A general approach to modifying preformed dendrimer-entrapped Au nanoparticles with different functionalities is proved to improve their biocompatibility.

Gold nanoparticles (NPs) have recently received immense scientific and technological interest because of their extensive applications in biology, catalysis, and nanotechnology. In most of these applications, the gold NP surfaces are modified with different functionalities. For instance, alkane thiol and alkylamine molecules are used for phase transfer of gold NPs, and oligonucleotide molecules are linked onto gold NPs for the subsequent spatial organization of nanocrystals and for the detection of particular DNA sequences. One unique approach to the preparation of gold NPs is through the use of poly(amidoamine) (PAMAM) dendrimers as templates. Although functionalized dendrimers have been used to prepare dendrimer-entrapped or dendrimer-stabilized gold or other noble metal NPs with different functionalities, in most circumstances dendrimer-entrapped gold NPs (Au DENPs) are prepared using amine-terminated PAMAM dendrimers. This is due to the commercial availability of this material, but this yields particles with high cytotoxicity and non-specific membrane binding due to the amine surface on the dendrimers, limiting the biological application of these particles. Preparation of non-toxic, biocompatible dendrimer-entrapped Au DENPs is of great importance for applications in various biological systems.

It is well documented that decreasing the surface charge of amine-terminated PAMAM dendrimers toward neutral reduces their toxicity. In this present study, we have developed a new, facile approach to surface modification of Au DENPs by replacing the terminal amine groups of the dendrimers after the entrapment of Au NPs. Au DENPs formed using ethylenediamine core amine-terminated generation 5 PAMAM dendrimers (G5NH2) as templates were reacted with acetic anhydride or glycidol molecules to form acetamide or hydroxyl-functionalized Au DENPs (see Scheme 1). The Au DENPs formed after surface functionalization are stable, water-soluble, and display similar sizes, size distributions, and optical properties as the original DENPs, however the surface charge changes and the biocompatibility is significantly improved. Using this approach, one can directly tailor the surface functionalities of preformed Au DENPs. To our best knowledge, this is the first example of modifying preformed Au DENPs and the corresponding dendrimer derivatives. See DOI: 10.1039/b612972b

**Scheme 1** Reactions for modifying Au DENPs prepared using amine-terminated G5NH2 dendrimers as templates.
the entrapped Au NPs under the pH conditions studied. This is in sharp contrast to dendrimer-protected Ag NPs, presumably due to the nature of metal Ag. The UV-Vis spectra of all Au DENPs dissolved in PBS buffer are similar to those of the Au DENPs dissolved in water, suggesting that they are stable under the ionic strength of the PBS buffer (ESI, Fig. S4). The corresponding surface modified dendrimers in the absence of the Au NPs do not show any absorption features at wavelengths above 250 nm (UV-vis spectra of G5-NH2, G5-NHAc, and G5-NGlyOH dendrimers are shown in the ESI, Fig. S5). The Au DENPs are stable in water, and no aggregates formed for at least 10 months after synthetic modification with either acetic anhydride or glycidol molecules (a photograph of the aqueous solutions of the Au DENPs is shown in the ESI, Fig. S6).

The morphology and size distribution of the synthesized Au DENPs were characterized by transmission electron microscopy (TEM) imaging (Fig. 2). All Au DENPs regardless of modification are relatively monodispersed and small, with sizes ranging from 2.0 ± 0.4 to 2.4 ± 0.5 nm. The size range of the Au DENPs is consistent with their UV-Vis absorption characteristics (Fig. 1) exhibiting a surface plasmon band around 510 nm. It is worthwhile to note that the plasmon peak of Au DENPs is assigned to the average contribution of the entire Au DENP population, while only the Au DENPs with diameters larger than 2.5 nm significantly contribute to the plasmon peak absorption at 510 nm. As is generally known, metal DENPs are usually smaller than 5 nm; in contrast, dendrimer-stabilized metal NPs (also sometimes referred to as interdendrimer particles) are always larger than 5 nm. The size of our particles suggests that the use of amine-terminated G5 dendrimers actually led to the formation of the Au NPs within the dendrimers. The size of the formed Au DENPs does not change significantly after the further organic functionalization process, suggesting that alteration of the DENPs or formation of Au interdendrimer particles after modification did not occur. High-resolution TEM images (ESI, Fig. S7) show that all Au DENPs are crystalline, as lattices of Au crystals are clearly observed. The crystalline nature of the Au DENPs was also confirmed using selected area electron diffraction (SAED). The (111), (200), (220) and (311) rings in the SAED patterns indicate the face-centered-cubic (fcc) crystal structures. Energy dispersive spectroscopy (EDS) of each of the Au DENP samples indicates the existence of Au elements.

