Biomacromolecules

ARTICLE

pubs.acs.org/Biomac

Simultaneous Synthesis of Temperature-Tunable Peptide and Gold Nanoparticle Hybrid Spheres

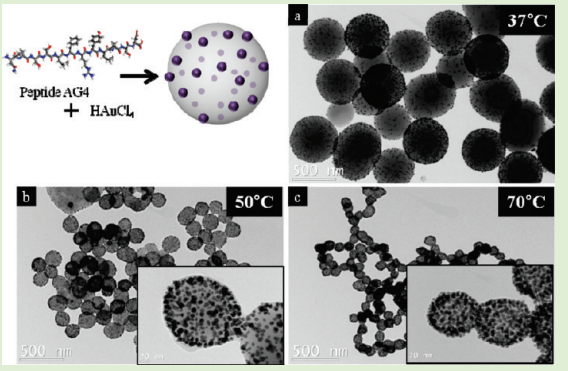
Jungok Kim,[†] Michael J. Sadowsky,[‡] and Hor-Gil Hur*[,†]

[†]Department of Environmental Science and Engineering and International Environmental Research Center, Gwangju Institute of Science and Technology, Gwangju, Republic of Korea

[‡]Department of Soil, Water, and Climate and BioTechnology Institute, University of Minnesota, St. Paul, Minnesota 55108, United States

Supporting Information

ABSTRACT: Hybrid spheres containing peptides and gold nanoparticles have been simultaneously synthesized in water using AG4 (NPSSLFRYLPSD) peptides that acted as a reducing agent to guide the nucleation and growth of gold nanoparticles and a precursor to form sphere-like structure by self-assembly where the size of hybrid spheres is precisely controlled by adjusting the operating temperature. The self-assembled peptide spheres remain stable even after selective removal of the gold nanoparticles by iodide etching. The amino acids containing the aromatic functional group in the peptide sequence significantly affect the construction of sphere structures. The surface of gold nanoparticles containing hybrid spheres has been functionalized using the thiol group linked to biomolecules. The ability to synthesize nanoparticle and self-assembled peptide structures with controlled size and composition in an environmental benign way will allow us to fabricate a new class of multifunctional organic−inorganic hybrid superstructures for various biomedical and electronic applications.



INTRODUCTION

Self-assembled biological molecules (e.g., peptides, proteins, DNA, etc.) have been investigated as excellent templates to assist the formation of hierarchical inorganic nanoengineered materials in environmental benign ways where typical self-assembly of biological molecules occurs via special recognition processes and mediates by noncovalent, electrostatic, hydrogen bonding, hydrophobic, and aromatic stacking interactions.[1,2] Among various biological molecules, peptide is one of the most attractive materials because it is able to (1) tune its chemical properties by adjusting primary amino acid sequence, (2) chelate with various inorganic ions and reduce them to form metallic, semiconducting, and insulating inorganic nanostructures under environmentally benign conditions,[3−7] and (3) control the crystal structure and morphology by adjusting the nucleation and growth mode of inorganic nanomaterials.[8,9] In addition, some peptides are able to self-assemble to form complex structures resulting in the formation of inorganic hierarchical structures including chains, sheets, and spheres.[10−14] Most of these heterostructures were synthesized using two-step processes involving the self-assembly of peptide to form the scaffolds/templates, followed by synthesis of inorganic nanostructures.

More recently, a single-step process has been developed that allows for the simultaneous formation of structurally complex and highly ordered nanoarchitectures. For example, a self-assembling cationic diphenylalinine peptide, which was previously shown to be useful for the synthesis of peptide nanotubes and nanospheres, can be used to direct the synthesis of spherical, peptide−polyoxoanion (phosphotungstic acid) hybrid nanostructures in water.[15−18] In addition, the aliphatic carbon-tailed peptide AG3 (AYSSGAPPMPPF), which has high binding affinity to silver and gold, can produce gold nanoparticle-coated double-helical structures in the HEPES buffer and control their structural characteristics by changing the reaction parameters.[14,19] Furthermore, it was newly reported that hollow spherical gold nanoparticle superstructures with a diameter ranging <52 nm were also prepared by modified peptide conjugates based on peptide AG3 in a simple reaction.[20] Despite these advances, there are still limited works on the simultaneous assembly of biological molecules and inorganic nanoparticles into well-designed supramolecular structures under aqueous conditions.

