Published in final edited form as:

Proc SPIE Int Soc Opt Eng. 2018 August ; 10723: . doi:10.1117/12.2500050.

A method for tethering single viral particles for virus-cell interaction studies with optical tweezers

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Abstract

Direct optical trapping of single viral particles allows characterization of individual particles in suspension with single-molecule sensitivity. Alternative to direct optical trapping of particles, individual particles may be tethered specifically in suspension for manipulation by optical tweezers indirectly, which could be useful for studies of virus-cell interactions. One specific example is the interactions between cell surface receptors and the envelope glycoproteins (Env) on the surface of human immunodeficiency virus type 1 (HIV-1). Env binds to cellular receptors and undergoes a series of conformational changes, culminating in fusion of the viral and cellular membranes that mediates viral entry into cells. In addition to being required for cellular infection, Env is also the sole target for neutralizing antibodies. Thus, significant research has focused on elucidating the structure of Env and the mechanism of HIV-1 entry. However, current methods are unable to resolve the dynamics and stoichiometry of Env binding to cellular receptors during the entry process. Fluorescence and electron microscopy have visualized Env clusters in the viral membrane, but the extent to which these clusters actually bind to cellular receptors, and the mechanism of cluster formation, remain unclear. We describe the development of an optical tweezers technique that can potentially address these questions by delivering a single HIV-1 virion to a live cell with minimal perturbation to the system. Our method can be used to quantitatively probe the physical interactions between Env and cellular receptors in their native environment, which may reveal critical parameters in HIV-1 entry. Furthermore, our method can be used to investigate other protein-protein interactions in the context of live cells, such as the recognition of particulate antigens by B cells, thus offering insight into fundamental features of protein-mediated receptor activation.

⁶.AUTHOR CONTRIBUTIONS

W. C. directed the project; K. S. conducted manipulation experiments; K. S. prepared and assayed HIV-1 virions; K. S. and W.C. wrote the paper.

Keywords

Optical trapping; HIV-1; virometry; manipulation; single-molecule biophysics; virus-cell interactions

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1. INTRODUCTION

Receptor binding on the cell surface underlines many important biological processes ranging from viral entry to signal transduction at the cell surface. Although the qualitative aspect of this fundamental event has been known for a long time, the quantitative aspect of this event remains incompletely characterized for many of these processes. One specific example is the process of HIV-1 entry. HIV-1 is an enveloped retrovirus that primarily infects T lymphocytes and macrophages *in vivo*. As the etiological agent of acquired immunodeficiency syndrome (AIDS), the HIV-1 replication cycle has been the subject of intense research over the last thirty-five years. Despite considerable advances, the mechanistic details for many steps in the cycle remain elusive due to inadequate techniques.

HIV-1 has a lipid bilayer that is derived from the host cell plasma membrane during viral assembly and budding. In HIV-1, 12 different viral-encoded proteins are present in the mature virion [2], together with numerous incorporated cellular proteins [1]. As an enveloped virus, HIV-1 requires a cellular entry scheme that will liberate its contents from the protective viral membrane in order to initiate infection. Entry is mediated by envelope glycoproteins (Env) in the viral membrane, which bind to cell surface receptors in a coordinated, sequential fashion and cause fusion of viral and cell membranes [3]. The viral core can then enter the cytoplasm and initiate replication.

Env functions as a trimer of heterodimers composed of noncovalently associated surface gp120 and transmembrane gp41 subunits [4–6]. Gp120 binding to a CD4 receptor on the target cell surface induces a conformational change in gp120 that exposes a binding site for a chemokine coreceptor, most commonly CCR5 or CXCR4. Gp120 binding to coreceptor causes additional conformational changes, which trigger the fusogenic machinery in gp41, leading to virus-cell membrane fusion and delivery of the viral payload into the cytoplasm.

