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High-Speed Mass Measurement of Nanoparticle and Virus

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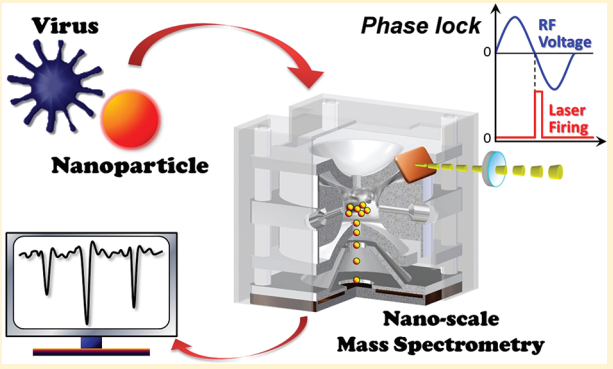
ABSTRACT: Until now, there have been no relatively easy methods to measure the mass and mass distributions of nanoparticles/viruses. In this work, we report the first set of measurements of mass and mass distributions for nanoparticles/viruses using a novel mass spectrometry technology. In the past, mass spectrometry was typically used to measure the mass of a particle or molecule with a mass less than 1 000 000 Da. We developed cell mass spectrometry that can measure the mass of a cell or a microparticle. Nevertheless, there is a gap for mass measurement methods in the mass region of a nanoparticle or virus (1 MDa to 1 GDa). Here, we developed a nanoparticle/virus mass spectrometry technique to make rapid and accurate mass and mass distribution measurements of nanoparticles/viruses. This technique should be valuable for the quality control of nanoparticle production and the identification of various viruses. In the future, this method can also serve to monitor drug delivery when nanoparticles are used as carriers. Furthermore, it may be possible to measure the degree of infection by measuring the number of viruses in specific cells or in plasma.



During the past decade, nanoparticle research has played a critical role in many different fields, including materials science, physics, electronics, energy research, and biomedical science. Businesses related to nanotechnologies have exceeded $100 billion U.S. Nevertheless, it is still extremely difficult to quickly measure the masses of nanoparticles. One approach is to use electron microscopy to measure the size of a nanoparticle and calculate the mass based on the density. However, this is a tedious and very time-consuming approach. For nanoparticles with an odd shape, such as the Au (core)/Ag (shell) nanoparticle, an error of 20% or more can be expected for the mass measurement by electron microscopy. Many physical and chemical properties of a nanoparticle are a strong function of the size and the size distribution. It is critically important to be able to quickly measure either the size or the mass of nanoparticles as well as their mass distributions. In this work, we report the development of a novel nanoparticle mass spectrometry method and the application of this method to quickly measure the masses of nanoparticles and their mass distributions.

Viruses play a critical role in infectious diseases, such as human immunodeficiency virus (HIV) and severe acute respiratory syndrome (SARS).[1] However, the mass of an individual virion is very difficult to measure, and the mass distributions of virus particles have not been reported. Therefore, it is highly desirable to be able to rapidly measure the mass and mass distributions of various viruses.[2] There are several established methods for nanoparticle and viral analysis, including a nanoscale cantilever,[3,4] quartz crystal microbalance (QCM),[5] charge reduced electrospray size spectrometry,[6] discrete conductance changes of binding and unbinding by an electrical detector,[7] and microscopy-based mass spectrometry.[8] Until now, these methods have not achieved rapid and convenient detection at the level of a single nanoparticle or virion, and they often require a relatively complex sample preparation that is particularly inconvenient for infectious materials, such as infectious viruses. In addition, the mass distribution of viruses cannot be obtained. Mass spectrometry has become an irreplaceable method in the analysis of biological molecules.[9] In this work, we report the development of charge-detection laser-induced acoustic desorption (LIAD) mass spectrometry (MS) for rapid mass measurements of nanoparticles and viruses.

