MECHANICS OF VIRAL DNA EJECTION
CLOSING REPORT (NKFI-ID: 124966)

In this project we made significant advancements towards the understanding of viral properties, behavior and infection mechanisms. The tempersture-dependent mechanical stability and topography of the T7 phage has been uncovered. Progress has been made in mechanically manipulating T7 phages while they are infecting E. coli cells. The DNA ejection process was visualized by TIRF microscopy combined with a microfluidics. A model system consisting of LPS, synthetic lipids and E. coli outer membrane proteins has been developed to investigate its interaction with individual T7 phage particles. A real-time T7 - E. coli infection analysis system was developed to visually monitor the process of phage-induced bacterial lysis as a function of time. We made important mechanistic insights into the host surface binding and recognition of T7 phages during their replication cycle and demonstrated that individual T7 particles find their target receptor by diffusion-driven rolling. We have also expanded our physical virology approaches to investigate the structure, dynamics and nanomechanics of SARS-CoV-2, the virus causing the COVID-19 pandemic. We discovered that the SARS-CoV-2 particles are highly compliant and mechanically resilient, and their spikes display high dynamics. Finally, we have made advances towards the characterization of the alpha and delta variants of the SARS-CoV-2 virus which may lead closer to understanding the mechanisms behind their increased infectivity.

1. Temperature-dependent nanomechanics and topography of bacteriophage T7

We measured the heat-induced changes in the properties of T7 bacteriophage particles exposed to a two-stage (65°C and 80°C) thermal effect, by using atomic force microscopy (AFM)-based nanomechanical and topographical measurements. We found that exposure to 65°C led to the release of genomic DNA and to the loss of the capsid tail (**Figure 1**); hence, the T7 particles became destabilized. Further heating to 80°C surprisingly led to an increase in mechanical stability, due likely to partial denaturation of the capsomeric proteins kept within the global capsid arrangement. IMPORTANCE Even though the loss of DNA, caused by heat treatment, destabilizes the T7 phage, its capsid is remarkably able to withstand high temperatures with a more or less intact global topographical structure. Thus, partial denaturation within the global structural constraints of the viral capsid may have a stabilizing effect. Understanding the structural design of viruses may help in constructing artificial nanocapsules for the packaging and delivery of materials under harsh environmental conditions. A paper was published on our results in J. Virology.

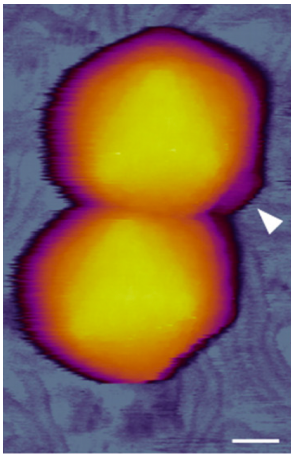

Figure 1. AFM of two T7 phages treated at 65°C. The white arrowhead points at the short, stubby tail complex visible on one of the particles, whereas there is no visible tail on the other one, indicating tail loss. Scale bar, 20 nm.

2. Characterization of LPS-lipid and bacterial outer membrane bilayers

The outer membrane (OM) of Gram-negative bacteria is a complex asymmetric bilayer containing lipids, lipopolysaccharides (LPS) and proteins. While it is a mechanical and chemical barrier, it is also the primary surface of bacterial recognition processes that involve infection by and of the bacterium. Uncovering the mechanisms of these biological functions has been hampered by the lack of suitable model systems. Here we report the step-by-step assembly of a synthetic OM model from its fundamental components. To enable the efficient formation of a supported lipid bilayer at room temperature, DMPC was used as the lipid component to which we progressively added LPS and OM proteins. The assembled system enabled us to explore the contribution of the molecular components to the topographical structure and stability of the OM. We found that LPS prefers solid-state membrane regions and forms stable vesicles in the presence of divalent cations. LPS can gradually separate from DMPC membranes to form independent vesicles, pointing at the dynamic nature of the lipid-LPS system. The addition of OM proteins from *E. coli* and saturating levels of LPS to DMPC liposomes resulted in a thicker and more stable bilayer the surface of which displayed a nanoscale texture formed of parallel, curved, long (>500 nm) stripes spaced apart with a 15 nm periodicity (**Figure 1**). The synthetic membrane may facilitate the investigation of binding and recognition processes on the surface of Gram-negative bacteria. A paper has been published in the international journal Nanoscale Advances.

