Thursday, March 24, Session II

L6

BASEPLATE STRUCTURE OF BACTERIOPHAGE Phi812 AND MECHANISM OF CELL WALL BINDING AND PENETRATION

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Antibiotic-resistant strains of *Staphylococcus aureus* cause human infections that are difficult to treat and lead to death [1]. Host-range mutants of bacteriophage (phage) phi812 infect 90 % of *S. aureus* isolates and, therefore, are promising phage therapy agents [2]. As the phage approaches its host cell, phage receptor-binding proteins attach to the cell wall. This interaction triggers a cascade of structural changes in the baseplate, resulting in phage tail contraction and genome ejection into the host cytoplasm [3]. Mechanistic description of the baseplate re-organization, however, remains unknown.

Using cryo-electron microscopy (cryo-EM), we reconstructed the phage baseplate in extended and contracted states. The quality of reconstructed maps enabled us to assign individual proteins to their densities. Selected proteins involved in the host cell wall binding and penetration were produced in recombinant form and their structures were solved using X-ray crystallography and cryo-EM single-particle reconstruction.

We present the first detailed structural characterization of a contractile phage infecting Gram-positive bacterium. Comparison of the two distinct baseplate states allows the description of the initial stage of phage infection on the molecular level. Finally, our results provide framework for engineering phage particles to combat *S. aureus* infections in humans.

1. D. M. Lin et al., *World J. Gastrointest. Pharmacol. Ther.*, **8**, (2017).
2. R. Pantůček et al., *Virology*, **246**, (1998).
3. J. Nováček et al., *Proc. Natl. Acad. Sci.*, **113**, (2016).

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L7

IN SITU CRYO-ELECTRON TOMOGRAPHY OF ENTEROVIRUS CELL ENTRY

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Enteroviruses from the family *Picornaviridae* are human pathogens that cause a range of diseases from the common cold to severe brain inflammation. Despite the societal and economic impact of enteroviruses, the available treatments are only symptomatic. The enterovirus cell entry and the release of the viruses from endosomes are potential targets for antiviral therapeutics. However, the details of these phenomena are not well understood.

Here, we used *in situ* cryo-electron tomography to visualize the cell entry and genome release of human rhinovirus 2. We observed endosome membrane remodelling and breakage followed by virus escape into the cytoplasm. We demonstrate that the endosome disruption is mediated by overactivation of a cellular mechanism by showing that endocytosis of very-low-density lipoprotein, the natural substrate of rhinovirus 2 receptor, also results in endosome disruption. The described mechanism of rhinovirus 2 cell entry is supported by data collected on other enteroviruses. Our results give evidence of the cellular mechanisms these viruses employ to enter cell hosts.

We acknowledge the Cryo-electron microscopy and Tomography Core Facility of CEITEC MU for their support in data collection and analysis.

L8

MECHANISM OF VIRION FORMATION OF THE *EMILIANIA HUXLEYI* VIRUS 201 ENVELOPED BY TWO MEMBRANES

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Emiliania huxleyi is a worldwide distributed unicellular marine alga whose cells are covered by calcite disks called coccoliths. By reflecting light, the coccoliths influence retention of heat in oceans, which impacts planetary climate [1]. *Emiliania huxleyi* virus 201 (EhV-201) and related nucleocytoplasmic large DNA viruses limit the population growth of *E. huxleyi* [2].

Virion of EhV-201 is pleiomorphic in shape, therefore we used localised reconstruction of small fractions of the virion edges to elucidate its complex ultrastructure, comprising an inner membrane, capsid, outer membrane, and surface protein envelope. Furthermore, we used focused ion beam milling and cryo-electron tomography to characterize the formation of EhV-201 virions in *E. huxleyi* cells. The particle assembly is initiated on membrane fragments, which separate from the endoplasmic reticulum. Assembly of the capsid proteins at the outer surface of the membrane fragment induces its bending and gradual formation of capsids containing a membrane sack. Virus DNA is packaged into the pre-formed particles through an opening in the capsid and inner membrane. The genome-filled intermediates bud into intracellular vesicles, and in this process, acquire the outer membrane and protein envelope. Virions are released from the cell by exocytosis or lysis of the infected alga. Our results give structural insight into the formation of EhV-201 – a pathogen that influences the Earth's climate.

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L9

BACTERIAL HELICASE-LIKE TRANSCRIPTION-ASSOCIATED FACTOR HELD

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Regulation of bacterial transcription performed by RNA polymerase (RNAP) is facilitated by various transcription factors (e.g. factors), which interact with RNAP. One of these factors is HelD, a transcription-associated protein unique for many Gram-positive bacteria (e.g. *Bacillus subtilis* or *Mycobacterium smegmatis*) [1]. This study focuses on the structure-function analysis of the complexes between RNAP and HelD. As there was no structure of HelD homologue known and full-length HelD itself resisted all attempts for crystallization, we used a combination of X-ray crystallography (one HelD domain), cryo-EM, small-angle X-ray scattering and homologous modelling [2, 3].

HelD interacts with RNAP in different stages of transcription. It penetrates deep into the RNAP primary channel, interacts with the critical active site residues and also binds tightly in the RNAP secondary channel. These channels are responsible for nucleic acids binding and substrate delivery. As these interactions are incompatible with the binding of DNA to the RNAP core, HelD effectively hinders the elongation process of transcription and can effectively clear RNAP of nucleic acids by dismantling RNAP-DNA complexes [3]. HelD itself is a multi-domain protein capable of structural changes both in solution [2] and in complex with RNAP [3]. These changes are linked to its function. The structural basis for the DNA-clearing function of HelD was explained but the recycling of RNAP inhibited by the HelD binding remains to be elucidated.