In addition to its essential role in productive cellular entry, Env is the only viral immunogen on HIV-1 surface, making it the sole target for neutralizing antibodies produced by the humoral immune system [7]. Therefore, it is a focus for the development of neutralizing antibody-eliciting vaccines and entry inhibitors. Moreover, Env-mediated cellular entry can reveal general features applicable to other systems, including receptor-mediated signaling, membrane fusion, and binding cooperativity.

Elegant structural and biochemical studies have provided significant insight into the sequence of receptor engagement and corresponding Env remodeling prior to and during cellular entry. However, several fundamental issues pertaining to virus-cell interactions and subsequent entry remain controversial. Clarifying these issues is crucial for understanding basic HIV-1 entry mechanisms and informing rational drug design.

Strikingly, HIV-1 Env appears to be sparsely distributed on the viral surface compared to glycoproteins on other enveloped viruses such as influenza [8]. Nevertheless, HIV-1 is still able to infect cells, raising the question of potential compensatory mechanisms. Recent studies suggest that maturation-dependent clustering of Env in the viral membrane is correlated with fusion competence [9], Superresolution fluorescence [9] and cryo-electron microscopy [10] can visualize Env distribution on virions but are unable to quantify the extent of receptor engagement. Thus, the dynamics and functional role of Env clustering remain unclear.

It is possible that spontaneous clustering occurs independently of virus-cell interaction, merely as a byproduct of Gag proteolysis during structural maturation of virions after budding from the cell; dissolution of the lattice could allow Env clustering driven by self-association of the gp41 cytoplasmic tail (CT). Alternatively, specific interactions with cellular CD4 receptors could induce Env clustering. Distinguishing between these two models motivates the development of a technique that can monitor high-resolution dynamics of Env clustering and probe cellular interactions while retaining the native states of the virus and cell.

Force spectroscopy has been used extensively to quantify receptor-ligand interactions [11]. By varying external loading rates, it is possible to link bond strength to molecular-scale chemistry. Atomic force microscopy (AFM) has been used to measure interactions between HIV-1 and cellular receptors [12]. However, this study used a cantilever with attached virions to force contact with a cell, which imposed artificial constraints on the geometry and number of bonds formed. They report that their virus functionalization, using LC-SMCC treatment of gp120, had no noticeable effect on viral infection. However, it is possible that modification of gp120 could still affect binding properties, and the long spacer arm in LC-SMCC has an unknown effect on force measurements. Furthermore, attaching virions to a cantilever via gp120 prevents an unknown number of Env from interacting with cells. Therefore, this study was unable to reliably investigate dynamical binding of multiple Env over long time courses.

Optical tweezers (OTs) offer significant advantages over AFM for this functional investigation; they can isolate a single virion in suspension that is free to rotate and interact with a cell diffusively, they have superior force sensitivity and dynamic range, and thus have the potential to allow monitoring of virion internalization.

We thus set out to design an optical tweezers-based technique with which we can manipulate a single virion in suspension by linking it to an optically trapped bead via a DNA tether. It will then be possible to measure near-native, physical interactions of the virion with the surface of a micropipette-immobilized cell, as schematically shown in Fig. 1. This technique opens up new ways for investigation of particle-cell interactions.

2. RESULTS

2.1 Metabolically biotinylating an HIV-1-incorporated cellular protein in virus-producing cells

Isolating a virion for cellular delivery requires a labeling scheme with high specificity and minimal structural perturbation. As new virions bud from the cell, they incorporate host cellular proteins [1]. In order to target single virions without disrupting Env structure or function, we hypothesized that we could utilize the natural viral budding process to incorporate a biotinylated cell surface protein into nascent HIV-1 virions. This would serve as an anchoring target in the virion, leaving viral proteins intact. We could then use dual-labeled DNA tethers to link biotinylated virions to an optically trapped bead. Steering this bead would allow us to move the virion close to a cell, but the flexible DNA tether would allow the virion to still diffuse and interact with the cell.