In the study, we demonstrated that peptide and gold nanoparticle hybrid spheres can be formed in aqueous solution by a simple single-step method and the size of the hybrid sphere structures can be easily tunable with temperature. This method,

Received: March 7, 2011
Revised: May 24, 2011
Published: May 26, 2011

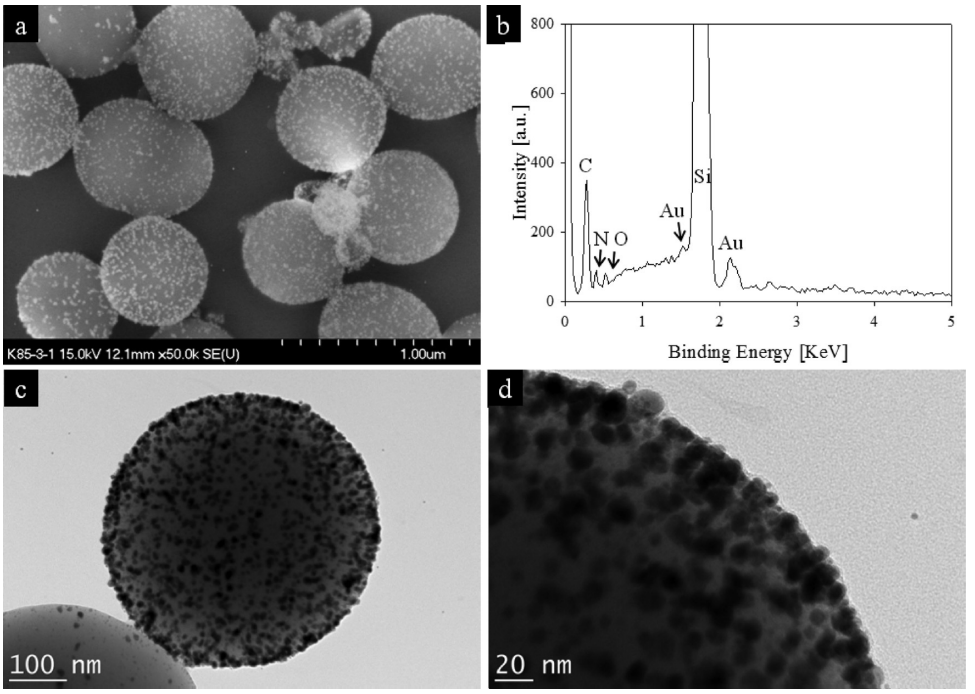


Figure 1. SEM image (a), EDX analysis (b), and TEM images (c,d) of the self-assembled peptide and gold nanoparticle hybrid spheres.

which combines peptide-based supramolecular structures with desirable functional inorganic materials, may contribute to a development of "bottom-up" fabrication of hierarchical structures with unique physical, chemical, and biological properties.

EXPERIMENTAL SECTION

Chemicals and Peptides. The $HAuCl_4 \cdot 3H_2O$ and $ClAuPMe_3$ were purchased from Aldrich Chemicals (St. Louis, MO), and HNO_3 was obtained from Duksan Pure Chemicals (Ansan, Korea). Nanopure water used was prepared by using the Milli-Q system (Millipore, Billerica, MA) and autoclaved prior to use to avoid microbial contamination. All other chemicals were reagent grade. All peptides used were purchased from Any Gen (Gwangju, Korea).

Synthesis of Peptide and Gold Nanoparticle Hybrid Spheres. Peptide AG4 (0.2 mg) and 0.1 mM of $HAuCl_4 \cdot 3H_2O$ were added in a final reaction volume of 1 mL, followed by incubation at 37 °C, in the dark, for 24 h. Mutated peptides, with specific amino acid changes in the peptide sequence, were functionally analyzed using the same reaction conditions as described above. The influence of temperature on the synthesis of gold nanoparticles with peptide spheres was determined by incubating reaction mixtures at 50 or 70 °C. The synthesized nanomaterials were collected by centrifugation at 9300 *g* for 5 min at 25 °C, washed twice with autoclaved deionized water, and resuspended in 100 μL of water for further analyses.

Gold Etching. The synthesized gold nanoparticles were removed from the surface of the peptide spheres by treatment with KI/I_2 gold etching solution. The KI/I_2 gold etching solution was prepared by the addition of 4 g of KI, 1 g of I_2, and 40 mL of H_2O, with constant stirring at 25 °C. We added 20 μL of etching solution to 100 μL of washed sample. The mixture was incubated for 5 min at 25 °C and washed two times with deionized water.

