

Quantifying cell-virus interactions using NanoTracker™ optical tweezers

Introduction

In a living cell, many complex mechanical processes govern its behavior, such as its interactions with the environment. A lot is still to be learned about these processes. Over the past years, quantitative and controlled manipulation using optical tweezers instrumentation has helped elucidate several of such processes [1]. In particular, the interaction of cell membrane receptors with their ligands can be studied in a straightforward manner using optical tweezers.

NanoTracker™ optical tweezers

Being designed as a highly sensitive force-sensing instrument, JPK's NanoTracker™ platform (figure 1) is perfectly suitable for single-molecule investigations such as motor protein tracking or biopolymer manipulation [2]. In addition, many biological processes in and around living cells can be explored by the use of the sensitive manipulation and force detection provided by optical tweezers. Advanced optical microscopy capabilities such as fluorescence and DIC microscopy are seamlessly integrated with optical tweezers manipulation and complement the experimental results.

Moreover, since experiments with live cells critically

depend on the ability to control their micro-environment, the PetriDishHeater™ (figure 2) is available to provide a complete solution for such experiments. It allows fine control of the temperature of the medium, as well as gas perfusion of samples mounted in Petri dishes.



Figure 2: The NanoTracker™ PetriDishHeater™ provides sample temperature control from room temperature up to 60°C with 0.1°C precision, as well as gas or buffer perfusion.

Below we demonstrate manipulation, force detection and imaging of membrane-bound receptor interactions with ligands using this advanced yet user-friendly research tool.



Figure 1: The NanoTracker™ optical tweezers platform.

Particle-cell interactions

In recent years, the interaction of nanoparticles with cells has come into focus of nano-research. The topics range from the study of nanotoxicity, where the impact of particle uptake into cells on the viability of the latter is studied, to the use of particles as carriers for drugs. With the help of optical tweezers and with particles as carriers, the delivery of agents or biomolecules to cells can be performed in a highly controlled manner. The force measurement capabilities of the NanoTracker™ further increases the field of studies by allowing investigations of the particle – cell interaction.

In figure 3, a 2-µm polystyrene particle is shown as it binds to a CV-1 cell. Under full 3-D positioning control, the bead

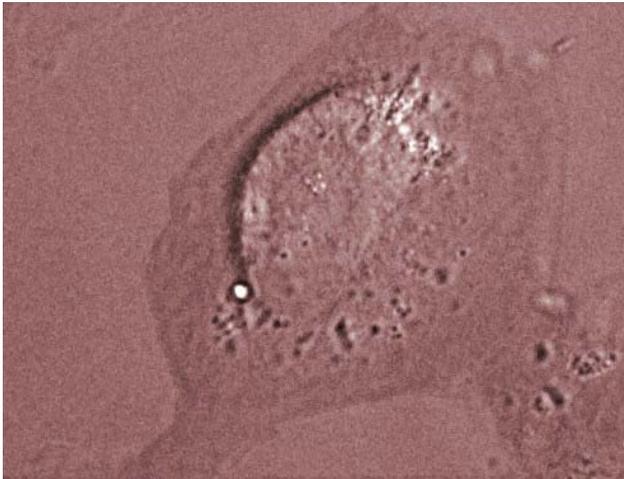


Figure 3: DIC image of a CV-1 cell with 2 μm polystyrene particle attached.

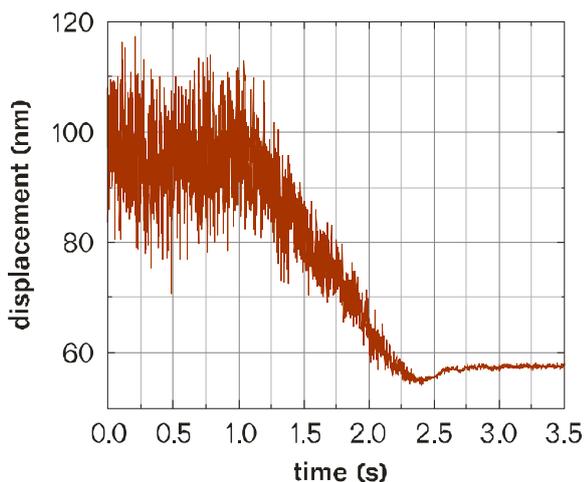


Figure 4: reducing displacement noise of a trapped particle attaching to a the cell, indicating membrane binding.

is steered towards the cell to allow it to interact with it. At the same time, the signal from the particle is recorded, showing the binding of the polystyrene particle to the cell: the signal shown in figure 4 clearly shows a drop in the amplitude of the Brownian motion exerted by the particle, revealing a restriction of the particle's motility as it binds to the cell. The signal can be further evaluated by performing an autocorrelation on it leading to time rates of different binding events [3,4].