Among other labeling methods that commonly rely on in vitro manipulation of the virus pool, we chose metabolic biotinylation because it enabled site-specific labeling within the cell to occur simultaneously with virion production. We wanted to maximize the strength of the bond between the protein anchor and the DNA tether so that upon pulling, we would measure protein-protein interactions between viral and cellular proteins rather than simply rupturing the DNA from the virion. The strong affinity between biotin and streptavidin would allow for this. The small size of biotin (244.3 Da) was also less likely to interfere with protein incorporation than larger tags and an excess would be efficient to remove.

Metabolic biotinylation technique uses an *Escherichia coli* biotin ligase, BirA, for sequence-specific ligation of biotin to a lysine in a 15 amino acid acceptor peptide (AP) [13, 14]. When AP is fused to the extracellular domain of a protein of interest, this metabolic approach facilitates specific, high-efficiency biotinylation of cell surface proteins [15, 16]. These biotinylated proteins can then be incorporated into budding virions when BirA and the fusion protein of interest are coexpressed in virus-producing cells [17]. This will allow high-affinity binding to a streptavidin-conjugated DNA tether linked to a trapped bead to manipulate single virions.

In order to select a cellular protein that is incorporated into HIV-1 virions for biotinylation, we wrote a MATLAB script to compare the expression library of 293T virus-producing cells (54,675 proteins) with the list of cellular proteins found in HIV-1 [18] (Cellular Proteins in HIV-1, https://ncifrederick.cancer.gov/research/avp/protein_db.asp) (303 proteins), narrowed to those localized to HIV-1 surface (131 proteins). We further restricted the list by protein structure and function, hypothesizing that transmembrane proteins with substantial cytoplasmic domains would be more difficult to pull from the viral membrane upon applied force. We also sought a protein whose overexpression has not been shown to influence viral infectivity. In the end, we chose the transferrin receptor (TfR) on the basis of its demonstrated incorporation into the viral membrane [19], endogenous expression in virus-producing cells, expected stability in the membrane, and functional insignificance to the virus (see Section 2.2). TfR has 67 amino acid in its cytoplasmic domain, which might stably anchor it in the viral membrane. By expressing transferrin receptor fused with AP at its C-terminal extracellular domain and BirA in virus-producing cells, it would be possible

for nascent HIV-1 virions to incorporate biotinylated transferrin receptors as they bud from the plasma membrane.

To test whether our HIV-1 virions had indeed incorporated biotinylated transferrin receptors, we first performed western blotting on iGFP virus preparations produced with BG505 Env. During virion production, varying amounts of TfR plasmid were transfected. The culture supernatants containing HIV-1 virions were harvested and assayed using streptavidin conjugated with alkaline phosphatase. As shown in Fig. 2A, our results indicate the presence of increasing biotinylated TfR in virus preparations with increasing plasmid input (Fig. 2A).

Although western blots show the presence of biotinylated TfR in bulk virus preparations, they do not necessarily indicate incorporation; TfR could be present in non-viral microvesicles that bud off from the plasma membrane. To test that individual virions indeed contain biotinylated TfR, we have used the optical trapping virometry technique that we recently developed in the lab [20]. This method works by direct optical trapping of individual HIV-1 virions in suspension and the coincident two-photon fluorescence that is resolved with single-molecule sensitivity provides a sensitive readout for the molecules associated with the trapped single-virion. Briefly, biotinylated virion preparations were incubated with Alexa-594 labeled streptavidin and flowed into a microfluidic chamber for trapping and measurement. The presence of enhanced green fluorescent protein (EGFP) fluorescence indicates the presence of individual virions while the associated Alexa-594 fluorescence unequivocally identifies the presence of biotinylated TfR associated with the virions. As shown in Fig. 2B, fluorescence measurements of Alexa 594 per virion comprise a broad distribution. Some virions have no Alexa-594 signal, but substantial fractions of virions showed Alexa-594 fluorescence. Importantly, HIV-1 produced in the absence of pTfR does not nonspecifically bind to fluorescent streptavidin when measured by virometry. Therefore, these results demonstrate that we were indeed able to metabolically biotinylate HIV-1 virions through incorporation of TfR.