Circular Dichroism (CD) Spectroscopy. The CD spectra of nanostructures were recorded from 190 to 250 nm by using a model J-810 spectropolarimeter (Jasco, Tokyo, Japan) at 298 K and a quartz cuvette with 1 mm path length. Analyses were performed using 0.2 mg/mL of peptide in water after 24 h of incubation at 37 °C under dark conditions.

Kinetic Studies. The change in gold ion concentration with time was examined in triplicate experiments using reaction samples consisting of 0.2 mg of peptide AG4 and 0.1 mM of $HAuCl_4 \cdot 3H_2O$. Samples were incubated at 37 °C and centrifuged after 0, 1, 3, 6, 12, or 24 h. The resulting supernatant solutions were diluted with 2% HNO_3, and the gold ion concentration was measured by inductively coupled plasma optical emission spectrometry (ICP-OES) using an Optima model 5300DV instrument (PerkinElmer, Waltham, MA).

Surface Functionalization with Fluorescent Dye-Labeled Peptide. The fabricated peptide and gold nanoparticle hybrid spheres were incubated with 1 mg of FAM-Si#6-C peptide for 24 h at 20 °C, followed by a previous report.[7] Samples were centrifuged and washed twice with deionized water. The intensity of the bound fluorescent-dye-labeled peptide with the gold nanoparticles on the hybrid spheres was analyzed by using confocal laser scanning microscopy (CLSM, LSM5, Zeiss, Germany).

Structural Characterization of Synthesized Nanomaterials. The formed nanostructures were characterized by field emission-transmission electron microscopy (FE-TEM), high-resolution TEM (HR-TEM), scanning electron microscopy (SEM), and energy-dispersive X-ray spectroscopy (EDX). TEM analyses were done using a Tecnai F20 FE-TEM (Philips Electron Optics, Eindhoven, The Netherlands) and a JEM-2100 HR-TEM (JEOL, Tokyo, Japan) at an accelerating voltage of 200 kV; the latter one was equipped for EDX analysis. The samples were prepared by depositing the nanostructures onto carbon-coated Cu support grids, followed by air drying. The samples for SEM analyses were analyzed at an accelerating voltage of 10 kV by using a Hitachi S-4700 SEM (Tokyo, Japan), equipped for EDX analysis. Samples were prepared by placing ~5 μL of a suspension containing nanostructures on a silicon wafer, followed by air drying.

RESULTS AND DISCUSSION

Simultaneous Synthesis of Peptide and Gold Nanoparticle Hybrid Spheres. Peptide AG4 was used as a precursor to

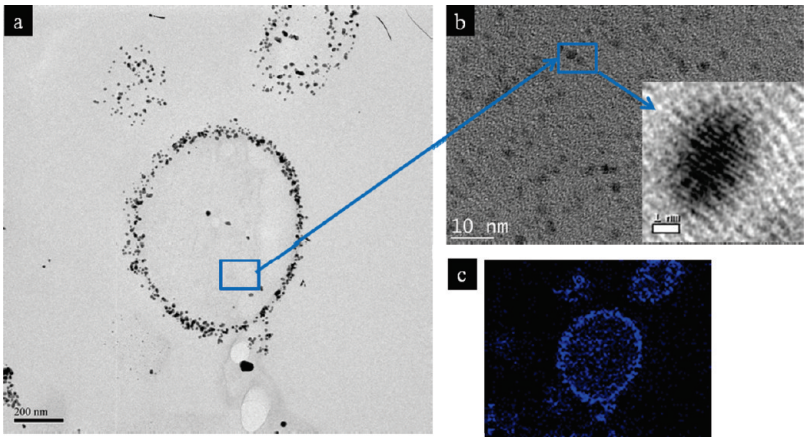


Figure 2. TEM image (a), HR-TEM images (b and inset), and EDX mapping (c) for cross-sectioned analyses of self-assembled peptide and gold nanoparticle hybrid spheres.