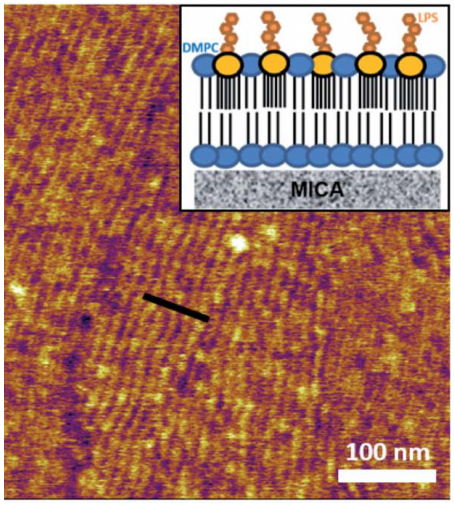

Figure 2. Supported Lipid Bilayer (SLB) formed by liposomes created with detergent mediated reconstitution. High-LPS-content OM-DMPC SLB surface prepared with detergents at 15 ˚C display a 2D crystalline wavelike pattern.

3. Imaging and mechanics of infectious DNA ejection by the T7 Bacteriophage

We explored how T7 recognizes and infects its target through the observation of single phage particles and its interactions with single E.coli bacterial cells. To investigate the mechanics of host-virus interaction, we pulled T7 away from the surface of individual E. coli by using an optically trapped microbead coated with anti-gp10. Single-phage DNA ejection kinetics were observed by using TIRF microscopy in a microfluidic flow cell. Following the ejection of most of the genomic DNA from T7, the DNA remained firmly attached to the capsid. Furthermore, to a surprise, the nearly completely ejected DNA could not be pulled out of the capsid even with forces up to 60 pN, suggesting that a mechanical latch mechanism controls the exit of DNA. Most likely, the viral

DNA retention is caused by an interaction between DNA and the internal surface of the proteins in the T7 tail, which functions as an injector complex. DNA ejection is probably controlled by the kinetics of tail extension that leads to the injector complex formation and the consolidation of interactions with the genomic DNA. The amount of initially released DNA is thus set by a competition between tail extension and DNA ejection, where the latter is tuned by a pressure gradient between the T7 capsid and its environment. How the mechanical latch becomes released and the entire T7 genome released, awaits further exploration. Our results show that single particle observation is an efficient tool to better understand the host recognition and DNA translocation processes of T7 phages, which is the result of a very precise harmony of molecular interactions. Finally, we have set up a system in which the nanomechanics of the direct interation between a single T7 phage particle and a single E.coli cell is monitored efficiently in our optical tweezers. In this assay, the single bacteriophage is held, with a microscopic bead, via an engineered, 3-micron-long DNA handle. This way the phage particle is allowed to position itself on the bacterial surface more freely. So far we have shown that the single virion can be captured and its DNA release can be followed by confocal imaging.

4. Real-time video-microscopic analysis of T7 phage surface docking and T7-induced bacterial lysis

We developed a microscopic assay that enables us to follow the time-dependent process of E. coli lysis induced by infection with the T7 bacteriophage. In this system a high-resolution phase contrast/epifluorescence video microscope was combined with a microfluidic chamber, in which surface-attached individual E. coli cells can be followed while being exposed to a well-controlled titer of T7 phage suspension. The presence of DNA, due either to the binding of T7 onto the E.coli surface or to an abrupt release from the bacterial cytoplasm during the final lytic step, can be monitored by measuring fluorescence generated upon the binding of DNA-intercalating dyes (e.g., Sytox Orange). We observed the emergence of luminescent spots on the bacterial surfaces, which are identified as individual phage particles. Bacterial lysis was observed to be delayed compared to lysis without Sytox Orange, suggesting that the dye either reduces phage titer (deactivates some phages), or the dye intercalation slows down nucleic acid-dependent enzyme processivity. The phage deactivating effect caused by Sytox might be also be enhanced by the constant sample illumination. Bright fluorescent spots appeared after lysis, which corresponds to the emergence of either bacterial or non-encapsulated viral DNA, or freshly released intact T7 viruses. We observed a bacterial bleb formation, which is probably caused by local disruption of the peptidoglycan layer in the bacterial cell wall. Finally, using AFM, we recently managed to capture individual T7virions on the surface of an E.coli bacterium, along with the instant of bacerial lysis. Our results represent an important step in the quantification of the temporal and spatial events related to T7 infection.

