# ABSTRACT

Molecular dynamics, manifested in backbone and side-chain motilities, plays a critical role in protein stability and function. Ligand binding, molecular recognition, enzyme catalysis, signal propagation and folding involve conformational dynamics covering a wide range of time scales and motional amplitudes. Solution NMR spectroscopy has a unique capacity to investigate dynamic properties of molecules over a range of different time scales with atomic resolution. Application of NMR methods to real protein systems has provided a compelling correlation between local motions and protein functions. This thesis focuses primarily on the characterization of dynamic features for several protein systems.

A $^{15}N$ backbone relaxation study of *Pseudomonas aeruginosa* azurin reveals motions in different time-scale regimes for several functionally important regions in the protein (*paper I*). In this work we try to understand the role of these motions for electron transfer (ET) processes. Presence of nanosecond mobility on the binding surface (called *hydrophobic patch*) of the protein together with a micro- to millisecond process around the Cu-site is consistent with the current view on intermolecular electron transfer (the *gated* ET paradigm). At the same time, the residues along the intramolecular electron transfer pathway show high rigidity that is remarkably conserved with increasing temperature.

NMR spectroscopy and computer simulations are used to examine the effects of binding of the extracellular ribonuclease barnase from *Bacillus amyloliquefaciens* with its natural inhibitor barstar (*paper II and III*). Although the spatial structure of free and bound barnase are very similar, subtle binding-induced variations in the structural ensemble of barnase result in changes of NMR chemical shifts and side-chains dynamics, as revealed by $^{2}H$ relaxation measurements. About one third of the affected residues are located in an extended β-sheet far from the binding interface, which forms an interface between *dynamic domains* in barnase. The observed changes in NMR parameters are rationalized in terms of changes in motions of the *dynamic domains*, underlining the role of such motions in propagation of conformational changes through the protein structure.

In the *paper IV*, we characterized the naturally unfolded cytoplasmic domain of the T cell receptor ζ subunit in the monomer and dimer forms using NMR diffusion measurements and analysis of chemical shifts.

*Paper V* presents the results of the backbone dynamics of the channel-forming peptide antibibiotic zervamicin IIB (Zrv-IIB). 'Model-free' analysis of relaxation data showed that the peptide is fairly rigid on a sub-nanosecond time-scale. The residues forming the polar side of Zrv-IIB helix are involved in micro-millisecond time-scale conformational exchange that might have a potential relevance for Zrv-IIB ion channel formation.

The results of these studies have provided a compelling correlation between local motions and function, confirming the essential role of internal motions for binding and signal transduction.



**Keywords:** NMR; relaxation; model-free analysis; electron transfer; signal propagation; binding; protein-protein interaction.

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