form simultaneously peptide spheres and a reducing agent to reduce gold ions to gold nanoparticles in water at physiological temperatures. Peptide AG4 (NPSSLFRYLPSD), which was isolated using a combinatorial phage-displayed peptide library, was previously shown to have high affinity to silver and be able to reduce silver ions to metallic silver nanoparticles in phosphate buffer at room temperature.[5] Incubation of peptide AG4 (0.2 mg) in a solution containing 0.1 mM $HAuCl_4$ for 24 h at 37 °C led to the formation of dark, violet-colored, precipitates, which indicated that gold nanoparticles were reduced by the peptides. TEM and SEM analyses indicated the formation of submicrometer spherical hybrid of peptide and gold nanoparticle conjugates with an average diameter of 473.8 ± 121.5 nm (Figure 1). EDX analysis confirmed that the submicrometer spherical structures contained gold nanoparticles (Figure 1b). Incubation of hydrochloroauric acid in the absence of peptide AG4 did not result in the formation of any precipitates or sphere-like structures. Similarly, no violet-colored precipitates and sphere structures were formed when water-insoluble organic gold ($ClAuPMe_3$) was incubated with peptide AG4 under the same reaction conditions (data not shown). This indicated that hydrochloroauric acid appears to play an important role in the self-assembly of peptide AG4 and the nucleation and growth of gold nanoparticles within the peptide structures. Whereas the analysis of the TEM image shown in Figure 1d indicated that the surface-associated gold nanoparticles had an average size of 8.6 ± 2.2 nm, the location of gold nanoparticles on the surface, or within the peptide spheres, was undefined by these analyses.

Cross-Sectional Analysis for the Location of Gold Nanoparticles. Cross-sectional analysis was performed to determine the distribution of the gold nanoparticles within these spherical hybrid structures. Results of cross-sectional TEM image analyses (Figure 2a) clearly show that the larger size gold nanoparticles (average diameter range of 8.6 ± 2.2 nm) were predominately located on the surface of peptide sphere, whereas HR-TEM analysis indicated that smaller gold nanoparticles (diameter range from 2.5 to 4.5 nm) were located inside of the hybrid sphere (Figure 2b). EDX elemental mapping analysis of gold confirmed the presence of gold nanoparticles throughout the spherical peptide structures (Figure 2c). These results indicate that peptide AG4 is able to self-assemble to form submicrometer spherical spheres that contained metallic gold nanoparticles by simultaneously reducing gold ions. It is considered that the small-sized gold nanoparticles formed during initial reduction by peptide AG4 were embedded inside of peptide spheres. Meanwhile, the surface gold nanoparticles are likely to be in the continuous growth process with exposing them to gold ions in the reaction solution. Recently, Song et al. reported a simultaneous fabrication of hollow spherical gold nanoparticle superstructures by peptide conjugates, which contain six carbons and two alanines in the N-terminal of peptide AG3, in the HEPES buffer acting as a additional reducing agent at room temperature.[20] The produced structures have an average diameter of 51.6 ± 0.8 with 8.3 ± 0.2 nm size of gold nanoparticles on the shell. However, the hollow spherical gold nanoparticle superstructures were not the structures with small-sized gold nanoparticles filled inside the spherical peptide structures, as we report in the current research.

Effect of Reaction Temperatures on the Size of Hybrid Spheres. Results shown in Figure 3 indicate that the size of peptide spheres was tuned by the reaction temperature. The average diameter of the peptide spheres decreased by approximately 56 and 76% when the reaction temperature increased from 37 (473.8 ± 121.5 nm) to 50 (to 208.2 ± 53.1 nm) and 70 °C (to 114.4 ± 19.6 nm), respectively (Figure 3). In addition, the size distribution of the hybrid spheres became more a monodispersed range with increased temperature. However, despite the reduction in the size of the peptide spheres with increased temperature, the size of the gold nanoparticles distributed on the surface of the sphere structures did not significantly change in response to reaction temperature. The mean diameter of the gold nanoparticles was 8.6 ± 2.2 nm at 37 °C, 8.3 ± 2.1 nm at 50 °C, and 8.1 ± 1.9 nm at 70 °C, respectively (Figure 3). This suggests that the fast nucleation and growth of the gold nanoparticles by increased temperature may affect to the self-assembly of the peptide and inorganic hybrid suprastructures, resulting in the reduced size of the hybrid structures rather than the final size of the surface gold nanoparticles.[19]

Peptide Spheres by the Dissolution of Gold. To obtain peptide spheres in the absence of gold nanoparticles, we accomplished the dissolution of gold nanoparticles from the surface and inside of the peptide sphere structures by treatment with a KI/I_2 solution. EDX analysis confirmed the absence of elemental gold nanoparticles inside, and on the surface, of the peptide sphere structures following KI/I_2 treatment (Figure S1 of the Supporting Information). The TEM images in Figure 4 show that KI/I_2

