# Friday, March 24, Session III

## L10

# MECHANISM OF RuvAB HOLLIDAY JUNCTION BRANCH MIGRATION

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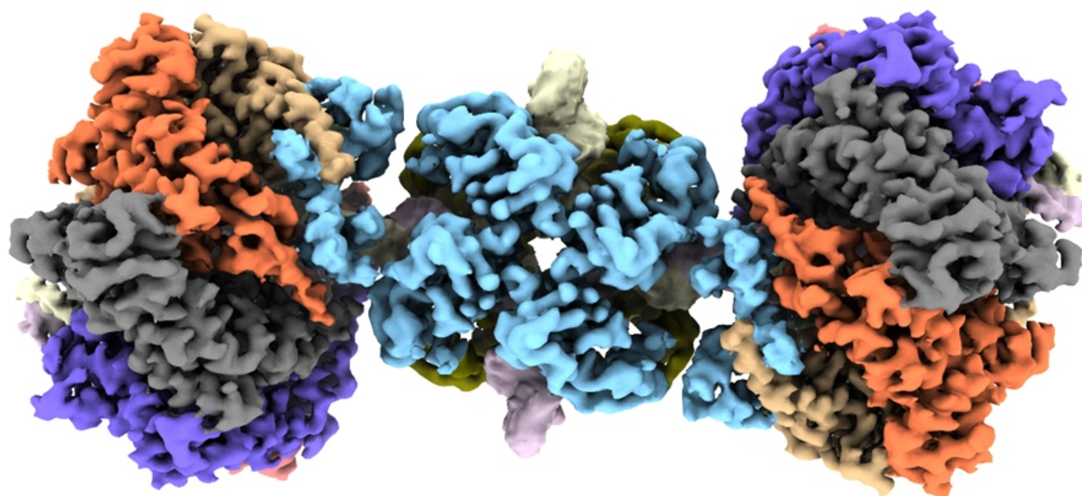
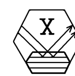
The Holliday junction (HJ) is a key intermediate structure formed during DNA recombination across all kingdoms of life. In bacteria, the HJ is processed by two homo-hexameric RuvB motors, which belong to the AAA+-ATPase family and assemble together with the RuvA-HJ complex to energize the strand exchange reaction. Despite its importance for chromosome maintenance, the structure and the mechanism by which this complex facilitates branch migration are unknown. Here, using time-resolved cryogenic electron microscopy (cryoEM), we obtained structures of the ATP-hydrolysing RuvAB complex in seven distinct conformational states at 2.9-3.4 Å resolution, captured during assembly and processing of a HJ. Five structures together resolve the complete nucleotide cycle of the RuvB motor and reveal the spatiotemporal relationship between ATP hydrolysis, nucleotide exchange and conformational changes in RuvB. We show how coordinated motions in a *converter* module, formed by DNA-disengaged RuvB subunits, stimulate ATP hydrolysis and nucleotide exchange. Immobilization of this module enables RuvB hexamers to convert the ATP-contained energy into a lever motion, which generates the pulling force driving the strand exchange reaction. We show structurally that the nucleotide cycle progresses around the ring, that RuvB motors rotate together with the DNA substrate and that the integration of both processes forms the mechanistic basis for DNA recombination by continuous branch migration. Taken together, our data decipher the molecular principles of homologous recombination by the RuvAB-HJ machinery, outline how hexameric AAA+ motors can generate mechanical force and provide a blueprint for the design of state-specific compounds targeting AAA+ motors.





**L11**

## CARPE PILI! HUNTING STRATEGY OF PHAGE JBD30 REVEALED BY COMBINATION OF CRYO-ELECTRON AND FLUORESCENT MICROSCOPY

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Increasing numbers of infections caused by multi-drug resistant bacteria have renewed the interest in phage therapy. However, the exact mechanisms of phage – bacterium interaction are not known for the most of them. Here, we present the virion structure and infection strategy of *Siphoviridae* bacteriophage JBD30 revealed by the combination of cryo-electron and fluorescent microscopy.

Bacteriophage JBD30 uses its tail fibres for recognising *P. aeruginosa* pili type IV. After the attachment to pili, the virion either diffuses along it or is pulled by pili retraction towards the cellular surface, where it irreversibly binds by the tripod complex of receptor binding protein trimers. Afterwards, the phage punctures the outer cellular membrane and degrades the peptidoglycan layer using the enzymatically active domains of receptor binding protein and tape measure protein C-terminal trimeric -helical coiled coil domain.

Bioinformatic analysis of tape measure protein showed that its N-terminal part is composed of three domains: hydrophobic transmembrane domain I (residues 57–79), cytoplasmic domain (residues 80–384) and hydrophobic transmembrane domain II (residues 385–409). Furthermore, the estimated size of these domains corresponds to the thickness of *P. aeruginosa* cellular membranes. We assume that the N-terminal part of the tape measure protein might form a channel spanning the whole cell wall facilitating DNA transition from the virion capsid into the host cytoplasm. New phage progeny is released approximately 80 minutes post ejection of phage DNA into the host cell.

The combination of cryo-electron microscopy analysis techniques, cryo-electron tomography and fluorescent microscopy allowed us to propose the mechanism of key stages of phage infection and describe it at time resolved molecular level.

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**L12**

## CRYO-EM STRUCTURE OF ASK1 SUGGESTS A ROLE OF INTER-DOMAIN INTERACTIONS AND TRX1 IN ITS REGULATION

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ASK1 (apoptosis signal-regulating kinase 1) is a member of MAP3K protein family, which directs cells towards inflammation or apoptosis through p38 or JNK signalling pathway [1,2]. Given its involvement in several serious diseases, ASK1 has been considered a promising target for therapeutic intervention [3]. Unfortunately, lack of relevant structural data and insufficient understanding of the intricate regulation of the kinase both hamper the search for effective drugs.