5. Topography, spike dynamics and nanomechanics of individual native SARS-CoV-2 virions

SARS-CoV-2, the virus responsible for the current COVID-19 pandemic, displays a corona-shaped layer of spikes which play fundamental role in the infection process. Cryoelectron microscopic structural data suggested that the spikes possess orientational freedom and the ribonucleoproteins segregate into basketlike structures. How these structural features regulate the dynamic and mechanical behavior of the native virion, however, remained unknown. By imaging and mechanically manipulating individual, native SARS-CoV-2 virions with atomic force microscopy, we demonstrated that their surface displays a dynamic brush owing to the flexibility and rapid motion of the spikes (**Figure 3**). The virions are highly compliant and able to recover

from drastic mechanical perturbations. Their global structure is remarkably temperature resistant, but the virion surface becomes progressively denuded of spikes upon thermal exposure. Thus, both the infectivity and thermal sensitivity of SARS-CoV-2 rely on the dynamics and the mechanics of the virus. Our work has been published in the prestigious international journal Nano Letters.

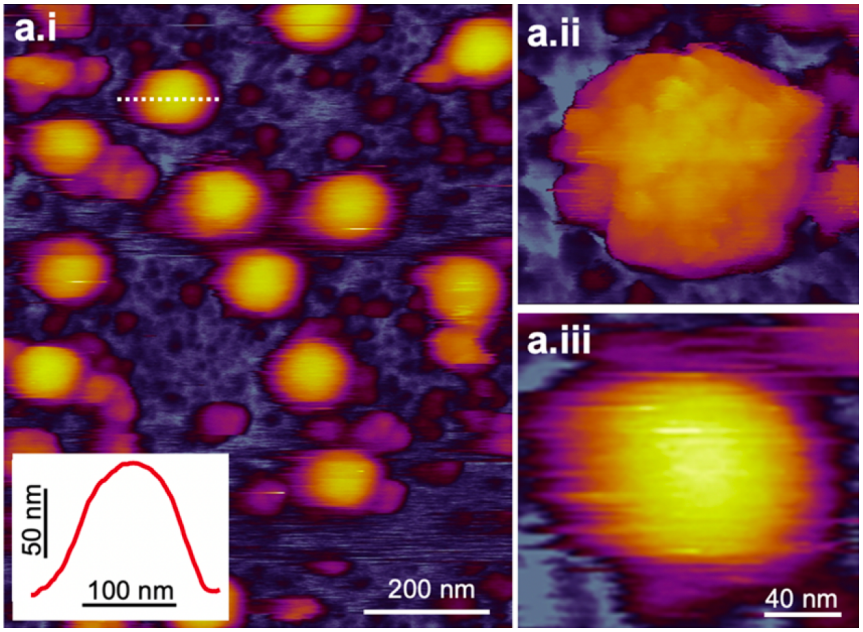

Figure 3. Topographical analysis of native, unfixed SARS-CoV-2 virions. (**a.i**) AFM image of an overview (0.8 Å~ 1 µm) sample area. Inset: Topographical profile plot measured along the horizontal diameter of one of the virions (dotted line), revealing a smooth surface. (**a.ii,iii**) Magnified image of a fixed and native virion, respectively, for better comparison. Surface protrusions are not resolved in the native virus particle, but a blurred, smooth topography is observed.

6. Topographical structure of SARS-CoV-2 variants

The alpha and delta variants of SARS-CoV-2 have been strongly contributing to the covid-19 pandemic due to their greater infectivity than the original Vuhan type of the virus. The mechanisms behind the increased infectivity are not understood. We have investigated the topographical structure of glutaraldehyde-fixed samples of the variants by using AFM. We have found that the size distribution of the variants is much greater than that observed in the case of the Vuhan type. Furthermore, the mean diameter of the variants was significanly smaller. These results indicate that the specific surface of the variants exceed that of the Vuhan type, considering that the volume of a spherical particle scales with the third power of the radius while its surface area with the square of the radius. It is plausible that the increased specific surface provides a simple physical explanation for the enhanced infectivity. A manuscript is currently in preparation about our results.