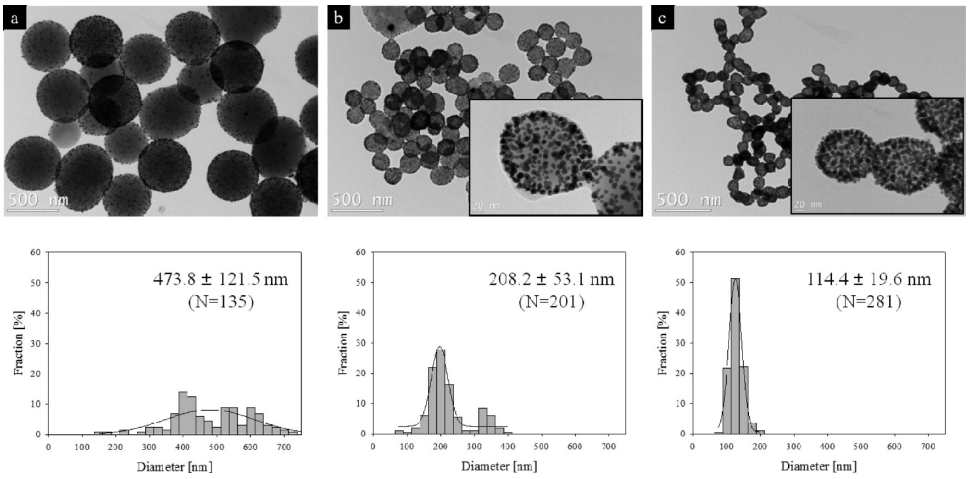


Figure 3. TEM images and size distribution of self-assembled peptide and gold nanoparticle hybrid spheres at 37 (a), 50 (b), and 70 °C (c).

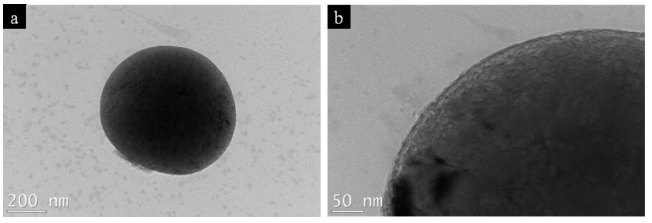


Figure 4. TEM images for the effect of gold etching by KI/I_2 on self-assembled peptide and gold nanoparticle hybrid spheres (a and b).

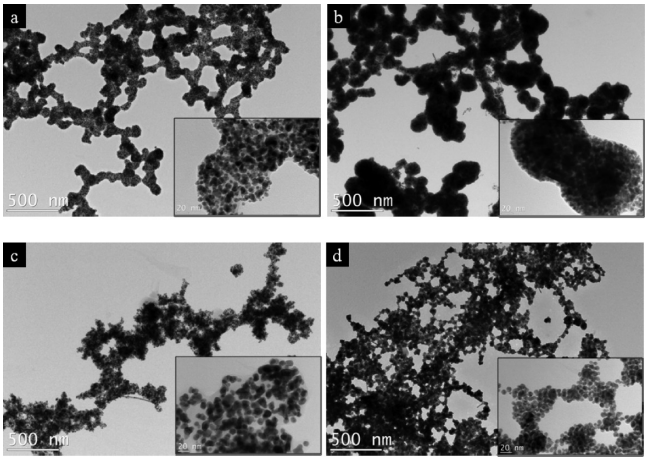


Figure 5. TEM images for the functional analyses of peptide sequences on self-assembled peptide and gold nanoparticle hybrid spheres by mutated peptides AG4_YtoG (a), AG4_YtoS (b), AG4_FtoG (c), and AG4_FYtoGS (d).

etching resulted in the successful removal of the gold nanoparticles from the sphere structures. In addition, these studies showed that peptide spheres were stably maintained in the absence of gold nanoparticles. High-resolution TEM images also show that the surface of the peptide spheres without the gold nanoparticles in contrast with the ragged surface of the hybrid structures with embedded gold nanoparticles (Figures 1d and 4b). Taken together, these results indicate that once the self-assembling peptide sphere structures are formed they remain stable without being affected by the gold nanoparticles. In addition, not only heterogeneous structures of peptide and gold nanoparticle hybrid but also homogeneous self-assembled peptide spheres without gold nanoparticles may provide extended potential applications such as use of antibacterial agents or gene and drug delivery system.[21,22]

