Supporting information

Fluorescent Tracking of Genome Release during Mechanical Unpacking of Single Viruses

A. Ortega-Esteban, K. Bodensiek, C. San Martín, M. Suomalainen, U. F. Greber, P. J. de Pablo, I. A.T. Schaap

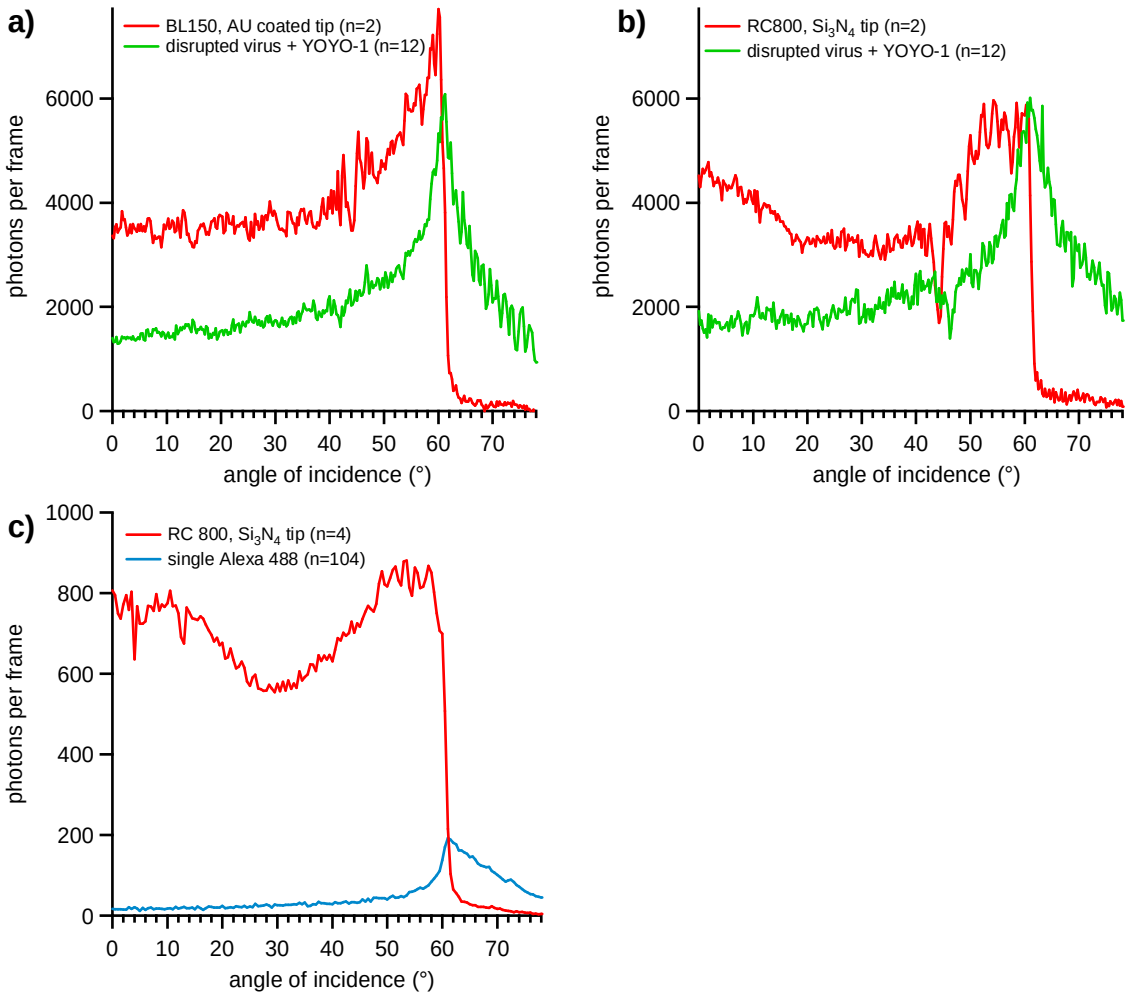

Figure S1. Fluorescence signal from AFM probe and sample at different illumination angles.