Virus-receptor interactions probed at different pulling speeds

The force and time scale associated with bond rupture or protein rearrangement is known to be a dynamic property that depends on the force loading rate applied [5]. If the force on a molecule is applied slowly, there is more time for the thermal fluctuations to allow rearrangement and bond rupture, and the breaking force will be lower on average. If, on the other hand, the force is increased more quickly (higher pulling speed), then there is less time for the thermal fluctuations to take place, and the breaking force will be higher on average. The different time scales for rearrangement associated with such *dynamic force spectroscopy* may further lead to the preference of certain processes to take place in the molecular complex under investigation. This relation was validated in the following experiments presented here, where under different pulling speeds receptor-ligand bond rupture (lower speed) and tether pulling (higher speed) could be observed.

Polystyrene microspheres were covered with influenza viruses [6]. The influenza virus binds via its envelope protein hemagglutinin (HA) sialic acid (SA) residues of glycoproteins on the plasma membrane of the host cell. A proper binding of the viruses to the microspheres was verified with fluorescence imaging.

The virus-coated microspheres were then approached to

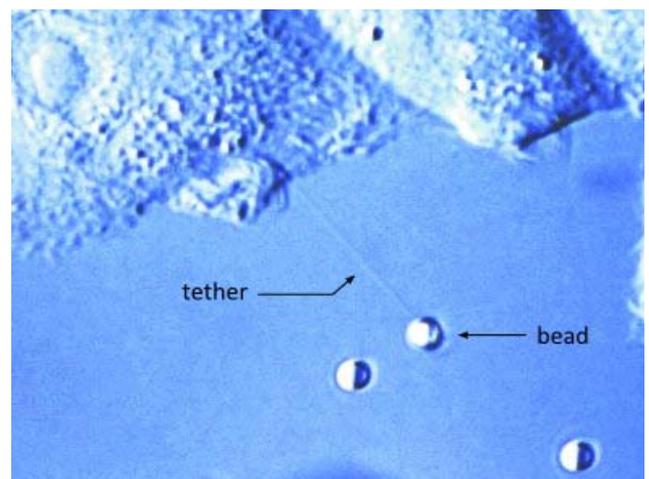


Figure 5: DIC image of a CHO cell with a membrane tether pulled by an optically trapped virus-coated microsphere.

the cells. After a short interaction time in the range of one to several seconds, the microsphere was retracted from the cell. Depending on the speed of retraction, membrane tethers were pulled from the cell before the membrane-virus interaction was ruptured [7, 8].

Figure 5 shows such a tether being pulled out from the membrane of a CHO cell. After an interaction time of two seconds, the virus-coated microsphere was retracted with a speed of 5 $\mu\text{m/s}$ by moving the piezo stage. At this relatively high pulling speed, the bond between the virus and its receptor was stable enough to allow tether formation, which includes plasma membrane - cytoskeleton separation and membrane reorganization.

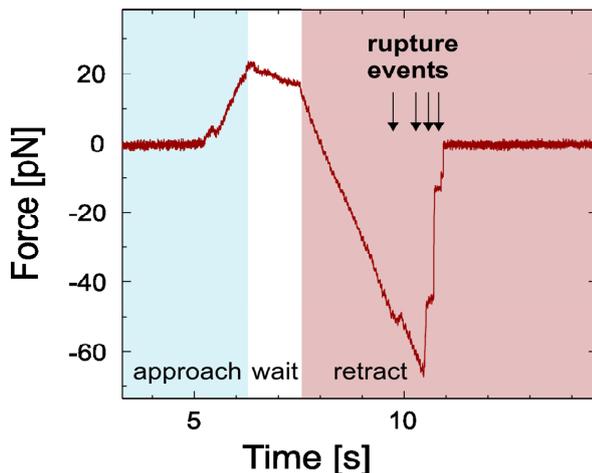


Figure 6: Measuring forces between cells and specifically coated microspheres. Approach and retract phases are shown. The retract phase shows bond ruptures in several steps.

In figure 6, the same experiment was performed at a lower retraction speed. The virus-coated particles were pushed into the cells with a force of about 20 pN (blue area in the graph). The viruses were let to interact with the cell (white area) and then retracted at 0.5 $\mu\text{m/s}$. From the stepwise unbinding seen in this phase we can deduce that multiple bonds had been formed.

These and similar studies allow to quantitatively dissect such cell membrane processes. As a complete solution including sample handling components like the PetriDishHeater™, the NanoTracker™ platform makes optical tweezers instrumentation available to a wide range of life science researchers.

Acknowledgment

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Literature

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