Imaging the replication of single viruses: lessons learned from HIV and future challenges to overcome

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The molecular composition of viral particles indicates that a single virion is capable of initiating an infection. However, the majority of viruses that comes into contact with cells fails to infect them. To understand what makes one viral particle more successful than others, one needs to visualize the infection process directly in living cells, one virion at a time. In this perspective, we explain how single virus imaging using fluorescence microscopy can provide answers to unsolved questions in virology. We discuss fluorescent labeling of virus particles, resolution at the sub-viral and molecular level, tracking in living cells, and imaging of interactions between viral and host proteins. We end this perspective with a set of remaining questions in understanding the life cycle of retrovirus, and how imaging a single virus can help researchers addressing these questions. While we use examples from the HIV field, these methods are of value for the study of other viruses as well.

THE IMPORTANCE OF IMAGING SINGLE VIRAL PARTICLES

Viruses are small genetic entities that utterly depend on infecting host cells to reproduce. Retroviruses are enveloped RNA viruses, between 80-150 nm in diameter, that contain two copies of a single-stranded 7-12 kb genome.[1–4] Due to their limited genome, retroviruses require a host for successful replication. By integrating a double-stranded DNA copy of their viral genome into the host genome, they ensure replication along with the host genome during each cell cycle.

The cycle of viral replication of HIV-1 is depicted in Figure 1. Most of the steps of the viral replication cycle have been studied via ensemble biochemistry. The knowledge obtained from these investigations has been crucial for the development of the viral inhibitors available today. However, it is generally accepted that only 0.1 to 10 % of the virions binding to the cell surface eventually integrate their genome into the host.[4] What makes a virus particle successful? Does a successful virus follow a different road within the cell? Which protein interactions determine

productive infections? Understanding the molecular mechanisms behind viral infections, and how these affect cells, is crucial in developing new treatments or vaccines. Only a single virus and single molecule analysis can reveal which different steps and host factors are required for a successful infection.

Fluorescent labeling of viral proteins and viral genome, in living cells, combined with sensitive fluorescence microscopic techniques provides an excellent approach towards a detailed and mechanistic understanding of the viral replication cycle and its underlying cell biology.[5–7] In this perspective we demonstrate how imaging single virions can provide unique insights into the hidden life cycle of a retrovirus. Without being exhaustive, we give some examples of single virus imaging applications in HIV research. In the final section, we will discuss some of the crucial remaining questions in virology, more specifically in the HIV field, for which single particle imaging can help researchers in finding the right answers.

CHALLENGES AND ADVANTAGES OF SINGLE VIRUS IMAGING

Fluorescent labelling of virus particles

The first step towards imaging single viral particles is the labeling of the viral components with high molecular specificity and efficiency. Additionally, the labeling degree needs to be carefully adjusted: it needs to be high enough to allow sensitive detection but low enough as to not influence functionality. Furthermore, the different imaging applications require fluorophores with distinct spectroscopic properties (molecular brightness, fluorescence lifetime, photostability, color, photo-switching capabilities, *etc*.). The choice of the molecule to label, how to label it and which tag to use, is linked to the biological question under investigation.

The exposed components of viral particles, namely envelope proteins and the lipid bilayer, are readily available for labelling using synthetic, photostable fluorophores[8]. A greater challenge is the labeling of internal components, which are typically inaccessible and require permeabilization of the virus for chemical labeling.

Over the years, different strategies have been developed to label the viral genome of HIV-1. For example, intracellular mRNA can be directly visualized by fusing a fluorescent protein to an RNA-binding protein which is expressed along with the target mRNA.[9,10] Alternatively, during reverse transcription, ethynyl-functionalized dNTPs are incorporated in the cDNA. These allow covalent linkage with azide reactive fluorophores via a copper-catalyzed azide-alkyne cycloaddition (CuAAC), a technology which is referred to as 'click chemistry'. Another approach relies on fluorescent *in situ* hybridization (FISH). This method exploits branched DNA technology to add a fluorescent probe to a specific nucleotide sequence and has been use to visualize either RNA (ViewRNA,[11,12]) or DNA (ViewHIV[13]) in virions.

