Nanocluster Assembly

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Bionanotube Tetrapod Assembly by In Situ Synthesis of a Gold Nanocluster with $(Gp5–His_6)_3$ from Bacteriophage T4**

Takafumi Ueno, Tomomi Koshiyama, Toshimitsu Tsuruga, Toshiaki Goto, Shuji Kanamaru, Fumio Arisaka, and Yoshihito Watanabe**

Utilization of biomolecules for the synthesis of novel materials is a very important strategy for the fabrication of new nanomaterials.[1–3] A number of proteins with a diverse range of structures (tube, ball, and cage) have been employed as frameworks for the construction of multidimensional

[*] Dr. T. Ueno
Research Center for Materials Science
Nagoya University
Nagoya 464-8602 (Japan)
Fax: (+81) 52-789-2953
E-mail: taka@mbox.chem.nagoya-u.ac.jp

T. Koshiyama, T. Tsuruga, Prof. Dr. Y. Watanabe
Department of Chemistry, Graduate School of Science
Nagoya University
Nagoya 464-8602 (Japan)
Fax: (+81) 52-789-2953
E-mail: yoshi@nucc.cc.nagoya-u.ac.jp

T. Goto
Department of Physics, Graduate School of Science
Nagoya University
Nagoya 464-8602 (Japan)

Dr. S. Kanamaru, Prof. Dr. F. Arisaka
Department of Biomolecular Engineering
Graduate School of Bioscience and Biotechnology
Tokyo Institute of Technology
B-39 4259 Nagatsuta-cho, Midori-ku, Yokohama 226-8501 (Japan)

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devices,[4–16] since proteins are useful materials for the deposition of metal composites and the mineralization of metal ions. Metal composites with important electronic, magnetic, and optical properties can be introduced either on the surface of or inside proteins by attaching peptide fragments to the proteins.[11–17] For example, native and synthetic histidine-rich peptide fragments exhibit a high affinity for metal cations and nanoclusters,[11,15,18–20] and have been used for this purpose: 1) synthetic His_6 fragments introduced into viruses and chaperonin have been applied for metal-cluster patterning[11,15] and 2) native histidine-rich fragments have been demonstrated to control the size distribution in nanocluster syntheses for various metals.[18,19]

However, it is still difficult to assemble proteins on metal composites and regulate their structures.[14,16] If we could control multidimensional protein assembly on metal composites, it would be a significant advance in the field of bionanodevice self-assembly. We have chosen gene product 5 (gp5) of bacteriophage T4 as an attractive model for work in this direction. Rossmann and co-workers, including two of the present authors, have reported the crystal structure of $(gp5)_3$ complexed with $(gp27)_3$ (Figure 1a).[21] Gp5 has three domains, namely the N-terminal domain (gp5N), which possesses an oligonucleotide/oligosaccharide (OB) fold, the lysozyme domain, and the C-terminal domain (gp5C), which forms a three-stranded β helix as $(gp5C)_3$ that may be called a bionanotube (Figure 1b and c). Gp5C functions as a needle to penetrate the outer membrane of *Escherichia coli*. Gp5 is one of the essential tail proteins in the assembly of bacteriophage T4. The complexes of $(gp27–gp5)_3$, $(gp5)_3$, and $(gp5C)_3$ were overexpressed and purified with the aid of a His_6 fragment introduced onto the C terminus of gp5 as described in previous reports.[21,22] We then studied the self-assembly of trimeric gp5 coupled with the in situ synthesis of Au nanoclusters (NCs). The three histidine-rich regions form a triad at the C termini of $(gp5–His_6)_3$ (Figure 1a). The triad of His_6 fragments is expected to assist in the synthesis of an Au NC at the C termini of the β-helical nanotube, $(gp5C–His_6)_3$. In this paper, we report the in situ preparation of Au NCs bearing a tetrapod assembly of the bionanotubes (Figure 2).

