DOI: 10.1002/asia.200900316

Biomodulation Approach for Gold Nanoparticles: Synthesis of Anisotropic to Luminescent Particles

Jadab Sharma,[a] Yian Tai,*[a] and Toyoko Imae*[b, c]

Control over the shape and functionalization of metal nanoparticles are two of the most important aspects for their widely anticipated applications in photonic devices, highly sensitive biological labels and therapeutics.[1–5] One of the requirements for many of the applications of such inorganic nanoparticles is to render them water dispersible and append with beneficial biofunctional molecules to avoid any undesired effects on the environment.[6–8] Therefore, it is often essential to prepare such nanoparticles by an environmentally friendly method so that the goal of addressing concerns of eco-friendly reducing agents, solvent system, and capping or stabilizing agents can be realized and also making such nanoparticles benign towards different biological conditions.[9] Herein, we report a simple biomodulation approach using extracts of apple (*Malus domestica*), lemon (*Citrus limonia*), tomato (*Lycopersicon esculentum*), and banana peel (*Musa cavendish*) to generate various nanostructures of gold, such as spherical, marigold-like, and triangular plates and demonstrate the synthesis of luminescent gold nanoparticles using fluorescent catabolites of chlorophyll (FCCs) available in banana peel extract.[10]

Although several methods have been developed for the preparation of anisotropic nanoparticles, most of them fail to explain the discrepancies associated with the growth of well-defined nanoparticles.[11] For example, contradictory results on the effect of additives, such as halide ions on growth of anisotropic nanoparticles by chemical and biosynthesis methods, have been reported recently.[12,13] Mirkin and coworkers have investigated the unique role of iodide ions on the growth of various anisotropic nanoparticles based on the preferential adsorption of iodide ions on specific crystal facets.[14] A similar unified framework system, however, does not exist for bio-based synthesis, and anisotropic nanoparticles have been synthesized randomly.[15–17] The reason is that biosynthesis involves a unique self-sustaining mechanism, and various shapes of nanoparticles can be generated simply by modulating different bio-additives.

Figure 1 A shows spherical nanoparticles (see the Supporting Information for details of synthesis procedures and TEM images of nanostructures) formed by adding aqueous $HAuCl_4$ (2 cm^3, 1 m**M**) to the apple extract (4 cm^3) at 80 °C (sample a). Transmission electron microscopy (TEM) and high-resolution TEM (HRTEM) images (Figure 1 A i and ii) clearly show that the nanoparticles are fairly monodisperse with an average size 10 (±3) nm. The dispersion of gold nanoparticles in water was light wine red (Figure 1 A iii), and its UV/Vis spectrum (Figure 1 A iv) exhibited a surface plasmon resonance (SPR) band centered at 530 nm.

Notably, the reaction was extremely slow at room temperature, indicating that slow reduction of gold ions could eventually lead to the growth of anisotropic nanostructures.[18] Figure 1 B shows that triangular nanoplates were formed when aqueous $HAuCl_4$ (0.5 cm^3, 10 m**M**) was treated with apple extract (3 cm^3) at room temperature for 12 h (sample b). Figure 1 B i shows a TEM image of nanoplates, and Figure 1 B ii shows the lattice fringes of a triangular nanoplate with an atomic *d* spacing of 0.23 nm, confirming the (111) plane of the face-centered-cubic (fcc) crystal structure of gold. The abundance of triangular nanoplates was 50 % and most of them were truncated at the apex. The largest edge dimension of such a triangular plate was 80 nm, and the smallest was 30 nm. Selected area electron diffraction (SAED) and X-ray diffraction (XRD) studies confirmed

[a] Dr. J. Sharma, Prof. Dr. Y. Tai
Department of Chemical Engineering,
National Taiwan University of Science and Technology,
43 Keelung Road, Taipei-10607 (Taiwan)
Fax: (+886) 2-2737-6644
E-mail: ytai@mail.ntust.edu.tw

[b] Prof. Dr. T. Imae
Keio Advanced Research Centers
Keio University, Hiyoshi, Yokohama 223-8522 (Japan)

[c] Prof. Dr. T. Imae
Graduate School of Engineering,
National Taiwan University of Science and Technology
43 Keelung Road, Taipei-10607 (Taiwan)
Fax: (+886)-2-2730-3627
E-mail: imae@mail.ntust.edu.tw

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/asia.200900316.