For labelling of the viral proteins, a panoply of methods is available. The method of choice of most research groups is the insertion of a gene encoding a fluorescent protein (FP) or a self-labelling protein tag (CLIP/SNAP/HaLo tag[1,14]) fused to the protein of interest. The large array of FPs available, including photo-switchable proteins, allied to a perfect labelling specificity, make this approach very attractive for (single) virus imaging.[15,16] Like all retroviruses, the HIV-1 genome encodes three major open reading frames: *gag* (group specific antigen), *pol* (polymerase) and *env* (envelope) polyproteins. *gag* encodes for the structural proteins matrix (MA), capsid (CA), nucleocapsid (NC) and p6. The viral enzyme protease (PR), reverse transcriptase (RT), and integrase (IN) are encoded by the *pol* gene. Due to small genomes, open reading frame overlaps and multifunctional proteins, incorporation of the coding sequence for a (fluorescent) protein into

the viral genome is accompanied with some challenges.[17] Consequently, most viral proteins cannot be labeled using this approach.

Another method to label viral proteins relies on the viral accessory protein (Vpr), which is incorporated into viral particles through specific interactions with the p6 domain of Gag. Vpr can be used to shuttle foreign proteins into the HIV-1 particles. FP-tagged versions of Vpr itself, as well as other viral proteins, have been successfully used to label the inner core of HIV-1 particles.[18,19]

Independently of the method chosen to incorporate a (fluorescent) protein into the viral particles, the tag itself might alter the labeled protein's function and/or affect viral morphology and infectivity. When the introduction of a protein tag yields mature virions, but with reduced infectivity, co-expression of modified and wild type protein can rescue virus infectivity (as shown for Gag[20–22]). Alternatively, the use of smaller fluorescent tags can be advantageous. An example is the introduction of a short peptide tag containing a tetracysteine motif (*e.g.* Cys-Cys-Pro-Gly-Cys-Cys). This tag interacts with cell-permeable biarsenical dyes (FlAsH, ReAsH), thereby allowing to specifically label the viral protein of interest. This approach has been used to image HIV-1 complexes in living cells.[8,23–27]

Another class of small labels for virus research are biorthogonal click labels.[17] Here, a non-canonical amino acid (ncAA) is built into the protein of interest at a specific site during viral synthesis, in amber-suppressed producer cells. During or after production, fluorescent labeling is carried out by adding an organic dye, designed to react specifically with the ncAA. This method has been used to study the nanoscale distribution of HIV-1 Env protein on the surface of viral particles using STED microscopy.[28]

Regardless of the fluorescent tag used, single-virus imaging will have to rely on quantitative biochemical/virology tools for functional characterization of labeled viral particles. The current trend is to label as few viral components as needed, with labels as small as possible, and preferentially without interference with the replication steps of the virus.

Resolution at the sub-viral and molecular level

The diameter of HIV-1 is between 120 to 145 nm.[2,29] The spatial resolution obtained with conventional fluorescence microscopy is, however, limited by the diffraction of the light to approximately 200-250 nm. The development of super-resolution fluorescence methods has made it possible to achieve lateral resolution down to 10-20 nm.[30] This has opened the possibility to visualize structural features of individual viral particles and to define the sub-viral protein distribution under physiological conditions.[6]