2.2 Incorporation of TfR preserves HIV-1 infectivity

For future applications of this technique, it is crucially important to maintain the infectivity of HIV-1 containing biotinylated TfR. To this end, we analyzed the effect of TfR overexpression and incorporation into the viral membrane by transfecting different amounts of TfR plasmid into 293T virus-producing cells along with fixed amounts of viral plasmids. We performed infectivity assays using TZM-bl cells, as described [21]. To assess the effect of TfR expression and the presence of exogenous biotin on virus production in 293T cells, we performed an enzyme-linked immunosorbent assay (ELISA) to detect HIV-1 p24 capsid protein. Figure 3A shows the concentration of virion particles as a function of TfR plasmid (pTfR) input during transfection for two strains of HIV-1 with two different Env. Although p24 concentration decreases with increasing TfR plasmid input, there is a concomitant decrease in the concentration of infectious virions; thus, the infectivity remains relatively stable (Figure 3B). The similar trend in reduction of virus production with increasing pTfR between the two strains suggests that this may be a fundamental feature of competition between different overexpressed proteins. Indeed, when we equalized the total DNA of two different pTfR conditions by adding carrier DNA (pcDNA3.1), the p24 also equalized.

Trypan blue staining of virus-producing cells indicated that 100 μ M biotin was not cytotoxic compared to complete media alone, regardless of when it was added (data not shown).

2.3 Removal of free biotin while preserving HIV-1 infectivity

According to the scheme shown in Fig. 1, in order to achieve efficient binding of biotinylated HIV-1 virions to streptavidin at the end of a biotinylated DNA tether, it was necessary to remove unbound biotin from virus preparations while still preserving viral stability. Among various methods that may serve this goal, we have specifically chosen dialysis for this purpose, which does not involve concentration of the virus that would otherwise violate the biosafety regulations of current lab setting. The dialysis was carried out at 4°C throughout in order to preserve the infectivity of HIV-1 virions. As shown in Fig. 4, the iGFP virus sample taken out at different time points after dialysis does not show significant drop in infectivity until 20 hours after the initiation of the dialysis procedure. Thus, we have limited the duration of dialysis to less than 24 hours in order to remove free biotin while preserving HIV-1 infectivity.

2.4 Biotinylated DNA tethers bind streptavidin

We have successfully made a double-stranded DNA (dsDNA) molecule that has functional groups on both ends (Fig. 5A). This DNA can be attached on one end to the surface of a polystyrene sphere coated by anti-digoxigenin through digoxigenin, and on the other to a single streptavidin through biotin. Because streptavidin has four biotin binding sites, this single streptavidin can be used as an ‘adaptor’ to attach additional biotinylated virion particles. We have tested this ‘streptavidin adaptor’ idea by probing this bead-DNA-streptavidin assembly on the surface of a biotin-coated polystyrene bead. We can obtain a single dsDNA tether between two beads reproducibly and robustly. No tethers were formed when there was no streptavidin. A single-DNA tether can be clearly distinguished from a multiple-DNA tether from the overstretch transition [22] observed when the molecule was pulled over 60 pN in force (Fig. 5B).

2.5 Bead-DNA tether-virion complex can be used to manipulate single HIV-1 virions in suspension

By combining biotinylated HIV-1 with dual-labeled DNA tethers and polystyrene spheres, it is possible to move the virion around in the flow chamber and deliver it to a live cell immobilized on a micropipette. In Video 1, an anti-digoxigenin antibody-coated polystyrene sphere is optically trapped and moved around the flow chamber. A fluorescent particle (virion) can be visualized at a distance from the bead approximately equal to the length of the DNA tether, 8 μ m. This video is a proof of principle of our technique. Upon placement of a live cell in the same channel atop of a micropipette [23], it would be possible to deliver the virion to the cell and measure early events in virion attachment. Future experiments under conditions that are conducive to virion internalization may allow observation of those events and measurement of their efficiency relative to virion attachment.

