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Controlled Encapsidation of Gold Nanoparticles by a Viral Protein Shell

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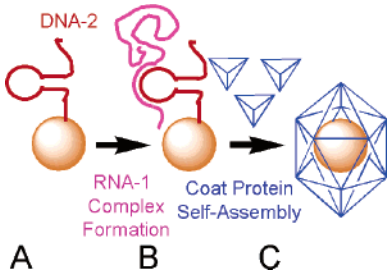
Plant virion capsids have desirable packaging and self-assembly properties that make them ideal structures for nanotechnology. For example, both host−guest chemistry[1,2] and supramolecular assembly have been demonstrated[3,4] using the self-assembly of icosahedral virus capsids as a scaffold. Herein we demonstrate the templated self-assembly of the *Red Clover necrotic mosaic virus* (RCNMV) coat protein (CP) around gold nanoparticles. RCNMV is an icosahedral virus composed of 180 CP subunits and a triangulation number of 3 (T = 3). The RCNMV genome is bipartite and comprised of a 3.9 kB polycistronic RNA-1 and a 1.4 kB monocistronic RNA-2. Although the X-ray crystal structure of RCNMV has not been determined, an 8.0 Å resolution cryoelectron microscopic reconstruction of a virion and a homology model of the CP have been generated.[5] Assembly of RCNMV and many other T = 3 viruses is stabilized by an internal protein/RNA cage.[9] Assembly of RCNMV begins with a specific recognition of a virion RNA sequence, the origin of assembly (OAS), by the CP. The binding of the CP to the OAS initiates the assembly process that results in the encapsidation of the viral genome. The RCNMV OAS consists of a complex of RNA-1 with an RNA-2 stem loop previously termed the trans-activator.[10] Insertion or attachment of the RCNMV RNA-2 stem loop to a foreign viral RNA results in packaging of the foreign RNA into virions possessing a wild-type morphology.

An artificial RCNMV OAS can be created on a nanoparticle by the attachment of a 20-base 5′-thiol deoxyuridine-modified DNA oligonucleotide analogue of the RNA-2 hairpin (d SH-5′-AGAG-GUAUCGCCCCGCCUCU-3′). This allows RNA-1 to hybridize to the stem loop, forming the functional OAS which then templates assembly of CP to the nucleic acid complex and formation of virus-like particles (VLPs). The use of cognate virion RNA to trigger encapsidation of a metal nanoparticle in RCNMV demonstrates a new principle of templated self-assembly, based on RNA−protein interactions. Templated self-assembly by RCNMV complements the use of DNA as a structural element in nanoassemblies.[6] The methods described here also have the potential to replace the use of proteins such as bovine serum albumin, widely used in cytochemistry applications, as a carrier that can be used to conjugate targeting peptides and proteins to the surface of nanoparticles.[7,8]

RCNMV undergoes reversible pH-dependent swelling. Structural analysis by dynamic light scattering (DLS) indicates that RCNMV swells from an average diameter of 35 ± 3 nm at pH < 6.5 to a diameter of 45 ± 3 nm in the range 6.5 < pH < 8, likely due to inter-CP charge repulsion in the virion (see Supporting Information). Utilizing a pH gradient, purified RCNMV CP can be assembled in vitro into VLPs. By solubilizing monomeric CP at pH < 9.5 and then reducing the pH by dialysis, VLPs are formed. The most efficient reassembly is observed when the final pH is 5.5. The assembly reaction in the absence of Au nanoparticles and RNA template yields VLPs with an average diameter of 29.8 ± 3 nm, as measured by DLS and transmission electron microscopy (TEM), which is smaller than native RCNMV (36.6 nm).[5]

Scheme 1. Encapsidation of Au Nanoparticles by RCNMV[a]



[a] The procedure mimics the encapsidation of the genome using an oligonucleotide (DNA-2) to capture the larger polycistronic RNA-1. The loop−RNA complex forms the origin of assembly that binds to CP. (A) Thiol-modified DNA conjugates to Au. (B) RNA-1 binds to DNA to form origin of assembly (OAS). (C) Coat proteins (CP) recognize OAS and polymerize around Au.

In this report, we have developed a strategy to encapsidate Au nanoparticles within an RCNMV CP shell. Scheme 1 shows the following steps: (A) DNA-2 was tethered to Au particles using conditions that limit the oligomers bound per particle. (B) Transcript RNA-1 to 100 conjugated Au, and the sample was incubated for 10 min. This incubation period allowed the RNA-1 to covalently attach to the complementary sequences on the modified oligonucleotides. (C) RCNMV CP was then added to the mixture. Particle assembly was initiated by dialyzing the sample against 50 mM Tris-HCl, pH 5.5, overnight at room temperature. The reaction was collected and pelleted through a 50% sucrose pad by centrifugation at 218000*g* for 20 min.