Matrix-assisted laser desorption/ionization (MALDI)[10,11] and electrospray ionization (ESI)[12] have been extensively used for biomolecular analysis. Biomolecular ions with a mass up to 1 MDa have been successfully measured. The mass measurement of particles with a mass higher than 1 MDa has been a challenge for a long time. Loo's group used gas-phase electrophoretic mobility molecular analysis (GEMMA) to measure the cowpea chlorotic mottle virus (CCMV) with the

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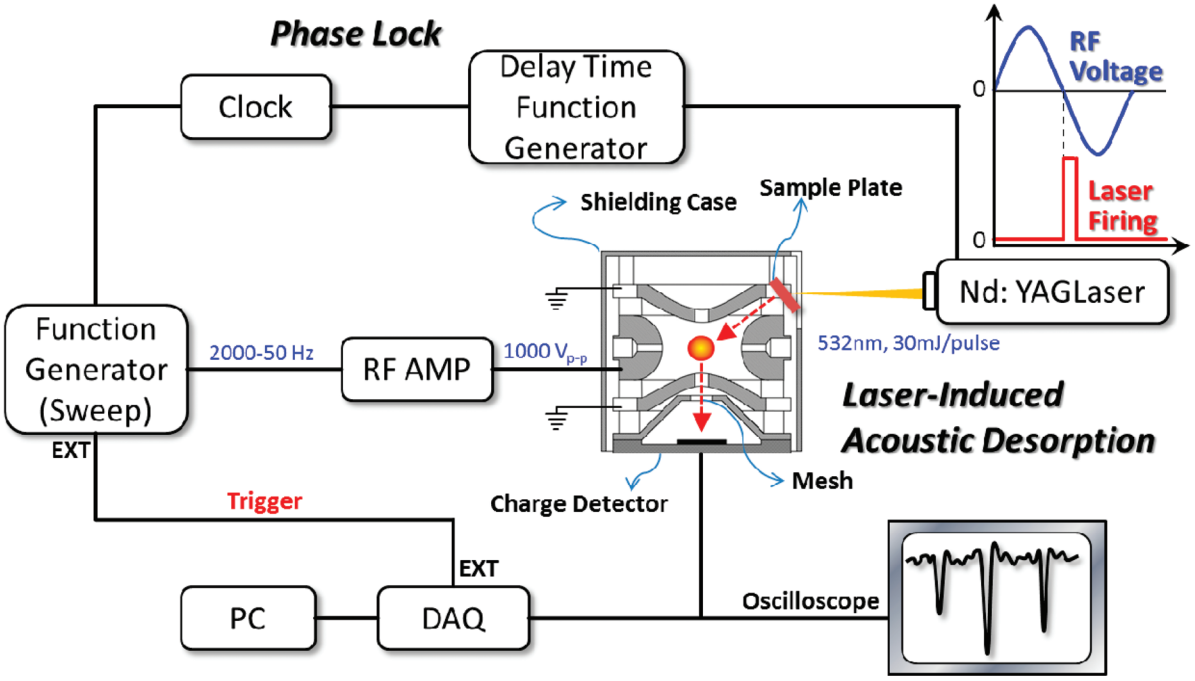

Figure 1. The block diagram of the experimental setup, including quadrupole ion trap, a pulsed Nd:YAG laser, a charge detector, a stainless steel shielding case, and an SiO_2 sample plate (400 nm thickness, high-resistance surface). An aliquot (10 μL) of the purified particles was placed on the front side of the sample plate. A frequency-doubled Nd:YAG laser beam (λ = 532 nm, 30 mJ/pulse) with a pulse duration of approximately 6 ns was shone directly onto the back side of the sample plate. A synchronization of laser firing with the phase of rf is installed for optimizing the trapping efficiency.

mass of 4.6 MDa and MVP-vault (9 MDa) by electrospray ionization (ESI) and mobility measurement.[13] Ions with m/z larger than 10^6 have mostly done by a cryodetector. Zenobi's group measured von Willebrand factor (vWF) protein masses between 5 and 20 MDa,[14] and Bier's group[15] has assayed polystyrene aggregates with a mass of ~4 MDa by cryodetection. The McKay group had determined masses of both 30S subunits and intact 70S ribosomes with the mass of 2.3 MDa from *Thermus thermophilus*.[16] In general, a cryodetector is bulky and expensive. In addition, the response time is somewhat long. In this work, we use a simple charge detector to achieve the detection of nanoparticles/viruses.

