Study essential viral processes through a dynamic single-molecule approach

Viral processes
Application note

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Study Essential Viral Processes through a Dynamic Single-Molecule Approach

Viral replication strategies and life cycles vary depending on the virus types and can differ throughout all stages, including virus entry, replication, latency, and shedding (Figure 1). Understanding infection and replication processes of viruses is an essential step toward the development of therapeutic strategies to mitigate or treat viral diseases.

Current approaches in virology rely on electron microscopy or RNA and protein quantification to detect viral properties. These methods are either limited to static images, which cannot record dynamic events, or ensemble methods, which are unable to characterize regulatory mechanisms of individual translation or replication processes. Instead, they provide an averaged readout.

In this application note, we show how single-molecule approaches, using optical tweezers correlated with fluorescence and label-free microscopy, can be applied to investigate viral replication processes in detail and aid the development of therapies and virus research as a whole.

Features of the C-Trap

Optical tweezers enable you to trap micron-sized objects, such as beads or viral particles with a highly focused laser beam. Trapping two or more beads will allow you to tether a biomolecule, such as DNA, between the beads and build complex biological systems. You can then manipulate the position and movement of the trapped object(s) by controlling the direction of the beam.

Real-time imaging lets you visualize the captured biomolecule (e.g., DNA or virus particles) or associated proteins interacting with it. For example, you can label different biomolecules involved in viral processes and monitor them through the integrated fluorescence microscopy system.

In this application note

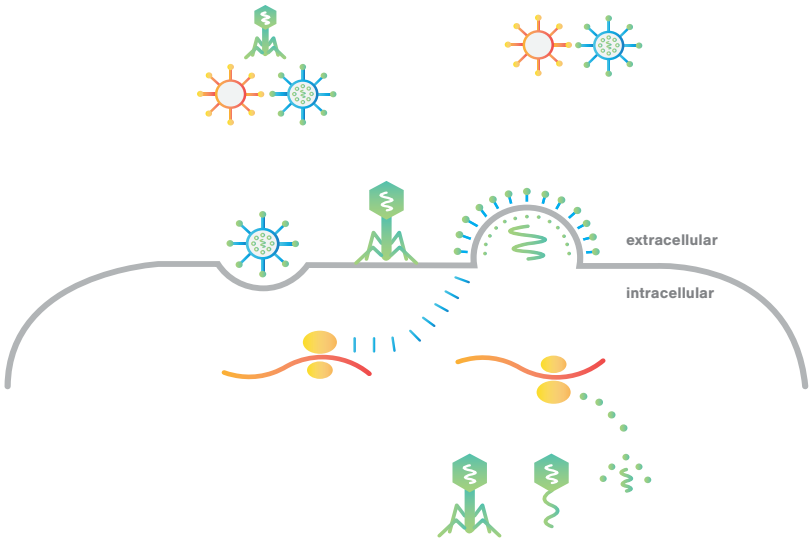
This application note demonstrates how you can use the C-Trap® Optical Tweezers – Fluorescence & Label-free Microscopy to gain insight into various processes associated with virus infection and replication. We list seven experimental designs that showcase how to analyze a wide range of aspects associated with virology in real time and at the single-molecule level.

Here, we present seven applications showing you how to:

- Detect and select single viral particles for optical tweezers experiments.
- Record the interaction forces between a virus and the host cell.
- Establish the kinetics and energy landscapes associated with conformational changes of viral proteins.
- Manipulate and visualize the autoregulation of viral RNA translation.
- Time and quantify packaging of viral DNA.
- Measure and visualize interactions between viral DNA and capsid proteins.
- Investigate and monitor protein-induced viral shedding and fusion.

Critical steps of the life cycle of viruses during host infection

1 Viruses interact with the membrane of a host cell. Recognition of host membrane proteins triggers the initiation of the viral cell cycle, including viral entry, replication, shedding, and in some cases latency.