In this study, we employed several methods to expand our understanding of ASK1 regulation and the role of thioredoxin 1 in its inhibition. Firstly, AUC showed that C-terminally truncated ASK1 tends to dimerize in solution and thioredoxin 1 appears to further promote this tendency. HDX-MS then allowed us to map the contacts between individual domains within a protomer as well as the dimerization interface. Also, it allowed us to identify the regions affected by thioredoxin 1 binding. Finally, single-particle cryo-EM analysis enabled us to determine the first structure of the C-terminally truncated ASK1 in its dimeric form.

Collectively, our results provide the first structural insight into dimeric ASK1 and suggest how binding partners might affect its activity. Presented findings may serve as a basis for the design of ASK1-specific inhibitors and represent a starting point for further experiments aiming at even fuller understanding of ASK1 regulation and functioning.

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**L13**

## COMPLEX ALLOSTERIC REGULATION OF MYCOBACTERIAL INOSINE-5'-MONOPHOSPHATE DEHYDROGENASE BY PURINE NUCLEOTIDES

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Inosine-5'-monophosphate dehydrogenase (IMPDH) is a crucial purine metabolism enzyme that is well established as a potential drug target against mycobacterial infections. However, most previous biochemical and structural studies were performed with IMPDH lacking its regulatory CBS domain, which binds allosteric regulators influencing the activity of IMPDH. This project aims to describe the allosteric regulation of full-length IMPDH and its underlying molecular mechanism. First, we isolated full-length and CBS variants of IMPDH from *Mycobacterium smegmatis* and performed a detailed in vitro biochemical characterisation. Testing the impact of selected purine nucleotides on IMPDH activity indicated an unexpected regulatory effect of nucleotide ligands at biologically relevant concentrations. Next, to overcome problems with the X-ray crystallography approach, we utilised single particle cryo-EM analysis and, up to now, successfully obtained a series of datasets of IMPDH in complex with its allosteric regulators. Preliminary data suggest structural changes in the active/inhibited forms of IMPDH, which are triggered by the mode of binding of nucleotide ligands to the CBS domain. This might enable us to unravel the mechanism of interdomain crosstalk that leads to changes in the catalytic core of the enzyme. Such a mechanistic insight could contribute to the design of novel antimycobacterial IMPDH-targeting drugs.

*The project National Institute of virology and bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) - Funded by the European Union - Next Generation EU*

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**L14**

## MECHANISM OF ANTIBODY-MEDIATED NEUTRALISATION OF THE TICK-BORNE ENCEPHALITIS VIRUS REVEALED BY CRYO-EM

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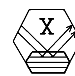
Tick-borne encephalitis (TBE), caused by the tick-borne encephalitis virus (TBEV), is a disease manifested by severe inflammation in the central nervous system, which may be fatal. Even though vaccination is available, only approximately 23% of Czech population has received it [1]. No targeted treatment is presently available for TBE, yet with cases continually rising the urge for one is ever-growing. This is where antibodies could be of use – it has previously been shown in a mouse model that an intravenous admission of TBEV-specific antibodies could serve as a treatment [2]. However, insight into the molecular mechanisms of TBEV neutralisation is limited.

Therefore, we studied the interactions between the TBEV strain Neudörfl and two neutralising mouse monoclonal antibodies, IC3 and A4, each binding to a different domain of the TBEV envelope protein [3]. TBEV was puri-



fied from infected tissue culture cells, mixed with Fab fragments prepared from the neutralising antibodies, and vitrified on grids for cryo-electron microscopy. Structures of the TBEV–Fab complexes were then solved using single particle analysis from the collected micrographs.

Deciphering the molecular basis of TBEV neutralisation by antibodies might aid with understanding the importance of different epitopes on the viral surface, possibly enabling tailored design of therapeutic antibodies or more specific vaccines in the future.

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**L15**

# THE GLACIOS 2 IS A HIGH-RESOLUTION STRUCTURE DETERMINATION TOOL, OPTIMIZED FOR GENERATING SUB 3Å SINGLE PARTICLE RECONSTRUCTIONS WITH UNPARALLELED AUTOMATION AND EASE OF USE

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*Thermo Fisher Scientific*

Cryo-EM has revolutionized the field of structure biology due to its capabilities resolving the three-dimensional structure of proteins, protein complexes and other biological macromolecules at high or even atomic resolutions. Particularly, the Cryo-EM single particle analysis (SPA) method and Cryo-Electron Tomography (Cryo-ET), have emerged where SPA is well-suited approach for the determination of native protein function and the dynamics of complex biological systems. CryoET is a cryogenic imaging technique that provides 3D datasets of larger structures such as large protein complexes, organelles and even parts of the cell. There is also electron diffraction method, MicroED, that allows for 3D structure determination of small chemical compounds and biological macromolecules.

Improved Thermo Scientific™ Glacios 2 is a 200kV FEG Cryo-EM built for delivering high throughput and high-resolution structures of proteins. Glacios is equipped with Selectris Imaging filter and Falcon 4i direct electron detector for the highest image quality and built-in fringe free imaging and aberration free image shift for high throughput data collection. Importantly, the Glacios is designed with ease of use for new users who are not experts in electron microscopy. Furthermore, it is optimized for supporting data collection on multiple grids which further optimizes the productivity of microscope time. We will show that using this system we achieved 1.6Å resolution with Apoferritin sample within 7hr of data collection and a throughput of ~650 movies/hr. We will also show the use of Glacios for cryo-ET method allowing cellular structure biology at high resolutions.