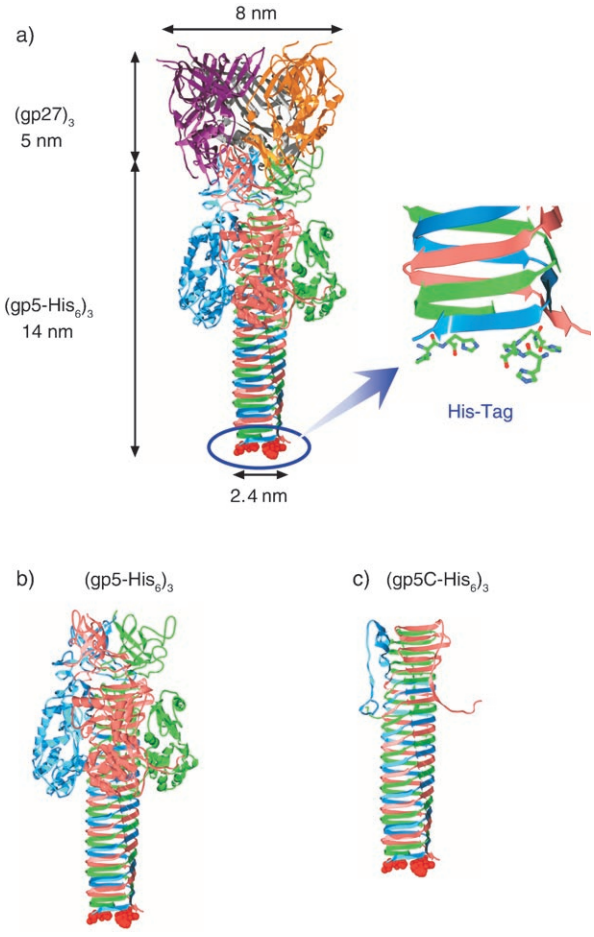

Figure 1. Structures of a) the membrane-puncturing bionanotube complexes of $(gp27–gp5–His_6)_3$ from bacteriophage T4 with a close-up view of the His_6 fragment region showing the histidine residues, b) $(gp5–His_6)_3$, and c) $(gp5C–His_6)_3$ (Protein DataBank file code: 1K28). The His_6 triad is displayed as a space-filling model in red.

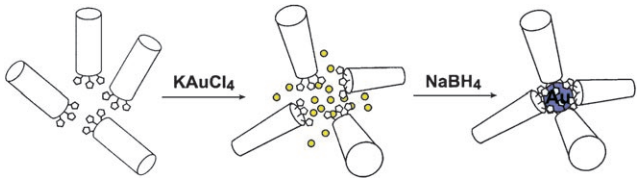

Figure 2. Self-assembly of $(gp5–His_6)_3$ through an Au nanocluster.

The $gp5–His_6$ trimer was treated with 300 equivalents of $KAuCl_4$ to obtain a homogeneous pale-yellow solution at pH 9.0 (20 mM tris(hydroxymethyl)aminomethane (Tris)/HCl and 0.2 M NaCl) and 4 °C. The Au^{III} ions were reduced with 2.5 equivalents of $NaBH_4$ to yield the Au NCs. Upon the reduction, the spectrum of the solution was changed significantly; there was the expected plasmon resonance absorption maximum for an Au NC at 530 nm (Figure 3a).[23] Under the same conditions, dark-purple precipitates were observed in a protein-free control experiment, while the solution containing only $(gp5–His_6)_3$ remained a clear purple color. These results suggest that $(gp5–His_6)_3$ can make Au NCs soluble in aqueous solution by covering the NCs, as previously reported for Au-NC-binding peptides.[24] Finally, the $Au/\{(gp5–His_6)_3\}_4$ composite was purified with size-exclusion chromatography (Sephacryl S300, fractionation range 1×10^4–1.5×10^6 Da) by monitoring the absorption both at 280 nm (protein) and at 530 nm (Au NC). The coelution of the protein and metal components through the column is a clear indication of the composite nature of the material (Figure 3b). The N- and C-terminal domains indicated in Figure 3c arise from processing, where $gp5–His_6$ is cleaved on the C-terminus side of Ser351, which is in a loop between the lysozyme and C-terminal domains. The two resultant fragments remain associated after the reaction with Au.[25]

We examined the structure of the $Au/(gp5–His_6)_3$ composites by transmission electron microscopy to understand the role of the His_6 fragments upon formation of the Au NCs. The TEM image of Au NCs with $(gp5–His_6)_3$ shows monodisperse NCs with a diameter of 2.7 nm (Figure 4a and the Au NCs histogram). The surface area of the Au NCs is 22.9 nm^2 on the

