

Synthesis of Gold Nanoparticles for In Situ Conjugation with Structural Carbohydrates**

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Gold nanoparticles (GNPs) have recently attracted much attention as innovative nanomaterials with unique properties in the fields of physicochemistry and biomedicine,^[1,2] because of their quantum-size effects. Surface modification of GNPs is essential for enhancing their functionality and versatility, hence extensive efforts have been devoted to methodological studies toward the synthesis of GNPs and modification of their surfaces with a variety of functional molecules.^[3–6]

Of the various substances used for surface modification of GNPs, carbohydrates have become a major target because of their specific molecular characteristics and actions in living systems.^[7] Many types of carbohydrate-related molecules have been used for conjugation with GNPs.^[8] However, there have been few reports on the related uses of structural carbohydrates because of their poor solubility and the considerable difficulties in their reaction with GNPs.

Cellulose, a β -1,4-linked D-glucopyranose polymer that is the major constituent of plant cell walls, is a typical structural polysaccharide.^[9] It has unique amphipathic and self-assembling properties because of the formation of regular intra and intermolecular hydrogen bonds.^[10] Such molecular features are expected to provide a high potential for gathering the functional carbohydrate moieties on GNP surfaces. However, cellulose that has a degree of polymerization (DP) greater than six is insoluble in both common aqueous and organic media because of its inherent, strong molecular interactions.^[9,11] Consequently, it is extremely difficult to apply to the glycomodification of GNP surfaces by using conventional approaches.

Herein we present the first preparation of GNPs that uses an ideal solvent for structural carbohydrates such as cellulose, namely hot 80% *N*-methylmorpholine-*N*-oxide (NMMO)/H₂O, and the in situ conjugation of the GNPs with thiolabeled cellulose through spontaneous chemisorption. We have previously reported the successful formation and resulting biofunctionality of cellulose nanolayers from cellulose thiosemicarbazones (cellulose-TSCs, Figure 1a). These nanolayers, which are formed through self-assembling S–Au chemisorption on a gold plate, have a parallel-chain alignment.^[12]

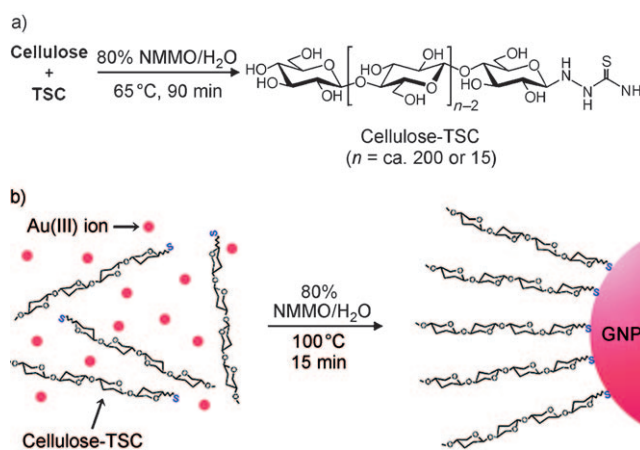


Figure 1. a) Schematic illustration of the synthesis of cellulose thiosemicarbazone (cellulose-TSC) and b) a possible structure of a cellulose-conjugated gold nanoparticle (GNP).

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Conjugation of cellulose-TSCs with GNPs that were prepared by using the conventional citrate-reduction method^[3] was initially attempted in an aqueous NMMO system, but this failed because of difficulties in redispersion of precipitated GNPs into the NMMO solvent (data not shown). However, we have found that GNPs are immediately formed in a hot NMMO solution by a facile redox procedure, which is the focus of the present study.

A dilute solution of tetrachloroauric acid (HAuCl₄) was added dropwise to a 80% NMMO/H₂O mixture that was stirred at 100 °C (see the Supporting Information). The solution immediately changed from a light-yellow to a reddish-purple color, the UV/Vis spectrum of which shows a surface plasmon resonance (SPR) band at 530 nm (Figure 2a). This is a strong indication that GNPs are directly formed from the ionic Au species in the hot NMMO/H₂O solution, without addition of reducing reagents. Transmission electron microscopy (TEM) confirmed that the precipitates from the GNPs/NMMO solution that occurred upon addition

