L19

INSIGHTS INTO THE STRUCTURE AND CONFORMATION OF BIOMOLECULES TO UNDERSTAND THEIR BIOLOGICAL FUNCTION FOR NEW DRUG SYSTEMS USING SAXS IN THE LABORATORY

Angelika Scheiflinger-Latal, Franz Pirolt, Heike Ehmann, Andreas Keilbach and Martin Medebach

Anton Paar GmbH, Anton Paar Straße 20, 8054 Graz, Austria
angelika.scheiflinger-latal@anton-paar.com

The development of new and better drugs, to overcome diseases like Alzheimer's disease, Parkinson or antibiotic resistance, are a main focus of research in the biological, pharmaceutical and medical field. In the last decades the interest in biological macromolecules and complexes providing properties to treat and cure diseases has tremendously increased. To understand their biological function in vitro especially small-angle X-ray scattering (SAXS) gains increasing attention offering complementary information to the traditionally used biological techniques. SAXS enables to study the structure and dynamics of biomolecules in solution where physiological key parameters can be tuned and tested.

SAXS is a versatile technique used for shape and size characterization of nanostructured materials between 1 nm and 200 nm. Biological samples, like proteins, peptides, monoclonal antibodies or viruses are already well known to be investigated with SAXS. Furthermore drug delivery systems like drug loaded vesicles, where size and shape parameters of the vesicle and the drug are found or granulate powders, where the internal surface obtained by SAXS correlates with the tablet hardness, are interesting examples of applications.

In the present contribution we show selected applications of biological macromolecules, using a multifunctional laboratory Small and Wide Angle X-ray Scattering (SWAXS) system, the SAXSpoint 2.0. The SAXSpoint 2.0 system is a compact lab-scale system with dedicated point collimation, which enables SAXS, WAXS and grazing-incident (GISAXS) scattering studies under ambient and non-ambient conditions, in-situ tensile SWAXS experiments, and RheoSAXS studies. It satisfies the advanced user with a wide range of dedicated sample stages, full experimental flexibility to meet the right environment for each sample, and highest resolution. The system provides simple operation, short measurement times and excellent angular resolution, enabled by a smart beam formation concept while maintaining a laboratory-friendly compact size and small footprint.

Different SAXS and WAXS studies on biological macromolecules and pharmaceutically relevant samples were performed on the presented SAXSpoint 2.0 system. Some of the samples required high resolution (very low minimum scattering angle) in order to resolve large structural dimensions. The unique sample-positioning mechanism enabled WAXS measurements to determine crystallinity without re-aligning any part of the SWAXS system. The presented studies clearly show that high-resolution and high-quality SWAXS data can be obtained for biological macromolecules and complexes investigated in their native state with a laboratory SWAXS system.

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L20

STRUCTURE AND DNA DELIVERY MECHANISM OF GENE TRANSFER AGENT OF *RHODOBACTER CAPSULATUS*

Pavol Bárdy[1] , Tibor Füzik[2] , Dominik Hrebík[2] , Roman Pantůček[1] , Pavel Plevka[2]

[1]Faculty of Science, Masaryk University, Kamenice 5, 625 00, Brno, Czech Republic
[2]Central European Institute of Technology, Masaryk University, Kamenice 5, 625 00, Brno, Czech Republic
bardy.pavol@mail.muni.cz

Gene transfer agents (GTAs) are extracellular particles that enable high-frequency horizontal gene transfer among prokaryotes and thus accelerate their evolution. GTAs are derived from phages that were independently acquired by several bacterial and archaeal lineages. In spite of their importance for adaptation and diversification of prokaryotes, the structure and mechanism of DNA delivery of GTA are unknown. Here we used cryo-electron microscopy to show that GTA of *Rhodobacter capsulatus* resembles bacteriophage from the family *Siphoviridae* with several unique features. The DNA-containing head of the GTA is shortened in the direction of GTA tail relative to the regular

