Molecular Biophysics

The main focus of our research is to study, understand and manipulate protein ligand interactions. The function of a protein is defined by its three-dimensional structure and its interactions with ligands (e.g. substrates, other proteins, nucleic acids). Nuclear magnetic resonance (NMR) spectroscopy is one of the most suitable tools to investigate protein structure, dynamics and ligand interactions. Besides NMR, we employ circular dichroism (CD), fluorescence spectroscopy, surface plasmon resonance (SPR), as well as molecular biology and biotechnology (e.g. phage display, two hybrid systems, recombinant protein expression) to learn more about protein ligand interactions.

The major research topics of our institute fall into two areas, namely viral proteins and their interaction with host cell proteins, and proteins that are relevant for function and dysfunction of the nervous system.

GABARAP

The $GABA_A$ receptor is the principal neurotransmitter receptor of inhibitory synapses. The $GABA_A$ receptor is decisive for regulation of sleep and mood. The $GABA_A$ receptor associated protein (GABARAP) binds to the $\gamma 2$ subunit of newly synthesized $GABA_A$ receptor molecules at the ER. It is involved in correct trafficking of $GABA_A$ receptors to GABAergic synapses and modulates $GABA_A$ receptor expression and function on the postsynaptic membrane. It also is responsible for the recycling of $GABA_A$ receptors via the Clathrin coated vesicle endosomal pathway.

The 117 amino acid GABARAP consists of an Ubiquitin-like fold with two additional amino terminal helices. The carboxy-terminal residues are an integral part of the globular fold. In vivo, the C-terminal Leu-117 of GABARAP is cleaved off leaving Gly-116 as a C-terminal residue that can be conjugated covalently to proteins and lipids via an E1 E2 E3 enzyme cascade.

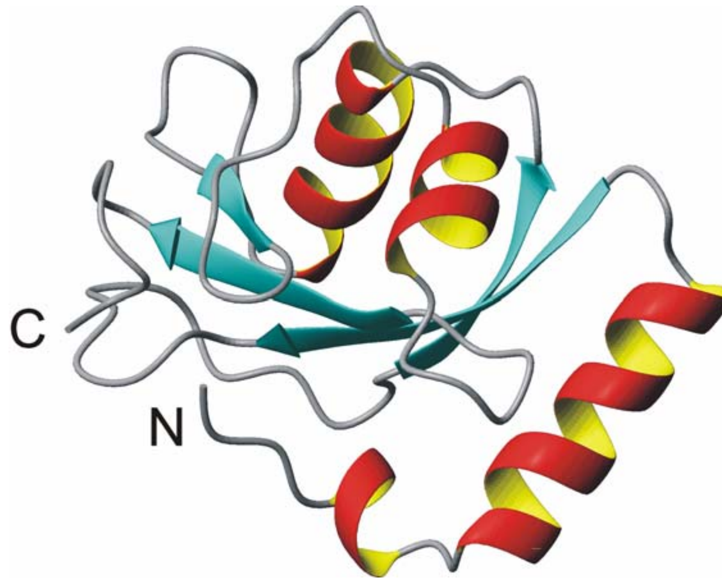

Fig.1: Ribbon presentation of the averaged GABARAP structure. Amino (N) and carboxyl (C) terminal ends are indicated.

Currently, we are studying the structural consequences of the C-terminal processing of GABARAP. We are interested in the structure of GABARAP with interacting proteins, and we aim to identify novel artificial ligands to study possible effects of inhibiting GABARAP's function.

Structure and function of HIV accessory proteins

Human immunodeficiency virus type 1 (HIV-1) is a member of the lentivirus family. In addition to the *gag, pol* and *env* genes present in all retroviruses, HIV-1 encodes two regulatory and four so called "accessory" proteins, which are dispensable for viral replication in cell culture but are known to be decisive for viral infectivity, replication and pathogenesis *in vivo*. One of these accessory proteins is virus protein R (Vpr). Vpr seems to be required at various steps of the HIV replication cycle and is therefore an interesting target for the development of antiviral agents. Virus protein U (Vpu) enhances virus particle release of infected cells, decreases tendency of syncytia formation and induces degradation of human CD4 receptor and some other host cell proteins. Nef ("negative factor") is a small protein of about 25 kDa that is essential for high titer viral replication and pathogenesis of

the acquired immunodeficiency syndrome (AIDS). Nef is produced at the earliest stage of viral gene expression and is a component of viral particles. Nef interacts with a multitude of host cellular proteins. We investigate the structure and function of these accessory proteins especially in complex with their host cell target proteins.