3. DISCUSSION

Receptor binding on the cell surface underlines many important biological processes. Although the qualitative aspect of this event has been known for many different systems, the quantitative and in-depth understanding of this event remains largely underdeveloped for many of these processes. In this work, we have developed a novel technique for study of the early events in virion-cell attachment. In this technique, a single virion was tethered to the end of a single DNA molecule, which allows manipulation of the virion and its potential delivery to a target host cell. This technique allows us to study virion interaction with its host cells, one at a time.

In this work, we have focused on a specific system of HIV-1 virions. HIV-1 entry into cells is a highly coordinated and dynamic process. Much has been revealed about the coordination of key players during entry, but limitations on techniques have hindered further mechanistic insight. These details may be crucial to entry inhibitor development. In the past, HIV-1 entry has mainly been studied in bulk, with many cells and many virions. Using optical tweezers to study single virus-cell interactions at the single-molecule level is unprecedented. The technique described here allows the possibility of probing virus-cell interactions via rupture force measurements at varying loading rates, allowing decomposition of multiple simultaneous interactions. By isolating selective steps with inhibitors, one could decipher the physical framework for different HIV-1 entry pathways. This experimental geometry could also reveal the probability of nonspecific interactions versus specific Env-receptor binding at a single-particle level; optical tweezers allow the virion to sample the surface of the cell without much constraint, maintaining physiological conditions.

The technique introduced here has unique potential to uncover the dynamics of Env clustering and cooperative binding. We expect that it will lend insight into essential viral entry mechanisms applicable to other viruses. It is also ideally suited to compare binding of different envelope glycoproteins across different viral strains at high resolution. Because it is able to directly probe the magnitude of interactions, our technique could be adapted to other fundamental questions in biology, such as protein-protein interactions and receptor-mediated B cell activation.

Lastly, this study also demonstrates the feasibility of using virometry [24] to quantify the copy number of cellular proteins incorporated into the viral membrane at a single-virion, single-molecule level. Although the incorporation of cellular proteins into HIV-1 has been well established, the identity, copy number, and heterogeneity among virions remain elusive due to lack of techniques available. The abundance of certain cellular proteins in HIV-1 may have a functional importance in enhancing cellular interactions, for example [25–27]. Thus, it is possible to use virometry to quantify protein copy number and associate this with physical interactions that our technique can directly measure.

In summary, we have described the development of a novel single-molecule technique that can be used to study interactions between single virion particles and live cells. This technique opens the door to many previously inaccessible questions about viral entry and more broadly, receptor-ligand interactions in the context of live cells. Overall, this technique

and the principles discovered will potentially elucidate mechanisms of viral entry, reveal fundamental information on the cooperativity of receptor-ligand interactions, and have broad applicability to other systems such as antigen-mediated B cell activation [28–31].

4. MATERIALS AND METHODS

4.1 Cell culture and production of biotinylated HIV-1 virions

Human embryonic kidney 293T cells were maintained at 37°C with 5% CO₂ in Dulbecco's Modified Eagle's Medium (DMEM, ATCC, Manassas, VA). TZM-bl cells were maintained at 37°C with 5% CO₂ in DMEM (HyClone, Logan, UT). Both cell lines were supplemented with 10% Defined Fetal Bovine Serum (HyClone, Logan, UT). Complete medium refers to DMEM with 10% Defined Fetal Bovine Serum.