Although the optical properties and sizes of all Au DENPs are very similar, their surface charges change. Zeta potential measurements show marked changes in the surface potentials of Au DENPs, with [(Au0)51.2-G5-NH2] at +36.86 mV, [(Au0)51.2-G5-NHAc] at +23.47 mV, and [(Au0)51.2-G5-NGlyOH] at +4.27 mV. The zeta potential changes reflect the successful surface modification of [(Au0)51.2-G5-NH2] DENPs, suggesting that the surface potentials of Au DENPs can be manipulated through conventional organic reactions with the dendrimers. Further PAGE analyses of the synthesized and modified Au DENPs and the corresponding dendrimer derivatives (Fig. 3) show that Au DENPs exhibit migration patterns similar to those of the corresponding dendrimer derivatives. [(Au0)51.2-G5-NGlyOH] migrates faster than G5-NGlyOH dendrimer, which is due to
the less complete hydroxylation reaction with Au NPs. This is consistent with our NMR results. Compared with corresponding dendrimers not associated with Au nanocrystals, the bands of Au DENPs are slightly broader, potentially the result of the increased polydispersity of Au DENPs. It is worth noting that we have tried to use matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry to quantify the degree of glycidol modification, but it is very difficult to ionize the metal core NPs. In order to determine the percentage of glycidol modification, it may be possible to react the remaining terminal amine groups of G5 dendrimers with an isocyanate dye to quantify the degree of dye conjugation. This work is currently on going in our lab.

The cytotoxicity of the synthesized Au DENPs was evaluated by an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay of KB cells (a human epithelial carcinoma cell line) (Fig. 4). Results show that all Au DENPs are non-toxic below a concentration of 1.0 μM. Above 1.0 μM, the cytotoxicity of Au DENPs follows the order: \( [(\text{Au}^0)_{51.2}-\text{G5-NH}_2] > [(\text{Au}^0)_{51.2}-\text{G5-NGlyOH}] > [(\text{Au}^0)_{51.2}-\text{G5-NHAc}], \) which is related to the degree of cationic surface charge. The toxicity of Au DENPs was also evaluated by visualizing the morphologies of KB cells treated with different surface-functionalized Au DENPs. At a concentration of 2.0 μM, the morphology of KB cells treated with \( [(\text{Au}^0)_{51.2}-\text{G5-NHAc}] \) DENPs is similar to the morphology of untreated KB cells, suggesting that \( [(\text{Au}^0)_{51.2}-\text{G5-NHAc}] \) DENPs display a very good biocompatibility (Fig. 5). Acetylation of \( [(\text{Au}^0)_{51.2}-\text{G5-NH}_2] \) DENPs neutralizes the surface charges of Au NPs, as confirmed by PAGE and zeta potential measurements, making them highly compatible with biological systems. In contrast to the acetylation reaction, much less complete hydroxylation of \( [(\text{Au}^0)_{51.2}-\text{G5-NH}_2] \) DENPs (as compared with hydroxylation of G5-NH2 dendrimers) cannot effectively neutralize their positive charges; therefore, the \( [(\text{Au}^0)_{51.2}-\text{G5-NGlyOH}] \) DENPs formed still display some cytotoxicity at high concentrations. The toxicity data of \( [(\text{Au}^0)_{51.2}-\text{G5-NH}_2] \) and \( [(\text{Au}^0)_{51.2}-\text{G5-NHAc}] \) DENPs are comparable with the corresponding dendrimer derivatives in the absence of Au NPs (ESI, Fig. S8). However, G5-NGlyOH dendrimers do not exhibit toxicity even at a concentration of 2.0 μM, suggesting that hydroxylation of G5-NH2 dendrimers to form G5-NGlyOH significantly decreases the surface charge of the dendrimers. These results imply that post-synthetic modification of Au DENPs is a straightforward approach to designing non-toxic Au NPs for biological applications. It is interesting to note that we also attempted to synthesize non-toxic Au NPs using preformed G5-NHAc and G5-NGlyOH dendrimers as templates under similar conditions. In both cases, black precipitates were formed (A photograph of the aqueous solutions of Au NPs synthesized using G5-NHAc and G5-NGlyOH dendrimers as templates is shown in the ESI, Fig. S9).† It seems that the complexation of \( \text{AuCl}_4^- \) ions with either the acetamide or glycidol hydroxyl-terminated G5 dendrimer is much weaker than that with amine-terminated G5 dendrimers, significantly decreasing the stability of the Au NPs.

In summary, we have discovered a new approach to functionalizing Au DENPs through dendrimer-mediated organic reactions. The resulting Au DENPs are crystalline and have an average size of 2.2 nm. The surface-functionalization of Au DENPs does not influence their sizes, size distributions, crystallites, water solubility, or stability. The surface potentials of the Au DENPs are varied depending on their surface functional groups. Importantly, the biocompatibility of Au DENPs is significantly improved after surface functionalization. We anticipate that various biological ligands can be conjugated onto Au DENP surfaces, followed by neutralization of the remaining amine groups of dendrimers to produce biocompatible functional Au DENPs for biological and therapeutic applications. The approach used for functionalization of Au NPs may be applied to a range of different dendrimer-templated metal NPs and different organic syntheses and opens a new avenue to tailoring the particle surface functionality for various applications.

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Notes and references