

Direct Growth of Shape-Controlled Nanocrystals on Nanotubes via Biological Recognition

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Abstract: The new biological approach was examined to fabricate shape-controlled Ag nanocrystals grown directly on surfaces, inspired by nature that various shapes of nanocrystals are produced accurately and reproducibly in biological systems. Here we demonstrate the direct growth of hexagon-shaped Ag nanocrystals on sequenced peptide-coated nanotubes via biological recognition. When the peptide, Asn-Pro-Ser-Ser-Leu-Phe-Arg-Tyr-Leu-Pro-Ser-Asp, recognizing and effecting the Ag nanocrystal growth on the (111) face, was sequenced and incorporated onto template nanotube surfaces, the biomineralization of Ag ions on the nanotubes led the isotropic hexagon-shaped Ag nanocrystal coating under pH control of the growth solution. Multiple Ag nanocrystal shapes were observed when the peptide mineralized Ag ions without the template nanotubes, and therefore the template nanotube has a significant influence on regulating the majority of Ag nanocrystals into the hexagonal shape. This biological approach, using specific peptide sequences on surfaces to control nanocrystal shapes, may be developed as a simple and economical method to produce building blocks with desired physical properties for new generation of electronics, sensors, and optical devices.

Introduction

Nanocrystals have been studied extensively due to their tunability in electronic structures by adjusting their shapes, which naturally leads to significant interest in developing them as building blocks in catalytic, optical, and electronic applications.^{1a} While spherical/rod-shaped nanocrystals have received intensive attention, other interesting nanocrystal shapes are of special interest due to their novel physical properties.¹ The shape control of nanocrystals has been achieved by inorganic approaches whereby the influence on the growth of particular nanocrystal faces at the nucleation stage controls the growth rate along different crystal axes and manipulates the shape of grown nanocrystals.^{1b,2} The influence toward particular faces of nanocrystals is mostly accomplished by capping these faces with organic surfactants. However, the binding specificity of organic surfactants toward particular crystalline faces is not well understood, and therefore it is still a challenge to choose appropriate capping agents to generate desired shapes of nanocrystals.^{2b} Another challenge in applying nanocrystals to nanometer-scale devices is that it is necessary to assemble nanocrystals in certain matrices or on certain surfaces.³ Because multiple shapes of nanocrystals are frequently produced in this

approach, this nanocrystal assembly requires an extra step to isolate the desired shape of nanocrystals.^{2b} Whereas direct growth of nanocrystals on surfaces will avoid those complications, the incorporation of nucleation sites and the shape control of nanocrystals are not trivial on surfaces.

Inspired by nature where various shapes of nanocrystals are produced accurately and reproducibly in biological systems,⁴ we report a biological approach to fabricate shape-controlled nanocrystals grown directly on surfaces. Here we demonstrate the direct growth of Ag nanocrystals in the hexagonal shape on sequenced peptide-coated nanotubes via biological recognition. The peptide sequence, Asn-Pro-Ser-Ser-Leu-Phe-Arg-Tyr-Leu-Pro-Ser-Asp (AG4), was found to recognize and effect the Ag nanocrystal growth on the (111) face via the combinatorial phage display peptide library.^{5a} When this sequenced peptide was sequenced and incorporated onto template nanotube surfaces, the biomineralization of Ag ions on the nanotubes led the isotropic hexagon-shaped Ag nanocrystal coating under pH control (Figure 1). In addition to the shape control of nano-

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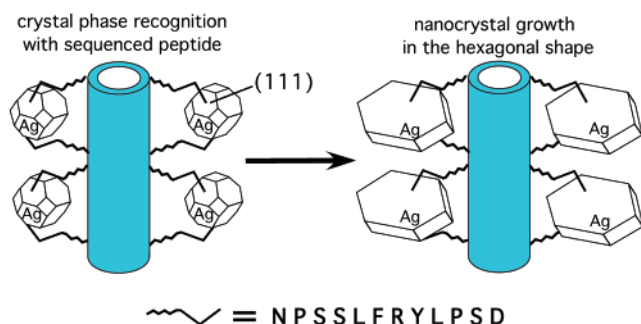


Figure 1. Scheme for Ag nanocrystal growth in the hexagonal shape on the template nanotubes. The sequenced AG4 peptide, incorporated on the nanotube surfaces, absorbs and reduces Ag ions to grow Ag nanocrystals. The AG4 peptide recognizes and effects the Ag growth on the (111) face and it controls the growth rate along different crystal axes and manipulates the shape of Ag nanocrystals.

crystals, nanocrystal-coated nanotubes are interesting in general due to their potential to serve as building blocks in optics, electronics, and sensor devices. Our strategy has an advantage that it does not require the self-assembling process of nanocrystals to organize them into the device configurations since the shape-controlled nanocrystals are grown directly on surfaces. Because the crystalline face recognition with peptides in particular sequences is much more specific than organic surfactants,⁵ the shape of resulting nanocrystals via this biological approach is also expected to be more controllable than the inorganic approach once the peptide sequences, which can recognize specific faces of metals/semiconductors, are identified.