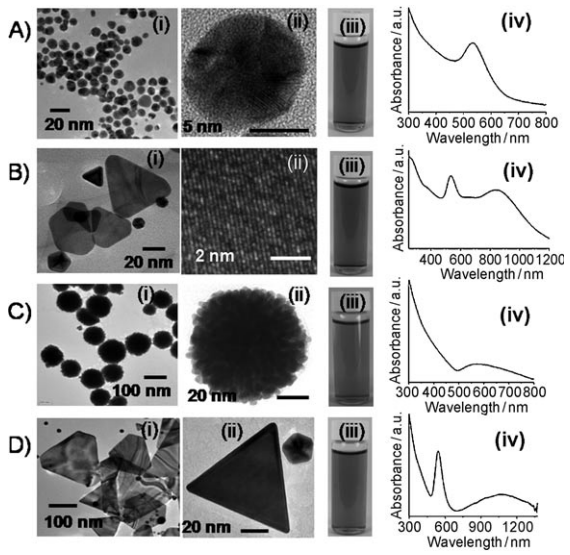

Figure 1. A) Gold nanoparticles obtained upon addition of aqueous 1 mM $HAuCl_4$ solution (2 cm^3) to apple extract (4 cm^3) at 80 °C under constant stirring for 1 h. i) TEM and ii) HRTEM images. B) Triangular nanoplates of gold obtained upon addition of aqueous 10 mM $HAuCl_4$ solution (0.5 cm^3) to apple extract (3 cm^3) and water (5 cm^3) at room temperature. i) TEM and ii) HRTEM images. C) Marigold-shaped nanostructures of gold obtained upon addition of aqueous 1 mM $HAuCl_4$ solution (5 cm^3) to tomato extract (10 cm^3) at room temperature. i,ii) TEM images. D) Triangular nanoplates of gold obtained upon addition of aqueous 10 mM $HAuCl_4$ solution (0.5 cm^3) to a mixture of lemon (1 cm^3) and tomato (5 cm^3) extracts at room temperature. i,ii) TEM images. iii) Photographs of sample bottles containing aqueous dispersions and iv) UV/Vis spectra for samples a–d.

that nanoplates were single crystals (see the Supporting Information). The remaining nanostructures were mostly pentagonal with sizes of 30–50 nm, which constituted 35% of the total particles estimated from the TEM studies. The aqueous dispersion of nanostructures was pale violet (Figure 1B iii). The UV/Vis/NIR spectrum (Figure 1B iv) showed two characteristic SPR bands centered at 530 and 850 nm, which could be attributed to the presence of pentagonal particles and in-plane dipole resonance of triangular plates, respectively.[19]

It should be noted that the reaction of aqueous $HAuCl_4$ (5 cm^3, 1 mM) with the extract (10 cm^3) of a ripe tomato generated gold nanostructures predominantly with marigold-like morphology (sample c), as is evident from the TEM images given in Figure 1C i and ii. The aqueous dispersion of the nanoparticles was light green (Figure 1C iii). The size of a representative nanostructure was 150 nm, and its HRTEM image showed that the marigold structure was formed from a large number of small (2–3 nm) nanoparticles (see the Supporting Information). The HRTEM images also revealed differently oriented lattice spacings from such small particles. The UV/Vis spectrum (Figure 1C iv) showed a very weak SPR band centered at 570 nm as a result of strong interparticle interactions of aggregated nanoparticles.[20]

A significant change in particle morphology was observed when a mixture of extracts was used. Figure 1D shows the triangular nanoplates obtained from the reaction of aqueous $HAuCl_4$ (0.5 cm^3, 10 mM) with a mixture of lemon extract (1 cm^3) and tomato extract (5 cm^3) at room temperature (sample d). The TEM images (Figure 1D i and ii) confirmed the formation of nanoplates (a few of the plates were micrometer sized) with an abundance of nearly 50%. Most of these nanoplates were truncated; the degree of truncation was different for each nanoplate, and they were single crystals, as confirmed from the SAED and XRD studies (see the Supporting Information). However, fully grown triangular nanoplates were also formed (Figure 1D ii) with a lateral dimension of 100–150 nm. The aqueous dispersion of these nanoplates was light magenta (Figure 1D iii), and its UV/Vis/NIR spectrum (Figure 1D iv) showed two characteristic SPR bands centered at 540 and 1070 nm corresponding to the dipole resonances of spherical nanoparticles and triangular nanoplates, respectively.[19] The reaction of aqueous $HAuCl_4$ (0.5 cm^3, 10 mM) with the lemon extract also generated triangular nanoplates in low abundance.

