Viruses as Optical Probes

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Abstract

We report here on two new methods, which have the potential to use the advantages of optical spectroscopy (physiological environment, chemical specificity, non-intrusiveness, time resolution) to accede, *in-vitro*, to the formation of virus capsids, phase transitions and cellular transit, one particle at a time. The first approach is the encapsulation of nanoparticles of different sizes and surface chemistry inside virus capsids. The nanoparticle cores act as spectroscopic enhancers and templates with tunable surface properties for the virus self-assembly from protein subunits. The second approach is to use the optical near-field to trap single particles in a physiological fluid inside nanochannels lithographically patterned on a surface. These two approaches, combined, will open the way to real-time monitoring of virus formation and intracellular imaging using viruses as optical probes.

Encapsulation of optically active nanoparticles in brome mosaic virus capsids

At present, a general goal in molecular materials is to preserve the chemical function of their molecular constituents upon self-assembly (e.g. DNA sensors, enzymatic assays). In the future, however, research inspired by complex molecular systems such as viruses, will take advantage of changes in the molecular characteristics occurring upon self-assembly. These changes may lead to a plethora of functional properties due to self-organization. As an example, a virus whose coat is made out of a single type of molecular building block, has a life-cycle composed of a sequence of stages, each stage being defined by a certain viral function (protection, recognition, etc.). These stages are orchestrated by symmetry changes in the self-assembled protein coat. Addressing the structural transformations, the assembly pathways and the cellular transit characteristics in a way that preserves the physiological environment, has enough chemical specificity and time-resolution to detect intermediates, and enough spatial resolution to investigate one virus at a time, remained an elusive goal until today. Our long-term strategy in overcoming these challenges is to associate the virus capsids with nanoparticles acting as spectroscopic enhancers and then use the advantages of optical spectroscopy (physiological environment, chemical specificity, non-intrusiveness, time resolution) to accede, *in-vitro*, to the formation, evolution and cellular transit of single virus capsids.

We have recently reported on the first example of Rayleigh resonance spectroscopy on single brome mosaic virus (BMV) capsids (28 nm) with Au particles (2.5-4.5 nm) inside, Fig. 1, [1][2].

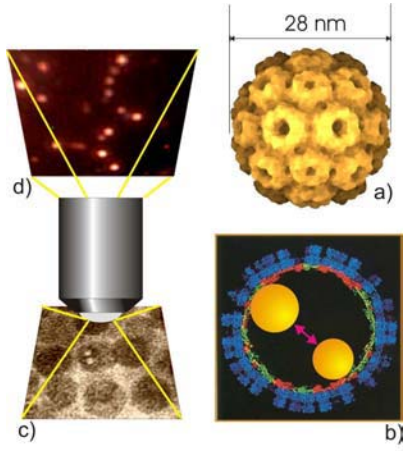

Figure 1: Collage showing pairs of Au particles incorporated during the self-assembly in virus capsids from protein subunits, and their optical signatures. (a) Reconstructed BMV capsid (adapted from [4]). (b) Schematic of two Au nanoparticles enclosed in a capsid. The distance between the particles is an important parameter for the optical spectral signature of the complex. (c) TEM picture of a pair of Au nanoparticles encapsulated in a capsid surrounded by reassembled capsids with RNA inside. (d) Dark-field scanning confocal microscopy of

individual virus capsids containing gold nanoparticles in aqueous buffer.

In this work, inspired by the natural virus particle formation, we developed a procedure to partially replace the negatively charged RNA inside the virions by gold particles. The protocol is adapted from the *in vitro* assembly of cowpea chlorotic mottle virus [3]. The association of negatively charged, citrate-covered, Au particles, 2.5-4.5 nm diameter, with the internal compartment of BMV capsids is done in three steps: disassembly of the BMV virions, reassembly in the presence of Au particles and purification of BMV particles.