The encapsidation of an Au nanoparticle by viral CP significantly increases the density of the resultant VLP. The increase in the percentage of the sucrose pad from the 20% utilized for native virion purification to the 50% used for VLP isolation assured that the pelleted material recovered does not contain unreacted CP or VLPs lacking an Au nanoparticle core. The purified sample was subsequently examined by DLS and TEM. Based on the results from these two methods, the average size for an assembled virus-like particle was 33.5 ± 3 nm. TEM images show that purification by sucrose density centrifugation eliminates 90% of the failed reaction products. Approximately 35% of the gold nanoparticles added to the assembly reaction were recovered as encapsidated particles. The ability of the encapsidated VLPs to withstand the pelleting process further suggests significant structural integrity of the particles. A negative stain of the sample with 2% uranyl acetate showed the presence of the complete protein shell encapsulating 10 nm Au nanoparticles (Figure 1).

The cryo-reconstruction indicated that the inner cage of RCNMV has a diameter of 17 nm. To determine if this inner diameter reflects the size of the nanocore that can be encapsidated, VLP assemblies were performed with 5, 15, and 20 nm Au nanoparticles. TEM images illustrate encapsidation of 5 and 15 nm but not 20 nm Au nanoparticles cores within viral CPs. Furthermore, the diameter of the Au core correlated with the VLP diameter (a table with diameter

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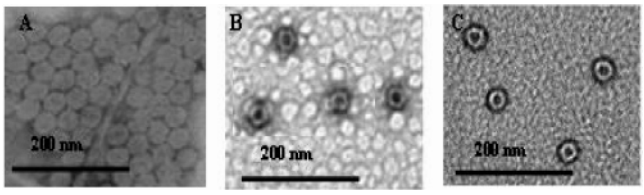

Figure 1. TEM images of (A) native RCNMV and encapsidation of 10 nm Au nanoparticles within RCNMV protein subunits (B) prior to and (C) after purification by sucrose centrifugation.

of the nanoparticle package and the outer diameter of the VLP is available in Supporting Information). These results indicate that RCNMV CP is able to encapsidate a range of gold core sizes. The lack of VLP formation with larger cores suggests that there is a maximum limit between 15 and 20 nm that, when exceeded, prevents formation of stable particles.

Control experiments were conducted to determine the requirement for the presence of the RCNMV OAS for encapsidation. In the first control, the assembly was conducted with 10 nm Au nanoparticles coated with bissulfonato(phenyl)phenylphosphine (BSPP) in the absence of RNA-1 or the DNA oligomer. While VLPs with encapsidated Au cores were observed by TEM prior to centrifugation, the pelleted fraction lacked them. The disintegration of the VLPs during centrifugation was likely due to a lack of structural integrity provided by an internal RNA/protein complex. A second control experiment was performed that utilized a random 20-nucleotide DNA oligomer attached to the Au nanoparticles. No VLPs were observed in the pelleted fraction after centrifugation. Finally, when a non-RCNMV RNA template was used in assembly reactions, no VLPs were obtained in the pelleted fraction. These results indicate the absolute requirement for the cognate RNA OAS to stabilize the encapsidation of the nanoparticle.

Nanoparticles have been widely investigated in cell-targeting applications. However, the colloidal stability of nanoparticle suspensions presents a limitation for intracellular targeting applications. To overcome this, a number of classes of biological molecules and polymers have been studied as steric stabilizers. For example, proteins and poly(ethylene glycol) can be used as surfactant to stabilize nanoparticles. Protein nanoparticle conjugates are often not very well defined in structure. It has been shown that 160 ± 8 bovine serum albumin (BSA) proteins were required to stabilize a 20 nm Au nanoparticle,[11] which is surprisingly close to the 180 protein subunits in RCNMV and many other $T = 3$ viruses. However, assemblies of hundreds or thousands of proteins around a metal nanoparticle have been observed.[12]

A virus protein capsid can function in two aspects important for the design of cell-targeting nanoparticles. First, the viral protein covering offers a constrained system that can be exploited as a container and provide protection for the encapsidated cargo. Second, the surface of the viral CP can be chemically engineered and modified to display specific targeting peptides or proteins.[13−15] Given that viral capsids can be engineered to target specific cells,[16,17] they have been investigated as a model system for drug delivery and material science applications. The self-assembled *Cowpea chlorotic mottle virus* (CCMV) has been studied as an imaging agent[18] and as a container for nanoscale synthesis of inorganic materials.[19] The experiments performed here demonstrate the reverse principle. The nanoparticle can be used as a template for viral assembly. With the nanoparticle acting as the nucleating point, multifunctional nanoparticles can be protected from degradation and avoid the colloidal stability problems characteristic of other approaches.

In summary, we have demonstrated a strategy that utilizes the origin of assembly sequences as a trigger to package nanoparticles. Association of these sequences on nanoparticles provides recognition to the CP. Analogous to the behavior of a wild-type RCNMV, self-assembly of CP then begins until a virion is formed with the nanoparticles fully packaged within the coat protein. The specific interaction between the CP and the sequences tethered on nanoparticles offers a great stability and specificity in nanoparticles encapsidation that can be furthered explore to package other cargo. The method reported here offers an approach that enables us to control the encapsidation of the desired materials by viral coat proteins.

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Supporting Information Available: Dynamic light scattering, TEM images, and Experimental Section describing the protocol for reassembly of empty capsid. This material is available free of charge via the Internet at http://pubs.acs.org.

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