All of the nanostructures discussed above had surface capping layers of various biomolecules, as confirmed by Fourier transform infrared (FTIR) absorption and X-ray photoelectron spectroscopic (XPS) analysis (see the Supporting Information). The FTIR spectrum exhibits several peaks in the regions 1000–2000 cm^{-1} and 2500–3500 cm^{-1}, indicating surface passivation by the biomolecules available in different extracts. In particular, characteristic peaks corresponding to the stretching frequency regions of the hydroxy (3400 cm^{-1}) and carbonyl (1722 cm^{-1}) groups are more prominent in all nanostructures depicted in Figure 1. The passivation is further supported by analysis of the XPS data (Table 1). The C1s binding energy region shows three peaks

Table 1. Binding energies of C1s, N1s, O1s, and Au4f electrons of samples a–d.

Binding energy[a]	Sample a [eV]	Sample b [eV]	Sample c [eV]	Sample d [eV]
C1s	285.0, 286.7, 288.3	285.2, 286.8, 288.4	285.1, 286.7, 288.4	285.2, 286.1, 287.9
N1s	401.6	401.2	401.1	399.6, 400.7
O1s	531.9, 532.9	531.7, 533.4	531.2, 532.7	530.7, 532.2
Au4f	83.9, 87.6	83.9, 87.6	83.9, 87.5	84.1, 87.7

[a] Spectral calibration was performed by assuming the Au4f peak at either 83.9 eV or 84.1 eV as internal standard.

mainly in the regions 285.0–285.2, 286.1–286.8, and 287.9–288.4 eV, indicating the presence of aliphatic/aromatic carbon (285.0 eV) with C–OH (286.7 eV) and COOH/O=C–N (288.4 eV) bonding. The N1s region shows a peak at 401.6 eV, which is higher in energy than the bonded amino group (amide) or free amino group and indicative of the presence of amino groups in its charged state.[20] The O1s region shows two peaks in the regions 531.2–531.9 and 532.2–533.4 eV, which could be assigned as COOH/NC=O and C–OH, respectively.

No well-defined anisotropic nanostructures were obtained by addition of seed particles, surfactants, or iodide ions, as previously reported for most of the chemical methods (see the Supporting Information).[14,21] This result indicates that the growth of anisotropic nanostructures by the biosynthesis approach involves a self-sustaining mechanism with no external shape-controlling factors.[12] Although temperature variation affects the reaction result, the addition mode of reagents (whether the reagents were added dropwise or all at once) to samples b–d had no drastic effect on the resulting nanostructures. Intuitively, the method could be extended to functionalize nanoparticles with luminescence properties. Figure 2 shows gold nanostructures obtained on addition of

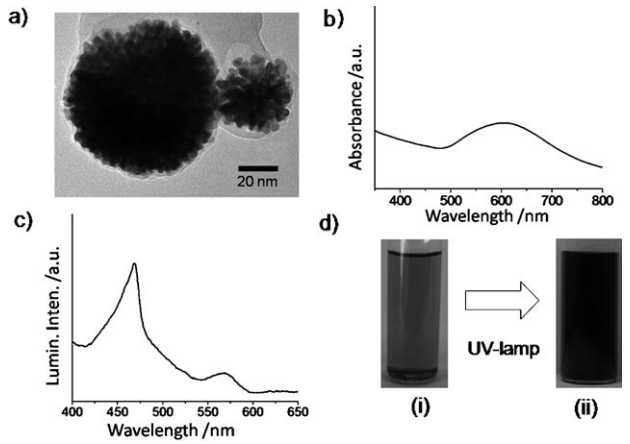

Figure 2. Gold nanoparticles obtained upon addition of an aqueous 10 mM $HAuCl_4$ solution (5 cm^3) to banana peel extract (10 cm^3) at room temperature. a) TEM image, b) UV/Vis spectrum, c) luminescence spectrum (excitation at 350 nm), and d) photographs of the dispersion of nanoparticles (in ethanol) in normal light (i) and under UV illumination at 366 nm (ii).