The most commonly used techniques to overcome the diffraction resolution limit are STimulated Emission Depletion (STED) microscopy and Single Molecule Localisation Microscopy (SMLM). These methods are briefly explained below (for a more complete review on super resolution fluorescence microscopy see reference [30]). STED microscopy is based on depleting the emission of fluorophores in the outer region of the diffraction limited spot with a red-shifted depletion laser pulse that is referred to as the STED beam. The selective deactivation of fluorophores through stimulated emission minimizes the effective fluorescent spot size. The increase in spatial resolution is dependent on the STED excitation intensity and, typically, a resolution of 30-50 nm can be obtained.[31,32] SMLM methods include photoactivated localization microscopy (PALM)[33] and (direct) stochastic optical reconstruction microscopy ((d)STORM).[34,35] These methods make use of fluorophores that can be converted in a stochastic manner from a “off” to a “on” state upon

illumination by a certain wavelength.[36] Stochastic light-induced switching of the fluorophores enables temporal separation of spatially overlapping molecules. The localization of each emitter can be determined by fitting a 2D Gaussian function to the point-spread function (PSF) of the detected signal, with a precision of 20-30 nm. Super-resolved images can be reconstructed from the calculated localizations.[37] Over the years, these methods have been used to elucidate the protein organization in isolated viral particles and within infected cells. [6,27,28,38–41]

An example is investigation of the organization and dynamics of viral assembly sites. During virus assembly, the Gag and Gag-Pol proteins, together with two viral RNA strands, are recruited to the plasma membrane (Figure 1, step 10). There, Env molecules are incorporated and the ESCRT machinery enables budding of the virus.[42] The nanoscale organization of different ESCRT components at HIV assembly sites was elucidated using 3D super-resolution microscopy.[43] Lippincott-Schwartz and co-workers showed that ESCRT subunits localized within the head of budding virions and that HIV release was triggered by the membrane association of ESCRT subunits.

SMLM has also been instrumental in resolving the spatial distribution of nascent Gag assemblies in relation to other viral or cellular proteins. Heilemann *et al*. have shown that Env glycoproteins are recruited to the viral budding sites in a ring-like formation, accumulating at the periphery rather than in the center of the budding sites. Env-rich membrane areas surrounding the budding sites were devoid of detectable Gag molecules, suggesting that Env proteins are recruited through Gag-induced alterations in the surrounding nascent viral bud.[38] Combined biochemical and super-resolution microscopy have been use to investigate the cellular restriction factor tetherin. This molecule inhibits the release of HIV-1 through direct incorporation into viral membranes, a process that is counteracted by the HIV-1 protein Vpu. Lehmann *et al.* have shown that during

viral assembly extended tetherin dimers incorporate their N-termini into the assembling virions and in this way restrict HIV-1 release (Figure 1).[39]

In other studies, STED has been used to investigate the distribution of Env molecules on the surface of individual HIV-1 particles.[28,40] Chojnacki *et al.* have used dual-color STED to show that membrane patches of the cellular receptor CD4 can induce clustering of mobile Env molecules on the viral particles, promoting efficient entry of the virus in the cell.[40] This result was later confirmed using ncAA labelling of Env proteins.[28] The authors suggested that Env trimers are initially recruited to viral budding sites in a random distribution, and after proteolytic maturation, they coalesce into a single Env focus to enhance the interaction with a CD4 patch on the target cell surface. The high mobility and clustering of Env proteins appears to be crucial for successful infection. Consequently, molecules disturbing Env cluster formation will reduce the infectivity of the viral particles.

One of these molecules is the serine incorporator protein 5 (SERINC5). In an article recently accepted in ACS Nano, Melikyan and co-workers have used 2D and 3D super-resolution microscopy to investigate the effect of SERINC5 on the distribution of Env proteins in single HIV-1 particles.[41] SERINC5 is a cellular restriction factor that can be incorporated into the progeny virus. It is known that SERINC5 incorporation into viral particles alters the Env structure, inhibiting the Env-mediated virus fusion with the target cell, and consequently reducing HIV-1 infectivity. Similar to previous studies, the authors found that Env proteins form clusters on the surface of the viral particles. Moreover, SERINC5 but not SERINC2, which lacks antiviral activity, was shown to disrupt the Env clusters. Poor colocalization of Env and SERINC5 molecules on the viral membrane implies an indirect mechanism of SERINC5-mediated inhibition

of HIV-fusion (Figure 1). This work is a beautiful illustration of how single molecule/particle and super-resolution studies can provide mechanistic insights into viral biology.