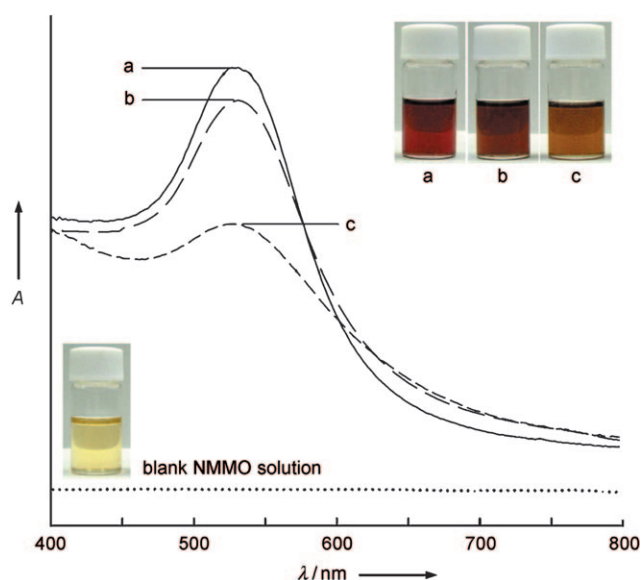
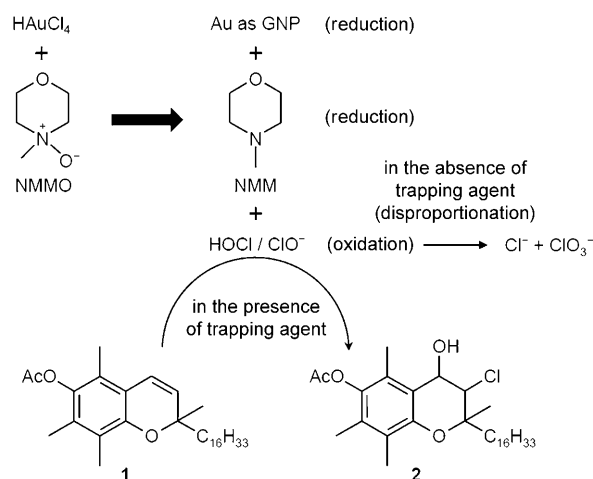


Figure 2. UV/Vis spectra and optical images of a GNP-free NMMO blank solution and GNPs/NMMO solutions. a) Cellulose-free GNPs, b) Cel₂₀₀-GNPs, and c) Cel₁₅-GNPs.

of methanol comprised several nanoparticles; these nanoparticles formed massive aggregates that were not redispersible (Figure 3a). These observations suggested that NMMO-related species are likely to play significant roles both in the formation and in the stable dispersion of GNPs in the NMMO/H₂O system.

Amine-*N*-oxides such as NMMO are frequently used in organic chemistry as oxidants and in oxidation reactions as

sacrificial catalysts because of their strong N–O dipoles.^[13] Nevertheless, in the present study, Au^{III} ions were immediately reduced to metallic Au⁰ in the aqueous NMMO system. In addition, the ¹H NMR spectrum of the chloroform extracts of the aqueous NMMO/HAuCl₄ solutions indicated that *N*-methylmorpholine (NMM) was formed to an extent that was approximately proportional to the concentration of HAuCl₄ (Figure S1 in the Supporting Information). Both Au⁰ and NMM are formed by reduction of their respective precursors (Au^{III} and NMMO), and, at a first glance, the mechanism of this process was unclear as there was no apparent reductant present in the GNP synthesis process (Scheme 1).



Scheme 1. Pathway of the NMMO/HAuCl₄ reaction system.

To elucidate the mechanism of the efficient GNP formation in the oxidative NMMO system, we carefully surveyed by-products and intermediates while paying particular attention to chlorine-derived species. In theory, gold (as Au^{III} and Au⁰) and chloride ions should be present in a stoichiometric ratio of 1:4; but only about 80 % of the theoretical amount of chloride ions were still present in this system after GNP formation was complete. Furthermore, formation of chlorate ions (ClO₃[−]) was proven by two independent methods that use reduction/AgNO₃ precipitation and MnSO₄, respectively (see the Supporting Information), while neither chlorite nor perchlorate ions were present (data not shown). These results prove that a fraction of the chloride ions served as a reductant in this reaction system and were finally oxidized to chlorate ions.