Effect of Amino Acid Changes on Peptide Primary Structure. To determine the relationship between the primary sequence of peptide AG4 and the self-assembly of peptide and gold nanoparticle hybrid spheres, phenylalanine and tyrosine residues in the sequence of peptide AG4, which have an aromatic functional group, were substituted with glycine and serine, respectively. The peptide sequences used in these analyses were NPSSLFRSLPSD (Y to S), NPSSLFRGLPSD (Y to G), NPSSLGRYLPSD (F to G), and NPSSLGRSLPSD (F and Y to G and S, respectively). Results in Figure 5 show that any of the tested amino acid substitutions in peptide AG4 resulted in the inability to form peptide sphere structures. Instead, all of the mutated peptides produced networked, wire-like, nonpatterned aggregates of peptides with gold nanoparticles (Figure 5). Interestingly, gold nanoparticles were still produced by the mutated peptides, even when tyrosine, which is known to act as an active reductant for gold, was replaced by glycine or serine.[23] Results of these studies demonstrated that the tyrosine residue alone is not sufficient for the reduction of gold ions, and several single amino acid changes within peptide AG4 inhibit the self-assembly of the nanosphere structures. Moreover, the primary structure of peptide AG4 appears to have a novel function that directs the synthesis of the nanosphere structures in the presence of gold ions. It should be noted that it was previously shown that changes in the internal amino acids in some peptides affect the synthesis, shape, and aggregation of gold nanostructures.[6] For example, substitution of one of the tyrosine residues with serine in peptide A3 resulted in the loss of the ability to form gold nanoparticles and self-assembling peptide nanoribbons with gold nanoparticles.[14,24] Despite this knowledge, however, the causal relationship between the primary structure of peptide AG4 and the sphere formation is currently unknown.

Kinetic Studies for the Synthetic Mechanism of Peptide and Gold Nanoparticles Hybrid Spheres. Kinetic studies were performed to determine the reaction rate of gold ions as a

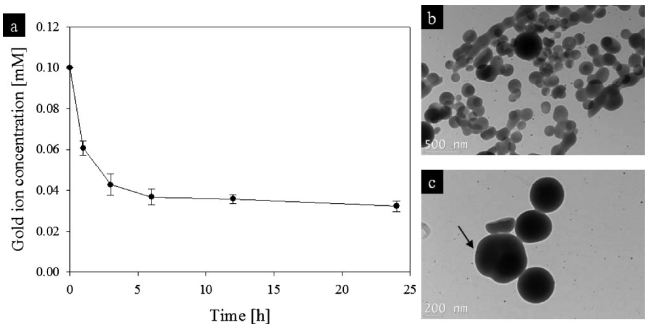


Figure 6. Reduction of gold ions concentration by peptide AG4 with time (a) and TEM images obtained at 3 h incubation (b,c).

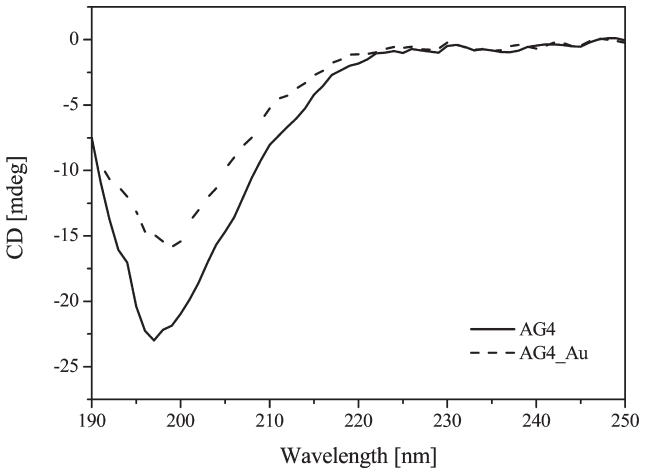


Figure 7. CD spectroscopy analyses for peptide AG4 with gold (dashed line) and without gold (solid line).

function of incubation time. ICP-OES analyses (Figure 6a) showed that gold ion concentration decreased from 0.1 to 0.04 mM after 6 h of incubation, followed by the slight reduction of gold ion concentration to ~0.03 mM after 24 h of incubation. These results indicated that peptide AG4 was involved in the rapid reduction of gold ions during early reaction stages. TEM images were taken to examine intermediate structures of peptide spheres that interact with the gold nanoparticles in the early stages of reaction. Results in Figure 6b show that neck-like structures and small-sized peptide spheres (90 to 400 nm diameter) were formed during the first 3 h of reaction.[25] In addition, results in Figure 6c show that hemisphere structures and incomplete peptide spheres, with different internal structures, are present and that small peptide spheres are likely incorporated into larger-sized ones via agglomeration.