The procedures to produce biotinylated HIV-1 virions are modified based on our procedures to produce HIV-1 virions as published previously [21]. Briefly, pTfR-AP-IRES-BirA-ER (hereafter pTfR, for simplicity) [32] was subcloned into the pcDNA3.1(+) vector for expression in 293T cells using EcoRI and NotI restriction sites. One of the plasmids encodes HIV-1 proviral DNA pNL4-3 that contains an EGFP flanked in between the matrix and capsid domains of the Gag protein (iGFP) [33] and carries a premature stop codon in gp120/gp41 coding sequence. 293T cells were seeded in 2 mL culture volumes in 35 mm wells the day before transfection. The following plasmid amounts were added to each 35 mm well. For EGFP-Vpr virions, 293T cells were transfected with 1 µg pNL4-3R-E-, 1 µg envelope plasmid encoding either a fully functional NL4-3 or BG505 envelope glycoprotein gp120/gp41 as indicated in the text, 0.3 µg pEGFP-Vpr [21] and varying amounts of pTfR with TransIT LT-1 transfection reagent (Mirus Bio, Madison, WI). For iGFP virions, 293T cells were transfected with 1 µg pNL4-3-iGFP2 and different envelope plasmids. Before transfection, medium was supplemented with 100 µM biotin, which was previously shown to be saturating for BirA in 293T cells (Nesbeth et al., 2006). Medium was changed 6 hours post-transfection, maintaining the 100 µM biotin supplement. 24 hours post-transfection, culture supernatant was collected and filtered through a 0.45 µm syringe filter. Virus preparations were then either aliquoted on ice and flash-frozen in liquid nitrogen, or dialyzed against PBS at 4°C in Spectra/Por Float-A-Lyzer G2 dialysis devices (Spectrum Laboratories, Inc., Rancho Dominguez, CA) with 1000kD molecular weight cutoffs (MWCOs) against PBS pH 7.4 for varying times. Immediately after collection, they were flash-frozen in liquid nitrogen. All viruses were stored at –80°C.

HIV-1 concentration was assayed with a p24 enzyme-linked immunosorbent assay (ELISA) as previously described [21] using the HIV-1 p24 Antigen Capture Kit (Advanced Bioscience Laboratories, Rockville, MD), following the manufacturer's instructions. p24 concentration was converted to virion concentration via the assumption of 10 million virions per ng p24 [21]. The concentration of infectious virions (titer) was measured using the TZM-bl indicator cell line, as described [21]. Infectivity was calculated by taking the ratio of infectious virion concentration to total virion concentration (titer/p24). Conditions were chosen with the goal of maintaining infectivity and maximizing biotinylated TfR incorporation.

4.2 Western blotting

Virus samples were mixed with 6x SDS-PAGE sample loading buffer containing 9% p-mercaptoethanol and cell lysates were mixed with 2x Laemmli sample buffer containing 5% p-mercaptoethanol. Samples were heated at 95°C for 5 minutes. After cooling to room temperature, samples were run on 10% SDS-PAGE. Bands were then transferred onto supported nitrocellulose membranes. Streptavidin-alkaline phosphatase conjugate (Invitrogen, Catalog #43–4322) at a concentration of 1.5 ug/mL was used to detect biotinylated protein. Protein bands were colorimetrically detected with nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl-phosphate, toluidine salt substrates (NBT/BCIP, Roche) in a buffer containing 0.1 M Tris-HCl, 0.1 M NaCl, and 0.05 M MgCl₂ at pH 9.5.

To test for expression of biotinylated TfR in 293T cells, cell lysates were also prepared. 293T cells were transfected with pTfR under the same conditions as virus-producing cells. Cells were washed with cold PBS and then incubated with 200 uL RIPA Lysis and Extraction Buffer containing protease inhibitor on ice for 15 minutes. Cells were collected and centrifuged at 12,000 rpm for 20 minutes at 4°C. The supernatant was collected and samples were stored at –80°C.