Optical techniques are nowadays pushing towards near-atomic resolution. An excellent example is single molecule Förster resonance energy transfer (smFRET).[44–47] FRET is the phenomenon of radiationless energy transfer between a suitable pair of fluorophores (donor, acceptor) in close proximity. [48] This method is very sensitive to the distance between two chromophores in the range of 2-10 nm, making it extraordinarily suitable to investigate molecular conformations and interactions.[49] When acceptor and donor fluorophores are placed in different locations on the same molecule, smFRET can be used to quantify protein structure and conformational dynamics in real time with Ångstrom precision. An example of smFRET in HIV research is the imaging of HIV-1 Env trimer conformational dynamics.[50] The analysis of FRET signals revealed the molecular events that underlie the two-step activation of HIV-1 Env by CD4 and co-receptor. Undoubtedly, smFRET might provide unique insights into other steps in the viral life cycle. The true challenge and relevance for single virus research lies in its application to study intracellular events.

Live imaging of the viral replication cycle

Only by tracking single viruses from cellular uptake until integration we can understand the different steps and host factors that are required for productive infection. Advances in labelling strategies and fluorescence microscopy methods have enabled imaging viruses within living cells in real time.[51–53]

MacDonald *et al.* used eGFP-Vpr labelled HIV-1 virus to visualize early post-entry events.[54] Tracking of single viral particles revealed that after entering the cytoplasm, HIV-1 uses cytoplasmic dynein and the microtubule network to migrate toward the nucleus. Later, FlAsH

labelling of integrase (IN) allowed the sensitive detection and tracking of viral complexes in both the cytoplasm and nucleus.[24] While the cytoplasmic HIV-1 complexes showed fast microtubule-directed movements toward the nucleus, intranuclear HIV-1 complexes showed only restrained diffuse movement within the nucleus, which could be indicative of interactions with chromatin.

For a productive infection of host cells, the viral capsid must be disassembled in a process referred to as uncoating. The debate on the exact mechanism, timing and localization of uncoating is still open.[55] The large size of the virus core (61 nm width, 120 nm length) in comparison to the nuclear pore (40 nm), requires active uncoating before nuclear import.[56] Using time-lapse imaging, Francis *et al.* showed that docking and uncoating of HIV-1 at the nuclear envelope is a prerequisite for nuclear import.[53,57] Ninety minutes post-infection and 35 min after docking to the nuclear envelope, the amount of CA in the viral particles decreased, which is indicative for uncoating.[57] A similar finding was obtained by Mamede *et al.,* which showed cytoplasmic uncoating HIV-1 particles within 90 min post-infection.[55] Recently Burdick *et al.* published a more controversial hypothesis on uncoating and reverse transcription of HIV-1 particles in the nucleus, close to their integration site.[20] After docking at the nuclear envelope for 1.9 hours, GFP-CA–labeled viral complexes were imported into the nucleus within ~4.4 hours post-infection. Importantly, while the exact location of the uncoating process is still under debate, several groups have shown that the number of CA moieties in nuclear complexes is lower than in those found in the cytoplasm.[21,27,58]

Despite the potential of fluorescence microscopy to investigate the molecular mechanisms underlying viral infection, its application in live imaging of single virus remains limited. This is linked to the photon yield of commonly used fluorophores, which limits the total number of image frames that can be recorded before photobleaching occurs. Additionally, the field of view that can

be recorded with high acquisition rate limits the number of particles that can be imaged simultaneously. Strategies to improve fluorophore photon yields, and a continued transition to automated high-content imaging, are therefore most promising in popularizing time-lapse single virus imaging.

