MS12-P9 An X-ray Crystallographic Approach to Obtain an Atomic Model of a High Mobility Group N Protein-Nucleosome Assembly

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Nucleosomes, comprising ~146 bp of DNA wrapped around a histone octamer along with adjoining linker DNA, form the basic repeating units of chromatin. Chromatin architectural proteins are nuclear factors that modulate genomic activities by modifying chromatin structure. Linker histone proteins are one type of chromatin architectural factor that interact with nucleosomes to foster compact states of chromatin, such as heterochromatin, which is generally associated with silenced genes. The ubiquitous and abundant High Mobility Group N (HMGN) proteins comprise a distinct family of chromatin architectural proteins that compete with linker histones to induce gene accessibility and maintain active states of chromatin. Unlike many transcription factors that recognize specific DNA sequences, HMGN proteins bind specifically to nucleosomes with high affinity irrespective of the underlying DNA sequence. This property has been attributed mainly to a highly conserved cluster of arginine residues, which bind to the acidic patch regions on the nucleosome. Moreover, under physiological ionic strength conditions, two molecules of HMGN bind co-operatively to a single nucleosome, and mixed pairs of variants will not associate with a given nucleosome. However, the mechanistic details of HMGN activity remain unclear. Here, we aim to obtain the first atomic model for an HMGN protein-nucleosome complex to shed light on the exact binding mode of HMGN proteins, their co-operative and homo-pairing association properties and potential structural alterations induced in the nucleosome.

HMGN proteins are unstructured prior to binding nucleosomes, but we have successfully overexpressed and purified the *Homo sapiens* HMGN1, HMGN2, HMGN3a, HMGN3b, HMGN4 proteins using a recombinant bacterial expression system. Biochemical assays have confirmed that all proteins are active in co-operatively binding to nucleosomes, with a variety of different DNA constructs tested. We have recently obtained crystals of an HMGN1-nucleosome complex that show diffraction beyond 4 Å, and we are currently optimizing growth, harvest and stabilization conditions to improve diffraction towards atomic model building resolution.

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MS12-P10 Characterization of RAGE-ligand interactions

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The Receptor for Advanced Glycation End products (RAGE) is a pattern recognition receptor and key in the innate immune response. It binds diverse ligands and initiates a downstream pro-inflammatory signaling cascade. Hyper-activation of RAGE has been linked to chronic inflammatory disorders, diabetic complications, tumor outgrowth, and neurodegenerative disorders. We are studying the structure of this unique receptor and of several of its ligands in order to understand the molecular basis for ligand recognition. Key ligands of RAGE are Danger-Associated Molecular Pattern molecules (DAMPs) like **HMGB1** and several members of the so-called S100 protein family, like **S100A9, S100B** and **S100A8/A9**. Depending on the nature of the ligand, its concentration and its affinity to the receptor, the cellular response evoked upon RAGE-ligand interaction is different. In order to characterize the RAGE-ligand interactions in more detail we analyzed their binding properties in vitro by microscale thermophoresis, surface plasmon resonance and isothermal calorimetry. Moreover, we elucidated the structural properties of S100A9, revealing that the metal ions Ca^{2+}- and Zn^{2+} induce major conformational changes exposing hydrophobic residues to the solvent, which are required for high-affinity binding to RAGE. Furthermore, we aim to develop strategies to interfere with RAGE activation. We study the binding of small molecules to S100A9 that are able to block S100A9-RAGE interaction. These data might guide the development of new strategies in the treatment of several disorders mentioned above.

Keywords: Receptor for advanced glycation end products, S100 Proteins, HMGB1