

Improved Biocompatibility of Surface Functionalized Dendrimer-Entrapped Gold Nanoparticles

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1. Experimental Section

Materials. Ethylenediamine core amine-terminated PAMAM dendrimers of generation 5 (G5.NH₂) with a polydispersity index less than 1.08 were purchased from Dendritech (Midland, MI). All other chemicals were obtained from Aldrich and used as received. Water used in all of the experiments was purified using a Milli-Q Plus 185 water purification system (Millipore, Bedford, MA) with resistivity higher than 18 MΩ.cm. Regenerated cellulose dialysis membranes (MWCO = 10,000) were acquired from Fisher.

Synthesis and post-modification of Au DENPs. The synthesis procedure is similar to that reported in the literature.^{1, 2} The Au DENPs were prepared using sodium borohydride reduction chemistry with the dendrimer terminal amine /gold atom molar ratio at 1: 0.4. Briefly, 5 mL HAuCl₄ solution (118.2 mM) was added into 20 mL G5.NH₂ aqueous solution (0.577 mM) under vigorous stirring. After 30 min, 6 mL NaBH₄ solution (197 mM) dissolved into water/methanol (2:1 in volume) mixture was slowly added to the gold salt/dendrimer mixture while stirring. The reaction mixture turned to a deep red color within a few seconds after addition of the NaBH₄ solution. The stirring was continued for 2 hours to complete the reaction. The reaction mixture was extensively dialyzed against water (6 times 4 liters) for 3 days to remove the excess of reactants, followed by lyophilization to get {(Au⁰)_{51.2}-G5.NH₂} DENPs.

The acetylation reaction procedure used to modify {(Au⁰)_{51.2}-G5.NH₂} with acetamide groups was followed from the literature.³ Briefly, 198 μL of triethylamine was added to a 10-mL methanol solution containing 107.86 mg {(Au⁰)_{51.2}-G5.NH₂} DENPs.

A methanolic solution (5 mL) of acetic anhydride (144.88 mg, 400% molar excess of the total primary amines of $\{(Au^0)_{51.2}\text{-G5.NH}_2\}$) was slowly added into the DENPs/triethylamine mixture solution while vigorously stirring, and the mixture was allowed to react for 24 h. The methanol solution of the reaction mixture was extensively dialyzed against PBS buffer (3 times 4 liters) and water (3 times 4 liters) for 3 days to remove the excess of reactants and by-products, followed by lyophilization to get $\{(Au^0)_{51.2}\text{-G5.NHAc}\}$ DENPs.

The hydroxylation reaction procedure used to modify $\{(Au^0)_{51.2}\text{-G5.NH}_2\}$ with hydroxyl groups was followed from the literature.⁴⁻⁶ To a 10-mL methanol solution containing 108.3 mg of $\{(Au^0)_{51.2}\text{-G5.NH}_2\}$ DENPs, a methanol solution containing 211.12 mg glycidol (400 % molar excess of the amine groups of $\{(Au^0)_{51.2}\text{-G5.NH}_2\}$) was added dropwise while stirring. The reaction was stopped after 24 h and the reaction mixture was dialyzed against water (6 times 4 liters) for 3 days to remove by-products and the excess of reactants, followed by lyophilization to get $\{(Au^0)_{51.2}\text{-G5.NGlyOH}\}$ DENPs.

For comparison, preformed G5.NHAc and G5.NGlyOH dendrimers were also used as templates to synthesize Au NPs under conditions similar to those that were used to synthesize $\{(Au^0)_{51.2}\text{-G5.NH}_2\}$. The G5.NHAc and G5.NlyOH dendrimers were synthesized and characterized according to our previous reports.^{4, 5, 7}

Characterization of Au DENPs. ^1H NMR and ^{13}C NMR spectra of Au DENPs were recorded on a Bruker DRX 500 nuclear magnetic resonance spectrometer. Samples were dissolved in D_2O before NMR measurements. UV-Vis spectra were collected using a

Perkin Elmer Lambda 20 UV-Vis Spectrometer. All samples were dissolved in water at the concentration of 1 mg/mL. The UV-Vis spectra of Au DENPs at different pHs and dissolved into PBS buffer were also collected in order to investigate their stability. Zeta potential measurements were performed using a PSS/NICOMP 380 ZLS particle sizing system (Santa Barbara, CA) with a red-diode laser at 635 nm in a multiangle cell. A JEOL 2010F analytical electron microscopy was performed at 200 kV with an EDS system attached. A 5- μ L aqueous solution of Au DENPs (1 mg/mL) was dropped onto a carbon-coated copper grid and air dried before measurements. Analysis of PAMAM dendrimers and Au DENPs by PAGE was performed on a Micrograd vertical electrophoresis system (Model FB-VE10-1, FisherBiotech, Pittsburgh, PA) with a commercial power supply (Model EC135-90; Thermo Electron Corporation, Milford, MA) according to a procedure described in a previous report.⁸ Precast 4–20% gradient express gels for PAGE were obtained from ISC BioExpress (Kaysville, UT). Tris-Glycine (TG) native buffer (pH = 8.3) was purchased from Invitrogen (Carlsbad, CA). It was diluted by a factor of ten to prepare the running buffer. PAGE separations typically required 50 minutes at 200 V. Reverse polarity was used for the analysis of the polycationic PAMAM dendrimers and Au DENPs. For comparison of the difference in migration behaviors, both PAMAM dendrimers (G5.NH₂, G5.NHAc, and G5.NGlyOH) and their corresponding Au DENPs were applied in PAGE analysis. Into each sample well 2 μ L of a sample solution composed of equal volumes of 1 mg/mL PAMAM dendrimers (amine, acetamide, hydroxyl-terminated G5 dendrimers) or Au DENPs and methylene blue sucrose dye solutions (50% sucrose, 1% methylene blue) was injected. Developed gels were stained with 0.025% Coomassie Blue R-250 in 40% methanol and