Quantifying interactions between viral and host proteins

Throughout the replication cycle, retroviruses depend on the host cell, from which they borrow functionalities such as active nuclear import (*e.g.* Christ *et al.*[19]) or chromatin tethering (*e.g.* Hendrix *et al.*[59]). Virus replication relies on specific interactions of viral proteins with themselves (oligomerization), or with other proteins, from the virus or present in the host cell. The most popular methods for studying molecular interactions in cells are fluorescence fluctuation/correlation spectroscopy and Förster resonance energy transfer (FRET).

Fluorescence fluctuation spectroscopy (FFS) is a powerful quantitative spectroscopic technique used to analyze the mobility and concentration of molecules, and eventually their interaction with other molecules.[60,61] Imaging variants of FFS methods include raster-image correlation spectroscopy (RICS)[62–66] and number and brightness (N&B) analysis.[67] In RICS, a raster pattern is scanned by the laser on a confocal laser scanning microscope, creating a space-time matrix of pixels within the image. The temporal information is included in each image as the obtained pixels are collected over time. From the spatial correlation function the diffusion coefficient and the binding processes can be identified.[68,69] N&B analysis is based on the intensity fluctuations present in the individual pixels over the course of the recorded image series. The effective molecular brightness and number of molecules for a single diffusing species is determined from the average value and variance of the fluorescence signal.[67]

FFS analysis of single virions revealed that the HIV-1 Gag stoichiometry varies between 750-2500 per virion, suggesting that the amount of Gag incorporated in single viral particles depends on the expression of the Gag protein in the cell.[70] Fogarty *et al.* have shown that the oligomeric state of cytosolic Gag also depends on the concentration of this protein during viral assembly.[71] A more recent investigation showed that a fraction of the cytosolic Gag partly diffuses as a monomer, frequently interacting with RNA, while another fraction diffuses much slower, as an oligomer. The latter species presumably functions as a seed for new assembly sites at the plasma membrane. [72] Derdowski *et al.* used FRET to demonstrate that most of the interactions between Gag proteins take place at cellular membrane. The authors also showed that Gag multimerization occurred at multiple distinct sites at the plasma membrane.[73]

FRET was also used by Borrenberghs *et al.* to probe the relative oligomeric state of HIV-1 IN inside single viral particles.[74] Small molecule inhibitors of the interaction between HIV-IN and the host factor LEDGF/p75, referred to as LEDGINs, were shown to stimulate IN oligomerization in the budding virus.[74,75] Increased IN oligomerization resulted in crippled virus with aberrant EM morphology. A similar labelling and FRET analysis of HIV-1 IN was later used to reveal how LEDGF/p75 alters IN stoichiometry upon nuclear import.[58] A similar mechanism was also observed for the IN from Moloney murine leukemia virus (MoMLV) upon interaction with BET-proteins.[76]

In general, during the retroviral replication cycle, multiple different components must interact with one another in a tightly time-space orchestrated manner. Fluorescence microscopy provides the means for quantifying all these interactions simultaneously and quantitatively. However, specific applications in single-virus research are scarce. With the ability of recording spectrally resolved microscopy images and videos, or even multiparametric data, it will be possible to unmix

multiple fluorophores, and thus monitoring the fate of different proteins simultaneously, disentangling intricate relations between different labeled viral and host components during the viral replication cycle. The real challenge lies in the analysis tools to convert such multidimensional fluorescence data to meaningful insights on viral biology.

REMAINING QUESTIONS IN HIV VIROLOGY

As shown in the previous section, imaging of single viral particles has already facilitated answering many biological questions concerning the retroviral replication cycle. However, much remains unknown.