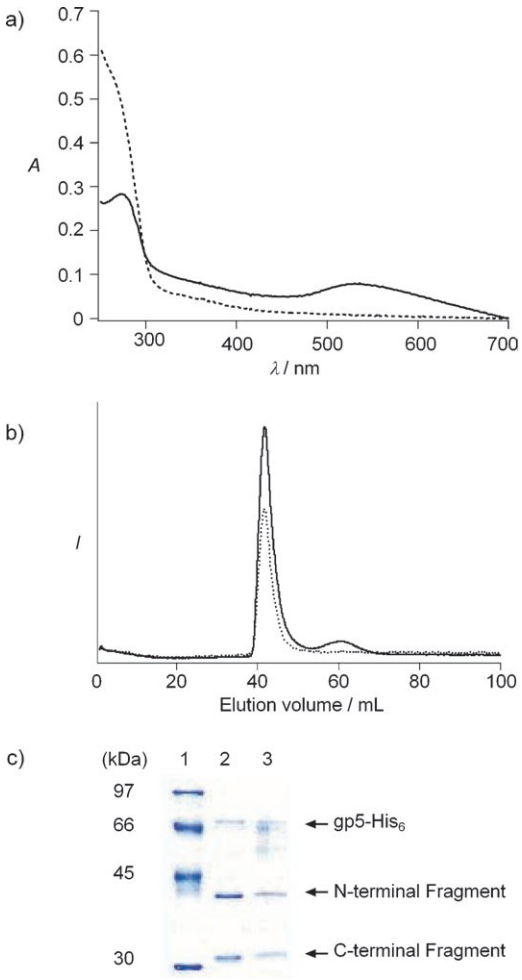

Figure 3. a) UV/Vis spectra of $KAuCl_4$/$(gp5–His_6)_3$ solution before (broken line) and after (solid line) reduction, b) size-exclusion-chromatography elution profile of Au/$\{(gp5–His_6)_3\}_4$ monitored at 280 nm (solid line) and 530 nm (broken line), and c) sodium dodecylsulfate (SDS) PAGE of a molecular-weight marker (lane 1), $(gp5–His_6)_3$ (lane 2), and Au/$\{(gp5–His_6)_3\}_4$ (lane 3). The gel (12.5%) was stained with Coomassie brilliant blue.

basis of the TEM results. The crystal structure of $(gp5–His_6)_3$ shows that the base area of the C-terminal region is 6.1 nm^2 when the $(gp5–His_6)_3$ tube is assumed to be a cylinder (Figure 1a). Thus, four $(gp5–His_6)_3$ units are expected to bind on the surface of each Au NC. In fact, close-up views of the composites indicate that an Au NC is covered with four proteins which are approximately 14 nm in length (Figure 4b). The protein size, which is identical to that of $(gp5–His_6)_3$ (Figure 1b), is in agreement with our proposed structure for composites consisting of four $(gp5–His_6)_3$ units and the Au NC in a tetrapod arrangement, as shown in Figure 4c.

The tetrapod assemblies were formed in a yield of 60% at pH 9.0, although only nonuniform aggregations of Au/protein composites occurred at pH 8.0 and 10.[26] The yield was decreased with addition of excess $KAuCl_4$ (500 equiv) because the Au NCs were formed with nonuniform size and shape.[26] In order to confirm that $(His_6)_3$ directs the composite formation and the Au NC size, $KAuCl_4$ was mixed with $(gp5)_3$ that had no His_6 fragments attached and then the mixture was reduced with $NaBH_4$ at pH 9.0. The prepared Au NCs were larger (diameter 3.3 nm) with polydispersion, and the proteins assembled more randomly around the Au NCs than in those composites prepared in the presence of $(gp5–His_6)_3$ (Figure 4d). Furthermore, $(gp27–gp5–His_6)_3$ and $(gp5C–His_6)_3$ also gave polydisperse and larger Au NCs under the same conditions (Figure 4e and f). In addition, these composites with Au NCs were also not shaped into uniform structures at pH 8.0 and 10.[26] These results clearly indicate that the specific Au/protein tetrapod composite was constructed by the reaction of $(gp5–His_6)_3$ with 300 equivalents of Au at pH 9.0 (Figure 4a).

The crystal structure of $(gp27–gp5–His_6)_3$ shows that the His_6 fragments located at the C-terminus of gp5 are 2.4 nm apart from each other in the trimer structure (Figure 1a). It has been previously reported that such periodical location of histidine-rich fragments results in the formation of size-regulated metal particles on an artificial peptide nanotube.[19] $(Gp5)_3$ with no His_6 fragments gave the polydisperse and larger Au NCs in the composites, with random binding of the proteins to the Au NCs (Figure 4d). Thus, the formation of the monodisperse Au NCs in Au/$\{(gp5–His_6)_3\}_4$ (2.7 nm) is expected to be restricted by the specific location of the His_6 fragments on the gp5 trimer.

The pH value of the reaction is also an important factor in generating the tetrapod assembly on an Au NC. Conformation change of histidine-rich peptides is induced in the presence of metal ions between pH 8.0 and 10.[27] The reduction of Au ions with $(gp5–His_6)_3$ at pH 8.0 gives random Au/$(gp5–His_6)_3$ assemblies with polydisperse Au-NC size. This is because the conformations of the His_6 fragments and the other surface residues are inappropriate for the formation of tetrapod assemblies at pH 8.0. At pH 10, few Au NCs were formed, due to low reactivity of $NaBH_4$ at high pH values.[26,28] It is expected that the structure of the His_6 fragment in $gp5–His_6$ at pH 9.0 is more suitable for binding Au atoms than that at other pH values.