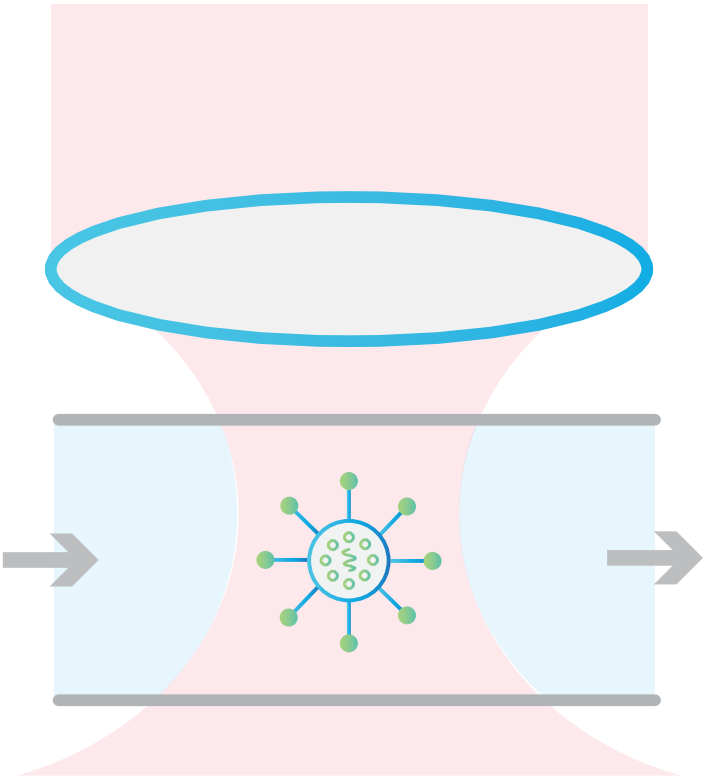


Experiments

Detect and select viral particles for optical-tweezers experiments

Optical tweezers are optimal tools for the detection and isolation of individual viral particles in a contact-free manner. While, in most cases, virus particles are small and aggregate in liquids which may confound the intended measurements, this technology enables you to catch virus particles in solution and differentiate single particles from aggregates (**Figure 2**).

The optical trapping of a single virus-particle enables you to control its location. On top of that, you can fluorescently label viral membrane proteins and quantify them based on fluorescence intensity in order to correlate protein levels with your desired measurements.



2 Schematic showing the experimental setup of an the optical trap capturing virions in a microfluidic chamber. The mobility of a virus or a virus aggregate is controlled and recorded by the optical trap. Fluorescently labeled membrane proteins of the virus offer multiple possibilities for imaging analyses.

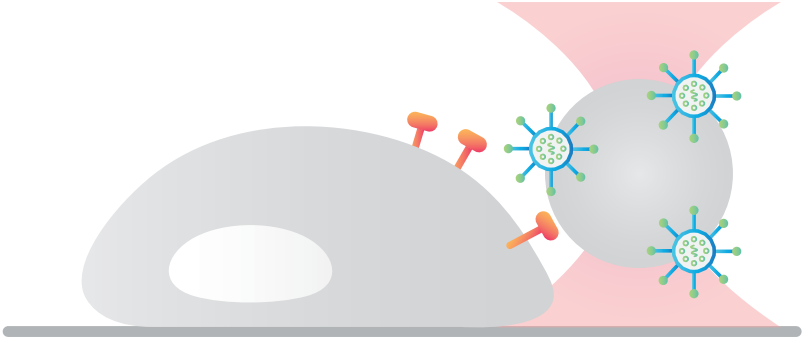
Read more

[1] Pang *et al.* (2014) *Nature Nanotechnology*

Record the interaction forces between a virus and the host cell

The interaction between a virus and the host cell is the first critical step in virus entry and infection. You can use the C-Trap to investigate the dynamic interactions between a single virus and the cell surface receptors of a host cell. This approach enables you to direct an optically-trapped bead, coated with your virus of interest, towards adherent cells. Subsequently you can push the bead against the cell membrane and pull it away after contact (**Figure 3**). The pulling force required to disrupt the established interactions between the virus and host cell indicates the strength of the interaction.