How many viral particles are required to productively infect a cell? This is perhaps one of the most intriguing questions in virology. The multiplicity of infection (MOI) is a term used in microbiology which refers to the ratio of agents (*e.g.* virus, bacteria, phage) to infection targets (*e.g.* cell). For example, when referring to a group of cells inoculated with virus particles, the MOI is the ratio of the number of virus particles to the number of target cells present. However, a MOI of 1 (one virus per cell) does not mean that all cells will be infected by one particle. For a specific MOI, the number of particles that a cell receives is described by the Poisson's distribution. Accordingly, at a MOI of 1, only 63% of cells will contain viral particles. To detect particles in more than 95% of the cells, a minimum MOI of 3 is required. Even then, not all cells that contain particles will be infected. Biologically speaking, it is extremely unlikely that one internalized virus particle will be able to yield one infection.

A more accurate way to determine how many viral particles are necessary to infect a cell is to measure a viral titer. Functional titers measure infectivity; one infectious unit represents the

number of viruses required to produce a cytopathogenic effect (*e.g.* a giant cell) or reporter read out in one cell (*e.g.* eGFP expression). Physical titers measure how much virus is present and can be obtained by measuring protein (p24) or RNA content, or by electron microscopy (particle count). Interestingly, from comparisons of functional titers with physical titers it appears that only 0.1 to 10% (1 in thousand to 1 in ten) of HIV-1 particles present during infection of cells yields an integrated provirus.[4]

The low efficiency of viral infection seems to be intrinsically linked to the high level of heterogeneity present between viral particles. The virus assembled in host cells may not all be functionally competent. This is true for virus produced *in vitro*, using cell culture methods, but less optimal production efficiency is also likely to happen *in vivo.* In a way, virus infection can be seen as 'the survival of the fittest'. Infection of cells is all about overcoming barriers; physical barriers such as the cellular membrane and the nuclear membrane, and cellular protection mechanisms such as restriction factors. Both barriers may inherently also select the fittest particles, particles that can cross membranes and nucleopores, and can overcome restriction factors. From an evolutionary perspective, redundancy may help selection if infection occurs with particles with different genomes. Another plausible justification for the observable low MOI is that the excess of particles entering the cell, even the non-functional ones, may work as a decoy against innate immunity/restriction factors.

Single virus imaging and live tracking can be used to pinpoint the 'superheroes', particles that cross membranes, avoid restriction factors and result in productive integration. Retro-tracking will identify the characteristics of these particles to discriminate them from non-integrating particles. Live imaging will reveal time dependent changes in functionality, *i.e.*, how virus that arrive earlier restrict the infection by late comers or rather prepare their efficient integration. [77,78] The examples

discussed in this Perspective highlight the potential of single virus imaging to address some of the open questions in virology.

Do (all) particles in the cytoplasm retro-transcribe? Not all particles enter the cell, and not all particles present in the cytoplasm enter the nucleus. Recently, it has even put into question whether reverse transcription of HIV-1 takes place in the cytoplasm or the nucleus.[20] Inefficient labelling of reverse transcription in cytoplasmic PICs may be linked to variable degrees of reverse transcription.[79,80] Is reverse transcription associated with uncoating? Fluorescent capsid labelling and live cell imaging should provide answers these questions.[20,21]

How are PICs selected to enter the nucleus? The integrase content of nuclear particles is lower than that of particles in the cytoplasm, implying morphological alterations at the nucleopore.[27,58] It is not yet known whether only particles with limited IN content pass the nucleopore or all particles need to be trimmed at the nucleopore from excess of IN. The answer to this will require live imaging and careful analysis of the amount of CA present during nuclear import. Which host factors are required to transport the PIC across the nuclear membrane is a also matter of debate. Super-resolution microscopy and FRET should provide sufficient resolution to identify the authentic protein-protein interactions.

Are all integrants transcriptionally active? From the particles that enter the nucleus, it is not known how many integrate into the genome of the host. In any case it is known that not all proviruses are transcriptionally active. HIV evolved to yield both productive and latent infections. Productive infections ensure spreading within the host and to other hosts. Latent infections ensure long time survival of the virus in the host. The chromatin environment of the provirus apparently determines transcriptional activity.[81,82] LEDGF/p75 plays an important role by selecting transcriptionally active regions of the genome through recognition of the H3K36me3 epigenetic

mark. DNA imaging of viral DNA and mRNA in the same cell can be used to discriminate latent and productive infections.

