Nanodumbbells

Self-Assembly of Metal–Virus Nanodumbbells**

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A challenge of nanoscale fabrication is the selective modification of nanoobjects at specific sites, for example, the selective chemical modification of the ends of a rod. Mokari et al. developed an elegant strategy for the functionalization of the ends of semiconductor nanorods,[1] while more complex assemblies based on proteins usually require tailor-made antibodies.[2]

We present here evidence that the presence of RNA allows for a simple and elegant method to produce gold nanostructures attached exclusively to the ends of the tobacco mosaic virus (TMV). We found that gold nanoparticles of 6-nm diameter bind specifically to the ends of the 300-nm-long viral tubes. We discuss mechanisms that may explain the surprising selectivity, especially the probable interaction of Au nanoparticles with the RNA strand[3,4a] that is partially liberated from the two ends of the TMV. The size of the Au nanoparticles can be enhanced to several tens of nanometers by electroless deposition of gold, thereby resulting in gold-virus-gold dumbbell structures.

Manipulation and assembly of particles on the nanometer scale is of great interest because the collective properties of nanoparticles arranged in patterns are in general different from the properties of a single nanoparticle.[1,5,6] DNA[5,7] and viruses[6] have highly symmetrical architectures and narrow size distributions which make them ideal templates for the synthesis of nanomaterials, often by using well-known nanoparticle-labeling techniques.[4] Rodlike viruses are attractive 1D templates for metal nanoparticles; the resulting structures

might be used advantageously to build novel materials and devices.[8–19]

An especially promising candidate is TMV[20] since it can aggregate end-to-end to form rods longer than the length of a single virion.[21] The known exact chemical and structural details allow for regioselective covalent linking[11] or localized chemical reactions with metal ions[15–18] or particles[19] to give nanowires and linearly assembled nanoparticles. However, the ends of the viral rods are only amenable to binding tailor-made antibodies,[2] which have various disadvantages—at least in terms of selectivity—for example, the production of antibodies in animals, and the attachment of metal clusters at a considerable distance from the orifice of the viral channel. Note that in its natural state, TMV contains no cysteine residues on its external surface, so Au nanoparticles cannot bind to the wild-type virus, although the residues can be introduced by genetic engineering,[22] as demonstrated with the cowpea mosaic virus.[6]

Our method is based on mixing a gold sol[4a] with a suspension of the virus (Au nanoparticles/TMV ratio ca. 4000:1, incubation overnight at RT, no agitation). Transmission electron microscopy (TEM) and atomic force microscopy (AFM) allow the selectively attached Au nanoparticles to be visualized. About half of the labeled virions carried a single Au nanoparticle (Figure 1a), the others bound two or more nanoparticles (Figure 1b). Altogether, about 90% of the TMV particles were labeled (determined from the TEM observations). We never observed particles bound to the exterior surface of the virion.

An important question is to which groups the Au nanoparticles actually bind. Protein sites are possible candidates—however, on the scale of the Au nanoparticles (gold facets of some nm extension), one cannot expect preference for certain parts of the protein, namely the surface of the four-barrel structure of the protein—which is available only at the virion ends. The only alternative is RNA, which binds to gold nanoparticles;[3] in fact, nanoparticle–RNA conjugates can be employed as tracers.[4b] The RNA helix is buried deeply inside the protein sheath,[20] but this is not the case at the ends of the virion. Indeed, the coat proteins can be removed, thereby exposing the RNA to solution.[23] We first tested whether free RNA can bind to Au nanoparticles by incubating RNA with the gold sol overnight. Figure 1d suggests that Au nanoparticles bind to RNA: The overall structure is reminiscent of the curled structure of RNA; however, it is densely coated with Au nanoparticles, in analogy to Au nanoparticles at the ends of the TMV (Figure 1b).

Gold sols are well-established tools, especially in biology.[4] In our case, the termination with citrate yields negatively charged particles with a zeta potential of about

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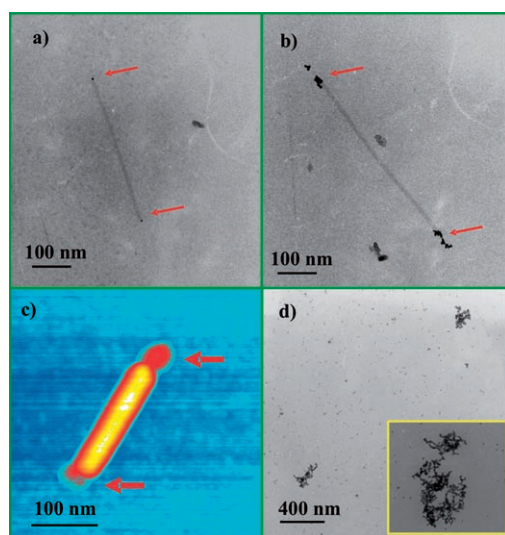
Figure 1. TEM (a,b,d) and, topographical AFM images (c) of Au nanoparticles (red arrows) bound to TMV and TMV-RNA. a) A single TMV containing one Au nanoparticle at each end. b) Two virions aggregated end-to-end; the ends of the rod contain bound Au nanoparticles. c) A single TMV (ca. 13 nm high) on a silicon substrate containing Au nanoparticles (5–8-nm high) at each end. d) Au nanoparticles bound to coiled TMV–RNA; the inset shows an expanded picture (350×350 nm).