In a previous study we reported that hypochlorous acid (HOCl) and NMM are formed under mild conditions by the reaction of NMMO with chlorinating agents such as cyanuric chloride.^[14] As we suspected that a similar mechanism might be operative in the NMMO/HAuCl₄ system, HAuCl₄ was added to a solution of NMMO that contained 3,4-dehydro- α -tocopheryl acetate (**1**) as a trapping agent for hypochlorite ions.^[14,15] The presence of **1** did not interfere with GNP synthesis, while the amount of generated chlorate ions decreased significantly with increasing amounts of **1**. Formation of the hypochlorite-captured product, 3-chloro-4-hydroxy- α -tocopheryl acetate (**2**) was confirmed by ¹H NMR

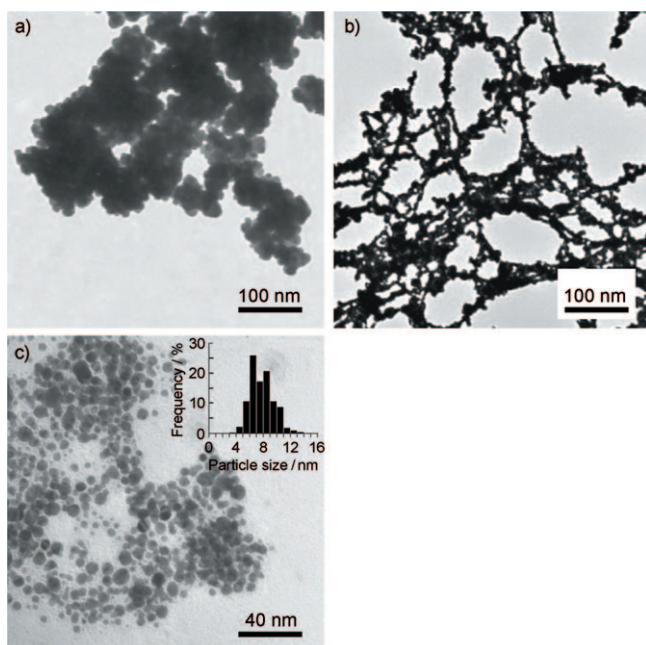
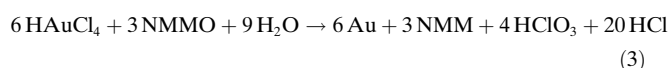
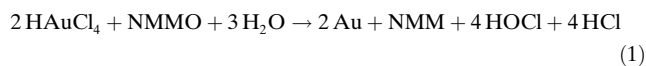


Figure 3. TEM images of GNPs and cellulose-GNPs deposited using methanol. a) Cellulose-free GNPs, b) Cel₂₀₀-GNPs, and c) Cel₁₅-GNPs. The inset shows a histogram of the diameter distribution of GNPs in the TEM image.

spectroscopy (see the Supporting Information) and comparison with an authentic sample. It was thus demonstrated that hypochlorite ions were formed as an intermediate in the chlorate generation/GNP formation process (Scheme 1).

On the basis of these observations, the following stoichiometric reaction was assumed to occur. Hypochlorite is initially formed as the directly oxidized product of chloride oxidation, which is directly coupled to the reduction of Au^{III} to Au^0 and NMMO to NMM. The chloride ions originate from the chloroaurate complex AuCl_4^- [Eq. (1)]. The thermodynamically and kinetically favored disproportionation of hypochlorite ions into chloride and chlorate ions is an immediate secondary reaction [Eq. (2)], which results in the overall NMMO/ HAuCl_4 redox process [Eq. (3)].



The incidental reductive GNP formation mechanism in the oxidative NMMO system was proved to a certain extent by the presence of hypochlorite species as an oxidized intermediate. It is well known that the N–O bond of NMMO becomes longer and less stable through interaction of the negative *exo*-oxygen atom with transition metal ions in the primary step of NMMO degradation/NMM formation.^[13] Consequently, it is likely that the aurate ion interacted with the dipole of NMMO in a similar way in this system, and the unique redox reaction may have been triggered by the unstable Au–NMMO complex, which finally led to the formation of GNPs. In light of these facts, the proposed reaction shown in Scheme 1 must be a major pathway for the NMMO-mediated GNP synthesis, although an alternative pathway cannot be ruled out. Furthermore, significant redshifts of the SPR bands were observed when the NMMO concentration was reduced (Figure S2 in the Supporting Information), which implies that the sizes of the GNPs may be controllable by variation of the concentration of NMMO.