4.3 Optical tweezers and virometry

The trapping of individual virions in solution was done as described previously [24] with a few modifications. Throughout, a home-made OTs instrument using a tapered amplifier diode laser at 830 nm (SYS-420–830–1000, Sacher LaserTechnik LLC, Germany) was used for optical trapping of individual HIV-1 virions [34]. Briefly, a laser power of 130.8 mW at the focus was used throughout for optical trapping and simultaneous TPF excitation. A Coherent OBIS solid state laser at 488 nm was used for epifluorescence imaging of EGFP-labeled virions. An electron-multiplying charge-coupled device (EMCCD) camera (Evolve, Photometrics) was used for all fluorescence detection including epifluorescence imaging. Individual HIV-1 virions were identified based on an EGFP positive signal and a measured diameter that lies between 96 and 216 nm as described previously [24]. Virometry to quantify biotinylated TfR copy number per virion was performed as described [20] with modifications. Alexa Fluor 594 streptavidin conjugate (Molecular Probes, Invitrogen) (SVD-Alexa 594) was used to detect and quantify biotinylated TfR in optically trapped virions. Briefly, dialyzed HIV-1 with incorporated biotinylated TfR was thawed from –80°C and incubated with 10 nM SVD-Alexa 594 for 1 hour at 20°C in the dark. The mixture was then diluted in PBS prior to injection into the flow chamber such that the final concentrations were 2 nM SVD-Alexa 594 and $0.8\text{--}1.3 \times 10^8$ virions/mL. These virus concentrations are higher than those found to aggregate in Pang et al., 2014 because here, dialysis removed much of the protein from the culture media that may have promoted aggregation in the previous study. This allowed higher-throughput measurements. All trapping experiments were conducted at $20.0 \pm 0.2^\circ\text{C}$.

4.4 Preparation of DNA tethers

Doubly-labeled, double-stranded DNA tethers were prepared as follows. To generate Digoxigenin handles, a 510 bp fragment of Lambda DNA (New England BioLabs (NEB), Ipswich, MA) was PCR amplified using Taq polymerase. Digoxigenin (Dig)-dUTP was

incorporated during PCR. The PCR product was purified with QIAquick PCR Purification Kit (Qiagen, Germantown, MD) and digested with XbaI (NEB). The digestion reaction was then treated with Antarctic phosphatase (NEB) and subsequently purified again with QIAquick PCR Purification Kit. To generate the long, biotinylated part of the tether, Lambda DNA was heated at 65°C for 5 minutes to melt the cos sites and immediately quenched on ice to form hairpins. Klenow Fragment (3' → 5' exo-) (NEB) was used to incorporate Biotin-14-dATP and Biotin-14-dCTP (Invitrogen, Carlsbad, CA), after which the reaction was passed through a Micro Bio-Spin P-6 Gel Column (Bio-Rad, Hercules, CA) equilibrated with 1x New England Buffer 2 (NEB). This product was then digested by XbaI and ligated with the phosphatase-treated Dig handle at a molar ratio of Dig handle : Lambda DNA of 2:1.

To form linkages for virion binding, doubly-labeled DNA tethers were incubated with 115 nM streptavidin, a 50-fold molar excess over DNA, for 30 minutes at 20°C and then 1 hour on ice. However, since unbound streptavidin would compete for virion binding, free streptavidin was removed by dialyzing DNA tethers against PBS for 87.5 hours at 4°C in 1000 kD MWCO membranes, with a total of four buffer exchanges.

4.5 Assembling bead-DNA tether-virion complex

Here, we report the conditions used to obtain Video 1, in which an optically trapped bead drags a fluorescent HIV-1 virion via a DNA tether in a microfluidic chamber. Dig antibody-coated beads, DNA tethers, and virions were mixed as follows. First, 1 volume dual-labeled DNA tethers was incubated with 1 volume Dig antibody-coated beads and 3 volumes PBS overnight at 4°C, which was a ratio of 1735 tethers/bead. The next day, biotinylated, dialyzed HIV-1 was added to the bead-DNA mixture at an excess of 100 virions/bead and incubated on ice 3.5 hours. To remove unbound virions, the mixture was then centrifuged 3 times at 6000g for 5 minutes, resuspended in 40 µL PBS after each spin, and resuspended in 10 µL PBS after the final spin. 5 µL of this was then diluted in 395 µL PBS before injection into the microfluidic chamber.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

