Session 20: New trends in structural biology

Lectures

L.20.1

Molecular mechanisms of bacterial DNA repair

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DNA is constantly undergoing chemical modifications, also called damage, that distort the genetic information. All living organisms have mechanisms to repair this damage. One major pathway of DNA repair is nucleotide excision repair (NER), which removes DNA helix-distorting lesions. Another is homologous recombination (HR), which repairs double-stranded DNA breaks. In this talk I will discuss structural and mechanistic studies of these two pathways in bacteria. I will show how UvrA, a DNA damage sensor in bacterial NER, finds the DNA lesion [1] and how the UvrB protein verifies its presence [2]. I will also describe the structure and mechanism of the RecF-RecO-RecR complex of bacterial HR [3]. Its role is to form a filament of RecA protein on single-stranded DNA. This nucleoprotein filament searches for a homologous DNA sequence to promote repair. Once this sequence is found, DNA strands are exchanged, leading to the formation of four-way DNA structures called Holliday junctions. I will also discuss the structure and mechanism of RuvC, a nculease that removes Holliday junctions to complete repair [4,5].

References

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L.20.2

Cryo-EM Facility at SOLARIS

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Cryo-EM Facility located in SOLARIS National Synchrotron Radiation Centre exists since 2019. In its four years history, starting from the stage of a drawing, the Facility has become a fully operational centre for cryo-electron microscopy, working with researchers from Poland and abroad. It offers two separate pathways of access to high-end microscopes - Glacios and Titan Krios G3i. The Facility allows to freeze and screen grids utilizing the Glacios microscope, thus offering a complete workflow for sample preparation and pre-characterization, required for the high-resolution data collection on a Krios microscope.
I will briefly present available equipment and techniques, with highlights from already published works. Moreover, I will show how the Facility operates, data collection routines, data flow and most importantly – how to access the microscopes.

L.20.3

Role of integrative structural biology in understanding the structure and dynamics of *S*-adenosyl-L-homocysteine hydrolase

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Methylation reactions are fundamental and ubiquitous in all living cells. *S*-adenosyl-L-methionine (SAM) is the most common methyl group donor. SAM-dependent methylation generates *S*-adenosyl-L-homocysteine (SAH), a potent inhibitor of SAM-dependent methyltransferases, and its intracellular accumulation suppresses the SAM-dependent processes. Therefore, SAH concentration must be controlled in the cell by *S*-adenosyl-L-homocysteine hydrolase (SAHase). SAHases form a homotetramer, with each subunit folded into three domains. Two principal domains (substrate- and cofactor-binding) are connected by a hinge element. During the hydrolytic cycle, subunits oscillate between two conformational states: closed (substrate-bound) and open (with a product released). However, the role of regions of the substrate-binding pocket in regulating SAHase dynamics has yet to be fully explained. Moreover, a mode of conformational changes of subunits within the tetramer during the catalytic cycle has been elusive. Thus, to understand in a more detailed manner the structure and dynamics of SAHases, we performed integrative structural biology studies, including, among others, crystallographic and Cryo-EM single-particle analyses of a bacterial SAHase. Our results reveal the high complexity of conformational changes of the enzyme during the catalytic cycle and the crucial role of protein dynamics in SAHase activity.

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L.20.4

Structural studies of the histidine biosynthetic pathway in plants toward the discovery of novel herbicides

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The cultivation of plant species important to agriculture depends on protection from weeds, usually attained by using herbicides. However, widespread herbicide usage has led to herbicide resistance in many weed species. Hence, we desperately need new herbicides to secure efficient agriculture and sustainably feed nearly eight billion people. The inhibition of the histidine biosynthetic pathway (HBP) in plants is one of the promising solutions because the HBP (i) is absent in animals, (ii) loss-of-function mutants are lethal, and (iii) single genes encode most HBP enzymes. The HBP is also integrated with other metabolic pathways, e.g., biosynthesis of nucleotides and folates.

The plant HBP consists of eleven reactions catalyzed by eight enzymes named consecutively HISN1-8 that localize to the chloroplast stroma. We have succeeded at solving the crystal and cryo-EM structures of HISN1, 2, 3, 5, 6, 7, and 8. We have also characterized the enzymes using biochemical, biophysical, and bioinformatic methods as well as studied the enzymes' evolution and conservation across species. The obtained data now enable structure-based drug design methods to rationally develop small molecules that would inhibit the HBP and could potentially serve as novel herbicides.

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Posters

P.20.1

Structural study of methylenetetrahydrofolate reductase from *Pseudomonas aeruginosa*

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Methylenetetrahydrofolate reductase (metF) from *Pseudomonas aeruginosa* is an important enzyme that regulates the *S*-adenosyl-□-methionine (SAM)-dependent methylation reactions crucial for bacterial metabolism. MetF is encoded in the same operon as the other proteins essential for the virulence of the bacterium: (i) *S*-adenosyl-□-homocysteine hydrolase (SAHase), which regulates SAM-dependent methylation reactions through the reversible decomposition of *S*-adenosyl-□-homocysteine (SAH) to □-homocysteine (Hcy) and adenosine, (ii) alarmone hydrolase, which decomposes (p)ppGpp/(p)ppApp compounds and (iii) a glycosyltransferase, which are necessary for biofilm formation. MetF catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is necessary to convert □-Hcy to □-methionine. Within this study, we propose that inhibition of MetF would disrupt cellular methylation reactions by preventing the synthesis of SAH by SAHase. Consequently, the inactivation of metF could be a valuable strategy for fighting infections caused by *P. aeruginosa*. For this purpose, we performed structural studies of the metF enzyme. The protein was expressed in *Escherichia coli*, purified as a tetramer and crystallized. Crystals diffracted X-rays up to 1.95 Å. The crystal structure was solved by molecular replacement. Within the tetramer, each subunit contains a bound FAD cofactor. Currently, the structure is under isotropic stereochemically restrained structure-factor refinement.