Understanding the dynamics associated with the initial interactions between a virus and a host cell will aid the development of novel therapies that prevent or mitigate viral infections. You can, for example, apply a similar approach to screen for drugs that prevent the virus from recognizing the host cell by comparing pulling forces under different treatment conditions.



3 The C-Trap can measure forces and dynamics associated with virus–host interactions. The correlated fluorescence imaging and optical tweezers can simultaneously characterize host cell changes and measure exerted forces upon interaction.

Read more

[2] Sieben *et al.*, (2012) *Proceedings of the National Academy of Sciences*

Extend your knowledge: LUMICKS Cellular Mechanics application note.

Experiments

Establish the kinetics and energy landscapes associated with conformational changes of viral proteins

Viral cell invasions commence once a virus recognizes and stick to a host cell through viral fusion proteins. The conformational changes of these proteins allow them to attach to the host cell and apply forces on the host cell's membrane. Understanding their dynamics can expand our knowledge about these procedures and subsequently help us to develop future antiviral therapies. However, the energy associated with the protein folding and unfolding is challenging to measure with conventional methods.

With optical tweezers instruments like the C-Trap, you can attach the ends of a viral protein between two beads, apply mechanical force to unfold the protein, and investigate the related kinetics (**Figure 4**). By studying these features at the single-molecule level, you can probe the intermediate conformations, a crucial detail that is commonly missed by other conventional methods. This kind of assay can, for example, provide you insights into how specific drugs disrupt viral proteins and their conformational dynamics during infections.

4 Two DNA handles tether the target protein between two beads. Moving the beads apart and releasing the forces, cause the protein and its domains to unfold and refold. Time, distance, and force signals are used to calculate the kinetics and reconstruct the energy landscape of protein unfolding.

Read more

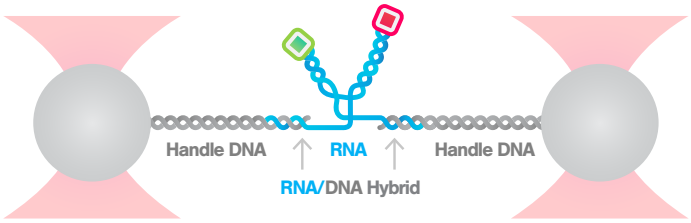
[3] Jiao *et al.* (2015) *Proceedings of the National Academy of Sciences*

Extend your knowledge: LUMICKS Protein Unfolding application note.

Manipulate and visualize the autoregulation of viral RNA translation

RNA pseudoknots are secondary structures of RNA that are critical in autoregulating translation and ribosomal frameshifting in many virus types. The C-Trap offers you the means to study the structural dynamics of RNA pseudoknots at the single-molecule level to reveal how secondary and tertiary RNA conformations regulate viral translation.

By creating and stretching a so-called dumbbell system including an RNA and two trapped beads (**Figure 5**), you can investigate forces associated with each step of structure unfolding and folding. The resulting readouts retrieved from these experiments also reveal the intermediate conformational states, providing you with a structure-specific energy landscape. These types of data offer you an in-depth understanding of the dynamics of the translational regulation of viral gene expression.



5 An RNA with a pseudoknot structure is attached to DNA handles on each end and tethered between two optically trapped beads. The optical tweezers can stretch the structure to detect forces associated with conformational changes of the pseudoknot. The smFRET experiment can reveal domain-dependent dynamics when two domains of mRNA are labeled with different fluorescent dyes.

Combining the force-induced measurements of the secondary structure with single-molecule fluorescence resonance energy transfer (smFRET) offers you a more complete picture. The combined approach enables you to correlate the folding mechanisms with the tertiary structure of the RNA and study mechanisms that, for example, inhibit the autoregulation of viral translation.

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[4] Zhong *et al.* (2016) *Scientific Reports*

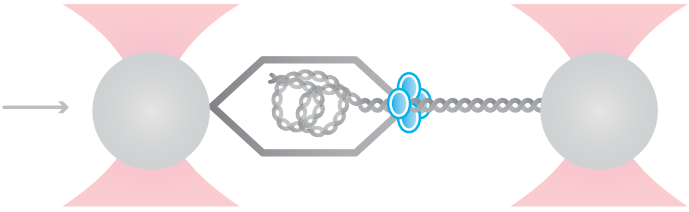
Extend your knowledge: LUMICKS RNA Dynamics application note.