Recently, we developed an ion trap mass spectrometer with a frequency-scanning laser-induced acoustic desorption (LIAD) and direct charge detection method for measuring the masses of cells and microparticles.[17] The LIAD method was used to desorb cells/microparticles without the need of a matrix to prevent a possible interference of particles from the matrix molecules produced by the MALDI technique.[18] Although matrix was not included in the microparticle/cell samples, most desorbed cell/microparticles are charged. The detailed mechanism is still not known. Most cells or microparticles in a vacuum were found to have more than 1000 charges. Therefore, a charge detector can be used to measure the number of charges of each cell or microparticle directly without the serious concern of the electronic background.[19] Since the mass-to-charge ratio (m/z) can be determined using the ion trap mass spectrometer and the charge detector can be used to determine the value of z for each particle, the mass of the cell/microparticles can be obtained.

The number of charges that can be directly detected by conventional electronics is limited by the background noise of 50−500 electrons and the number of charges on a nanoparticle or virus that are expected to be less than 50, thereby the measurements of the masses of the nanoparticles/virus is a major challenge. Therefore, there is a gap in the mass region that cannot be measured by mass spectrometry.

Once the measurements of nanoparticles/viruses are successfully achieved, mass spectrometry can be used to measure particles from atoms to cells; namely, with masses ranging from a few Daltons to ~10^{16} Da. A microbalance and/or a micro cantilever device can be used to measure a particle with a mass higher than this range. In this work, we successfully demonstrate the measurements of nanoparticles/viruses to fill this technical gap of mass spectrometry.

■ INSTRUMENTATION

The scheme for the experiment is shown in Figure 1. The instrument is a modified version of the previous facility which has been reported before.[19] Therefore the structure is briefly reported with the emphasis on the difference from the previous one. LIAD was used to desorb the nanoparticles/viruses. A quadrupole ion trap was used to trap the desorbed and charged particles. The phase lock system to enhance trapping efficiency and collect more desorbed particles was installed.[20] The laser firing is synchronized with the zero radio frequency (rf) voltage by the four channel digital delay/pulse generator (Stanford Research System, Inc., model DG535, CA). The scheme of electronic design is also shown in Figure.1. With this approach, the analysis speed is increased by a factor of ~10. We also gave a shielding to the charge detector to reduce the electronic background. The experimental setup of MS consisted of LIAD for desorption of nanoparticles and viruses without any matrix. Nanoparticle and virus samples were deposited onto a silicon wafer (thickness of approximately 400 μm). A frequency-doubled Nd:YAG (neodymium-doped yttrium aluminum

