of our collaboration is obtaining a molecular level understanding of the mechanism of action of human ileal bile acid-binding protein (I-BABP), a member of the family of intracellular lipid binding proteins associated with metabolic diseases and cancer. Bile salts are amphipathic molecules synthesized from cholesterol in the liver, which in the small intestine facilitate the absorption of dietary lipids, cholesterol, and fat-soluble vitamins. Besides their role in digestion, they are also known as signal molecules. By interaction with receptor proteins and activating various signalling pathways, they have a role in the regulation of metabolic processes. Human I-BABP, a protein playing a key role in the cellular trafficking of bile salts, is known to function by interacting with the cell membrane of enterocytes in the distal small intestine. Preliminary results and the study of homologous proteins suggest that interaction with the cell membrane is accompanied by a partial unfolding of I-BABP, which is modulated by the presence of ligands. Our study is aimed at obtaining a better understanding of the I-BABP-membrane-bile salt interaction on the molecular level. To achieve our goal we use an integrated biophysical approach combining the tools of structural biology (NMR) with calorimetry (ITC) and fluorescence spectroscopy. Currently, we are focusing on three main questions. Primarily, we are interested in elucidating the thermodynamics and kinetic mechanism of I‑BABP unfolding in aqueous buffer and in the presence of liposomes. Another important goal for us is revealing the structural changes occurring in I-BABP upon the interaction with lipid bilayers. Finally, our third question is concerned with the effect of ligands on the I-BABP-membrane interaction. The proposed experiments should provide us with a mechanistic insight of ligand binding and dissociation in human I-BABP and may open up new possibilities for the treatment of metabolic diseases as well as the development of potential I-BABP-based drug carrier systems. In addition, the studies we propose will contribute to a better understanding of molecular recognition mechanisms in general, with particular emphasis on transient protein-membrane interactions as well as the coupling between folding and binding in proteins.