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L10

THE STRUCTURAL BASIS OF COMPLEMENT INHIBITION BY THE HUMAN PARASITE *TRYPANOSOMA BRUCEI GAMBIENSE*

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African trypanosomes have developed elaborate mechanisms to avoid clearance by the human immune system. While many of the defence systems have been described in detail, the basis of alternative pathway inhibition remains obscure. Trypanosomes are unaffected by complement-mediated lysis but the molecular processes that prevent formation of the membrane attack complex are currently not understood. Recently we have identified the trypanosome surface receptor that interacts with complement factor 3, the central hub of the complement cascade, and determined its complex structure by single particle cryo-EM. Using an interdisciplinary approach combining biochemical, biophysical, structural and cell biological methods we provide detailed insight into the unusual molecular mechanism of complement inhibition by an important human pathogen.

L11

CHARACTERIZE YOUR MOST CHALLENGING INTERACTIONS. THE NEW MONOLITH

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Knowing the strength of the interactions between key players is crucial to get the insights you need to understand the details behind how a given biological event occurs.

The new Monolith is the latest solution we provide to our customers who wish to quantify their biomolecular interactions of any kind in any experimental conditions. Monolith utilizes two proprietary technologies- MST as well as our newest addition to the portfolio- isothermal spectral shift.

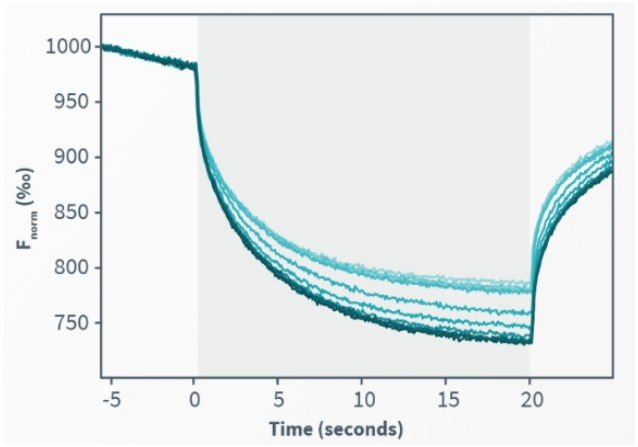
MST technology allows for quantification of molecular interactions between a target and ligand by detecting changes in fluorescence intensity while a temperature gradient is applied over time. The fluorescent signal comes from the target that is either fluorescently labeled or has intrinsic fluorescence and becomes an extremely sensitive reporter for the interaction.

tions utilizes an experimental procedure during which a fluorescently labelled target generates a particular emission spectrum, and if a ligand binds to this target, the fluorophore's local chemical environment is changed, causing a shift in its fluorescence spectrum. This particular Monolith detector exploits this phenomenon by performing ratiometric measurements at two emission wavelengths of a labelled target in the presence of various concentrations of a ligand. Isothermal spectral shift in order to quantify a molecular interactions utilizes an experimental procedure during which a fluorescently labelled target generates a particular emission spectrum, and if a ligand binds to this target, the fluorophore's local chemical environment is changed, causing a shift in its fluorescence spectrum. This particular Monolith detector exploits this phenomenon by performing ratiometric measurements at two emission wavelengths of a labelled target in the presence of various concentrations of a ligand.

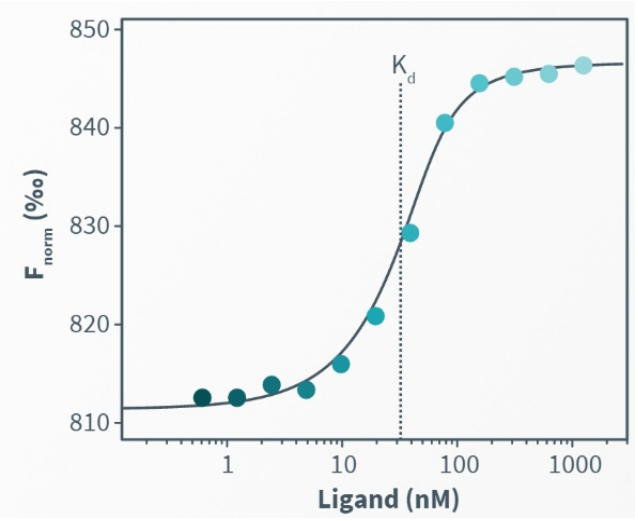
In both of the detectors types which can be combined in a Monolith instrument- the binding affinity is automatically determined at the end of each run without additional and lengthy data analysis. (figure 1.)

Monolith enables characterization of in solution interactions for a wide range of biomolecules, even for challenging samples such as membrane proteins, intrinsically disordered proteins, small molecules and cell lysates. Since the binding partners are in solution, there is no lost activity due to immobilization, and evaluation is size independent. Measurements can be performed in any buffer, including detergents, using low sample volumes and concentrations. The collected data analysis also facilitates the evaluation of competition assays and ternary binding events.

Monolith provides a valuable orthogonal method to validate your results from other biophysical methods and to characterize your most challenging interactions.



A)



B)

Figure 1. The affinity constant (Kd) is calculated from a fitted curve that plots normalized fluorescence against concentration of ligand.