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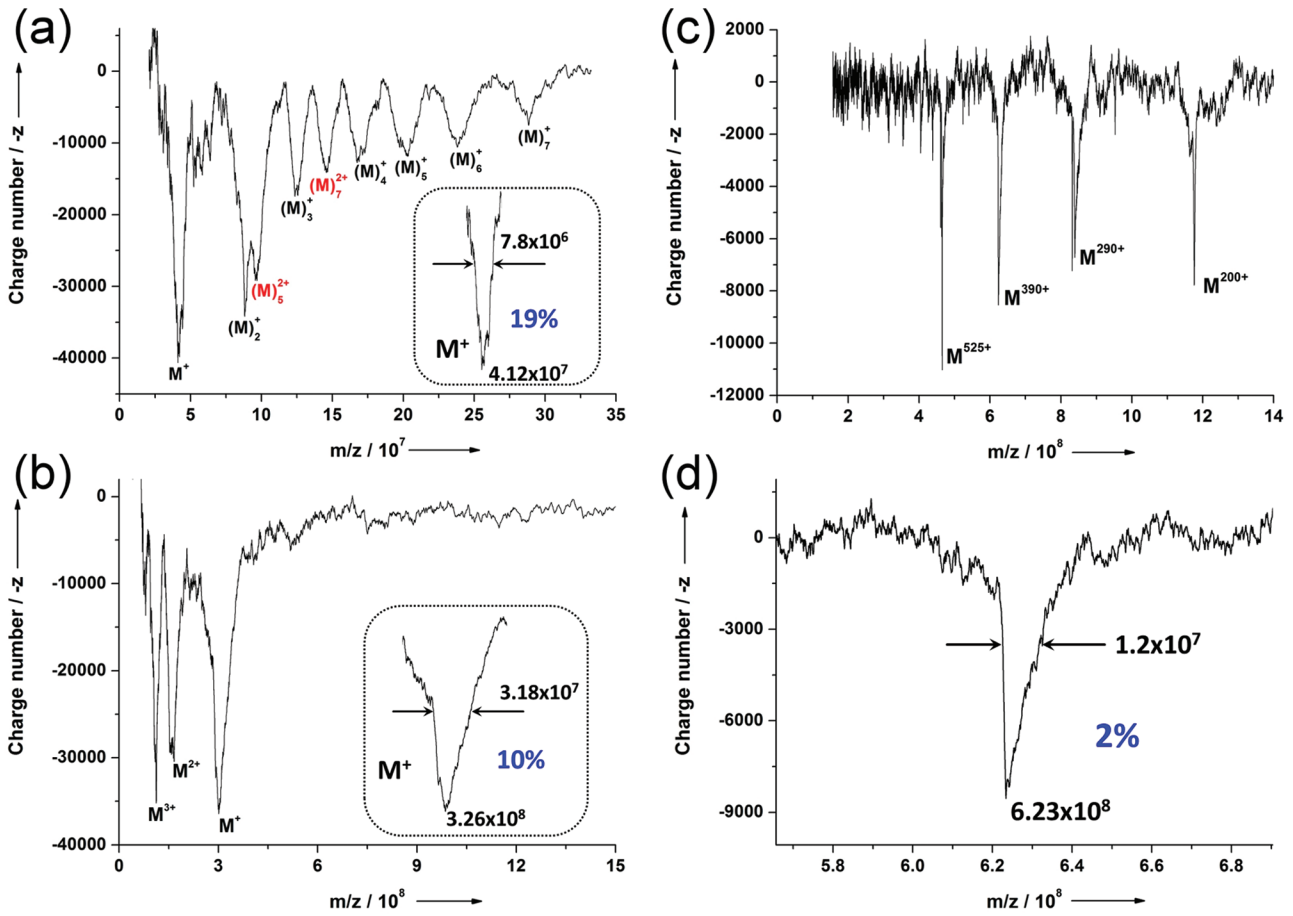

Figure 2. Mass spectra of 50, 100, and 900 nm standard polystyrene spherical particles, and the HIV were measured by our MS instrument. The typical trapping parameters ($\Omega/2\pi$ and $V_{p\text{-}p}$) used in each measurement were (a) 2000 Hz and 1000 V for particles of 50 nm, (b) 1500 Hz and 1000 V for particles of 100 nm, (c) 800 Hz and 1500 V for particles of 900 nm, (d) the mass distribution was approximately 2% ($\Delta M/M$) of 900 nm. Since the mass distributions for the inserts in parts a and b are significantly higher than 2%, they should reflect the mass distributions of the particles in the samples. All of the mass spectra were summed up by twenty individual single-scan mass spectra.

garnet) laser beam (wavelength 532 nm, 30 mJ/pulse, Laser Technique, Berlin, Germany) with a pulse duration of approximately 6 ns was shone directly onto the back side of the sample plate to desorb the nanoparticles/viruses by LIAD with a power density of approximately 10^8 W/cm^2. The trapping rf voltage was set at 1000 $V_{p\text{-}p}$ (peak-to-peak voltage) (TReK, Inc., model 5-80, NY). The nanoparticles and viruses released by laser desorption were trapped with an adjustable alternating-current (ac) field (~2 kHz, depending on the particle size) and damped to the trap center by helium gas at ~60 mTorr to retard the kinetic energy of the desorbed particles for more efficient trapping. The ion trap was operated under an axial mass-selective instability mode by scanning the trapping frequency in the range 2000−200 Hz. A voltage of 1000 $V_{p\text{-}p}$ was initially applied with a high-voltage transformer driven by an rf voltage power amplifier (TReK, Inc., model 5-80, NY) and a function generator (Stanford Research Systems, model DG345). The frequency was scanned with a functional generator so that particles with an unstable trajectory were ejected along the axial direction and subsequently detected by a charge detector. This instrument can rapidly measure the mass of a nanoparticle/virus and a microparticle/cell. On average, it takes less than 1 min to finish the measurement of one sample. The analysis speed is more than a factor of 10 faster than that of our cell mass spectrometer reported in the past.[19] Using this instrument, the speed of mass measurements for nanoparticles/viruses can be increased by orders of magnitude relative to microscopy-based mass spectrometry.[8]

