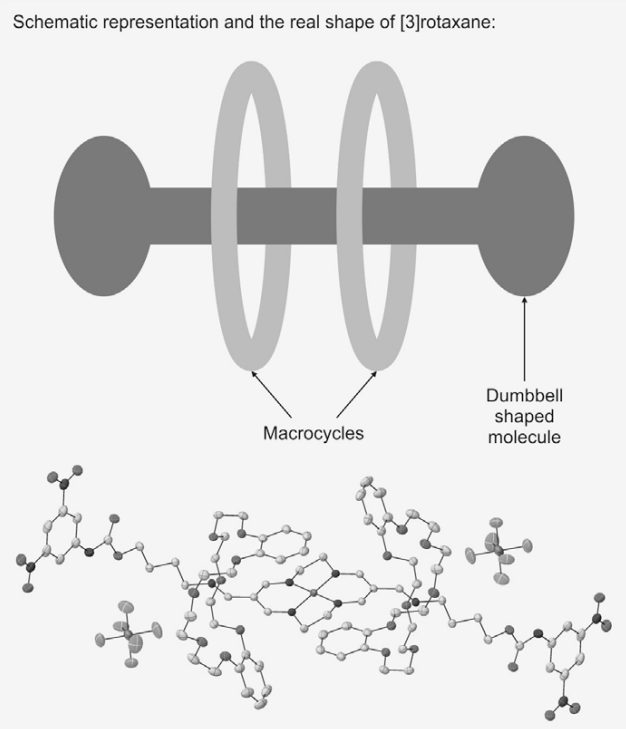


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**Keywords: [3]rotaxane, polyrotaxanes, mechanically interlocked molecular compounds**

# MS06-P02

## Structural basis of ASPL-mediated regulation of p97 methylation by METTL21D

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The ATPase p97/VCP (valosin containing protein) belongs to the family of AAA+ proteins (ATPases associated with diverse cellular activities). This essential and conserved protein family is involved in a broad range of cellular processes, such as DNA repair, cell cycle regulation, transcriptional activation, recombination, organelle biogenesis, ubiquitin-mediated protein degradation and homotypic membrane fusion (1). The function of p97 is regulated by a number of adaptor proteins and post-translational modifications (2). One example is the adaptor protein ASPL (alveolar soft-part locus), that has been investigated in further detail in our group, biochemically and structurally, showing that ASPL regulates p97 activity by reassembling it from a hexamer into a heterotetrameric complex containing two monomers of p97 and two molecules of ASPL (3).

The METTL21 family of methyltransferases is a novel class of methyltransferases dedicated to methylation of chaperones. METTL21D, also known as VCP-lysine methyltransferase (VCP-KMT), trimethylates p97 specifically at Lys315 (4). Overexpression of METTL21D has been shown to be present in number of human tumor tissues and able to increase the metastatic migration capability of several METTL21D knockout cancer cell lines (5). Trimethylated Lys315 is ubiquitously present in the intact hexameric form of endogenous p97 inside the pore, but it is inaccessible to METTL21D. ASPL promotes methylation (6) by disassembling the hexameric form of p97, thereby creating a new interaction interface for METTL21D.

Here we present the first crystal structure of a methyltransferase from the METLL21 family, METTL21D, bound to its target chaperone p97 in presence of its remodeling adaptor protein ASPL, adenosine-diphosphate (ADP) and the cofactor S-adenosyl-L-methionine (SAM). The structure reveals that METTL21D binds to the highly conserved second region of homology (SRH) motif of monomeric p97, in close proximity of the target site Lys315. The SRH motif serves as a recognition sequence for METTL21D, but the interaction extends to a larger surface of the D1 domain of p97. The structure shows the importance of p97 remodeling by ASPL and potentially other remodeling adaptor proteins to enable modification of inaccessible residues and to create new interaction interfaces.

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**Keywords: p97 regulation, reassembly, methylation**

## MS06-P03

# Structure-functional studies of protein P4 from bacteriophage φ8

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The dsRNA bacteriophages of *Cystoviridae* family infect gram-negative *Pseudomonas* species bacteria [1]. The bacteriophages package their genome into empty capsid – procapsid, which protects the genome from degradation inside as well as outside host cell. The genome packaging is performed by a molecular motor - P4 proteins, which are components of procapsid [1, 2]. The P4s possess an NTPase activity that converts the chemical energy from ATP hydrolysis to a mechanical movement of packaging ssRNA precursors into a procapsid, where the replication and transcription of dsRNA occurs [1, 3]. The P4s are RNA helicases belonging to the Superfamily 4 of helicases with characteristic presence of conserved sequence motifs (H1, H1a, H2, H3 and H4) [2, 3]. The RNA helicases cause the distribution of RNA-protein complexes and carry out RNA unwinding [2]. The P4 assembles into hexameric ring (Fig.1), which has on the outer perimeter NTP-binding sites and the nucleic acid binding sites are located in the central channel. Each P4 monomer consist of N-terminal, core NTPase domain with sequence motif and C-terminal domain. The C-terminal domain is inserted into the central channel of hexamer and its conformational changes regulate ring stability and ATPase activity of P4s [3]. Here we report our crystallization experiment results of the φ8 P4 protein crystals.

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