Optical Trapping and Sorting of the Human Immunodeficiency Viruses at Single-Molecule Level

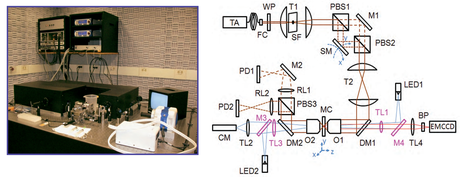
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Abstract

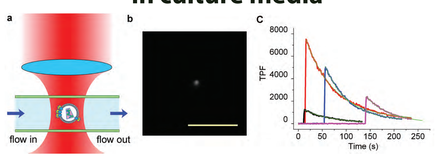
Optical tweezers (OTs) use the momentum of photons to trap and manipulate microscopic objects contact-free in three dimensions. Although this technique has been widely used in biology to study molecular motors, biopolymers, cytoskeletons, and for sorting of microparticles, its application in pathogenic viruses has been very limited largely due to the small size of these particles. Using an improved OTs instrument that can simultaneously resolve two-photon fluorescence (TPF) at single-molecule level, here we show that individual human immunodeficiency virus type 1 (HIV-1) internally tagged with green fluorescent proteins (GFP) can be optically trapped and manipulated in culture media. The hydrodynamic radius of each trapped virion can be directly measured. The wide intensity distribution of TPF from individual virions interrogated by the optical trap reveals that each HIV-1 incorporates a random number of Vpr molecules fused to GFP, with a range of 3-128 molecules per virion. By using fluorescent-labeled monoclonal antibodies specific for HIV-1 envelope glycoprotein gp120, we measured the envelope spike protein content for single virions in culture media. The quantity of spike proteins per virion varies widely and as low as a single envelope spike can be clearly detected. This broad distribution implies substantial heterogeneity of these virions in transmission and infection at single-particle level. Analogous to flow cytometry for cells, this fluid-based technique may allow detection and multi-parameter analysis of viruses and other nanoparticles within heterogeneous populations at single-particle and single-molecule resolution.

Optical tweezers/two-photon fluorescence microscope

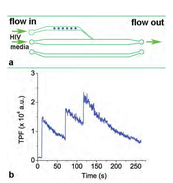


Schematic layout of the OTs/TPF microscope using TA diode laser. FC, fiber collimator; WP, wave plate; T1, T2, telescopes; SF, spatial filter; PBS1, PBS2, PBS3, polarizing beam splitters; M1, M2, M3, M4, reflective mirror; SM, steerable mirror; DM1, DM2, dichroic mirror; O1, O2, objectives; MC, microfluidic chamber; TL1, TL2, TL3, TL4, tube lens; RL1, RL2, relay lens, PD1, PD2, position-sensitive detectors.

Optical trapping of HIV virions in culture media

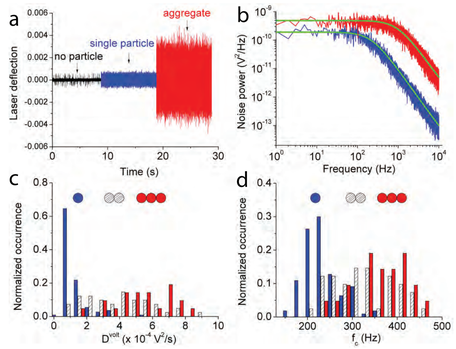


(a) HIV-1 virions were delivered into a microfluidic channel and trapped by the IR laser focused at the center of the channel. (b) TPF image of a trapped HIV virion recorded by an EMCCD camera. The scale bar is 10 μm. (c) Representative TPF time trajectories from individually trapped HIV virions. All traces were fitted with single exponential decay (green), with time constants as follows: 50.7 (red), 49.5 (blue), 51.5 (magenta) and 54.1 s (black).