Experimental Section

Ag nanocrystals in the hexagonal shape were grown on template nanotubes, self-assembled from bis(*N*- α -amido-glycylglycine)-1,7-heptane dicarboxylate. The chemical process to synthesize and assemble the monomers into the template nanotube was described previously.⁶ Those nanotubes in diameters of 20–200 nm were used as templates to grow shape-controlled Ag nanocrystals on the nanotube surfaces. After the template nanotubes were washed with distilled water twice, 0.5 mL of the template nanotube solution was incubated with 0.02 mL of the sequenced AG4 peptide (Asn-Pro-Ser-Ser-Leu-Phe-Arg-Tyr-Leu-Pro-Ser-Asp, 25 mM) in a buffer solution (pH = 7) for 24 h to immobilize it on the nanotubes. Since the template nanotube contains well-defined binding sites for proteins and peptides via hydrogen bonding,⁷ the AG4 peptide can be coated on the nanotubes in the simple incubation method. The immobilization of the AG4 peptide on the nanotube was verified by a confocal Raman microscope (LabRam, Jobin Yvon/Horiba). The AG4 peptide was sequenced by Applied Biosystems peptide synthesizer 432A and purified with Beckman 110 HPLC with the C-18 reverse phase column at the CUNY Gene Center. After the AG4 peptide immobilization on the nanotubes, 40 μ L of AgNO₃ solution (30 mM) was incubated with the stirred peptide nanotube solutions between pH 4 and 10 to grow Ag nanocrystals on the nanotubes. The Ag nanocrystal growth on the nanotubes was monitored via the Ag ion incubation time from 0.5 to 96 h by TEM and UV–vis spectroscopy.

Results and Discussion

When the template nanotubes were incubated with the AG4 peptide in a pH 7 buffer solution for 1 day, the AG4 peptide was immobilized on the nanotube surface, confirmed by a

Raman microscope (Supporting Information). Ag ions were then absorbed and reduced simultaneously by the AG4 peptide on the nanotubes when AgNO₃ was incubated in the nanotube solution.^{5a} The resulting Ag nanocrystals on the nanotubes were observed to be isotropic hexagonal plates at pH = 7. The Ag nanocrystals appeared on the nanotubes within 0.5 h after Ag ions were incubated in the nanotube solution, and the size of Ag nanocrystals increased as the ion incubation time increased (Figure 2a). Ag nanocrystals were grown to 14 nm in diameter after 14 h, as seen in transmission electron micrograph (TEM) in Figure 2b. The magnified TEM image in the inset of Figure 2b shows the isotropic hexagonal shape of Ag nanocrystals on the nanotube. Figure 2c and the tilted TEM image of Figure 2b indicate that those Ag nanocrystals are hexagonal plates, and the thickness is determined as 3 nm from the edge image shown in the right side of the Ag nanocrystal in Figure 2c. It should be noted that other Ag nanocrystal shapes were also observed when the AG4 peptide mineralized Ag ions without the template nanotubes in suspension.^{5a} Therefore, the nanotube surface seems to have an influence on regulating the majority of Ag nanocrystals into the hexagonal shape,⁷ while the exact shape-controlling mechanism on the nanotubes is not clear at this point. Hexagon-shaped Ag nanocrystals were grown to 50 nm in diameter after 72 h (Figure 2d), and then further nanocrystal growth was not observed at longer ion incubation, as shown in Figure 2a. Hexagon-shaped Ag nanocrystals were also observed to grow on the nanotubes whose diameters were equivalent to the diameter of Ag nanocrystals after 72 h of the Ag ion incubation (Figure 2e). The electron diffraction pattern of Ag nanocrystals in the hexagonal shape (inset in Figure 2e) shows the highly crystalline structure. While the plasmon band of hexagon-shaped Ag nanocrystals on the nanotubes red-shifted as the size of nanocrystals increased (Figure 3), those spectra indicate the distinctive electronic structures of hexagon-shaped Ag nanocrystals compared to spherical Ag nanocrystals in the similar size domains.^{1f,8}

