Universität Basel

Research Project

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

Third-party funded project

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Biological function results from time-dependent interactions between biomolecules. 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 structure, dynamics and function. It is the goal of this proposal to apply and further develop these strengths of NMR technology in two subprojects.

Subproject A is directed towards the determination of structure, dynamics, and interactions in several medically important systems, for which we have made significant progress in recent years: (1) two G-protein coupled receptors (GPCRs), i.e. the beta1-adrenergic receptor (b1AR) and the chemokine receptor CCR5, which is also the coreceptor for HIV. For b1AR, we could show that it is possible to follow signal transduction by ^{1}H-^{15}N backbone resonances. We now want to carry out a full NMR dynamics characterization comprising the binding of drug, G protein and arrestin ligands in different membrane environments. Beside solution NMR, this will also entail the study in full lipid bilayers by solid state NMR. Thus we hope to establish b1AR as a reference system for studies of GPCRs by NMR. We have recently obtained solution spectra for CCR5 of comparable quality to b1AR. We could also detect many resonances of the engineered chemokine ligand 5P12-RANTES in complex with CCR5 by solid state NMR. 5P12-RANTES is currently in clinical trials as an HIV entry inhibitor. We want to carry out a solution and solid state NMR analysis of this receptor with 5P12-RANTES and engineered RANTES ligands that elicit different arrestin and G-protein signaling activities with the aim to provide the structural and dynamical explanation for this differing signaling behavior. (2) Interactions of the bacterial virulence factor cyclic di-guanosine-monophosphate (c-di-GMP). We have solved the structures of several c-di-GMP protein complexes and c-di-GMP oligomers as well as determined the kinetics of c-di-GMP oligomer formation in solution. In particular, we could recently solve the structure of the c-di-GMP recognition region of the chemotactic protein CleD in complex with c-di-GMP. C-di-GMP binding to CleD induces formation of a ternary complex with the flagellar motor switch protein FliM thereby controlling bacterial flagellar motor function. We now want to solve the structure of the full c-di-GMPcomplex and study the formation of the ternary c-di-GMPcomplex. (3) Abl kinase, which is an important leukemia drug target. We have shown that binding of different classes of inhibitors to a large, multidomain Abl construct induces distinct domain rearrangements, which shed light on the mechanism of kinase regulation. We have now assessed the influence of a number of medically relevant point mutations and studied a larger class of inhibitors. In particular, the opening of the multidomain structure is correlated to the conformation of the activation loop in the catalytic domain. We now want to obtain an in-depth dynamic description of the catalytic domain, which is possible due to an advance in labeling techniques, as well as continue to develop suitable labeling of Abl for single molecule FRET studies.

Subproject B is directed towards NMR technique development. Often proteins from higher eukaryotes cannot be expressed in functional form in *E. coli*, but only in higher eukaryotic expression systems. The difficulties of isotope labeling in such systems presents a bottleneck for the analysis by modern heteronuclear NMR. We have recently developed a robust method for isotope labeling in insect cells by feeding isotope-labeled yeast extract. The method currently yields 90ă% ^{15}N and ^{13}C as well as >60 % ^{2}H labeling with very good yields. We want to improve this method by increasing the level of ^{2}H incorporation and cost efficiency for ^{13}C labeling as well as extend it to other eukaryotic systems. We also want to obtain an in-depth understanding of the often adverse effects of ^{2}H incorporation to cellular behavior by a proteomics analysis of the response to growth on $^{2}H_2O$ for *E. coli*, yeast and higher eukaryotes.

Keywords Biophysics, Structural Biology, GPCR, kinase, NMR, FRET
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