If the viral particles are not completely disassembled, but only swollen by the pH change, the association of the Au with the capsid does not occur.

Negatively charged Au sols are obtained by using the method of citrate and tannic acid, which allows for synthesis of particles of uniform and controlled diameter (min. 3 nm ± 12%, max. 17 nm ± 7%) by varying the ratio between the rapid and slow reductants, the tannic acid and the sodium citrate, respectively.

Our procedure, based on self-assembly of protein subunits and metal cores, is novel. As a consequence of the self-assembly principles, this method should be quite general, allowing for incorporation of different types of extant nanoparticles such as quantum dots, and superparamagnetic beads.

Here we ask the question: are the protein-protein interactions still the main driving force for the capsid self-assembly, or the Au-protein interaction is now dominant? To address this issue, we have varied the diameter of the nanoparticle to be incorporated and measured by transmission electron microscopy (TEM) the outer diameter of the nanoparticle/capsid complex. If the Au-protein interaction were the dominant one, one would expect an increase in the capsid diameter proportional to the size of the incorporated particle. We have found that this is not the case. Instead, the diameter of the capsid/nanoparticle complex is constant. Moreover, there is a cutoff size of 12.5 nm for the encapsidated Au particle, which further supports a dominant protein-protein interaction dictating the capsid formation. However, the Au particle encapsidation yield does depend on the Au particle diameter. We have obtained an optimum diameter of 9±1 nm for citrate-covered Au particles incorporation. The existence of an optimum diameter may point to the existence of a large protein precursor from which capsids form.

A 5-fold improvement in the yield of nanoparticle incorporation has been obtained by replacing the citrate with biotinylated DNA containing the high affinity capsid-binding site. The same DNA and biotin/avidin linker has been used to encapsidate CdSe quantum dots whose luminescent properties make them interesting candidates for tracking of particle-modified viruses in the cell. Fig. 2 shows TEM pictures of DNA-coated Au particles and quantum dot particles incorporated in brome mosaic viruses.

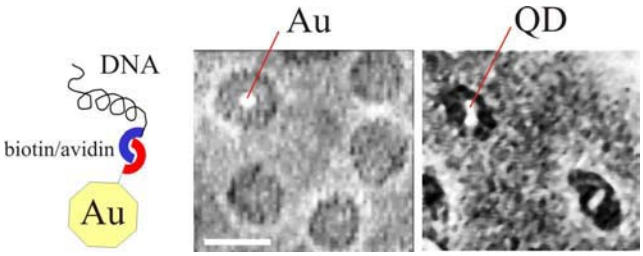

Figure 2: Core/capsid interaction can be adjusted to resemble more those in the wild-type virions by functionalizing the nanoparticle surface with DNA containing the high-affinity capsid-binding site. The DNA-modified nanoparticles can be then encapsidated as shown here for Au cores and CdSe quantum dot cores.

More research on the nature of nanoparticle core virus capsid interactions will unveil ways to make this interaction more similar to that occurring between the RNA molecules and the capsid, thus preserving the functional attributes of the capsid.

An important step forward would be to have access to the capsid formation at the scale of a single virus in real-time and in a physiological environment. Single-virus studies avoid the ensemble averaging of other methods and have direct access to intermediates. In the following, we introduce a novel method that is not limited by diffraction in the size of trapped particles, is non-intrusive and allows for parallel manipulation.

Near-field optical trapping of small particles inside nanochannels

When a small particle with index of refraction greater than that of its environment passes through the waist of a focused Gaussian beam of light, forces resulting from scattering of photons on different regions of the particle tend to immobilize the particle in the most intense part of the focal zone,