To confirm the effect of the AG4 peptide nanotube on the shape control of Ag nanocrystals, two control experiments were examined. As the first control experiment, Ag nanocrystals were grown on the template nanotubes without the AG4 peptide, reduced by hydrazine hydrate for 14 h at pH = 7. Under this condition, Ag nanocrystals were observed to coat the nanotube surfaces only partially (Figure 4a), whereas Ag nanocrystals fully coated the AG4 peptide-functionalized nanotubes at pH = 7 in the same Ag ion incubation time (Figure 2b). For the second control experiment, a different peptide with the same number of amino acids as the AG4 peptide whose sequence is not designed to recognize the (111) face of Ag nanocrystals was immobilized on the template nanotubes, and the shape of Ag nanocrystals on those nanotubes was compared to that of nanocrystals grown on the AG4 peptide nanotubes. When the HG12 peptide (His-Gly-Gly-Gly-His-Gly-His-Gly-Gly-His-Gly) was sequenced and immobilized on the template nanotubes in the same condition as the AG4 peptide coating, both spherical and hexagonal shapes of Ag nanocrystals were observed on the HG12 peptide nanotubes after the 72 hr-incubation of Ag ions and reduction with hydrazine hydrate (Figure 4b), while the shape of Ag nanocrystals was regulated to hexagonal shapes on the AG4 peptide nanotubes in the same experimental

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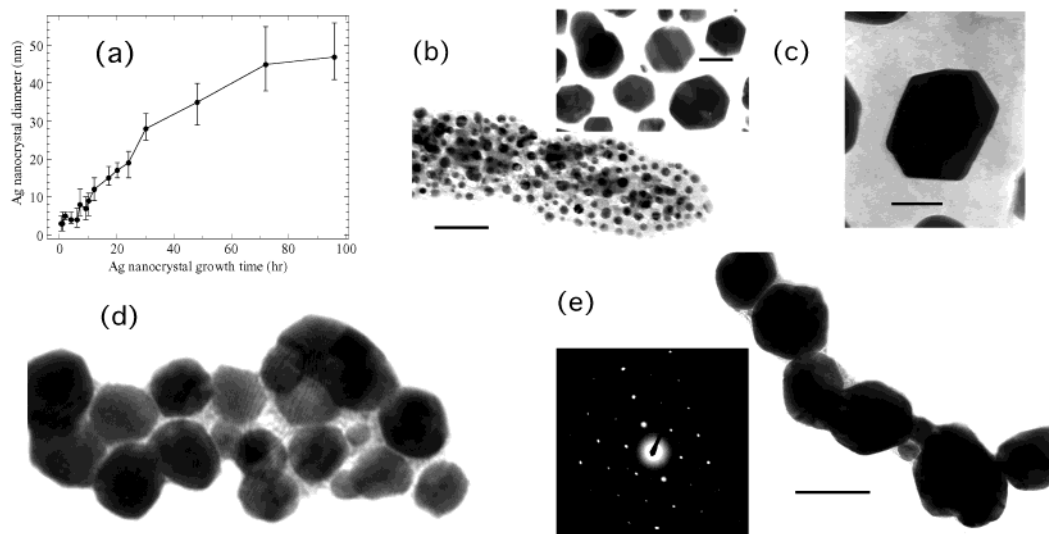


Figure 2. (a) The correlation between the size of Ag nanocrystals and the nanocrystal growth time in the pH 7 growth solution. (b) TEM image of hexagon-shaped Ag nanocrystals on the nanotube after 14 h in the pH 7 growth solution. Scale bar = 100 nm. Inset: Highly magnified TEM image. Scale bar = 15 nm. (c) Tilted TEM image of the hexagon-shaped Ag nanocrystal on the nanotube after 14 h in the pH 7 growth solution. Scale bar = 10 nm. (d) TEM image of hexagon-shaped Ag nanocrystals on the nanotube after 72 h in the pH 7 growth solution. Scale bar = 50 nm. (e) TEM image of hexagon-shaped Ag nanocrystals on the smaller nanotube after 72 h in the pH 7 growth solution. Scale bar = 50 nm. Inset: the electron diffraction pattern showing (111), (011), and (100) faces.

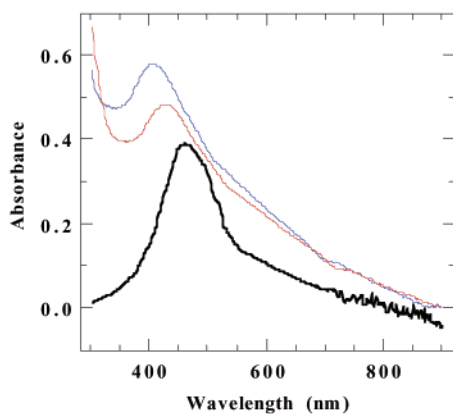


Figure 3. UV/vis spectra of hexagon-shaped Ag nanocrystals on the nanotubes in the diameters of 5 nm (blue), 14 nm (red) 50 nm (black).

conditions (Figure 2d). It should be noted that the longer incubation did not affect the shape and the size of the Ag nanocrystals. Therefore, these comparisons indicate that the AG4 peptide certainly has a significant effect on the growth and the shape of Ag nanocrystals on the nanotubes. Because the free-standing AG4 peptide without the nanotube produced multiple Ag nanocrystal shapes,^{5a} the shape control on the nanotube may be observed by the reduction of the AG4 peptide aggregation due to the peptide immobilization at the discrete binding sites on the nanotube.⁷