CD Analysis for the Peptide Secondary Structural Changes. The change in the secondary structure of peptide AG4 upon the addition of gold ion was examined using CD spectroscopy. The peptide AG4 in the absence of gold ion showed no ordered secondary structure, but a strong negative peak at 197 nm indicated a random coil conformation of the peptide in water (Figure 7, solid line). When gold ion was introduced to the AG4 solution, the CD absorption was reduced without changing shape (Figure 7, dashed line). This result suggests that the peptide AG4 possesses the random coil conformation regardless of the presence of gold ions. However, the

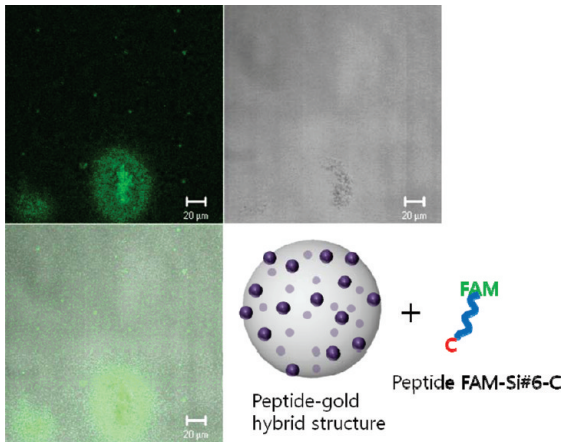


Figure 8. CLSM image of peptide FAM-Si#6-C bounded peptide and gold nanoparticle hybrid spheres.

presence of gold ions causes a decrease in the intensity of CD signal, which is related to the formation of peptide sphere structures with specific interaction with gold ions.[26]

Surface Functionalization of Hybrid Spheres. The surface functionalization of peptide and gold nanoparticle hybrid spheres was exploited by using a previously designed peptide (FAM-Si#6-C) with the fluorescent dye NHS-fluorescein linked to the N-terminal end and cysteine linked to the C-terminal end, respectively (Figure 8).[7] This peptide was previously shown to bind gold. Confocal laser scanning microscopy was used to measure the intensity of light emitted by the fluorescent-dye-labeled peptide that is specifically bound to the gold nanoparticles in the hybrid structure. Results shown in Figure 8 indicate that the FAM-Si#6-C peptide binds onto the surface of gold nanoparticles containing hybrid spheres. This suggests that the gold nanoparticles exposed on the surface of the hybrid structures can be easily modified with bioactive molecules containing thiol groups and that these hybrid structures may be useful for a variety of applications.

CONCLUSIONS

In conclusion, we have demonstrated the fabrication of temperature-tunable, peptide, and inorganic hybrid spherical structures composed of a self-assembling peptide with gold nanoparticles. The hybrid spheres formed under aqueous acidic conditions at physiological temperature. The gold nanoparticles that formed in this reaction were localized both inside and on the surface of the peptide sphere, indicating the simultaneous self-assembly of peptides and nucleation of gold ion via the specific interaction of both reactants. The size of peptide spheres with gold nanoparticles was tunable and inversely related to reaction temperature. The gold removal experiment showed that once formed the peptide spheres were stable in the absence of gold nanoparticles. The assembly of the spheres was dependent on the primary amino acid sequence of the peptide and was specifically influenced by amino acids containing aromatic functional groups. The method used here to form multifunctional hybrid biomolecules may be useful to produce a variety of nanostructures that may find wide application in the biomedical, electronic, and nanotechnological areas for the production of drug delivery, bioimaging, and biosensor systems.

■ ASSOCIATED CONTENT

Supporting Information. Experimental procedures, supplementary description, SEM images and EDX analysis after gold etching, TEM images of stability analyses for the effect of acidic, basic, and enzymatic treatment, and a schematic illustration for the formation of self-assembled peptide and gold nanoparticle hybrid spheres. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
*Tel: 82-62-715-2437. Fax:82-62-715-2434. E-mail: hghur@gist.ac.kr.

■ ACKNOWLEDGMENT

This work was supported by the National Research Foundation of Korea (NRF: 2010-0029224) grant and the 21C Frontier Microbial Genomics and Applications Center Program (11-2008-10-001-00) funded by the Korea government (MEST). We acknowledge Prof. Nosang V. Myung at University of California at Riverside, Riverside, CA, for his editing comments and criticism in the preparation of this manuscript.