An aqueous NMMO system that can dissolve slightly soluble structural carbohydrates would also be promising for the glycomodification of GNP surfaces. In this study, an 80% NMMO/ H_2O solution at 100 °C was used both for the GNP synthesis and for in situ surface modification with cellulose-TSC molecules (Figure 1b, also see the Supporting Information). Cellulose-TSCs were presumed to possess a ring-closed (possibly β -pyranose) structure, while a substantial amount of the nonmodified cellulose molecules remained (Figure 1a and Figures S3 and S4 in the Supporting Information). Negligible side reactions (e.g., partial oxidation of hydroxyl groups) occurred during TSC modification in a hot NMMO solution (Figures S3–S6 in the Supporting Information). The GNPs were successfully synthesized in the presence of cellulose-TSCs in 80% NMMO/ H_2O solution (Figure 2), and simultaneously conjugated with the cellulose-TSCs (Figure 3). However, there was a possibility of noncovalent

attachment of TSC-free cellulose to the GNP surface as such crude cellulose-TSCs were used (Figure S6 in the Supporting Information). SPR bands at 530 nm were unambiguously assigned in the UV/Vis spectra of the cellulose-TSCs, with approximate DP values of 200 (Cel_{200} -TSC) and 15 (Cel_{15} -TSC). The TEM image of the GNPs synthesized in the Cel_{200} -TSC/NMMO solution presented a unique nanonetwork morphology composed of GNPs connected by nanofibrous cellulosic strings (Figure 3b). Such an architecture was found neither in the gold-free Cel_{200} -TSC deposit nor in the GNPs prepared in the TSC-free cellulose/NMMO solution (Figure S7 in the Supporting Information). This specific assembly and entanglement of the cellulose chains may be attributed to their restrictive terminal immobilization by S–Au bonds on the GNP surfaces. The Cel_{15} -GNPs with relatively uniform size (average diameter (6.8 ± 1.7) nm) were well-scattered and quasi-regularly placed at a certain distance between particles (Figure 3c), which suggests that aggregation of the GNPs during the NMMO removal process (shown in Figure 3a) was inhibited by the Cel_{15} -TSCs conjugated to the GNP surfaces. The SPR intensity is strongly dependent on the interfacial circumstances of the nanoparticles,^[1] which possibly results in the relatively broad SPR profile of Cel_{15} -GNPs/NMMO suspension. At this stage, the sugar ligand density of cellulose–GNP conjugates was unknown. However, quartz crystal microbalance analysis indicated that, in a model case, water-soluble cellohexaose-TSCs could be densely chemisorbed onto an Au plate with a coverage value of $0.79 \text{ chains nm}^{-2}$ (Figure S8 in the Supporting Information). Consequently, a relatively high density of carbohydrate ligands on the GNP surface was expected for the cellulose-conjugated GNPs. Moreover, as-prepared cellulose–GNP conjugates were stable and well-dispersed in an NMMO solution over a long period (Figure S9 in the Supporting Information).

This unique approach allowed us to design a diverse array of carbohydrate–GNP conjugates by tailoring functional carbohydrates, for example, chitosan, cellohexaose, cellobiose, maltose, and lactose (Figures S10–S12 in the Supporting Information). In particular, lactose-conjugated GNPs that have bioactive galactose residues at the nonreducing ends of the ligand enabled visualization of cell morphology and variation by a specific adsorption to rat liver cells, on which asialoglycoprotein receptors that can recognize galactose residues were present (Figure S13 in the Supporting Information). The lactose–GNPs were also very stable, even in a serum-containing tissue culture medium (Figure S12 in the Supporting Information). Furthermore, silver NPs were successfully synthesized in a similar manner by the NMMO-mediated GNP synthesis (Figure S14 in the Supporting Information), which could potentially have wide applications for various functional carbohydrate-conjugated metal NP materials.

In conclusion, the discovery of a facile, direct synthesis of GNPs in an aqueous NMMO/ HAuCl_4 system has been reported. This was the first achievement of a one-pot, one-step preparation of cellulose–GNP conjugates. The proposed unusual mechanism of the redox process is consistent with chloride ions being oxidized to chlorate ions via intermediate

hypochlorite ions, while both Au^{III} and NMMO were reduced in turn. This novel and simple protocol for preparation and conjugation of GNPs is expected to find application in designing unique structural carbohydrate–GNP conjugates for nano and bioengineering functions.

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