Structure and function of SARS-associated coronavirus accessory proteins

A novel coronavirus (CoV) has been shown to be the etiologic agent of the severe acute respiratory syndrome (SARS) epidemic, which affected about 30 countries in late 2002. The viral genome is almost 30 kb in length and contains at least 11 open reading frames, whereas the exact number depends on the strain and the minimal count of coded amino acid residues. Coronaviruses are positive-strand RNA viruses that code for the characteristic proteins replicase (R), spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins. In addition, SARS-CoV codes for subgroup-specific accessory proteins that are thought to be dispensable for viral replication in cell culture, but may be important for virus-host interactions and thus contribute to the virus' fitness.

Recently, we determined the solution structure of the soluble ectodomain of the type-I transmembrane protein X4 by nuclear magnetic resonance spectroscopy. The fold of the protein is the first member of a further variation of the immunoglobulin like beta-sandwich fold. Because X4 does not reveal significant sequence homologies to proteins in the data bases, we carried out a structure based similarity search for proteins with known function. High structural similarity to D1 domains of ICAM-1 and ICAM-2, and common features in amino acid sequence between X4 and ICAM-1, suggest X4 to possess binding activity for the α_L integrin I domain of LFA-1.

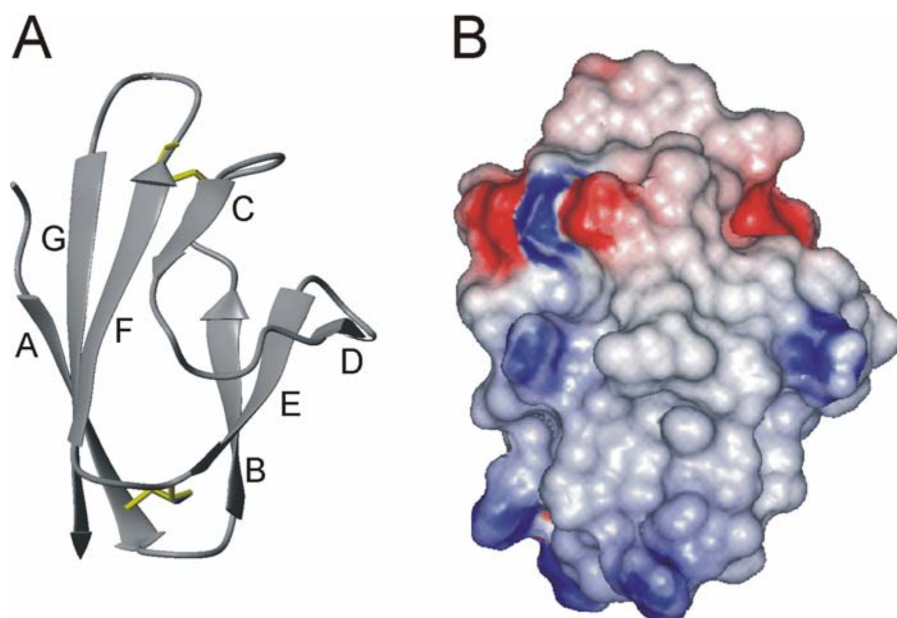

Fig.2: Ribbon representation (A) and surface charge distribution of (B) of the lowest-energy solution structure of the SARS-CoV X4 ectodomain. Secondary structural elements are accentuated and labelled according to their sequential arrangement. Heavy atom sidechains of Cysteines 8, 20, 43 and 52 are shown in yellow to visualize the disulfide bridges. Red and blue surfaces represent negative and postive electrostatic potentials, respectively.

Diagnosis and therapy of Alzheimer's disease

Alzheimer’s disease (AD) affects millions of people. So far, there is no clear way to a successful therapy. Possibly there might never be a perfect therapy, because it seems quite hard to replace the function of neurons that are gone. Another approach to the problem certainly is prevention. But currently, it is almost impossible just to assay potential preventive properties of a given treatment. So, there is a strong need for a correct and reliable diagnosis of AD at stages as early as possible.

Some years ago, we attempted to identify ligands that would strong and highly specific bind to Amyloid peptide Aβ to prevent Aβ self aggregation as a potential therapeutic approach. Using phage display, we screened a peptide library with more than one billion different 12mer peptides. Because we used a mirror image version of the phage display approach, we obtained peptides that solely consist of non-proteinogenic D-enantiomeric amino acid residues. Such D-peptides are known to be protease-resistant and non-immunogenic, which is important for use in animals or humans. The hereby obtained D-peptide binds

tightly and with high specificity to Aβ deposits. Currently, we are developing this D-peptide into a probe for on line monitoring of the Aβ plaque load in the living brain.