Furthermore, the assembly of proteins on an Au NC is influenced by the electrostatic potential of the tube proteins. Au NCs are stabilized in solution when they are covered with negatively charged molecules, such as citrate anions.[29] The electrostatic potential map of $(gp5–His_6)_3$ (calculated with the Grasp software) clearly shows a negatively charged region located at the C termini of the $gp5–His_6$ units (Figure 5b). On the other hand, $(gp5C–His_6)_3$ has negative patches at the N and the C termini and the negative charge at the N terminus prevents the specific binding of the C terminus with an Au NC (Figure 5c). In the case of $(gp27–gp5–His_6)_3$, the negatively charged regions are located only at the C terminus (Figure 5a); however, the gp27 fragment has many exposed methionine and cysteine residues with high affinity for Au^{III} cations and Au NCs.[29] Thus, Au NCs nonspecifically bind on the $(gp27–gp5–His_6)_3$ surface (Figure 4d). Furthermore, the isoelectric point of $(gp5–His_6)_3$ is estimated to be 6.0 (calculated with the Protein Calculator v3.2 software).[30] These considerations suggest that the tetrapod structure, Au/$\{(gp5–His_6)_3\}_4$, is held through the cooperative effects of

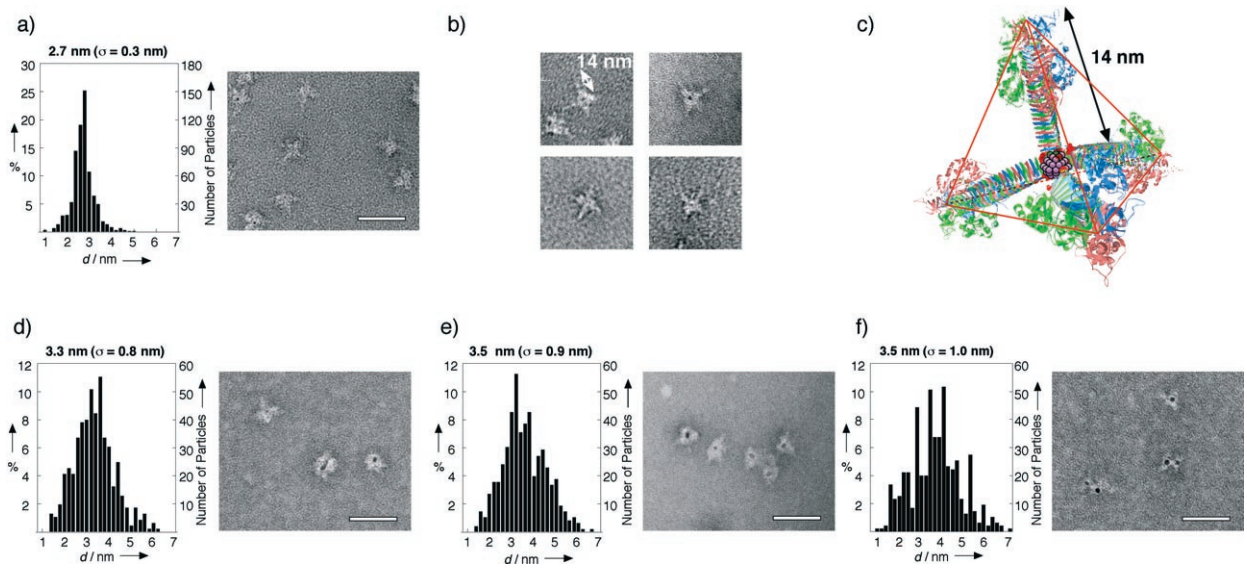

Figure 4. Au nanocluster size distribution and TEM images for a) Au/{(gp5–His_6)$_3$}$_4$, d) Au/(gp5)$_3$, e) Au/(gp27–gp5–His_6)$_3$, and f) Au/(gp5C–His_6)$_3$. b) Close-up TEM image of single Au/{(gp5–His_6)$_3$}$_4$ composites. Scale bar: 50 nm. All reactions were carried out at pH 9.0. TEM grids were stained with 2% uranyl acetate. c) The proposed structure of the composite.