When Ag nanocrystals were grown on the peptide nanotubes in the pH < 7 growth solutions, hexagon-shaped nanocrystals were still grown with more polydispersity. However, a striking difference was observed when the pH of growth solutions was over 7. Under this basic condition, Ag nanocrystals grew faster and smaller on the nanotubes, whose diameters were around 15 nm after 9 h of the Ag ion incubation (Figure 5), and further growth of Ag nanocrystals was not observed for the longer growth period (Supporting Information). Because the efficiency of electron donation from the amino acids in the AG4 peptide toward Ag ions generally increases as pH increases, the shape

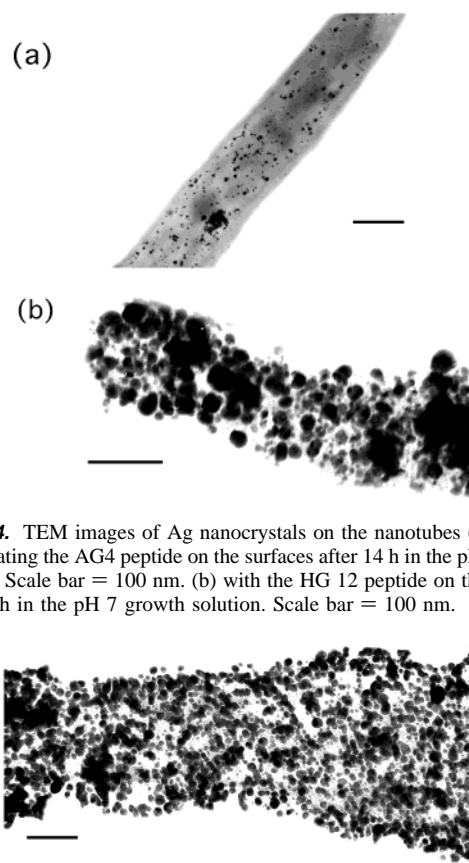


Figure 4. TEM images of Ag nanocrystals on the nanotubes (a) without incorporating the AG4 peptide on the surfaces after 14 h in the pH 7 growth solution. Scale bar = 100 nm. (b) with the HG 12 peptide on the surfaces after 72 h in the pH 7 growth solution. Scale bar = 100 nm.

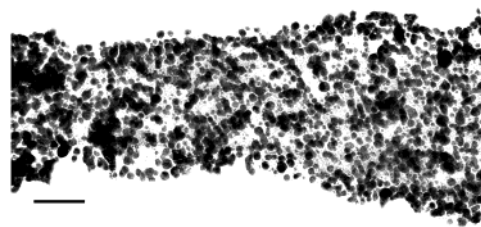


Figure 5. TEM image of Ag nanocrystals on the nanotubes after 72 h in the pH 9 growth solution. Scale bar = 50 nm.

and the size of Ag nanocrystals are not under control due to the faster growth rate in basic conditions.⁷ The hexagon-shaped nanocrystal formation around pH = 7 is also explained by the crystal growth kinetics that Ag nanocrystals grow slowly enough to form into larger hexagonal plates around the isoelectric pH = 6.09 under the influence of the AG4 peptide.^{5a} This

mechanism is supported by a control experiment to accelerate Ag nanocrystal growth in the presence of an excess reducing agent in the growth solution. When Ag nanocrystals were grown at pH = 7 for 72 h with hydrazine hydrate, hexagon-shaped Ag nanocrystals in the diameter of 50 nm were no longer observed. Instead, Ag nanocrystals were limited to grow only up to 15 nm in diameter under this growth condition (Supporting Information). This outcome suggests that the acceleration of Ag ion reduction rate due to the addition of the reducing agent decreases the size of Ag nanocrystals on the nanotubes via the faster growth rate, which is consistent with the proposed growth mechanism.

Conclusions

Hexagon-shaped Ag nanocrystals were grown directly on the peptide nanotubes by means of biological recognition and biomineralization against Ag ions with the sequenced AG4 peptide on the nanotube, which has a significant influence in the Ag nanocrystal growth on the (111) face. The pH value of

the growth solution is necessary to be controlled around 7 to form isotropic Ag nanocrystals in the hexagonal shape on the nanotubes because the pH value has a significant effect on the growth rate of Ag nanocrystals.

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Supporting Information Available: Raman spectrum of the AG4 peptide on the nanotube, growth of Ag nanocrystals on the nanotubes as a function of time at pH = 9, TEM image of Ag nanocrystals on nanotubes in the presence of an excess reducing agent, hydrazine hydrate, at pH = 7 (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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