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Gold Nanoparticles as Spectroscopic Enhancers for in Vitro Studies on Single Viruses

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The physicochemical principles that underlie the ability of viruses to be successful disease agents can also provide interesting prospects for drug delivery,[1] materials synthesis in confined environments,[2] molecular entrapment,[3] and the tracing of the intracellular transport pathways.[4]

All viruses contain two basic units: a core of nucleic acid plus a surrounding layer of proteins, known as the capsid. The viral capsid is generally formed from a large number of protein subunits, which enables the capsid to perform a variety of functions: protection of the genome, molecular recognition, nucleic acid release upon infection, reassembly and externalization of progeny virions.[5] Profound structural changes of the capsid are required to achieve successful infection, including changes in capsid protein composition, secondary and tertiary structural changes, and covalent cross-linking between neighboring subunits.[6] While these highly coordinated, large-scale, structural changes of fundamental importance to viral infectivity are known to occur,[7] little is presently known about how these transitions are triggered.

Cryoelectron microscopy combined with image reconstruction[8] and X-ray diffraction have started to reveal a process of fascinating complexity encompassing rigid subunit rotations, refolding, and phase transitions during virion assembly.[5,9] However, these static methods require a synchronous population of viruses, making it difficult to detect intermediates. Furthermore, the sample preparation conditions are usually far from a physiological environment. Traditional optical spectroscopic methods are much less intrusive and provide time resolution but also suffer from averaging effects over an ensemble of viruses.

One way to obtain time-resolved structural information in a controlled physiological environment and avoid averaging over different subpopulations is to study single virus particles. However, the extremely small amount of sample in a single virus requires techniques of spectroscopic enhancement.

The encapsulation of Au nanoparticles offers two possibilities in terms of spectroscopic enhancement: plasmon polariton or Rayleigh resonance (RR), and surface enhanced Raman scattering (SERS). Both are expected to occur due to the large polarizability of Au particles in the optical region.[10] RR spectroscopy can be done on single particles, while SERS is thought to require aggregates of several particles.[11] In this communication we report on the first example of RR spectroscopy on single brome mosaic virus (BMV) capsids (28 nm) with Au particles (2.5−4.5 nm) inside.

BMV offers a simple and convenient system to study the dynamics of macromolecular structure.[12] The past three decades of research on BMV have led to the development of a variety of tools to study basic processes of viral replication, protein−RNA interaction, RNA-dependent RNA replication, and viral capsid assembly and disassembly.[13,14] The BMV icosahedral capsid is composed of 180 protein capsomeres that are stabilized by weak interactions.[15] While the assembly of the capsid in the absence of nucleic acid is possible,[16] the virus particle formation in the infected cell requires concerted interactions between the positively charged amino-terminal portions of the capsomeres present within the inner cavity of the capsid and negatively charged RNA.[16]

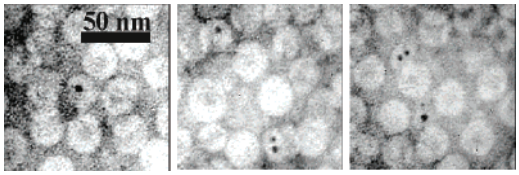

Figure 1. Transmission electron micrographs of dissociated and reassociated BMV virions enclosing Au nanoparticles (dark spots) with diameters between 2.5 and 4.5 nm.

Inspired by these facts, 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.[12] 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 (see Supporting Information for details). Negatively charged Au sols are obtained by using the method of citrate and tannic acid,[18] which allows for synthesis of particles of uniform and controlled diameter (minimum 3 nm ± 12%, maximum 17 nm ± 7%) by varying the ratio between the rapid and slow reductants, the tannic acid and the sodium citrate, respectively.

After reassembly, the BMV/Au particles were stained with 1% uranyl acetate and examined under the transmission electron microscope. Figure 1 shows a single Au particle and a pair of Au particles, approximately 4 nm diameter, enclosed inside the BMV capsids. Functionalized capsids usually maintain the same diameter and aspect as empty ones; however, distorted functionalized capsids were occasionally found. A better tolerance to Au particle encapsulation should be possible by tuning the Au particle surface properties to resemble properties of RNA. Due largely to the purification procedure, we have never observed Au particles stuck on the outer surface of the capsid, using this or other staining methods after purification, which supports the idea of electrostatic association between the negatively charged metal particle and the positively charged inner surface of the protein coat.

Purified BMV capsids containing Au particles exhibit a red coloration due to the characteristic RR of the Au particles.[10] On the basis of TEM, approximately 2% of the viral capsids are seen to be Au functionalized.

