STUDY OF PROTEIN CONFORMATIONAL AND INTERACTION DYNAMICS BY MOLECULAR DYNAMICS SIMULATIONS AND NUCLEAR MAGNETIC RESONANCE EXPERIMENTS

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An Abstract of a Thesis Submitted to the Graduate

Faculty of Rensselaer Polytechnic Institute

in Partial Fulfillment of the

Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject: BIOLOGY

The original of the complete thesis is on file
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July 2009
(For Graduation August 2009)

ABSTRACT

The function of proteins is inherently linked to their atomic motions. Such fluctuations may occur at a range of timescales from femptoseconds to minutes and involve a variety of spatial components. In order to identify, study and eventually understand these processes we must implement a variety of techniques that report on the different timescales and atomistic details of relevance. Molecular Dynamics (MD) simulations of proteins and other biomolecular systems have been long proven to be a powerful methodology for the calculation of thermodynamic averages and the characterization of structural/functional relations. Such methods allow the study of protein dynamics occurring on a broad range of timescales and atomic resolution that may not be directly accessible by common experimental techniques, thus providing complementary information to experiments. In the present Thesis, we describe the application of all-atom MD simulations as well as combination of such simulation methods with NMR experiments, as a paradigm for the study of protein dynamics. We focus on the dynamics of protein-protein interactions, whether these take place within a single polypeptide chain or involve distinct protein domains. We examine three cases of systems with great interest from a biological and pharmacological perspective. The first is a transmembrane protein complex composed of the G-protein coupled receptor (GPCR) rhodopsin and its G-protein intracellular counterpart transducin. The structure of this very important signaling complex has not been experimentally determined in atomic resolution. Based on the analysis of our μsec-timescale simulation trajectory starting from a docked conformation of the complex, we report a highly dynamic interface that is alternating between distinct interdomain orientations. We propose the general structural features of the interface and relate our results with experimental measurements from electronic paramagnetic resonance (EPR) experiments and high resolution models of activated rhodopsin states. We further suggest novel mutagenesis experiments that can be used to investigate the stability and correlated atomic motions of this model membrane protein receptor system. The second part is a comparative study of two

intrinsically disordered peptides, Aβ(1 -40) and Aβ(1 -42), which are the main constituents of amyloid plaques found in the brain of patients with Alzheimer's disease (AD). An atomic-detail characterization of these peptides is crucial since it would provide the starting point for modeling oligomerization and fibril assembly, which are key events in the pathology of AD. We use enhanced-sampling simulations to describe the conformational ensembles adopted by these flexible peptides and relate our simulation results with experimental results from NMR. We find that the ensembles of the monomeric species are highly diverse, yet possess unique structural features that may affect the early stages of fibril assembly, and further provide a structural basis for the difference in the amyloidogenic profiles between the two peptides. The third study focuses on a fusion protein designed to study the structure, dynamics and thermodynamics of the interactions between ubiquitin and ubiquitin-interacting motifs (UIMs). UIMs are single helical domains that act as ubiquitin recognition modules for a variety of ubiquitin receptor proteins. We use standard NMR spectroscopy techniques to solve the solution structure of the complex and a variety of NMR relaxation methods to characterize its plasticity at a range of timescales from picoseconds to milliseconds. Our results indicate a highly dynamic interaction interface at the milliseconds timescale that has not been reported in previous solution studies. Dynamics at the ubiquitin-UIM interface may affect the way UIM recognizes different types of ubiquitin chains in the context of complex interaction patterns that are observed in the cell. In the future, MD simulations will be crucial in deciphering the atomic details of these processes thus providing a rationale for the design of protein domains with optimum interaction properties.