aqueous $HAuCl_4$ (0.5 cm^3, 10 mM) to banana peel extract (10 cm^3) at room temperature. The nanostructures exhibited significant blue luminescence properties owing to the presence of FCCs. Figure 2a shows a TEM image of such nanostructures with marigold-like morphology. The UV/Vis spectrum (Figure 2b) showed a broad SPR band centered at 600 nm as a result of strong interparticle interactions. The luminescence spectrum (Figure 2c) exhibited a peak at 468 nm, which was significantly shifted from the original position (430 nm for unattached FCC) as a result of the immobilization on nanoparticles (see the Supporting Information).[10] The observation of fluorescence from these typical nanostructures is significant because fluorescence quenching would be the dominant effect on metal nanoparticle surfaces depending on the orientation of the dipole; notable exceptions are the superlattice structures of metal nanoparticles.[22,23] Controlled experiments, carried out by adding various amounts of seed particles to the extract, confirmed that the intensity of fluorescence spectra was marginally dependent on the amount of nanostructures. The dispersion of such nanoparticles became intensely blue upon irradiation by a 366 nm UV lamp (Figure 2d). The aqueous dispersions of all the above nanostructures were stable for up to 30 days when kept at low temperature, whereas dispersions in ethanol settled within 24 h.

In essence, our findings open up a new possibility of synthesizing differently shaped nanoparticles by the judicious modulation of different bio-additives. There are several potential bio-ingredients with complex molecular structure responsible for various biological reduction reactions, which can be used for the preparation of anisotropic and luminescence nanoparticles. It will be of genuine interest to utilize such ingredients under different mixing conditions for the synthesis of nanoparticles with various shapes and functionalities. Apart from the aesthetic appeal, biomodulation will be of interest to address the environmental concerns associated with the hazardous chemicals used in the chemical methods.

Experimental Section

Preparation of extracts: Apple extract: A medium sized (172.8 g) apple was cut into small pieces and crushed in a 1000 cm^3 beaker. Apple juice was finally extracted with 150 cm^3 deionized water and filtered through a sintered funnel. The pH of the extract was 4.7 at 25 °C. Tomato extract: A medium sized (132.3 g) tomato was cut into small pieces and crushed in a 250 cm^3 beaker. Tomato juice was finally extracted with 100 cm^3 deionized water and filtered through a sintered funnel. The pH of the extract was 4.4 at 25 °C. Lemon extract: A medium sized (122.3 g) lemon was squeezed in a 250 cm^3 beaker and mixed with 40 cm^3 deionized water and then filtered through a sintered funnel. The pH of the extract was 2.4 at 25 °C. Banana peel extract: Extract of banana peel was obtained by the deep freezing method using two banana peels each time in 80 cm^3 of ethanol. Water (20 cm^3) was added to the extract, which was then filtered through a sintered funnel (pH 6.4 at 25 °C). All these extracts were stored at low temperature (4 °C).

Synthesis of spherical nanoparticles using apple extract (sample a): Aqueous 1 mM $HAuCl_4$ solution (2 cm^3) was added dropwise to a 50 cm^3 round bottom flask containing apple extract (4 cm^3) at 80 °C under constant stirring. The reaction mixture turned wine red after 5 min, and the reaction was allowed to continue for 1 h. The reaction mixture was cooled to room temperature and deionized water (10 cm^3) was added. **Synthesis of triangular nanoplates using apple extract (sample b)**: Aqueous 10 mM $HAuCl_4$ solution (0.5 cm^3) was added at once to a 30 cm^3 sample bottle containing apple extract (3 cm^3) and then deionized water (5 cm^3) was added at room temperature. The reaction mixture was stirred gently for about 30 s and then kept undisturbed for 12 h. On completion of the reaction, deionized water (10 cm^3) was added. **Synthesis of marigold-like nanoparticles using tomato extract (sample c)**: Aqueous 1 mM $HAuCl_4$ solution (5 cm^3) was added at once to a 30 cm^3 sample bottle containing tomato extract (10 cm^3) at room temperature. The reaction was initiated by gentle shaking for about 30 s and then kept undisturbed for 12 h. **Synthesis of triangular nanoplates using mixed extracts of tomato and lemon (sample d)**: Aqueous 10 mM $HAuCl_4$ solution (0.5 cm^3) was added at once to a 30 cm^3 sample bottle containing lemon extract (1 cm^3), deionzied water (4 cm^3), and tomato extract (5 cm^3) at room temperature. The reaction was initiated by gentle shaking for about 30 s and then kept undisturbed for 12 h. On completion of the reaction, deionized water (10 cm^3) was added. All the above nanostructures were collected by centrifugation at 10000 rpm for 15 min and redispersed in deionized water (15 cm^3) using an ultrasonic bath (30–60 s). **Synthesis of luminescence nanoparticles using banana peel extract (sample e)**: aqueous 10 mM $HAuCl_4$ solution (0.5 cm^3) was added at once to a 30 cm^3 sample bottle containing banana peel extract (10 cm^3) at room temperature. See the Supporting Information for further details.