Since the RR is highly sensitive to changes in the dielectric constant of the medium within a few nm from the metal surface,[19,10] we compared the spectra of bare Au particles and those reassembled

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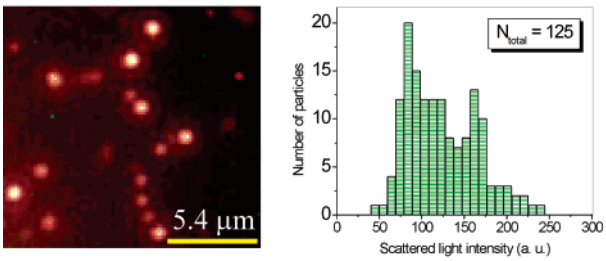

Figure 2. Dark-field optical micrograph of BMVs, decorated inside with Au nanoparticles (left). The population distribution of the scattered intensity (right) is bimodal, with an approximate ratio of the most frequent intensities of ~1:2.

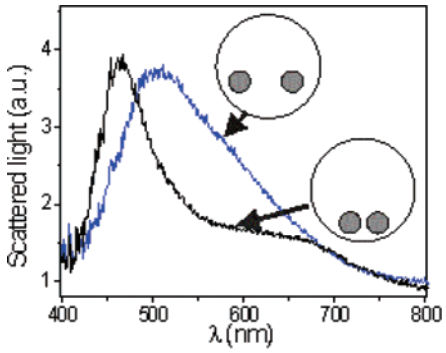

Figure 3. Rayleigh resonance spectra from two different selected BMVs, likely to include pairs of Au nanoparticles. A double-peaked spectrum (black) can be explained through particle coupling, while a single-peak spectrum (blue) is assigned to particles being too far apart to couple.

within BMV capsid proteins, both in the buffer used for virus reassembly (pH = 5.5). The RR peak has been found at 524 nm for bare Au particles, in good agreement with theory.[10] The RR peak for BMV capsids containing Au nanoparticles was broadened with respect to the one for bare Au and also blue-shifted to 518 nm.

The main differences between spectra of free and encapsulated Au nanoparticles are expected to come from (1) an increase in the index of refraction due to the protein layer[20] ($n \approx 1.34-1.55$) and (2) variations of the spectrum corresponding to pairs of electromagnetically coupled nanoparticles[10] (Figure 1). None of these changes predicts a blue shift. On the contrary, a red shift is expected. At this point, we do not have a rigorous explanation for the blue shift. We can only speculate that the shift may be caused by variations in the nature of bonding between the surface ions and the metal,[20] with the consequence of a change in the free electron distribution. Only a strong interaction between the basic residues on the capsid and the metal surface could result in such an effect.

Insights in the origin of the spectral broadening can be gained by measuring the RR spectrum of single Au-functionalized BMVs. We used confocal dark-field microspectroscopy to visualize isolated BMVs in an acidic buffer (pH 5.5) underneath a microscope cover glass (Figure 2).

A histogram of the intensity distributions qualitatively confirms the observation that there are two different populations of scattering centers, one bright and one dim, on the micrograph in Figure 2. This can be understood if we compare with Figure 1, showing two types of Au particle incorporation: single particles or pairs of particles. Moreover, the scattered intensity for the brighter spots is roughly the double of the dimmer ones (Figure 2). This supports the conclusion that the dark-field microscopy is done on single virus capsids containing either a pair or a single Au particle.

It is interesting to inquire into the heterogeneity of the spectra from single-virion enclosing pairs of Au particles. This question is relevant because one important source of heterogeneity should be the distance between the individual gold particles. This parameter dominates the spectral features when the two particles are at closer distances than one radius. Two emerging SPR peaks are expected from two in-phase plasmon polariton modes.[10] Figure 3 shows two extreme differences between spectra taken from scattering centers, which, based on scatter intensity, correspond to encapsulated pairs of particles. As suggested by the significant variations in Figure 3, the interparticle distance can be used to quantify the amount of capsid swelling, since the Au particles seem to be strongly attached to the viral capsid walls. Attempts to monitor the spectral changes associated with pH-induced capsid transformations using this method are in progress.

In conclusion, we present here the first example of Au particle incorporation in BMVs and their use as spectroscopic markers. RR spectroscopy done on single viruses should open new opportunities for in vivo monitoring of viral capsid transitions.

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Supporting Information Available: Experimental details of the dissociation/reassociation protocol (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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