■ REFERENCES

(1) Lim, Y.-B.; Moon, K.-S.; Lee, M. *Chem. Soc. Rev.* **2009**, *38*, 925–934.
(2) Gazit, E. *Chem. Soc. Rev.* **2007**, *36*, 1263–1269.
(3) Dickerson, M. B.; Sandhage, K. H.; Naik, R. R. *Chem. Rev.* **2008**, *108*, 4935–4978.
(4) Sarikaya, M.; Tamerler, C.; Jen, A. K.-Y.; Schulten, K.; Baneyx, F. *Nat. Mater.* **2003**, *2*, 577–585.
(5) Naik, R. R.; Stringer, S. J.; Agarwal, G.; Jones, S. E.; Stone, M. O. *Nat. Mater.* **2002**, *1*, 169–172.
(6) Kim, J.; Rheem, Y.; Yoo, B.; Chong, Y.; Bozhilov, K. N.; Kim, D.; Sadowsky, M. J.; Hur, H.-G.; Myung, N. V. *Acta Biomater.* **2010**, *6*, 2681–2689.
(7) Kim, J.; Myung, N. V.; Hur, H.-G. *Chem. Commun.* **2010**, *46*, 4366–4368.
(8) Chen, C.-L.; Rosi, N. L. *Angew. Chem., Int. Ed.* **2010**, *49*, 1924–1942.
(9) Zhao, X.; Zhang, S. *Chem. Soc. Rev.* **2006**, *35*, 1105–1110.
(10) Fu, X.; Wang, Y.; Huang, L.; Sha, Y.; Gui, L.; Lai, L.; Tang, Y. *Adv. Mater.* **2003**, *15*, 902–906.
(11) Yan, X.; Cui, Y.; He, Q.; Wang, K.; Li, J. *Chem. Mater.* **2008**, *20*, 1522–1526.
(12) Euliss, L. E.; Grancharov, S. G.; O'Brien, S.; Deming, T. J.; Stucky, G. D.; Murray, C. B.; Held, G. A. *Nano Lett.* **2003**, *3*, 1489–1493.
(13) Yan, X.; Cui, Y.; Qi, W.; Su, Y.; Yang, Y.; He, Q.; Li, J. *Small* **2008**, *4*, 1687–1693.
(14) Chen, C.-L.; Zhang, P.; Rosi, N. L. *J. Am. Chem. Soc.* **2008**, *130*, 13555–13557.
(15) Yan, X.; Zhu, P.; Fei, J.; Li, J. *Adv. Mater.* **2010**, *22*, 1283–1287.
(16) Reches, M.; Gazit, E. *Science* **2003**, *300*, 625–627.
(17) Reches, M.; Gazit, E. *Curr. Nanosci.* **2006**, *2*, 105–111.
(18) Gazit, E. *FEBS J.* **2007**, *274*, 317–322.
(19) Chen, C.-L.; Rosi, N. L. *J. Am. Chem. Soc.* **2010**, *132*, 6902–6903.
(20) Song, C.; Zhao, G.; Zhang, P.; Rosi, N. L. *J. Am. Chem. Soc.* **2010**, *132*, 14033–14035.
(21) Liu, L.; Xu, K.; Wang, H.; P.K., J. T.; Fan, W.; Venkatraman, S. S.; Li, L.; Yang, Y.-Y. *Nat. Nanotechnol.* **2009**, *4*, 457–463.
(22) Yan, X.; He, Q.; Wang, K.; Duan, L.; Cui, Y.; Li, J. *Angew. Chem., Int. Ed.* **2007**, *46*, 2431–2434.
(23) Ray, S.; Das, A. K.; Banerjee, A. *Chem. Commun.* **2006**, *26*, 2816–2818.
(24) Slocik, J. M.; Stone, M. O.; Naik, R. R. *Small* **2005**, *1*, 1048–1052.
(25) Yan, X.; Zhu, P.; Li, J. *Chem. Soc. Rev.* **2010**, *39*, 1877–1890.
(26) Alfonso, I.; Bru, M.; Burguete, M. I.; Garcia-Verdugo, E.; Luis, S. V. *Chem.—Eur. J.* **2010**, *16*, 1246–1255.

■ NOTE ADDED AFTER ASAP PUBLICATION

This article posted ASAP on June 9, 2011. Sentence 8 in the second paragraph of the Results and Discussion section has been revised. The correct version posted on June 21, 2011.