■ SAMPLE PREPARATION

An aliquot (10 μL) of the purified particle suspension was deposited onto a ~400-μm-thick silicon wafer and then air-dried in a desiccation box. The standards were spherical polystyrene nanoparticles with sizes of 50, 10, and 900 nm, which were purchased from Thermo Scientific (Fremont, CA). The concentrations were 2×10^{14}, 3×10^{13}, and 5×10^{10} particles/mL, respectively. Polystyrene spheres were thoroughly washed with deionized water, recovered by centrifugation, and resuspended in filtered (0.2-μm pore size filter) and distilled water. The purification process is important because the presence of sodium azide or any residual salt could produce background particles during laser desorption, rendering the analysis difficulty. For the virus particle, lentiviruses were produced by cotransfecting a 15-cm dish, followed by additional purification with dialysis. Then, the viruses were resuspended in filtered (0.2-μm pore size filter) and deionized water at a concentration of approximately 1×10^9 particles/mL. The viruses were concentrated by ultracentrifugation (36 mL/tube, 25 000 rpm, 1.5 h, 4 °C, low brake, SW28 rotor). The minimal packaging G glycoprotein of the vesicular stomatitis

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virus (VSV-G) with pseudotyped lentiviral vector has been adopted to increase vector biosafety.[21] An important approach for alleviating such concerns is the use of self-inactivating transfer vectors.[22,23] These vectors contain a deletion in the downstream long terminal repeat (LTR) that when transduced into target cells result in the transcriptional inactivation of the upstream LTR and substantially diminish the risk of vector mobilization and recombination.

To check the feasibility of this nanoparticle/virus mass spectrometer and accurately detect the masses of nanoparticles/viruses, we first measured standard polystyrene spheres with sizes of 50 and 100 nm. The calculated masses are 4.14×10^7 and 3.3×10^8 Da for 50-nm and 100-nm polystyrene particles, respectively. A typical mass spectrum of the two polystyrene particles using this MS method is shown in Figure 2.

In Figure 2a, the m/z of the 50-nm polystyrene spherical particles were measured to be 4.12×10^7 (M^+, monomer), 8.3×10^7 (M_2^+, dimer), 1.25×10^8 (M_3^+, trimer), 1.66×10^8 (M_4^+), 2.05×10^8 (M_5^+), 2.48×10^8 (M_6^+), and 2.89×10^8 (M_7^+). The identification of these peaks was based on a similar approach of ESI with the assumption that the integer number of the charge with the monomer and clusters of the monomer. Therefore, the particle numbers are determined by dividing the intensity of the charge detector by the charge number of the particle. The number of particles carrying double charges was measured to be 1.02×10^8 (M_5^{2+}) and 1.45×10^8 (M_7^{2+}). The result indicates that the larger clusters carry more charges. These results are also in good agreement with a 50-nm polystyrene spherical monoparticle with a mass of 4.14×10^7 Da. In Figure 2b, the m/z of 100-nm polystyrene particles were measured to be 3.26×10^8 (M^+, single charge), 1.65×10^8 (M^{2+}, double charges), and 1.12×10^8 (M^{3+}, triple charges). These values are consistent with the calculated mass of 100-nm polystyrene spherical monoparticles at 3.3×10^8 Da. In Figure 2c, the commercial 900-nm polystyrene particle sample was with a narrow diameter distribution of ~0.5% given by the manufacturer. Our result for the mass distribution was approximately 2% ($\Delta M/M$). Therefore, the resolution is approximately 50 for our instrument.

