MS7-P5 Application structures of nucleotide-protein complexes to study RNA recognition by bacterial and archaeal Lsm proteins

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Sm-like proteins (Lsm) exist in all three domains of life and are defined by the so-called Sm fold, which is comprised of an N-terminal α-helix and five anti-parallel β-strands. Bacterial Lsm proteins Hfq is a post-translational regulator of gene expression that binds small non-coding RNAs (sRNA) and promotes their interaction with mRNAs. Eukaryotic Lsm proteins act as chaperone for mRNAs and non-coding RNAs through various steps in metabolism. Function of the archaeal Lsm proteins have studied poor but it is known they bound archaeal sRNA.

Structures of Lsm proteins in complexes with short oligo-RNAs promote understanding involvement of the proteins in the RNA metabolism greatly. Recently we have used complexes of proteins with single ribonucleotides to determine the RNA-binding sites on the protein surface [1]. The complexes can be obtained before crystallization or by soaking of the protein crystals in the ribonucleotides solution. Typically, preparation of nucleotide-protein crystals is easier that the crystallization of large RNA-protein complexes. Using this technique we have identified three different RNA-binding sites on the Hfq surface, one of them have been located for the first time. Now we are using this technique to study archaeal Lsm proteins from *Methanococcus jannaschii* and *Sulfolobus solfataricus* and our last results will be demonstrated. This method can be used to study any RNA-binding protein interacting with single-stranded RNA.

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[1] V. Murina, N. Lekontseva, A. Nikulin, Hfq binds ribonucleotides in three different RNA-binding sites, Acta Crystallogr, D69 (2013) 1504-1513.

Keywords: Lsm, Hfq, archaea, RNA-protein interaction, single-stranded RNA, ribonucleotide-protein complexes

MS7-P6 Structural biology of 5hmC-specific endonuclease PvuRts1I

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PvuRts1I is a prototype for a larger family of restriction endonucleases that cleave DNA containing 5-hydroxymethylcytosine (5hmC) or 5-glucosylhydroxymethylcytosine (5ghmC), but not 5-methylcytosine (5mC) or cytosine. Here, we report a crystal structure of the enzyme at 2.35 Å resolution. Although the protein has been crystallized in the absence of DNA, the structure is very informative. It shows that PvuRts1I consists of an N-terminal, atypical PD-(D/E)XK catalytic domain and a C-terminal SRA domain that might accommodate a flipped 5hmC or 5ghmC base. Changes to predicted catalytic residues of the PD-(D/E)XK domain or to the putative pocket for a flipped base abolish catalytic activity. Surprisingly, fluorescence changes indicative of base flipping are not observed when PvuRts1I is added to DNA substrates containing pyrrolocytosine in place of 5hmC (5ghmC). Despite this caveat, the structure suggests a model for PvuRts1I activity and presents opportunities for protein engineering to alter the enzyme properties for biotechnological applications.

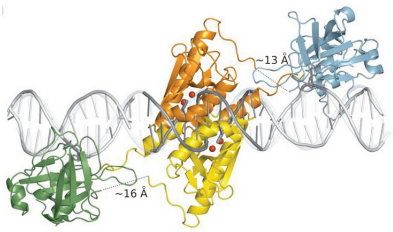

Figure 1. Model for PvuRts1I dimer binding to 5hmC containing DNA

Keywords: PvuRts1I, endonuclease, SRA, PD-(D/E)XK, 5-hydroxymethylcytosine