doi:10.1246/cl.2012.113
Published on the web December 30, 2011

Virus Capsid Coating of Gold Nanoparticles via Cysteine–Au Interactions and Their Effective Cellular Uptakes

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This manuscript describes the synthesis of virus capsid protein-coated Au nanoparticle (VP–AuNP) without the use of the inherent self-assembly of virus proteins into virus particles. Covalent binding between Au and cysteines in the virus proteins keep the cell-surface binding sites on the external surface. Based on this method, various sizes of VP–AuNP can be created in a similar manner to native virus particles. We clarified the optimum size of the VP–AuNP for internalization into cells.

The explosive growth in nanoscience has led to the development of a large number of novel nanomaterials for application to the biomedical and pharmaceutical fields. In particular, inorganic nanoparticles have received much focus as potential nanomaterials due to their unique optical and magnetic properties.[1–3] For the transportation of such nanomaterials into cells in vivo, nanomaterials should be modified to provide several crucial features, such as highly stable dispersibility in serum, molecular recognition enabling their internalization into the cells, and an optimal size for efficient uptake into cells.[4–6] Virus capsid proteins (VPs) are ideal building blocks for the coating of nanoparticles (NPs) as they possess binding sites to cell surface receptors and simultaneously provide NPs with stable dispersibility in serum.[7–10] There have already been a number of reports on the encapsulation of nanoparticles by virus capsid proteins. For example, Dragnea et al. have reported that AuNPs and Fe_3O_4 can be encapsulated by VPs during the VP self-assembly process to generate AuNP-templated virus capsids.[11,12] In this approach, the enclosed AuNPs assist in the self-assembly of VPs to form virus capsids (Figure 1a). Therefore, the size of the virus particle is similar to, or smaller than, the original virus size, and NPs larger than the native virus cannot be functionalized as a template.

Herein, we prepared gold nanoparticles coated with VPs (referred as VP–AuNPs) through the covalent linkage of AuNPs and cysteine residues (Cys) on a virus protein (Figure 1b). Importantly, the covalent attachments between Au and Cys residues orient the binding sites toward the external surface, so that the VP–AuNPs can be considered as *pseudo*-virus capsules. In this approach, the size of the VP–AuNPs could be controlled independent of the size of the original virus particles. Our aim in this study is to clarify the size dependency of VP–AuNP uptake into mammalian cells. The cellular uptake of quantum-dot encapsulated virus capsules has been reported,[13] however, there has been no discussion of size dependency of virus capsules on their cellular uptakes. Our results would lead to the design of a variety of nanoparticles coated with VPs that maintain their function in cells.

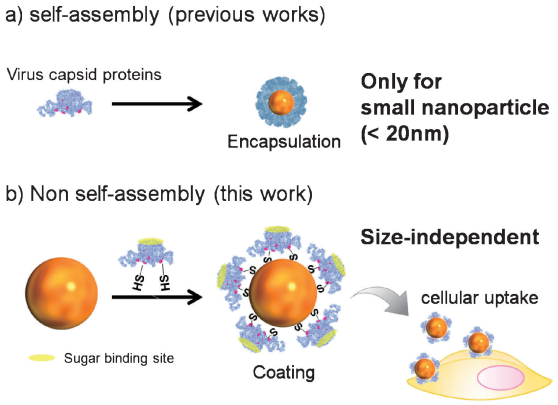

Figure 1. Schematic illustration of virus capsid-coated AuNPs using (a) self-assembly or (b) non-self-assembly system.