After successfully measuring nanoparticles, we pursued the measurements for the mass of HIV-based lentiviruses. The HIV-based lentivirus is a spherical enveloped virus of approximately 90−120 nm in diameter with a three-layer structure and a virion buoyant density of 1.16−1.18 g/cm^3 in sucrose.[24] There are two identical copies of single-stranded RNA (9.2 kb each) in HIV, each with a center and a capsid (p24) followed by a host cell membrane (Figure 3b).[25] The m/z of the HIV lentivirus was measured to be 3.51×10^8 (M^+, monomer), 7.12×10^8 (M_2^+, dimer), 1.08×10^9 (M_3^+, trimer), 1.46×10^9 (M_4^+), and 1.76×10^9 (M_5^+). The clusters with multicharges were measured to be 5.01×10^8 (M_3^{2+}), 2.75×10^8 (M_3^{4+}), and 2.1×10^8 (M_3^{5+}), as shown in Figure 3a. These results are in good agreement with the calculated mass range of single HIV lentivirus particles. The mass distribution of the HIV virus was approximately 10% ($\Delta M/M$, monomer). Major components of a virion, such as the type of nucleic acids and proteins, are well-known; it is expected that the mass distribution of the same virion should be narrow. The mass distribution of ~10% can imply that the number of each protein and nucleic acid can be different. It can also be from the various host proteins packaged into the virus particles.[26,27] In a mass spectrometry measurement, the majority of the water inside of a cell or a virion is depleted in the vacuum system. Our measurements indicate that the mass is very close to the estimate of size by an electron microscope, which may imply the presence of a small amount of water (<1−2% of the mass of virion) inside of the measured virion. In Figure 3b, the accuracy of the mass measurement by our MS is ~1%, and the resolution is ~2%. Therefore, the observed mass variety should exhibit the mass distribution of the virus particles. Here, we demonstrate the first rapid and reliable measurement of both the mass of a nanoparticle/virus and its mass distribution.

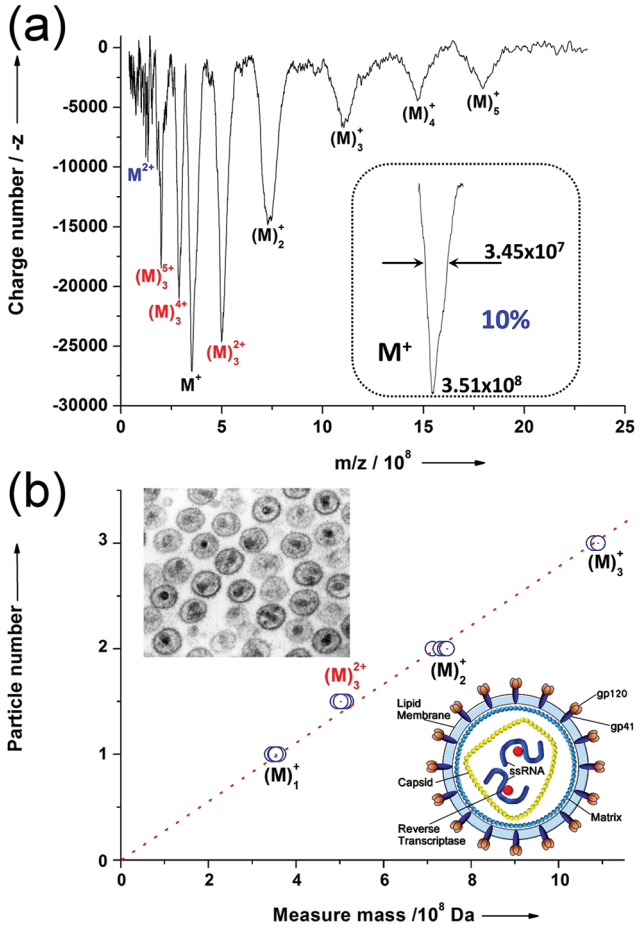

Figure 3. (a) The mass spectrum of HIV was measured by our MS. The typical trapping parameters ($\Omega/2\pi$ and $V_{p\text{-}p}$) used in each measurement were 1500 Hz and 1000 V. The mass of monomer was measured to be 3.51×10^8 Da, which is in good agreement with the calculated mass range of single HIV lentiviruses $2.67-6.43 \times 10^8$ Da, and the HIV mass distribution is approximately 10% ($\Delta M/M$, monomer). The scan time was 100 ms. (b) Plot of measured masses versus those assigned for the particle numbers for HIV. Upper inset: electron micrographs of HIV.[30] Lower inset: schematic of the single HIV structure.

In conclusion, we developed a mass spectrometry technology capable of making rapid and accurate mass and mass distribution measurements of nanoparticles/viruses. We provide the first report for the measurement of the mass distribution of a virus. This technology should be valuable for quality control of nanoparticle production in the future. It can also be used to monitor drug delivery when nanoparticles are used as carriers. The degree of infection may be measured by measuring the number of viruses in specific cells or plasma.[28,29]

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Notes

The authors declare no competing financial interest.

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