Experiments

Time and quantify packaging of viral DNA

During viral assembly, double-stranded DNA viruses require the assistance of ATP-dependent packaging motor complexes. Using optical tweezers, you can study the processes by which viruses pack large amounts of genetic material in confined environments and uncover critical steps and requirements in viral DNA packaging.

For example, you can tether a DNA packaging motor and one end of the DNA substrate between two optically trapped beads (**Figure 6**). Next, you can directly measure distance changes between the two trapped beads during DNA packaging. Both the cycle duration (period of fast packaging and pause) and the amount of packed DNA reveal the functions of the specific motor. The procedure lets you measure and compare mechanochemical functions of DNA packaging motors depending on, for example, ATP concentrations or capsid capacities, as well as different forces on the DNA.



6 Setup of single-virus DNA packaging assessment. One bead tethers a capsid with prepacked DNA, and the other bead tethers the free DNA end. Once these are tethered, the C-Trap can measure distance changes between the beads, as well as the associated forces, to study motor activity.

Read more

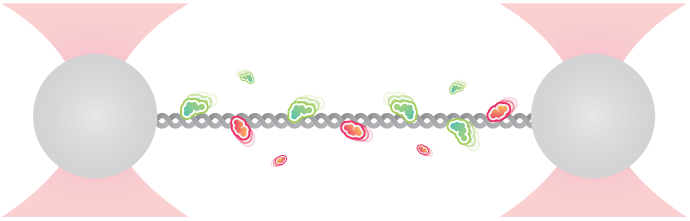
[5] Liu *et al.* (2014) *Cell*

Measure and visualize interactions between viral DNA and capsid proteins

Viral capsids typically consist of limited types of viral particles – some merely contain a single type. However, these particles can assemble in a wide range of geometries and shapes that protect and deliver the genetic material.

Using optical tweezers correlated with fluorescence microscopy, you can study the nucleation and growth process of viral particles while capsid proteins interact with nucleic acids. For instance, the C-Trap can tether a double-stranded DNA between two beads and subsequently expose the single molecule to viral capsid proteins (**Figure 7**). As these proteins bind to the DNA, they shorten the nucleic acid through compaction. The fluorescently labeled capsid proteins reveal progressively compacted protein–DNA complexes as the fluorescence intensity increases.

This information, in turn, uncovers how viral capsids compact and store genetic material like DNA. Knowing how proteins interact with DNA helps understand how and in what conformation the DNA is packed inside the capsid.



7 The number of protein complexes interacting with DNA can be quantified using fluorescence intensity. Simultaneously, the force on the DNA can be modified to study its interaction with the viral proteins.

Read more

[6] Marchetti *et al.* (2019) *Nano Letters*

Extend your knowledge: LUMICKS C-Trap brochure

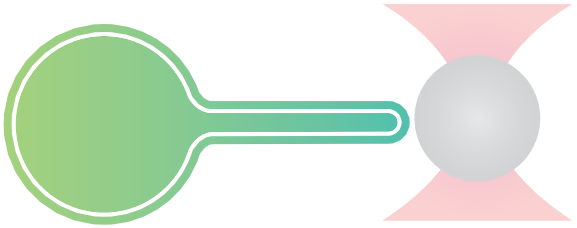
Experiments

Investigate and monitor protein-induced viral shedding and fusion

Shedding of progenies from the host cell is a vital step in the viral life cycle. One example of shedding is the viral budding from a cell, which involves cell membrane tension and several virus-associated proteins.

Optical tweezers are optimal to simultaneously apply and measure forces associated with membrane deformation upon viral shedding (**Figure 8**). The fluorescence imaging provided in the C-Trap further enables you to visualize the curvature of the host cell's membrane as well as the distribution of labeled membrane proteins while simultaneously assessing the forces associated with shedding.