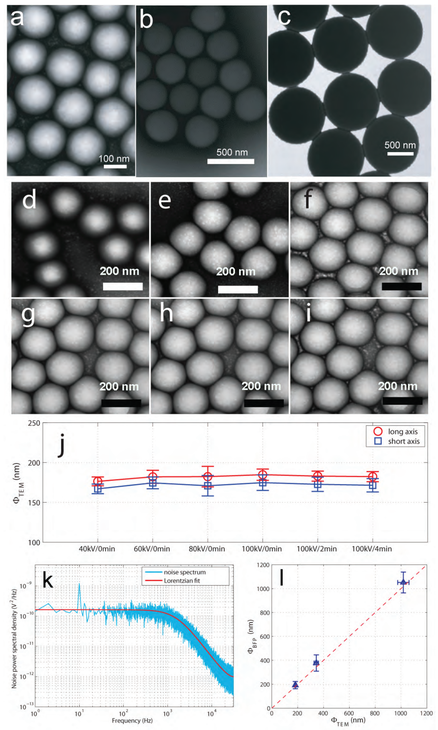


Sequential trapping of individual HIV-1 virions in microfluidic chamber. (a) Experimental design to deliver HIV-1 virions through microfluidic channels for sequential trapping of individual virions in culture media. HIV was delivered from the top channel while the complete media was injected through the middle channel. (b) Representative TPF intensity time course from sequentially trapped virions. The abrupt increase of TPF shown as sawtooth pattern indicates the sequential arrival and trapping of HIV-1 particles.

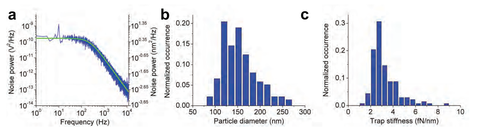
BFP measurement to sort HIV-1 virions based on size



(a) The laser deflection signal measured in real time using BFP interferometry. (b) Power spectra calculated from the data shown in (a). The blue and red spectra were fit to aliased Lorentzian. Histograms for electronic diffusion coefficient Dvolt (c) and corner frequency fc (d) derived from Lorentzian fitting parameters for one (blue), two (hashed) and three (red) virions being trapped.

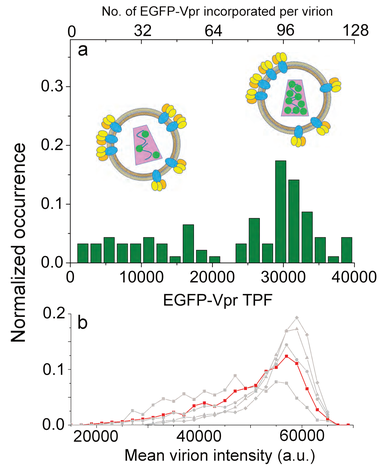


Comparison between TEM and BFP laser interferometry in the measurement of the diameters of reference polystyrene particles.

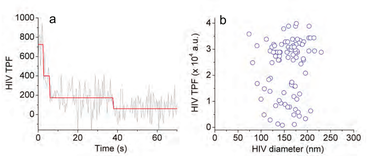


Measurement of diameter for single HIV-1. (a) The power spectrum of a trapped virion when the chamber was wiggled at 10Hz with amplitude of 176 nm along y-axis of the sample plane. The green curve is fitting of the thermal noise background to aliased Lorentzian. (b) Size histogram for single fluorescent particles from chamber wiggling along y-axis (N=137). (c) Distribution of the optical trap stiffness for each virion trapped.

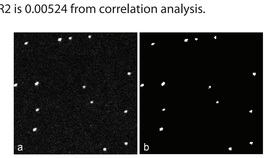
Quantitate # of EGFP-Vpr inside virions



HIV-1 randomly incorporates EGFP-Vpr into virions. (a) Distribution of EGFP-Vpr TPF intensity from single virions. (b) Mean virion intensity distributions based on confocal images of virions prepared with various amount of pEGFP-Vpr plasmids, at 0.1 (gray square, N=888), 0.3 (red square, N=2130), 0.5 (gray circle, N=2791), 1 (gray triangle, N=4894) and 2 μg (gray diamond, N=2786), respectively.

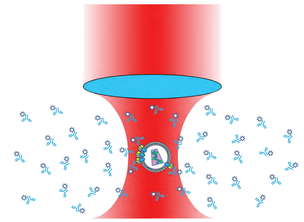


Fluorescence analysis of EGFP-Vpr incorporation. (a) For HIV-1 virions with low TPF intensity, clear steps could be identified from the TPF time course while the virion was trapped. (b) The TPF intensity of individual HIV-1 was plotted as a function of the virion diameter measured for each trapped particle based on laser deflection. The adjusted R2 is 0.00524 from correlation analysis.