We used the virus capsid protein VP1 derived from JC virus, which belongs to the Polyomavirus family, for coating AuNPs. VP1 monomer (VP1m) spontaneously forms VP1 pentamers (VP1ps) in buffered solution.[14] Each VP1m contains six cysteine residues at position 42, 80, 97, 200, 247, and 260.[15] The solvent accessibility of cysteine residues on VP1p were calculated on the basis of previous literature.[16,17] A structural model of the JCV VP1p was created by aligning the primary sequence of JCV VP1m with the coordinates of VP1m from SV40 (PDB: 1SVA). On the basis of this calculation, we found that only the cysteine at position 97 in a VP1p, which is located on the internal surface of the virus particle, was exposed to the solvent (Figure 2). Each VP1m has a binding site for α2,6-linked sialic acid (yellow region in Figure 2) located on the face opposite the cysteine residue at position 97.[18,19] Thus, the linkage of five cysteines on VP1p to the AuNP surface would orient the binding sites outward, allowing efficient binding to the cell surface.

We selected bis(*p*-sulfonatophenyl)phenylphosphine (BSPP) as the ligand agent for the AuNPs. BSPP is a water-soluble derivative of triphenylphosphine that has been often used as a stabilizing agent for AuNPs under high salt concentrations.[20,21] After incubating citrate-modified AuNPs (diameters of 20, 30, 40, and 50 nm) with 5 mM BSPP for 6 h, the AuNPs were then purified by multiple centrifugations to remove excess BSPPs to yield BSPP–AuNPs.

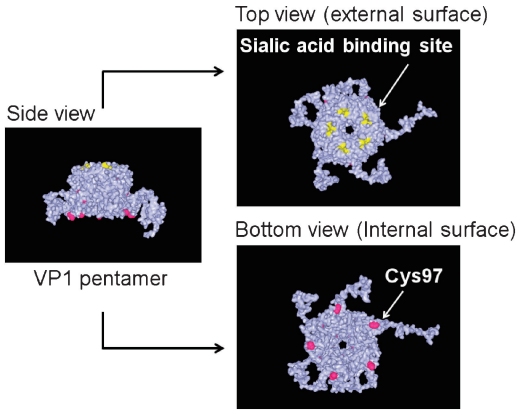

Figure 2. Distribution of cysteine residues and sialic acid binding sites on the surface of a VP1 pentamer of JC virus. Red dots and yellow regions show cysteine residues and sialic acid binding sites, respectively.

VP1ps were recombinantly expressed in *Escherichia coli* as described in our previous report[22] and purified by HPLC on a size exclusion column (see ESI[26]). A VP1p solution (0.02 mg mL^{-1}) was added to the BSPP–AuNPs (1.0×10^{11} particles) in HEPES buffer (pH 7.4, 20 mM) and subsequently incubated for 2 h at room temperature. The AuNPs solution was purified by several centrifugations to remove excess VP1ps. Figure 3a shows the hydrodynamic diameter determined by DLS before and after the treatment with VP1p. As a control experiment, carboxyl lipid-modified AuNPs (COOH–AuNPs), whose ζ-potential (−30 mV) is the same as that of BSPP–AuNPs (−28 mV), were used. The hydrodynamic diameter of BSPP–AuNPs treated with VP1ps increased by approximately 8 nm compared with the untreated BSPP–AuNPs. This increase in value is in agreement with the estimated size of the VP1p monolayer. On the contrary, the addition of VP1ps to the COOH–AuNPs did not cause any increase in particle size, indicating that there was interaction between the VP1ps and COOH–AuNPs. This result suggests that the VP1p cysteine residues cannot access the COOH–AuNPs due to blocking by packed alkyl chains. The binding of VP1ps to BSPP–AuNPs, but not COOH–AuNPs, was also confirmed by STEM images (Figure 3b). Images clearly indicate that VP1ps accumulated around the BSPP–AuNPs, but it appears that there were no proteins around the COOH–AuNPs. Since the COOH–AuNPs have a similar ζ-potential to BSPP–AuNPs, these data also indicate that VP1ps did not bind to the BSPP–AuNPs via nonspecific electrostatic interactions, but via thiol–Au bindings.

