Research Proposal

Dr. Maruthi Krishna Mohan Poluri
Rutgers University, NJ

NMR Investigations on Structure, Stability and Folding of Core Histone Protein Complexes and their Interactions with Histone Chaperons and Histone-modifying enzymes

Origin of Proposal

Solution Nuclear Magnetic resonance (NMR) spectroscopy is a powerful technology for the study of molecular structure, dynamics and interactions at the atomic level. Recent methodological developments complemented with the isotope labeling techniques have greatly reduced the size limitations that have traditionally prohibited detailed NMR investigations of many biologically important molecules. Recent studies demonstrated that NMR can be successfully applied to large protein machineries (hundreds of kDa) to obtain high-resolution structural and dynamics data [1-3]. In this regard, I would like to work on “Histone proteins” that constitute the Nucleosomes/ Nucleosome Core Particles (NCP) (Figure 1) and their interactions with “Histone chaperons and histone-modifying enzymes. [4-6].
Histone proteins are among the most highly conserved proteins in eukaryotes, emphasizing their important role in the biology of the nucleus. Histones are structurally and functionally bipartite. These are highly alkaline proteins found in eukaryotic cell nuclei that package and order the DNA into structural units called nucleosomes. Two copies of each of the four histone proteins: H2A, H2B, H3, and H4 forms the fundamental unit called the “nucleosome core particle” (NCP), represents the basic repeating structure in chromatin, and play a chief role in gene regulation. The Histones H2A and H2B exist as hetero-dimer (Fig. 2A), whereas as the histones H3 and H4 exist as a hetero-tetramer. The structured histone-fold domains are responsible for DNA binding, whereas the flexible histone tails function as signaling and docking platforms for a diverse range of protein factors such as “histone-modifying enzymes” (ex, histone methyltransferases, histone acetyltransferases, histone deacetylases etc.) [6]. Although histones are highly conserved, specialized histones called the “histone variants” (for example H2A.Z-H2B hetero dimer, Fig. 2B) co-exist in a given species with the major histone types in the nucleus [5,7]. They have the potential to locally alter chromatin surface and hence change the equilibrium between different types of nucleosome–nucleosome interactions. Furthermore, Histones are closely escorted by “histone chaperones” from their point of synthesis up to their delivery site [8,9] (Fig. 2C).

**Objectives**

I will address central questions regarding the fundamental mechanisms underpinning histone functionality and complex macromolecular formation using the histones H2A-H2B dimer, H3-H4 dimer and tetramer at residue level. More specifically, I will determine the structural stability, folding behavior and intrinsic dynamic properties of the H2A-H2B hetero-dimers (Fig. 2A), and H3-H4 hetero dimers and hetero tetramers. Further to envisage the regulatory role of the nucleosome core particle, structural and dynamics studies will be performed with the histone complexes using different histone variants. As NMR is best suited for the study of the site-specific motional properties of these biological systems [1] that are critical to their function and for the characterization of low-affinity interactions that often cannot be probed using other structural biophysical
methods, such as X-ray diffraction or electron microscopy, I intend to study the association and dissociation mechanisms of the core histone complexes with their respective histone chaperons that aid in the formation, storage and transport of the histone dimers and tetramer complexes (Fig. 2C) [10] and also with the histone-modifying enzymes such as histone acetyltransferases, methyltransferases etc [11] as described above.

Methodology

An integrated biophysical approach will be employed using multi dimensional solution state protein Nuclear Magnetic Resonance (NMR) as a major technique to obtain mechanistic insights into fundamental biological processes. Residue level assignments of the proteins will be achieved using the solution state protein triple resonance experiments. The use of chemical shift perturbations, hydrogen exchange studies and
relaxation experiments will be performed to enable the structural, energetics and dynamic information of various histone complexes at different states of the folding energy landscape and during their interaction with different macromolecular complexes (Histone-Histone and Histone-DNA). In order to characterize the transiently populated conformational states that may be intimately related to the proper function of the histones paramagnetic relaxation enhancement (PRE), relaxation dispersion and temperature dependent analysis of amide proton chemical shifts will be employed \[3,12\]. Optical spectroscopy and isothermal calorimetric techniques will be used to obtain the global stability features and energetic parameters of the histone complexes. Molecular modeling and protein/protein-DNA docking studies will be performed to complement the structural and functional data obtained using the biophysical techniques. Biochemical and molecular biology techniques such as protein expression, purification, recombinant DNA technology methods will be used rigorously to in order to produce different histone complexes with altered chain lengths and stabilities and modifications.

**Significance of Work**

Although folding energy landscapes and stability features of small globular monomeric proteins are well understood, still the principles that dictate the folding of the complex homo and hetero protein complexes are poorly understood. The present structural and folding study of histone complexes attempt to understand such a complex scenario as none of the monomeric proteins can fold independently. The structural and dynamic information provided with the histones and their interacting chaperons threw invaluable perspectives and mechanistic insights into the complex macromolecular energy landscapes. Furthermore as the histones are the basic building blocks of the nucleosome core particle, the fundamental units of the chromatin, the structural and dynamic information of these proteins are crucial to understand the basic processes such as gene regulation, DNA replication, DNA repair and transcription. Moreover as these histones undergo post-translational modifications such as methylation, acetylation, phosphorylation etc, they interact with several classes of enzymes which are the basic drug targets to control the gene regulation, transcriptional repression etc \[11\]. Hence forth these structural and function studies have utmost biophysical and biological significance.
**Long term Prospective**

These aims are intimately related to my long-term research objectives that are primarily focused to ultimately: (i) determining the role of the H2A-H2B dimer and H3-H4 tetramer in the formation of nucleosome core particle (NCP) along with the post-translational modifications; (ii) determining the interactions of histones and its variants with the DNA segments; (iii) determining the structural basis for the assembly and interaction of core histones with different classes of histone chaperons and histone modifying enzymes; (iv) integrating structural, dynamic and thermodynamic information to understand the mechanisms of action of large protein machineries; (v) establishing NMR as a routine tool for the characterization of supra-molecular biological systems.

**References**