7. Imaging the infection cycle of T7 at the single virion level

We used TIRF microscopy to uncover the spatial dynamics of the target recognition and binding by individual T7 phage particles. In the initial phase, T7 virions bound reversibly to the bacterial membrane via two-dimensional diffusive exploration. Stable bacteriophage anchoring was achieved by tail-fiber complex to receptor binding which could be observed in detail by atomic force microscopy (AFM) under aqueous buffer conditions. The six anchored fibers of a given T7 phage-displayed isotropic spatial orientation. The binding of the T7 phage particles was followed by the transfer of their genomic DNA into the host E.coli cell, which we were able to follow at the single virion level (**Figure 4**). DNA transfer took place on a time scale of approximately one minute. The viral infection led to the onset of an irreversible structural program in the host which occurred in three distinct steps. First, bacterial cell surface roughness, as monitored by AFM,

increased progressively. Second, membrane blebs formed on the minute time scale (average ~5 min) as observed by phase-contrast microscopy. Finally, the host cell was lysed in a violent and explosive process that was followed by the quick release and dispersion of the phage progeny. DNA ejection from T7 could be evoked in vitro by photothermal excitation, which revealed that genome release is mechanically controlled to prevent premature delivery of host-lysis genes. A paper was published about our results in the International Journal of Molecular Sciences.

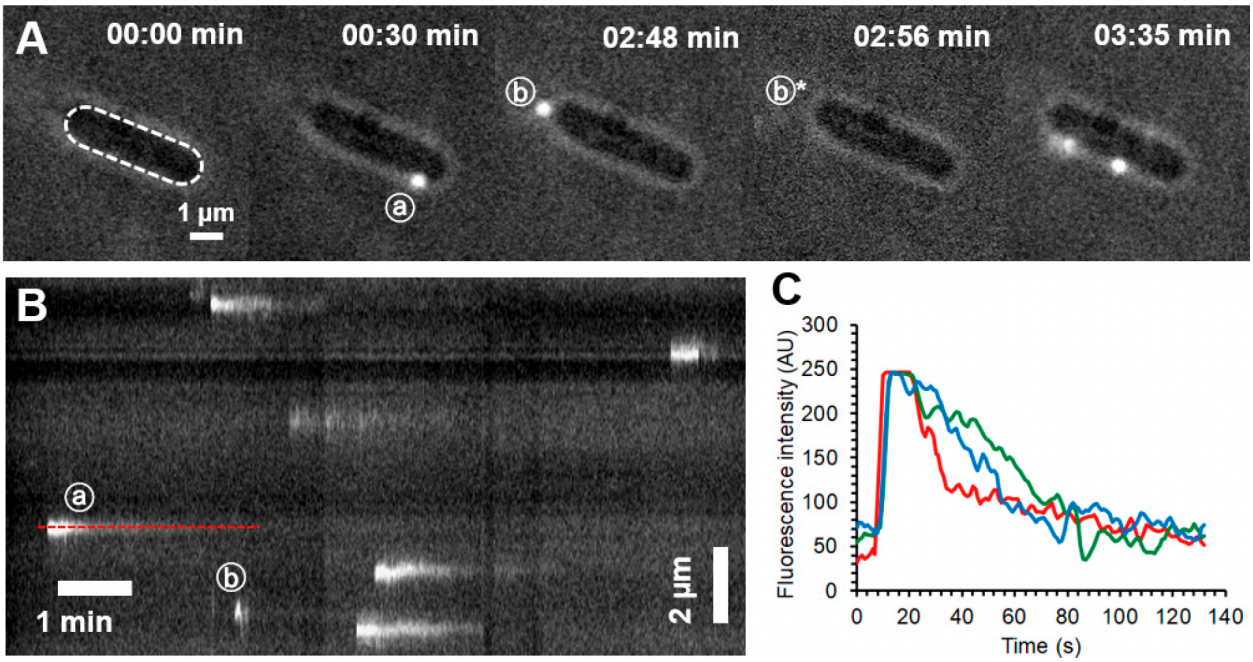

Figure 4. Single-phage infection process. (**A**) Snapshots of the early moments (0–10 min) of a single bacterium infection highlighting the docking of bacteriophages. The bacterial outline is slightly visible due to low levels of phase-contrast illumination. Dashed white line indicates the bacterial periphery along which a kymogram (**B**) was constructed. The same two phages are marked with circled (a) and (b) in the snapshots (A) and the kymogram (B). (b*) marks the position where phage (b) was visible 8 s earlier. Red dashed line marks the location of the relative intensity profile of a single phage (a) shown in (**C**) in red. Blue and green lines show the injection kinetics of two additional phages.