This work was supported by NIH Awards 1R21AI135559-01A1 and 1DP20D008693-01 to WC, NSF CAREER Award CHE1149670 to WC and also in part by Research Grant No. 5-FY10-490 to WC from the March of Dimes Foundation. We thank Professor Allen Liu for kindly providing the pTfR-AP-IRES-BirA-ER plasmid. We thank Professor Benjamin Chen for kindly providing the provirus plasmids encoding iGFP virions. We thank Professor John Moore for kindly providing the plasmid encoding HIV-1 BG505 envelope. The following reagents were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH): pEGFP-Vpr from Warner C. Greene; pNL4-3 from Dr. Malcolm Martin; TZM-bl cells from Dr. John C. Kappes, Dr. Xiaoyun Wu and Tranzyme Inc.

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Figure 1.

Schematic design to tether a single HIV-1 virion at the end of a single DNA. The virion can then be manipulated through the DNA tether to probe the interaction between the virion and the cell immobilized atop of a micropipette.

Figure 2.

Metabolic biotinylation of individual HIV-1 virions. (A) Western blotting of virion preparations with varied inputs of TfR (indicated in the figure) and BirA expression plasmid detected with streptavidin-alkaline phosphatase conjugate. Loaded samples were diluted to have equal p24 concentrations. Lysate from 293T cells transfected with pTfR-AP-IRES-BirA-ER (pTfR) serves as a positive control. (B) Optical trapping virometry results for individual HIV-1 virions: Left: histogram of Alexa 594 fluorescence, proportional to the number of biotinylated transferrin receptors, in individually trapped virions. Right: histogram of EGFP fluorescence in individually trapped virions. $N = 28$ virions trapped on the same day.

Figure 3.

Effect of TfR incorporation on HIV-1 production and infectivity. (A) Concentration of physical virion particles as a function of pTfR plasmid input, as measured by p24 ELISA. Open circles: iGFP backbone with 1 μ g BG505 pEnv; black circles: EGFP-Vpr backbone with 1 μ g NL4-3 pEnv. (B) Infectivity of EGFP-Vpr virions as a function of pTfR plasmid input. The concentration of infectious particles is measured by TSM-bl cell assay. Infectivity is the percentage of apparent infectious virions in a sample.

Figure 4.

Effect of dialysis on HIV-1 stability. iGFP virions (with 1 μ g BG505 pEnv and 2 μ g pTfR) as a function of dialysis time in PBS using 1000 kD MWCO membrane. (A) concentration of physical virion particles as measured by p24 ELISA. Error bars are standard deviations of duplicate trials. (B) infectivity of virions. Error bars are propagated uncertainties using standard deviations of p24 and titer. The right y-axis is normalized by the sample diluted in complete media at 0 h of incubation.

Figure 5.

Biotinylated DNA tether binds streptavidin. (A) Schematic of the double-stranded DNA tether prepared from half lambda DNA that contains functional groups as indicated on both ends of the molecule. (B) A representative force-extension curve for a single DNA tether formed between an anti-digoxigenin coated polystyrene particle and a biotin-coated polystyrene particle. The length of the overstretch transition in this figure measures 5.4 μm , consistent with that of a half-lambda DNA.

Video 1.

Bead-DNA-virion complex can be used to manipulate single HIV-1 virions in suspension using optical tweezers. In this video taken in fluorescent view, a polystyrene sphere was optically trapped close to the center of the view, a fluorescent particle is tethered around 8 μm (the tether length) away, being dragged around the micro fluidic chamber.

<http://dx.doi.org/doi.number.goes.here>