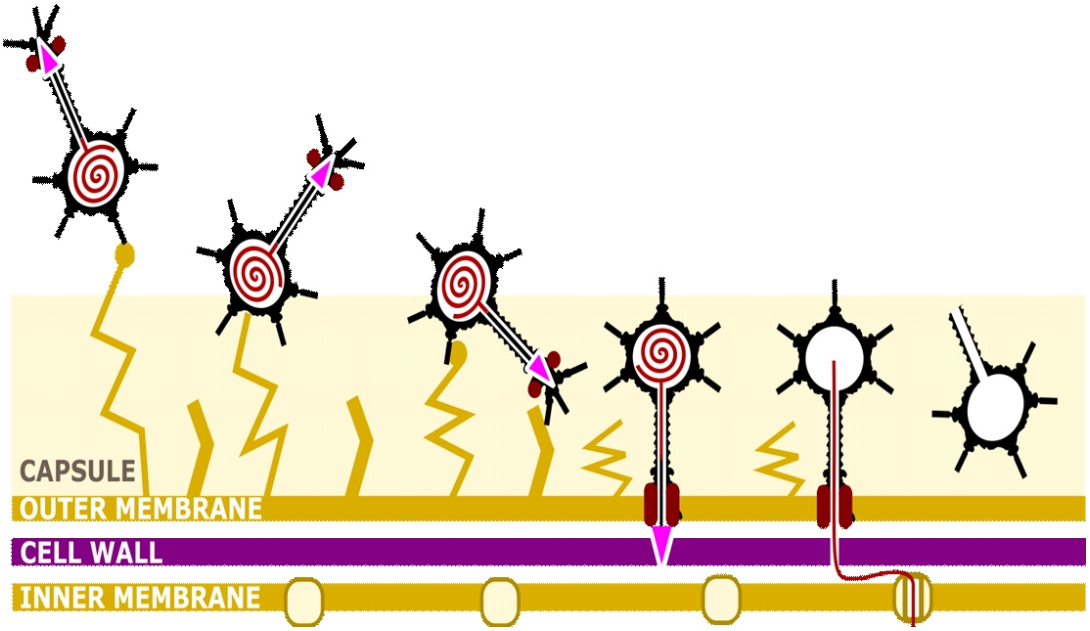

Figure 1. DNA-delivery mechanism of *Rhodobacter capsulatus* gene transfer agent (GTA).

icosahedral heads of phages. This results in T3 compressed icosahedron with ~50% reduction of the DNA packaging capacity. It represents the smallest possible size of a capsid, which can be assembled in portal-containing viruses. Unlike in the previously studied phages, attachment of the GTA tail to the head is reinforced by the interaction of the neck adaptor protein with the capsid. Tape-measure protein, which determines the length of the GTA tail, exhibits similarities to the structural features of tape-measure proteins of phages with both short and long tails and may, therefore, correspond to their common ancestor. DNA-delivery commences by the GTA attaching to the host capsule by head-spike and tail-fibres. Subsequently, the baseplate interacts with the outer membrane, irreversibly disassembles and releases the cell-wall hydrolase, located inside the baseplate. The tape-measure protein is no longer held in the virion and ejects together with DNA into the periplasm, from where DNA is uptaken to the cytoplasm by cell transformation complex. Our results reveal important structural features in tailed phage virion and describe novel DNA-delivery mechanism present in GTAs and related phages.

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L21

GENOME RELEASE OF ECHOVIRUS 18

David Buchta[1], Tibor Füzik[1], Dominik Hrebík[1], Yevgen Levdansky[1,2], Lukáš Sukeník[1], Liya Mukhamedova[1], Jana Moravcová[1], Robert Vácha[1], Pavel Plevka[1]

[1]Central European Institute of Technology, Masaryk University, Brno, Czech Republic
[2]Max Planck Institute for Developmental Biology, Tübingen, Germany

Viruses from the genus *Enterovirus* are important human pathogens. Receptor binding or exposure to acidic pH in endosomes converts enterovirus particles to an "activated" state that is required for genome release. However, the mechanism of enterovirus genome release is not well understood. Here, we used cryo-electron microscopy to visualize virions of human echovirus 18 in the process of genome release.