We confirmed the thiol–Au binding between VP1ps and BSPP–AuNPs by XPS analysis (Figure 4). The original spectrum of BSPP–AuNPs showed two peaks corresponding to the binding energy of electrons in Au 4f orbitals at 87.0 (Au $4f_{5/2}$) and 83.0 eV (Au $4f_{7/2}$). After coating the VP1ps, these peaks shifted approximately +1.0 eV. This represents a change in orbital energies from Au–Au or Au–BSPP (Au^0) to Au–S (Au^{+1}), indicating that the direct binding of Cys97 inside the VP1ps to the BSPP–AuNPs surface.[23] These results suggest that VP1p-coated AuNP (VP1p–AuNP) binding sites to α2,6-linked sialic acid remain on the external surface as in the native virus.

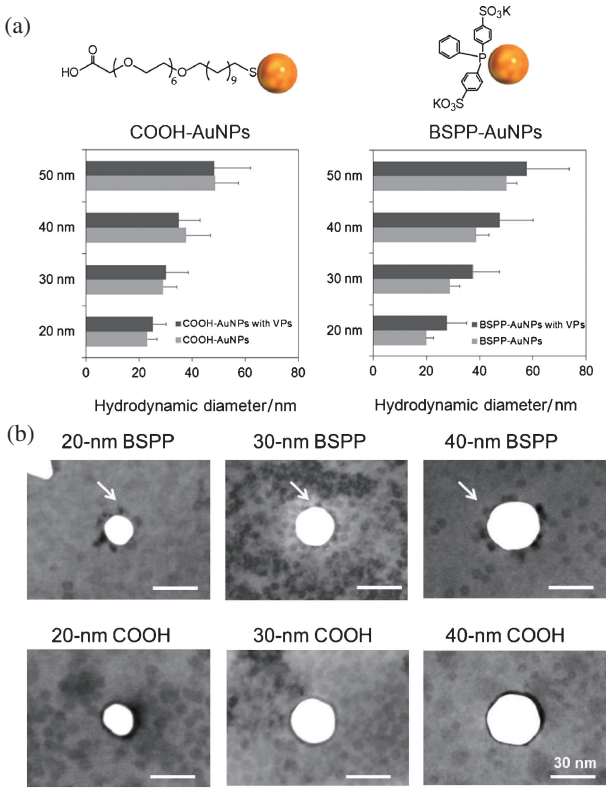

Figure 3. (a) Hydrodynamic diameter of AuNPs before and after the treatment with VP1 pentamers (VP1ps). (b) STEM images of BSPP–AuNPs with VP1ps (upper) and COOH–AuNPs with VP1ps (lower). White arrows show VP1ps.

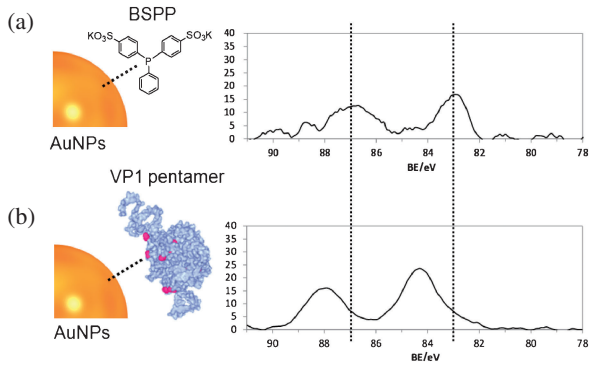

Figure 4. XPS spectrum of 40-nm AuNPs cast on a silica substrate. (a) BSPP–AuNPs and (b) VP1p-coated AuNPs.

We investigated the effect of AuNP size on cellular uptake using NIH3T3 cells. The cells were incubated in a VP1p–AuNP solution (1.0×10^{11} particles) in Dulbecco's Modified Eagle Medium (DMEM) for 1 h at 37 °C and collected by centrifugation. The average number of AuNPs in a single cell was calculated using an inductively coupled plasma atomic emissions spectrometer (ICP-AES) analysis (Figure 5a). As a control, we used BSPP–AuNPs. VP1p–AuNPs were largely taken up into cells compared to the BSPP–AuNPs for all diameters. These results support the idea that VP1p–AuNPs retained the inherent ability to recognize sialic acids. We added BSA-coated AuNPs into the culture medium of NIH3T3 cells as a control and

