Universität Basel

Research Project

Biomacromolecular structures, dynamics and interactions by NMR and new developments in NMR technology

Third-party funded project

Project title Biomacromolecular structures, dynamics and interactions by NMR and new developments in NMR technology
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Biological function results from time-dependent interactions between biomolecules. It is ultimately encoded within the primary chemical structure of molecules resulting in defined three-dimensional atom positions and movements. NMR spectroscopy is the only experimental method, which yields both structural and dynamical information on biomolecules at atomic resolution with minimal invasiveness and at close to natural conditions. As such it can provide unique information to understand the connection between primary structure, tertiary structure, dynamics and function. It is the goal of this proposal to apply and further develop these strengths of NMR technology with the aim to reveal general principles of protein structure function relations. The proposal is divided into two subprojects:

Subproject A is directed towards the determination of structure, dynamics, and interactions in four medically important systems, for which we have made significant progress in recent years: (1) Abl kinase, which is an important leukemia drug target. We have obtained assignment of a large, multidomain Abl construct that is the minimal autoregulatory fragment. Our data show that binding of different classes of inhibitors induces distinct domain rearrangements, which shed light on the mechanism of kinase regulation. We now want to reveal the atomic causes of these allosteric rearrangements, investigate several medically and functionally relevant mutants, and extend the studies to single molecule FRET. (2) the HIV-1 coreceptor and G-protein coupled receptor (GPCR) CCR5. We have obtained large-scale functional expression of CCR5 in insect cells and E. coli, which permits effective isotope labeling. We seek to apply new solid-state and solution NMR techniques for structure determination and to develop general strategies for GPCR analysis by a comparison to other available GPCRs. (3) Interactions of the bacterial virulence factor cyclic di-guanosine-monophosphate (c-di-GMP). We have determined the structure of the PilZ homolog PA4608 in complex with c-di-GMP and the kinetics of c-di-GMP oligomer formation in solution. We now want to solve the structures of a complex c-di-GMP with the chemotactic protein CC3300 and of higher c-di-GMP oligomers. (4) Lipopolysaccharide (LPS), the causative agent of endotoxic shock. We have developed a method to study LPS by solution NMR and obtained expression of the lipopolysaccharide binding protein, which is the first receptor of the endotoxic recognition cascade. We intend to study its interaction with LPS.

Subproject B is directed towards the NMR characterization of unfolded states of proteins and their relation to the folded structure with an emphasis on pressure denaturation and structural modeling of unfolded ensembles. The overarching goal is to rationalize protein folding by correlating high resolution experimental data on unfolded states with primary, secondary and tertiary structure information. (1)

We have obtained unique data on the pressure/cold-denatured state of ubiquitin, which shows similar subpopulations of partial native structures as an alcohol-denatured state. By addition of non-denaturing concentrations of alcohol, full pressure-induced unfolding can be achieved at room temperature in a completely reversible way. This allows following the unfolding transition at very high resolution. We want to extend this analysis to a larger set of about 10 proteins representing different folds and sequences with the aim to correlate unfolding behavior with sequence or structure and to understand the nature of pressure unfolding. (2) We have developed an effective method to calculate structural ensembles of unfolded proteins from large sets of NMR data. We want to continue these efforts with the aim to include FRET and chemical shift data in order to obtain highly defined quantitative models for larger sets of unfolded proteins.

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