MS1-P11 **Structure of eukaryotic 2'-O-ribose methyltransferase in complex with mRNA cap analogue.**

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The 5' cap structure is characteristic for the eukaryotic mRNA. This modification is critical for mRNA stability and translational efficiency. It contains 7-methylguanosine (m^7G) linked by inverted 5'-5' triphosphate bridge to the rest of mRNA [1]. There are also additional methylations 2' oxygens of the riboses of the first two transcribed nucleotides - Cap01 and Cap02 structure [2]. Here we present the crystal structure of a methyltransferase which is responsible for the formation of Cap01 structure in eukaryotes.

The full-length protein was produced in E.coli expression system. Based on bioinformatics predictions and limited proteolysis assays a stable and soluble truncation variant of the protein was identified, overexpressed and purified. Co-crystallization trials with m^7GpppG and S-adenosylomethionine (SAM) as the methyl group donor were performed and yielded crystals that diffracted X-rays up to 1,95 Å. The structure of the protein together with m^7GpppG and SAM was solved using anomalous signal for selenomethionine derivative. In comparison to previously published structure of viral orthologs of 2'-O-ribose methyltransferases [3] interesting differences in the cap binding pocket were observed. To verify the structure, we substituted with alanines the residues potentially responsible for cap structure binding. The proteins containing the substitutions were tested in methylation activity assays. Significant differences in activity were observed and they were dependent on localization of amino acids responsible for interactions with particular atoms of mRNA 5′ cap analogue.

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MS1-P12 **Structural and Functional Studies of FNE, a Bacterial Adhesion Protein of Streptococcus Equi: FNE**

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Streptococcus equi is a gram-positive bacteria responsible for diseases of the upper respiratory tract in horses that can be fatal such as strangles. Exceptionally, this bacteria is responsible for meningitis in humans. FNE is a protein involved in adhesion and virulence of *Streptococcus equi* by interacting with fibronectin in the extracellular matrix of the cell. Fibronectin is a long-glycoprotein (250 kDa) organized into functional domains that interact with different partners such as bacterial adhesion proteins. FNE Interacts with the Gelating Binding Domain of fibronectin (GBD), located near the N-terminus. We study the structural and functional aspects of this interaction as a model for bacterial adhesion to the GBD. We were unable to crystallize FNE or a truncated version lacking the disordered C-terminal peptide. We therefore developed artificial proteins that bind to FNE with the objective to create complexes of these proteins in complex with FNE and amenable to crystallization. We made use of a library coding for artificial protein constructed by repetition of a pattern designed HEAT from a thermophilic archaeal protein [1]. Three artificial proteins interacting with the FNE have been obtained by phage-display and the corresponding complexes with FNE were tested for crystallization. We will present this new innovative cristallogenesis technique and the structure of the complex FNE / artificial partner obtained at 1.83 Å of resolution.

[1] Urvoas, Guellouz et al ; J Mol Biol. 2010, 404b307-27

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