The approach is unique in that it reveals the relationship between membrane curvature and viral proteins during budding, which can be correlated with its shedding efficiency.



8 Optical tweezers trapping a streptavidin-coated bead and directing it to a membrane vesicle with fluorescently labeled membrane proteins. Retracting the bead upon contact with the membrane creates a nanotube from the membrane vesicle that can be visualized through fluorescence microscopy to validate the curvature.

Read more

[7] Moreno-Pescador *et al.* (2019) *ACS Nano*

Extend your knowledge: LUMICKS Membrane Fusion application note.

"I see it very valuable to study virus with single-molecule techniques. There's a big potential in studying the budding force during virus membrane fusion. I think optical tweezer is the right tool for it."

- Prof. Poul Martin Bendix, Niels Bohr Institute

"Single-molecule manipulation and visualization enable unprecedented insights into the mechanisms and mechanics of the viral life cycle, which are surprisingly adaptable and sophisticated. These insights could inform us of new ways to curb virus infection and spreading in the host."

- Prof. Shixin Liu, Rockefeller University

Conclusion

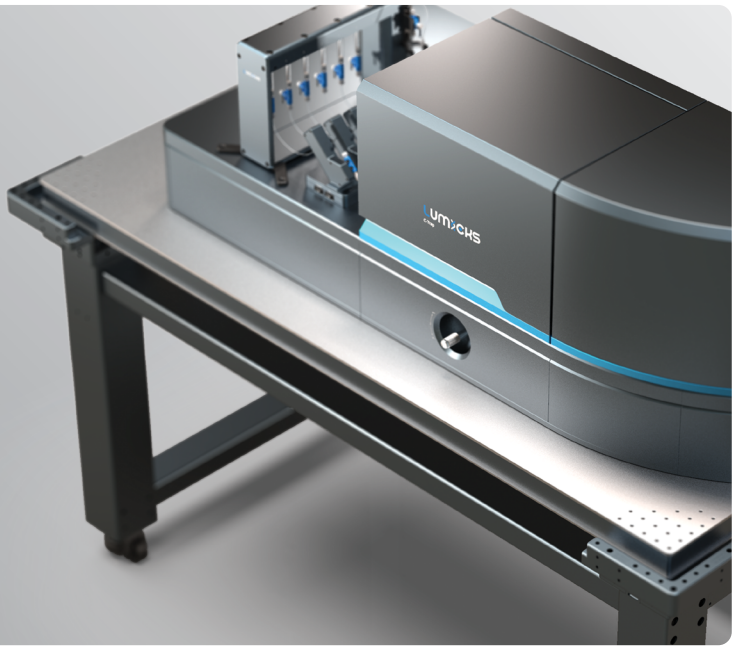
While conventional assessments of viral properties, for example, electron microscopy and expression profiling, characterize viral infections of virus populations, they are restricted to static or averaged outcomes. **Studying the viral life cycle through single-molecule approaches, such as optical tweezers, enables you to detect specific and stochastic mechanistic events associated with virus infections.**

The C-Trap® is a unique tool that offers correlated optical tweezers and state-of-the-art imaging techniques. The all-in-one approach provides you with simultaneous force measurements and fluorescence visualization of specific viral events.

You can use dynamic single-molecule approaches to uncover specific viral life cycle steps from the initial interaction between a virus and its host cell to the shedding of the viral progeny. These detailed analyses of viral infections, ultimately, serve to develop new therapies against viruses and, potentially, keeping us one step ahead of the pathogen.

C-Trap specifications

- Force resolution: < 0.1 pN at 100 Hz (1 µm beads at ≥ 0. 35 pN/nm trap stiffness)
- Minimal incremental step size: 2 Å | Absolute position
- Type of microscopy supported: Confocal/STED or Widefield/TIRF/IRM
- Confocal colors: up to 3 colors (default: 488 nm, 532/561 nm, 639 nm)
- Widefield/TIRF colors: up to 3 colors (default: 488 nm, 561 nm, 639 nm)



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