−42 mV.[24,25] TMV is negatively charged (isoelectric point ca. 3.5) at the pH value employed (ca. 5).[26] Therefore, electrostatic interactions between the Au nanoparticles and the TMV–RNA can be excluded. Furthermore, interactions between the RNA charge and the induced dipoles in the Au nanoparticles[27] cannot operate here because the nanoparticles would then also bind to the external surface of the TMV; although the coat protein may offer some positively charged residues close to the exterior surface, the nanoparticles do not bind there. Recently, Cardenas et al. proposed that the hydrophobic effect of the DNA bases resulted in the binding of Au nanoparticles to DNA.[28] It is also known that pyridine replaces citrate bound on gold surfaces.[29] We expect a similar mechanism in our case (Figure 2): The aromatic bases of the RNA replace surface-bound citrate, hence Au nanoparticles selectively bind to RNA.

We proved the presence of RNA bases at both ends of the virions indirectly by partial enzymatic removal of the RNA
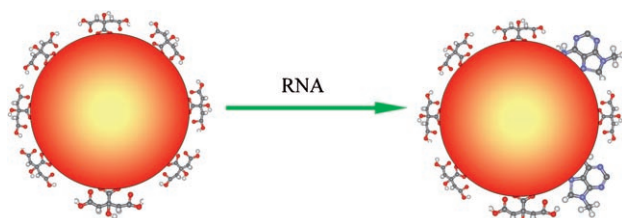

Figure 2. Schematic view of the selective attachment of Au nanoparticles to RNA (drawing not to scale). The heterocyclic aromatic bases in the RNA replace carboxylate groups on the gold surface (O red, C gray, N blue, H white). The RNA can be free RNA, or RNA protruding from TMV.

with RNase A. In parallel experiments, an aliquot of a TMV suspension was treated with RNase A and incubated with the gold sol, while another aliquot was incubated only with the gold sol. Approximately 20% of the TMV particles treated with RNase A were labeled with Au nanoparticles, while without RNase A treatment about 90% of the virions were selectively labeled at their ends. We presume that the Au nanoparticles are able to remove several coat proteins and thereby expose the RNA; indeed, the average length of the gold-labeled virions is approximately 25 nm lower than the length of native virions.

To expand the range of functionality of the Au nanoparticles, electroless deposition of gold was used to enlarge the size of the Au nanoparticles.[30] Silicon wafers with adsorbed TMV–Au nanoparticle conjugates were immersed in gold baths, and dumbbell-shaped structures with heights of (69 ± 13) nm formed (Figure 3).
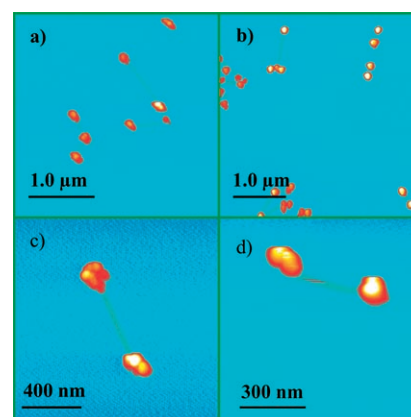

Figure 3. Topographical AFM images of gold nodules at the ends of TMV rods (adsorbed on silicon substrates). a), b) TMV rods with gold nodules at their ends. c), d) Details of the dumbbell structures. A viral rod can contain a single virion, two virions, or three virions aligned end-to-end.

In conclusion, Au nanoparticles can selectively bind to the ends of TMV and their size can be enhanced to yield gold-virus-gold dumbbells. It is likely that a section of the viral RNA is accessed, and its heterocyclic aromatic bases interact specifically with Au nanoparticles by replacing citrate on the gold surface. Therefore, excellent spatial selectivity can be achieved without chemical or genetic modification of TMV. The interaction of unmodified (for example, not thiolated) RNA with Au nanoparticles can be used as a novel method for assembly on the nanoscale of molecules containing nucleobases, such as peptides and enzymes bound to nucleic acids, or other viruses, or for RNA sequence detection.[3] Furthermore, selective growth of Au nanoparticles on the viral ends shows some possibilities for future work: for example, their application as nodes for establishing reliable electrical contacts on the nanoscale; binding with magnetic metals could allow for macroscopic manipulation of the TMV with external magnetic fields; and optical effects should result in local field enhancements of the nanoparticles.

Experimental Section

Plasmid DNA containing a full-length copy of TMV cDNA was employed to mechanically infect *Nicotiana tabacum* var. Samsun nn plants (for details about the isolation of TMV from the plants and their storage see Ref. [17]). Gold sol (typically 200 μL of 0.8 mg mL^{-1}; Aurion, Wageningen, NL) was added to dialyzed TMV suspension (800 μL of 0.025 mg mL^{-1}). Au nanoparticles were selectively size-enhanced with an established electroless deposition method:[30] An oxidized silicon wafer with TMV–Au nanoparticles was immersed into a mixture of 0.06 M cetyltrimethylammonium bromide (CTAB, 150 μL; Fluka, 99.8%), 0.25 mM HAuCl$_4$·3H$_2$O (200 μL, Aldrich), and 0.1 M ascorbic acid (10 μL, Merck), adjusted to pH 4. The coat proteins were disassembled by mixing TMV (10 μL of 10 mg mL^{-1}) with 90 μl of proteinase K (100 μg mL^{-1}) in 0.1 M Tris buffer (Tris = tris(hydroxymethyl)aminomethane; Fluka, 99.8%) at pH 8.3 (2 h, RT, no agitation); then the gold sol (200 μL of 0.8 mg mL^{-1}) was added. TEM was performed on a Philips CM 200 operated at 200 kV. Thermomicroscopes Autoprobe M5 and CP were operated in non- and intermittent contact mode with Nanosensors (NCHR) or Budgetsensors cantilevers.

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