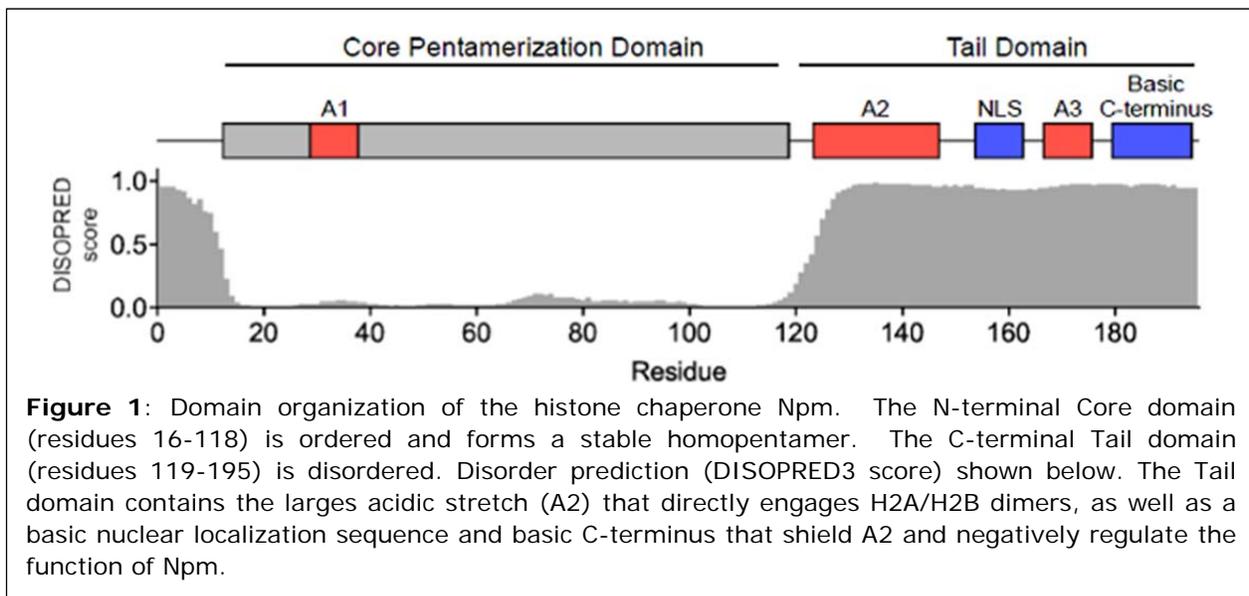


Structural Mechanisms of Histone Recognition by Histone Chaperones

Chromatin is the complex of DNA and proteins that comprises the physiological form of the genome. Non-covalent interactions between DNA and histone proteins are necessary to compact large eukaryotic genomes into relatively small cell nuclei. The nucleosome is the fundamental repeating unit of chromatin, and is composed of 147bp of DNA wrapped around an octamer of histone proteins: 2 copies of each H2A, H2B, H3 and H4.