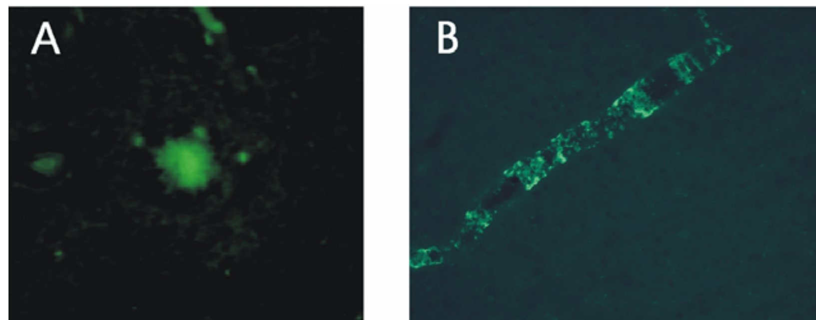

Fig.3: Fluorescence microscopy of a brain tissue section derived from a patient who suffered from AD. (A) Stain of a plaque deposit with the identified fluorescein-labelled D-peptide (excitation 450 to 490 nm, emission 515 to 565 nm). (B) Fluorescence microscopy of a brain tissue section derived from a transgenic APP/PS1 mouse and stained *in-vivo* with the D-peptide. Shown are Aβ deposits in blood vessels.

Structural basis of SH3 domain binding specificity

Protein tyrosine kinases (PTK's) are involved in signal transduction pathways that regulate cell growth, differentiation, activation and transformation. Src-familiy kinases show a common architecture consisting of an N-terminal unique domain, followed by the regulatory SH3, SH2 domains and by the tyrosine kinase domain containing the active site. The SH3 domain binds to polyproline motifs and is required for association of the kinase with many intracellular substrate or binding partners. Some viruses (HIV-1, Hepatitis C) evolved proteins (Nef, NS5A) that interact with the SH3 domains of specific Src-family kinases, leading to perturbations of cellular signalling pathways, and that way ensure their own replication and/or persistence.

The main topic of this project is the structural investigation of selected SH3 domain-ligand complexes at high resolution to gain insights into the structural basis of the observed specificity and affinity of ligand and SH3 domain. In the long run, novel therapeutic approaches based on the manipulation of specific SH3-ligand interactions may be possible.

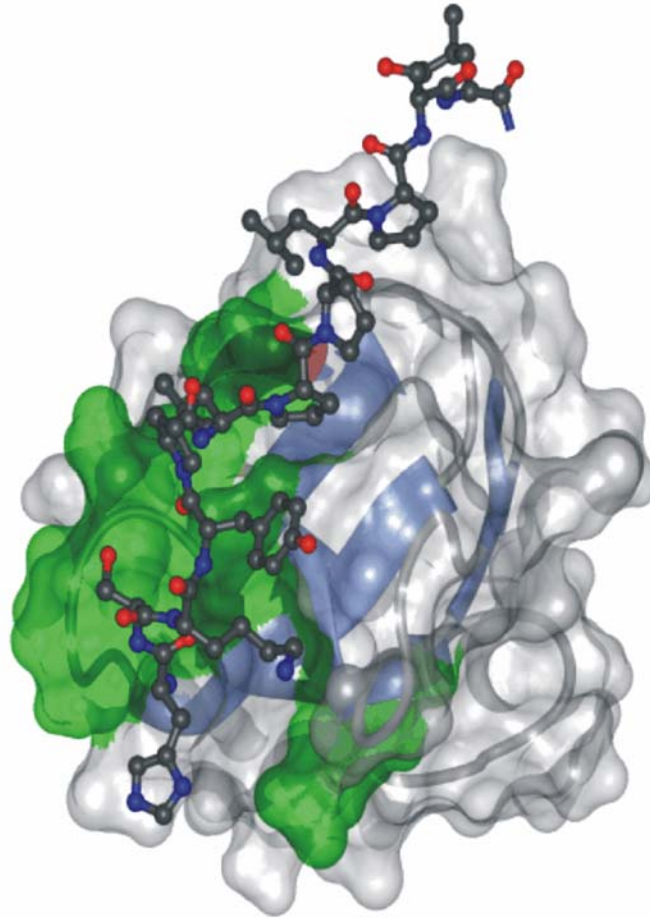

Fig.4: Ribbon and surface diagram of the high resolution structure of human Hck SH3 in complex with an artifical peptide ligand (PDB entry 2A4Y). The surface of residues that show the most prominent chemical shift changes upon peptide binding are colored in green. The bound peptide is shown in a ball and stick representation.