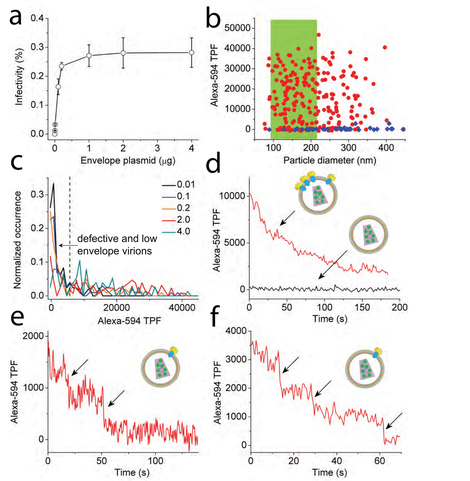


(a) Confocal images of EGFP-Vpr tagged HIV-1 virions immobilized on poly-L-Lysine coated coverslip. (b) Virion particles identified from the image shown in (a) using a custom-written Matlab image analysis script.

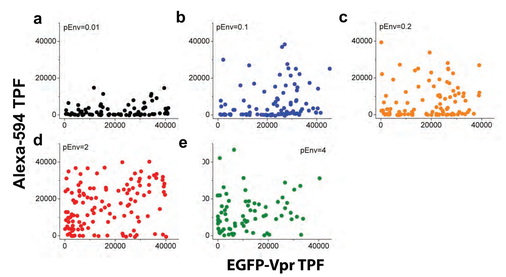
Optical Trapping 'Virometry'



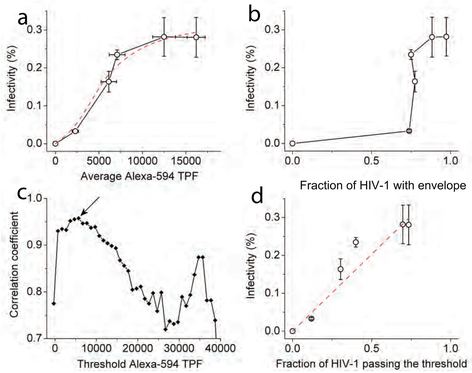
Schematic of optical trapping 'virometry' to monitor HIV envelope glycoproteins using optical tweezers at single-molecule and single-particle level.



(a) Infectivity of HIV-1 virions as a function of pEnv. The error bars are standard deviations from three independent replicates. (b) Alexa-594 TPF as a function of particle diameter. Red circles are for HIV-1 from 2 μg pEnv (N=231) while blue diamonds are for virions without envelope (N=42). (c) TPF intensity histograms for Alexa-594 from single HIV-1 particles, with black, blue, orange, red and olive curves for virions from 0.01 (N=84), 0.1 (N=102), 0.2 (N=100), 2.0 (N=128) and 4.0 μg pEnv (N=76), respectively. (d)-(f) representative Alexa-594 TPF time courses from single virions bound with fb12, where individual photobleaching steps are indicated.



Two-color correlation analysis for EGFP-Vpr and envelope glycoproteins in single HIV-1 virions. Alexa-594 TPF was plotted as a function of EGFP-Vpr TPF for virions prepared with 0.01, 0.1, 0.2, 2 and 4 μg pEnv, respectively as indicated.



Correlation of HIV-1 infectivity with envelope glycoprotein content. (a) HIV-1 infectivity as a function of average Alexa-594 TPF from virometry. The error bars for TPF are standard errors of the mean. The error bars for infectivity are standard deviations from three independent replicates. (b) HIV-1 infectivity as a function of the fraction of viruses with envelope glycoproteins. (c) Correlation coefficients derived from the threshold analysis for viral infectivity as a function of the threshold Alexa-594 TPF. (d) HIV-1 infectivity well correlated with the fraction of viruses whose envelope content passed the threshold denoted by the arrow in (c). The red dashed line shows the best linear fit.

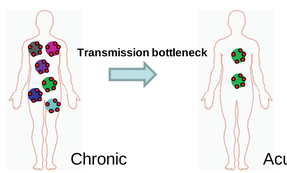
Conclusions

(1) Single HIV-1 can be optically manipulated in culture media 'contact-free'.
(2) Optical trapping 'Virometry' allows multi-parameter analysis of single HIV-1.
(3) Individual HIV-1 dramatically differs in gp120 and Vpr contents.
(4) A minimum of two trimeric spikes is required for the optimal infectivity of HIV-1.

Implications

Individual virions differ in their ability to infect host cells, 'elite' viruses

Facts:
(1) 76% infection due to a single virus (Keele et al. PNAS, 2008, 105: 7552)
(2) Transmitted virus is enriched for higher Env content (Parrish et al. PNAS, 2013, 110: 6626): "Such excess is likely of advantage during the earliest stages of HIV-1 Infection"



Acknowledgment

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