a, b) Background signal from two different types of AFM probe (in surface contact) compared to the intensity from single viruses, which became fluorescent in presence of 300 mM YOYO-1 due to capsid rupture. Sufficient signal-to-noise is only available in the TIRF regime, which starts at 61°. Only then the signal from the AFM probe drops to a level that is much smaller than the fluorescence signal from the virus. Laser intensity at 64° is 0.34 µW/µm², excitation beam diameter at 0° is 82 µm. The used emission band pass filter: 512/25. Both silicon nitride AFM cantilevers were from Olympus: BL150 (0.03 N/m, both sides gold coated) and RC800 (0.06 N/m, back side gold coated).

c) To prove single molecule sensitivity the same experiment as in b) was performed with a ribosome that was labeled with a single Alexa488 fluorophore. For this experiment the laser intensity was reduced by 5.6 x. The used emission band-pass filter: 525/50.

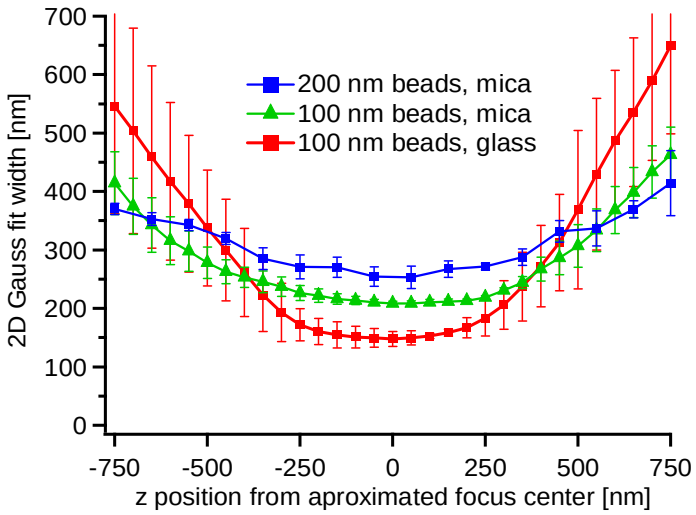

Figure S2: Fluorescent spot size at different focus positions.
To measure if the exact focus position had a large effect on the measured spot size we attached 100 nm diameter fluorescent beads (Tetraspeck™, Life Technologies, CA, USA) to the surface and changed the z-focus in steps of 50 or 100 nm. The spot size was obtained by using the width of a Gauss fit. For glass (red, n=3) the minimum width was 148 nm, larger than the theoretical spot size of a point source (71 nm) due to the convolution of multiple point sources that result from the use of a 100 nm diameter bead. For mica (green, n=3) the width increased to 208 nm, due to the refractive index mismatch. When 200 nm diameter fluorescent beads (Fluoresbrite®, Polysciences Europe GmbH, Germany) were used on mica, the fitted width further increased to 253 nm (blue, n=3), which shows that the fitted width scales with the size of the object. In all cases a change of focus of ± 250 nm had little effect on the spot size.

Movie S1: Slow unpacking of wild-type adenovirus.
Three-dimensional rendered AFM images show the temporal evolution of the virus integrity correlated with fluorescence microscopy emission images. The shown virus capsid is the same as the one shown in figure 3. The small changes in the vertical dimensions of the capsid is a consequence of the alternating scan direction (up-down scan direction is always followed by a down-up scan). The movie is sped up; the whole experiment took 40 minutes for the 21 AFM frames.

Movie S2: Fast unpacking of wild-type adenovirus.
Fluorescence emission while executing a nano-indentation on an intact virus capsid. On the left side, the whole field of view of the camera is presented. It can be observed that there are already some spots of light corresponding to broken viruses or dsDNA deposited on the substrate. On the right side, a zoom shows how the fluorescence emission increases while the viral particle is disrupted as presented in figure 4. 100 frames are shown that correspond to approximately 13 s of real time.