i.e. the center. This force can exceed the Brownian forces at the room temperature if the field gradient across the diameter of the particle is significant [5]. This method of three-dimensional position control of a particle in a fluid, known as optical tweezers, found an enormous number of applications ranging from fundamental physics of ultracold atoms to subcellular dynamics. The wide acceptance gained in the biology community by the optical tweezers technique, greater than any other proximity probe technique, is due to its non-intrusiveness, quasi three-dimensional character, and possibility of measuring very small forces 10^{-13}-10^{-10} N, relevant for biological matter. However, the requirement that the light intensity gradient across the whole diameter of the particle is non-negligible, implies that particles smaller than the diffraction limit (~250 nm) are difficult to trap. Because of this limitation, only relatively large viruses, such as the tobacco mosaic virus (~500 nm in length), could be trapped with mild-enough light beams to preserve their integrity. To alleviate the particle size problem, several researchers proposed to use near-field optical tweezers relying on the strongly enhanced electric fields present close to metal nanostructures [6][7]. The near-field close to such a metal structure consists of evanescent components, which decay on much shorter distances than the wavelength of the light.

This idea, although very promising, has not been realized in practice yet, with either aperture-based or apertureless near-field scanning optical microscopes, probably because it would be difficult to find in water a rapidly diffusing nanoparticle with a single probe tip.

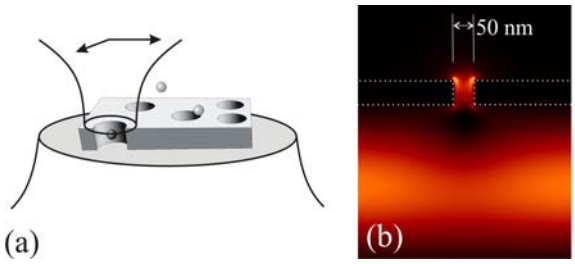

Figure 3: (a) Schematic picture of the nanochannel optical trap method: the incident beam illuminates from the bottom a few nanoholes at a time in an opaque metal screen. The nanoholes, with a diameter from 50 to 200 nm, are generated by nanosphere lithography and spaced widely enough to allow single nanohole selection by high numerical aperture collection optics from the top. (b) Vertical cross-section, with calculated density map of the electric field intensity, inside a one-dimensional channel through a 50 nm thick gold film. The incident field polarization is perpendicular to the channel walls; the laser wavelength is 800 nm.

Instead of using a near-field microscope tip to catch a nanoparticle, we have used a large ensemble of nanoholes, or channels, fabricated in a 50-200 nm thick metal film. For fabrication, we are currently employing the nanosphere lithography method, Fig. 3 (a). The particles for study are dispersed in solution in contact with the metal film. Some of them diffuse into the nanochannels. When a laser beam is shone onto the metal film, the particles located inside the nanochannels are trapped due to strong near-field intensity gradients present in the subwavelength cavity, Fig. 3 (b). Although a large ensemble of nanochannels are simultaneously illuminated, they can be "read" one at a time, with a confocal scanning microscope from the other side of the film. We can thus choose a trap that was incidentally populated at the time when the laser was turned on.

Initial experiments on the near-field trapping efficiency have been done on commercially available 200 nm fluorescent latex beads and 500 nm diameter nanochannels in a 250 nm thick Au film deposited on silica. The realization of this initial step in itself is a very promising result since despite a significant number of theoretical papers predicting near-field optical trapping effects, there are no experimental reports to confirm its achievement, with the exception of Ng et al. who observed coherent motion of colloidal particles close to the walls of a waveguide [8]. From measurements of the fluorescence emission from an occupied trap we have found that a) single beads are "sucked" inside the hole and remain there for the time the laser is on, b) the amplitude of the position fluctuations of a bead inside a nanochannel depends on the trapping laser intensity, c) stable trapping is obtained at a fraction of laser power required for the same bead diameter in the classical optical tweezers case, d) a 10-fold fluorescence enhancement is observed as a result of the interaction between the fluorescent bead and the near-field of the metal walls.

In the future, we will couple the nanoparticle/virus approach with the near-field optical scheme to monitor the self-assembly process between a nanoparticle core and protein subunits to make a viral particle with a nanoparticle core

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