MS1-P3 **Structure-function studies of proteins operating on nucleic acids.** Jan Dohnalek,[ab] Tomas Koval,[b] Jarmila Duskova,[a] Tereza Skalova,[a] Jan Stransky,[a] Libor Krasny,[c] Jana Korelusova,[c] Petra Lipovova,[d] Tomas Podzimek,[d] Jaroslav Matousek,[e] Jindrich Hasek[a] *[a]Institute of Macromolecular Chemistry AS CR, Heyrovskeho nam. 2, 16206 Prague 6, Czech Republic, [b]Institute of Physics AS CR, Cukrovarnicka 10, 16200 Prague 6, Czech Republic, [c]Institute of Microbiology AS CR, Videnska 1083, 14200 Prague 4, Czech Republic, [d]Institute of Chemical Technology, Technicka 5, 16628 Prague 6, Czech Republic, [e]Institute of Plant Molecular Biology, Biology Centre AS CR, Branisovska 31, 37005 Ceske Budejovice, Czech Republic*
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Two groups of proteins with functions related to nucleic acids are studied in our laboratory: plant nucleases and subunits belonging to *Bacillus subtilis* RNA polymerase. Both represent uneasy targets of structure-function studies and require specific approaches to achieve reliable results. Tomato nuclease TBN1 expressed recombinantly in tobacco leaves is a 37 kDa glycoprotein capable of hydrolysis of both ds and ss RNA and DNA substrates, and with confirmed tumor growth suppression *in vivo* [1]. To determine its x-ray structure hundreds of badly diffracting crystals were screened, and initial phases were determined for the *H*3 form, using a combination of MAD and MR. Deglycosylation, post-crystallization treatment, lysine methylation and other approaches were employed without much effect on data quality [2]. The quality of the *H*3 data disabled further structure refinement and so data from later grown $P3_121$ crystals were used to adopt the initial raw model for further refinement to arrive at the final structure at 2.16 Å resolution. The structure of plant nuclease type I from tomato brings very important answers regarding its stability, glycosylation, oligomerization and surface properties. At the same time it raises new questions as to the specificity of the enzyme towards structured nucleic acids.

Proteins and complexes of *B. subtilis* RNA polymerase require careful selection of purification protocols to identify and separate the desired subunits or various sub-complexes. A combination of size exclusion chromatography, ion exchange, dynamic light scattering, and use of solubilization agents leads to defined soluble sub-complexes of RNAp for further structural studies. Native RNAp from *B. subtilis* undergoes relatively fast degradation and aggregation in solution at 4 °C and so for structural studies reproducible purification approaches for small protein amounts are being developed.

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MS1-P4 **The high resolution structure of the assembly domains of the S-layer protein SbsC.** [1]Anđela Đordić, [1]Tea Pavkov-Keller, [2]Eva Maria Egelseer, [2]Uwe B. Sleytr, [1]Walter Keller *[1]Institute of Molecular Biosciences, K.F. University Graz, Austria*, *[2]Center for Nanobiotechnology, University of Natural Resources and Applied Life Sciences, Vienna, Austria*
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Monomolecular paracrystalline surface layers (S-layers) are composed of a single (glyco)protein and are the most commonly observed cell surface structures of bacteria and archaea. Because of their diverse properties S-layers have various potential applications in nanobiotechnology [1]. However, detailed structural information on S-layer proteins is very scarce. In order to determine the structure-function relationship of SbsC, the S-layer protein from *Geobacillus stearothermophilus*, deletion mutants were produced. It was shown that the N-terminal part is responsible for binding to the secondary cell wall polymer (SCWP) and that the C-terminal part is essential for self-assembly [2]. Recently, the crystal structure of the C-terminally truncated form $rSbsC_{(31-443)}$ was solved at 2.4 Å [3]. We continued the work with different N-terminal truncations. Biophysical characterization of all constructs was performed and crystals of 3 different truncated forms were obtained. The high resolution structures of all domains involved in self-assembly could be determined. Combined with electron microscopy data obtained from 2D crystals of the full length SbsC we propose the model for self-assembly.

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