NMR studies of protein-small molecule and protein-peptide interactions

Guan, J.

Summary

The work described in this thesis focuses on the application of various NMR techniques to the study of interactions between proteins and small molecules and proteins and peptides, including the well-established classical NMR approaches, and the recently developed paramagnetic NMR methods. Computational tools have been used to complement and visualize the experimental data.

Chapter 1 presents an overview of NMR techniques for studying protein-ligand interactions. Due to its superior sensitivity in detecting weak interactions, NMR is the most used technique for fragment-based drug screening and the power of NMR is not restricted to primary screening only.

The work presented in Chapter 2 gives detailed information on the characterization of fragment hits binding to FKBP12 using 1D-^{1}H NMR. Recent advances in paramagnetic NMR spectroscopy have evolved a wide range of applications in studying protein-protein complexes but few cases of protein-ligand complex studies have been reported. To look for suitable ligands used in a paramagnetic NMR study (Chapter 4), fifteen FKBP12 ligands with diverse binding affinities and chemical structures were selected for the pilot study. The suitabilities of the ligands were assessed based on their binding affinity, chemical structure, isomeric purity, and changes of chemical shifts upon binding to the target protein. Based on the characterization of the bound ligands, the general rules of an ideal ligand were established as: (1) the ligand must be in fast exchange on the NMR time scale; (2) must contain sufficient number of proton resonances that are well-separated in the 1D-^{1}H NMR spectrum; (3) must be isomerically pure; (4) must show clear and measurable chemical shift changes upon binding to the protein. The binding pose of the most suitable ligand was subsequently characterized in detail in Chapters 3 and 4.

Chapter 3 describes the determination of the binding pose of a small molecule bound to FKBP12 based on NOE restraints. Chemical shift perturbation (CSP) analysis indicated that a large region of the protein, spanning both binding sites 1 and 2 on FKBP12, was affected either directly or indirectly by ligand binding. The CSP map therefore presents a challenge to identify the actual binding site. Thus, intermolecular NOE restraints acquired from standard isotope filtered/edited NOESY experiments were used to determine the binding site and ligand binding mode. Although not all NOE restraints were satisfied in the final structure, due to dynamics in the complex, the results show a promising hydrogen bond network that has also been found in other FKBP12 ligands.

Chapter 4 presents an orthogonal NMR methodology to determine the structure of the above-mentioned complex based on paramagnetic NMR pseudocontact shifts (PCSs). The two-armed lanthanide binding tag, CLaNP5, was attached on three different double cysteine variants of FKBP12. The results indicate that it is possible to identify the ligand binding site and obtain a low resolution structure using bound ligand PCSs from simple 1D-^{1}H NMR spectra. Due to the rigid structure of CLaNP5, optimization of the tensor frame and the lanthanide position was not necessary but could improve the quality of the structure. Therefore, the methodology can be particularly valuable for studying proteins that are very large or difficult to be labeled isotopically.

The weak interaction and dynamic behavior of transient encounter complexes is challenging to study using existing experimental tools. The binding partners in such complexes present in nature, mostly being proteins, have low specificity and can interact with multiple partners in cascades. Chapter 5 describes the characterization of dynamics in artificial encounter complexes formed by plastocyanins and short charged peptides. Using CSP and PRE NMR spectroscopy, the dynamics in the encounter complexes were visualized and compared with the results from electrostatic Monte Carlo (MC) simulations. The highly similar CSP maps and the small shifts among different complexes strongly suggest a high degree of dynamics. In addition, the scattered PREs indicate the presence of multiple orientations. The overall results suggest that the complexes have multiple orientations and are dominated by electrostatics, and evidence for weak short-range interactions is provided by the PRE data.