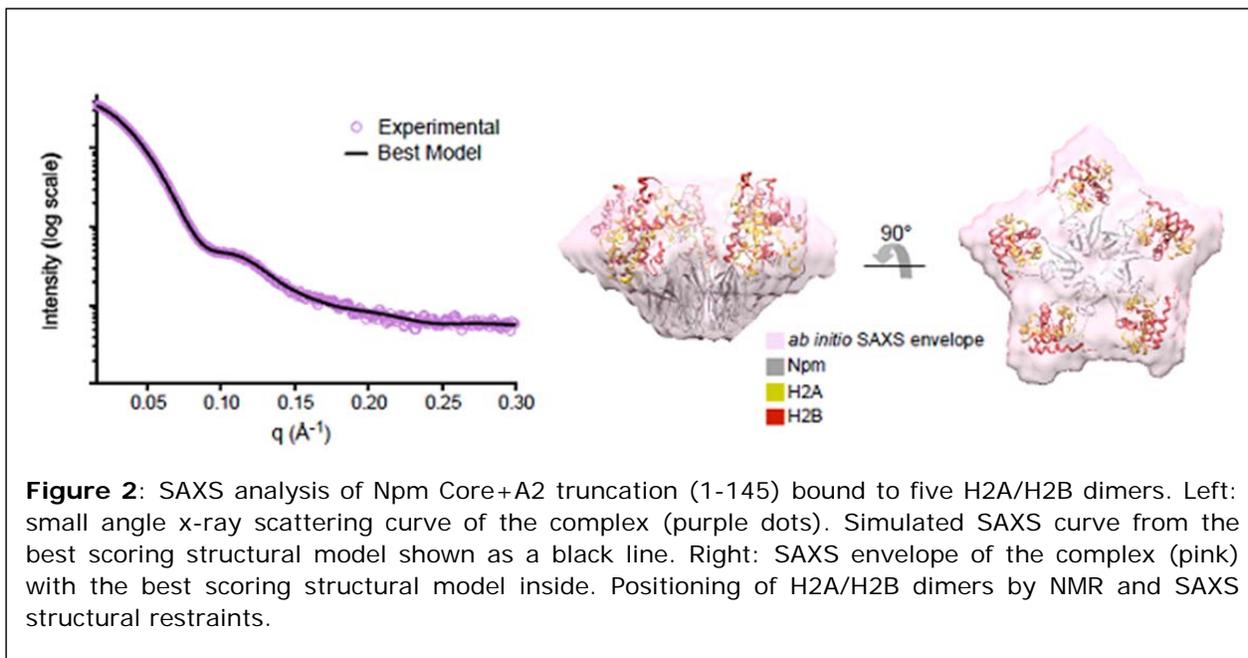
Assembly of nucleosomes in the cell requires the coordinated effort of many proteins, including ATP-dependent chromatin remodeling enzymes and ATP-independent histone chaperone proteins. Histone chaperones are a large class of proteins responsible for binding the highly basic histone proteins, shielding them from non-specific interactions, facilitating nuclear import of histones, and finally depositing histones onto DNA to form nucleosomes. Despite performing many overlapping functions, histone chaperone proteins are highly structurally divergent. However, nearly all histone chaperones contain highly charged intrinsically disordered regions (IDRs)¹. In many cases truncation of these conserved regions results in loss of histone affinity and deposition functions.

Nucleoplasmin (Npm) is a highly conserved H2A/H2B-specific histone chaperone expressed during the early stages of vertebrate development. Npm is tasked with storing large amounts of H2A/H2B dimers in the oocyte (an immature cell in the ovary), and releasing them upon fertilization to keep up with the rapidly dividing embryo. Structurally, Npm contains a pentameric N-terminal Core domain and an intrinsically disordered C-terminal Tail domain composed of alternating acidic and basic stretches (Figure 1). Previous mass-spectrometry analysis showed that Npm is heavily modified mainly along the Tail domain during development, and that these modifications alter its histone affinity and deposition rates². Complete removal of this IDR indicated that it is necessary for histone binding, however smaller truncations of the C-terminus resulted in increased histone affinity and deposition rates. This suggested that this region acts as both a major site of histone interaction and a regulator of Npm function.



A study led by Christopher Warren and Dr. David Shechter at the Albert Einstein College of Medicine utilized NMR and biochemical assays to show that the intrinsically disordered Npm Tail domain negatively regulates histone binding by dynamic intramolecular shielding of a key acidic stretch (A2)³. Paramagnetic Relaxation Enhancement NMR (PRE-NMR) was used to gain structural information on the monomeric Tail domain alone and in complex with H2A/H2B. A stable pentameric complex of Npm bound to five H2A/H2B dimers was able to be formed by removal of this C-terminal autoregulatory region, though this complex was far too large for NMR structural analysis and resisted all crystallization attempts.

In a collaboration with Dr. Tsutomu Matsui, SSRL, size-exclusion chromatography coupled SAXS (SEC-SAXS) data were measured at BL4-2 for this complex (Figure 2). The SEC-SAXS data confirmed that the pentameric complex was stable and without significant flexibility. The oblate, star shaped SAXS envelope calculated from these data indicated that the H2A/H2B dimers rest on the upper portion of the lateral face of the Npm pentamer. Using the relative positioning of the Tail domain and H2A/H2B obtained by PRE-NMR, Dr. Matsui was able to build novel models of the pentameric complex that satisfied structural restraints from both NMR and SAXS data. These NMR-restrained SAXS hybrid models provide the highest resolution insight to date on the architecture of a complex that is critical setting up the chromatin landscape in the earliest stages of embryonic development. These models also help to partially explain the vital role that Npm plays in histone storage and deposition processes during vertebrate development. Future collaborative studies will target the structural basis for understanding the roles of many histone chaperones in both normal and cancerous cells.



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