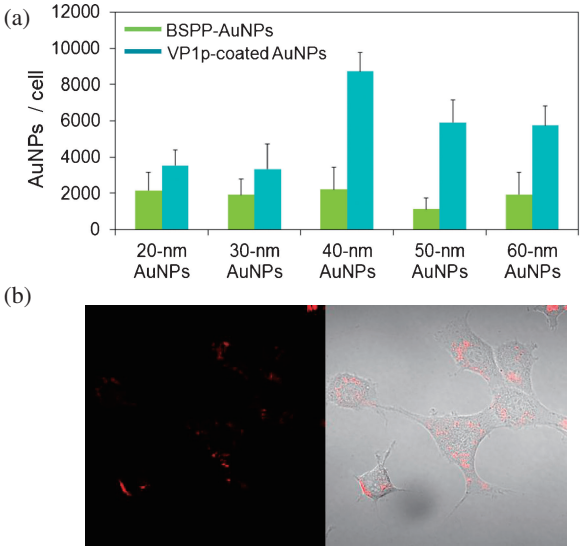

Figure 5. Cellular uptake of VP1p–AuNPs into NIH3T3 cells after incubation for 1 h at 37 °C. (a) Effect of the core size of VP1p–AuNPs on uptake. (b) CLSM images show fluorescence image (left) and merged fluorescence and DIC image (right). Alexa Fluor 647 was conjugated to VP1p–AuNPs (40 nm).

confirmed that they were not internalized into the cell (data not shown). This supports the uptake of VP1p–AuNPs into cells through the specific recognition of sialic acid on the cell surface. A graph of the number of AuNPs in/on cells versus AuNP size shows that cellular uptake was largely dependent on size. It was found that 40 nm was the most efficient core size for cellular uptake. The size of the VP1p–AuNPs (47.5 ± 12.6 nm) corresponded to that of native JC virus (≈50 nm).[24] This implies that the size of the JC virus has been tuned for efficient cellular uptake. The 40-nm VP1p–AuNPs showed most readily internalization probably due to the best combination between the binding affinity to the cell surface and the endocytosis efficiency. The self-assembly process of animal virus proteins tends to yield virus capsules smaller in size than the original virus,[13,17] however, our result clearly demonstrates that the construction of virus capsules of the same size as the original virus is important to their use in efficient drug-delivery carriers. Micro BCA assay showed the surfaces of AuNPs were completely covered with VP1ps and the surface density was almost identical for all size of AuNPs (Table S2[26]). This indicates that the cellular uptake of AuNPs is not dependent on their surface coverage of virus proteins, but on the size of AuNPs. The internalization of VP1p–AuNPs into cells was supported by confocal laser scanning microscopy (CLSM, Figure 5b). For visualization, Alexa Fluor 647 was conjugated to the lysine residues on the outside of the VP1p after construction of the VP1p–AuNPs. The results show that the VP1p–AuNPs were detected in cells, mainly from endosome-like intracellular vesicles. These dot images suggest that these AuNPs were internalized into cells by a pathway similar to that of virus capsules.[25]

To the best of our knowledge, this is the first report of nanoparticle-encapsulated virus capsules prepared using a non-self-assembly approach, through the direct binding of cysteine and Au. We clarified the optimum size of VP1p–AuNPs for internalization into cells. Our approach, based on the covalent binding of virus protein and AuNPs so as to retain the cell-surface binding sites on the external surface, has widened the possibility of transfecting various sized and shaped nanomaterials, thereby facilitating the plasmonic features of nanomaterials in cells for application to photothermal therapy and raman scattering-based diagnostic tools.

We gratefully acknowledge the Japan Society for the Promotion of Science (JSPS) for financial support through FIRST Program. KN is thankful for a research fellowship of JSPS for young scientists. A part of this work was conducted at Hokkaido Innovation through Nanotechnology Support (HINTS), supported by "Nanotechnology Network JAPAN" Program of the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

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