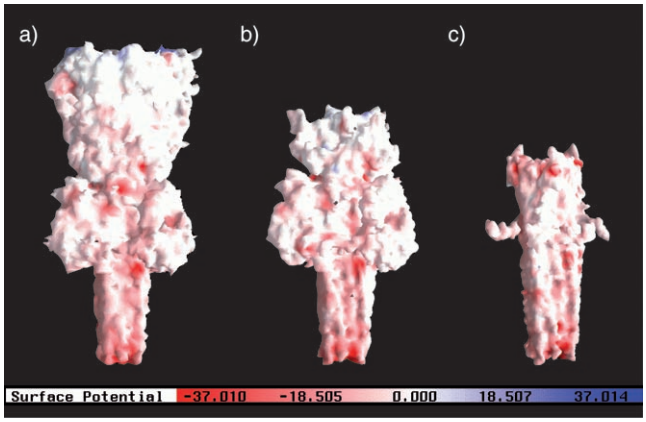

Figure 5. Electrostatic potential maps for a) (gp27–gp5–His_6)$_3$, b) (gp5–His_6)$_3$, and c) (gp5C–His_6)$_3$. The surface is colored according to its potential (blue positive, red negative). The maps were generated by calculations with the Grasp software.[31]

the His_6 triad, the negative charge of the C termini, and the electrostatic repulsion between each (gp5–His_6)$_3$ on an Au NC at pH 9.0 (Figure 4a).

In summary, we have prepared a tetrapod assembly of bionanotubes by in situ synthesis of Au NCs with (gp5–His_6)$_3$ from bacteriophage T4 (Figure 2). The three-dimensional structure is regulated cooperatively by the location of His_6 fragments and the surface electrostatic potential on the (gp5–His_6)$_3$ nanotube. The results indicate that if His_6 fragments are introduced at desired positions in proteins, it could be possible to design various architectures of protein assembly on metals by using in situ metal nanocluster formation. Modification of the surface charge may further facilitate the specific assembly of the tetrapod. This presents a novel strategy for the fabrication of nanoscale metal composites.

Experimental Section

Reagents were purchased from Nacalai Tesque and Sigma–Aldrich, and used without further purification. The expression and purification of proteins containing His_6 fragments were performed as reported previously.[21,22] The proteins were checked by SDS PAGE and MALDI-TOF MS (Voyager instrument, PE Biosystems). All reactions were carried out at 4 °C.

Preparation of gp5 without a His_6 fragment: Gp5 without a His_6 fragment was isolated by the glutathione *S*-transferase (GST) fusion method. Cell pellets that expressed the GST–gp5 fusion proteins were resuspended in buffer solution (10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 140 mM NaCl, 2.7 mM KCl, pH 7.3) and lyzed by sonication. The mixture was centrifuged at 17500 rpm for 20 min. The supernatant was loaded onto a GSTrap FF column (Amersham Biosciences). GST–gp5 was eluted from the column with 50 mM Tris/HCl and 10 mM glutathione buffer at pH 8.0. The eluted solution was dialyzed against 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 140 mM NaCl, and 2.7 mM KCl at pH 7.3. The GSTs of the GST–gp5 proteins were digested with thrombin protease for 6 h. The free GSTs were then trapped with the GSTrap FF column. Finally, the gp5 proteins were purified with a Superdex G200 column on an ÄKTA explorer 100 FPLC system (Amersham Biosciences).

Preparation of Au/{(gp5–His_6)$_3$}$_4$: A $KAuCl_4$ solution (1 mM, 480 μL in 20 mM Tris/HCl and 0.2 M NaCl buffer, pH 9.0) was added to a solution of (gp5–His_6)$_3$ (0.4 μM, 4 mL in 20 mM Tris/HCl and 0.2 M NaCl buffer, pH 9.0). The mixture was stirred for 30 min, and then a solution of $NaBH_4$ (2.5 mM, 480 μL in 20 mM Tris/HCl and 0.2 M NaCl buffer, pH 9.0) was added. After stirring for 30 min, the mixture was centrifuged at 15000 rpm for 10 min to remove precipitate. The resulting solution was purified on a size-exclusion column (Sephacryl S300) equilibrated with 20 mM Tris/HCl and 0.2 M NaCl buffer (pH 9.0).

Transmission electron microscopy: A solution of Au/{(gp5–His_6)$_3$}$_4$ (3 μL) was applied to a copper grid covered with a thin amorphous carbon film for 1 min, and then excess solution was removed with a filter paper. The grid was washed with water (3 μL) for 1 min and then stained with 2% uranyl acetate (3 μL) for 1 min. The images of the negatively stained samples were recorded on a

JEM-4010N microscope (300 kV). Other samples were prepared and observed with the same procedure.

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