We discovered that viral RNA exits the echovirus 18 particle through a hole that forms in the capsid upon loss of one, two, or three adjacent capsid-protein pentamers. The resulting hole, which is more than 120 Å in diameter, enables the release of the genome without the need to unwind its putative double-stranded RNA segments. Thus, our findings uncover a novel and conserved mechanism of enterovirus genome release that could become target for antiviral drugs.

L22

CRYO-EM STRUCTURE OF BACTERIOPHAGE P68: FROM HEAD TO TAIL

Hrebík, Dominik[1]; Štveráková, Dana[2]; Škubník, Karel[1]; Füzik, Tibor[1]; Pantůček, Roman[2]; Plevka, Pavel[1]

[1]Central European Institute of Technology, Masaryk University, Kamenice 5, 625 00, Brno, Czech Republic, email: dominik.hrebik@ceitec.muni.cz
[2]Department of Experimental Biology, Faculty of Science, Masaryk University, Kamenice 5, 625 00, Brno, Czech Republic

Bacteriophages from the family *Podoviridae* use short non-contractile tails to eject their genomes into bacteria. However, there is limited high-resolution information about structure and mechanism of genome delivery of *Podoviruses* that infect Gram-positive bacteria such as *S. aureus*. Here we used cryo-electron microscopy and X-ray crystallography to determine the structures of *S. aureus* phage P68 in its native form, genome ejection intermediate, and empty particle. The structure of the native phage was solved to 3.3 Å and 3.9 Å for capsid and tail respectively. We show that residues from N-terminus of the major capsid protein enable incorporation of P68 portal complex into phage head by forming a special interface. P68 head contains seventy-two subunits of an inner core protein, which are positioned between the portal complex and phage genome. Fifteen of the inner core proteins bind to and alter the structure of adjacent major capsid proteins and thus specify attachment sites for head fibers. Unlike in the previously studied phages, head fibers of P68 enable positioning of its virion at cell surface for genome delivery. P68 genome ejection is triggered by disruption of interaction of one of the portal protein subunits with phage DNA. The inner core proteins are released before the DNA and probably enable translocation of phage DNA across bacterial membrane into cytoplasm. The genome translocation mechanism and the portal assembly mechanism is likely to be conserved among bacteriophages infecting gram-positive bacteria.

L23

STRUCTURAL STUDY OF HUMAN ENTEROVIRUS 70 USING CRYO-ELECTRON MICROSCOPY

Tibor Füzik, Jana Moravcová, Sergei Kalynych, Pavel Plevka

Structural Virology, Central European Institute of Technology, Masaryk University, Kamenice 753/5, 62500 Brno, Czech Republic

Enteroviruses, belonging to the group of *Picornaviridae*, are non-enveloped, positive-sense-stranded RNA viruses. These animal and human pathogens cause variety of diseases ranging from mild respiratory illnesses to life-threatening meningitis. Human enterovirus 70 (EV70), which is transmitted by eye secretions, causes acute hemorrhagic conjunctivitis, a serious eye disease.

We used cryo-electron microscopy to determine the molecular structure of EV70 particles. EV70 was propagated in human hTERT-RPE1 cell line and purified by PEG precipitation and ultracentrifugation in CsCl gradient. Purified virus solution was vitrified on grids covered with holey carbon. Cryo-electron microscopy was done using an FEI Titan Krios transmission electron microscope equipped with Falcon II/III direct electron detector. The processed data resulted in near-atomic resolution electrostatic potential maps, suitable for building of the atomic structure of the EV70 capsid proteins.

After successful construction of the model of full and empty particle, we focused on the region of the capsid that might be occupied by a pocket factor, a small molecule that regulates genome release of the virus. This pocket factor might be replaced by artificial inhibitors that over-stabilize the virus and prevent its infection. Knowledge of the EV70 virion structure will allow development of specific capsid-binding inhibitors that may be used to treat the disease caused by this virus.