CHEMISTRY
AN ASIAN JOURNAL

Acknowledgements

We thank Yi-Chun Tsai for assistance during the experiments. This work was supported in part by the National Science Council of Taiwan (96-2113M-011-002-MY2).

Keywords: biomodulation · biosynthesis · gold · nanoparticles · optical properties

[1] M. A. El-Sayed, *Acc. Chem. Res.* **2001**, *34*, 257.
[2] R. Jin, Y. Cao, C. A. Mirkin, K. L. Kelly, G. C. Schatz, J. G. Zheng, *Science* **2001**, *294*, 1901.
[3] O. V. Salata, *J. Nanobiotechnol.* **2004**, *2*, 3.
[4] L. R. Hirsch, R. J. Stafford, J. A. Bankson, S. R. Serchen, B. Rivera, R. E. Price, J. D. Hazle, N. J. Halas, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 13549.
[5] C. Burda, X. Chen, R. Narayanan, M. A. El-Sayed, *Chem. Rev.* **2005**, *105*, 1025.
[6] E. Katz, I. Willner, *Angew. Chem.* **2004**, *116*, 6166; *Angew. Chem. Int. Ed.* **2004**, *43*, 6042.
[7] D. L. Feldheim, B. E. Eaton, *ACS Nano* **2007**, *1*, 154.
[8] M. De, P. S. Ghosh, V. M. Rotello, *Adv. Mater.* **2008**, *20*, 4225.
[9] P. Raveendran, J. Fu, S. L. Wallen, *J. Am. Chem. Soc.* **2003**, *125*, 13940.
[10] S. Moser, T. Müller, M-O. Ebert, S. Jockusch, N. J. Turro, B. Kräutler, *Angew. Chem.* **2008**, *120*, 9087; *Angew. Chem. Int. Ed.* **2008**, *47*, 8954.
[11] C. Lofton, W. Sigmund, *Adv. Funct. Mater.* **2005**, *15*, 1197.
[12] A. Rai, A. Singh, A. Ahmad, M. Sastry, *Langmuir* **2006**, *22*, 736.
[13] T. H. Ha, H. J. Koo, B. H. Chung, *J. Phys. Chem. C* **2007**, *111*, 1123.
[14] J. E. Millstone, W. Wei, M. R. Jones, H. Yoo, C. A. Mirkin, *Nano Lett.* **2008**, *8*, 2526.
[15] S. S. Shankar, A. Rai, B. Ankamwar, A. Singh, A. Ahmed, M. Sastry, *Nat. Mater.* **2004**, *3*, 482.
[16] B. Liu, J. Xie, J. Y. Lee, Y. P. Ting, J. P. Chen, *J. Phys. Chem. B* **2005**, *109*, 15256.
[17] Y. He, Z. Zhang, Z. Guo, N. Gu, *Biotechnol. Prog.* **2008**, *24*, 476.
[18] X. Liu, N. Wu, B. H. Wunsch, R. J. Barsotti Jr. , F. Stellacci, *Small* **2006**, *2*, 1046.
[19] J. E. Millstone, S. Park, K. L. Shuford, L. Qin, G. C. Schatz, C. A. Mirkin, *J. Am. Chem. Soc.* **2005**, *127*, 5312.
[20] J. Sharma, S. Mahima, B. A. Kakade, R. Pasricha, A. B. Mandale, K. Vijayamohanan, *J. Phys. Chem. B* **2004**, *108*, 13280.
[21] N. Jana, L. Gearheart, C. J. Murphy, *Chem. Mater.* **2001**, *13*, 2313.
[22] F. E. Hernández, S. Yu, M. Garcßa, A. D. Campiglia, *J. Phys. Chem. B* **2005**, *109*, 9499.
[23] N. Nishida, E. S. Shibu, H. Yao, T. Oonishi, K. Kimura, T. Pradeep, *Adv. Mater.* **2008**, *20*, 4719.

Received: July 27, 2009
Published online: November 24, 2009