How is Gag-Pol involved in the onset of assembly? HIV-1 assembly is controlled primarily by the Gag polyprotein. Gag orchestrates HIV-1 assembly by recruiting all the building blocks required for the formation of a fully infectious viral particles and the synthesis of Gag precursor protein alone is sufficient to produce virus-like particles. However, since the viral enzymes, PR, RT, and IN, are produced from the Gag-Pol polyprotein, its incorporation into virions is required for infectivity.[83] This is likely to occur via 'co-assembly' with Gag.[84] During and after release of virions from cells, the Gag precursor protein is cleaved by the viral protease (PR) into mature proteins. As dimerization of PR is essential for PR activity, oligomerization of the Gag-Pol polyprotein is a prerequisite for the formation of mature virions. Does Gag-Pol form oligomers because of IN dimerization? When are these oligomers formed? Are the changes in the Gag-Pol oligomerization induced by LEDGINs responsible for the observed morphological defects of the virus?[75] This question could be addressed with quantitative FFS and super-resolution microscopy.

In conclusion, the exciting technological advances in the field of fluorescence microscopy with super-resolution, more advanced fluctuation imaging methods and innovative labeling strategies have opened windows that will yield more detailed insights into the viral replication cycle. While we described examples from the HIV field, the methods presented here have been applied to other viruses. For instance, live cell imaging has revealed that simian virus 40 (SV40) utilizes endocytosis through caveolae for infectious entry into host cells[85] and that Ebolavirus is internalized via micropinocytosis.[86] More recently, fluorescent labelled viral ribonucleoprotein complexes were used to track the uncoating of single influenza A virus in living cells.[87] These

viruses, responsible for some of the epidemics that occur in the 20th century, have been around for decades. Yet, imaging of single viral particles is still unravelling new information concerning their *modus operandi*. The methods are well established, can be readily adapted and applied to investigate other, new viruses. To the best of our knowledge, at the date of publication of this Perspective, there are no records of single virus imaging of SARS-CoV-2. However, we believe that single virus imaging approaches will be of uttermost importance to understand the molecular mechanisms involved in the infection caused by SARS-CoV-2 and the subsequent development of new treatments or vaccines.

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ABBREVIATIONS

BET, bromo- and extraterminal domain; CA, capsid; CD 4, cluster of differentiation 4; DNA, deoxyribonucleic acid; eGFP, enhanced green fluorescent protein; Env, envelope; ESCRT, endosomal sorting complexes required for transport; FFS, fluorescence fluctuation spectroscopy; FISH, fluorescence in situ hybridization; FLIM, fluorescence lifetime imaging microscopy; FP, fluorescent protein; FRET, Förster resonance energy transfer; Gag, group specific antigen; HIV, human immunodeficiency virus; IN, integrase; LEDGF, lens epithelium derived growth factor; LEDGIN, LEDGF/p75-IN interaction inhibitor; MA, matrix; MoMLV, Moloney murine leukemia virus; MOI, multiplicity of infection; N&B, number and brightness; NC, nucleocapsid; NPC, nuclear pore complex; PALM, photoactivated localization microscopy; PIC, pre-integration complex; PIE, pulsed interleaved excitation; PR, protease; PSF, point-spread function; RICS, raster image correlation spectroscopy; RNA, ribonucleic acid; ROI, region of interest; RT, reverse transcriptase; STED, stimulated emission depletion microscopy; STORM, stochastic optical reconstruction microscopy; S/N, signal-to-noise; TIRF, total internal reflection fluorescence; TRN-SR2, transportin-SR2; Vpr, viral protein R; Vpu, viral protein Unique; WT, wild type;

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