7% acetic acid aqueous solution overnight. The gels were destained with an aqueous solution containing 7% (v/v) acetic acid and 5% (v/v) methanol.

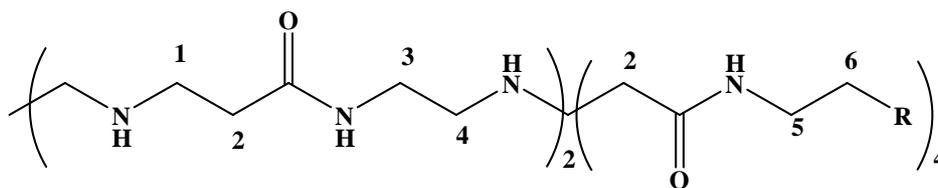
Cytotoxicity assay and cell morphology observation. The KB cells (a human epithelial carcinoma cell line) were purchased from the American Type Tissue Collection (ATCC, Manassas, VA) and grown in regular RPMI 1640 medium. An MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used to quantify the viability of cells. Briefly, 1×10^4 KB cells per well were seeded into a 96-well plate. After overnight incubation, Au DENPs at concentrations ranging from 0 nM to 2 μ M in PBS (pH 7.4) was added. After 24 h incubation with Au DENPs and the corresponding dendrimer derivatives at 37 °C, MTT reagent in PBS solution was added. The assays were carried out according to the manufacturer's instructions. For each concentration of Au DENPs, 5 wells of cells were analyzed. In parallel, after treatment with Au DENPs for 24 h, the cell morphology was observed by Leica DMIRB fluorescent inverted microscope. The magnification is set at 200x for all samples.

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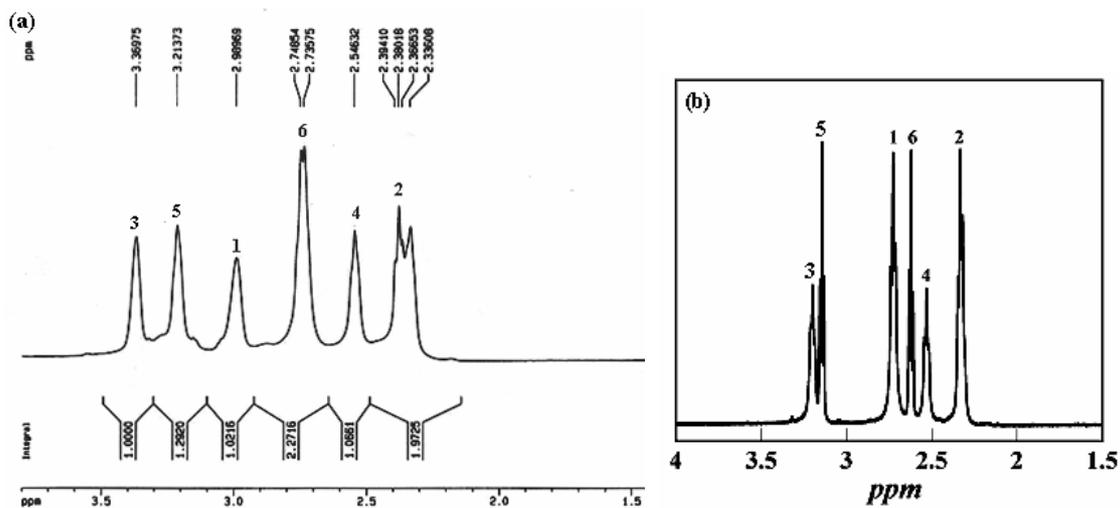
2. ^1H NMR and ^{13}C NMR spectra of $\{(\text{Au}^0)_{51.2}\text{-G5.NH}_2\}$, $\{(\text{Au}^0)_{51.2}\text{-G5.NHAc}\}$, and $\{(\text{Au}^0)_{51.2}\text{-G5.NGlyOH}\}$ Au DENPs and the corresponding dendrimer derivatives.



A-R = NH_2

B-R = NHCOCH_3

C-R = $\text{N}(\text{CH}_2\text{CHOHCH}_2\text{OH})_2$



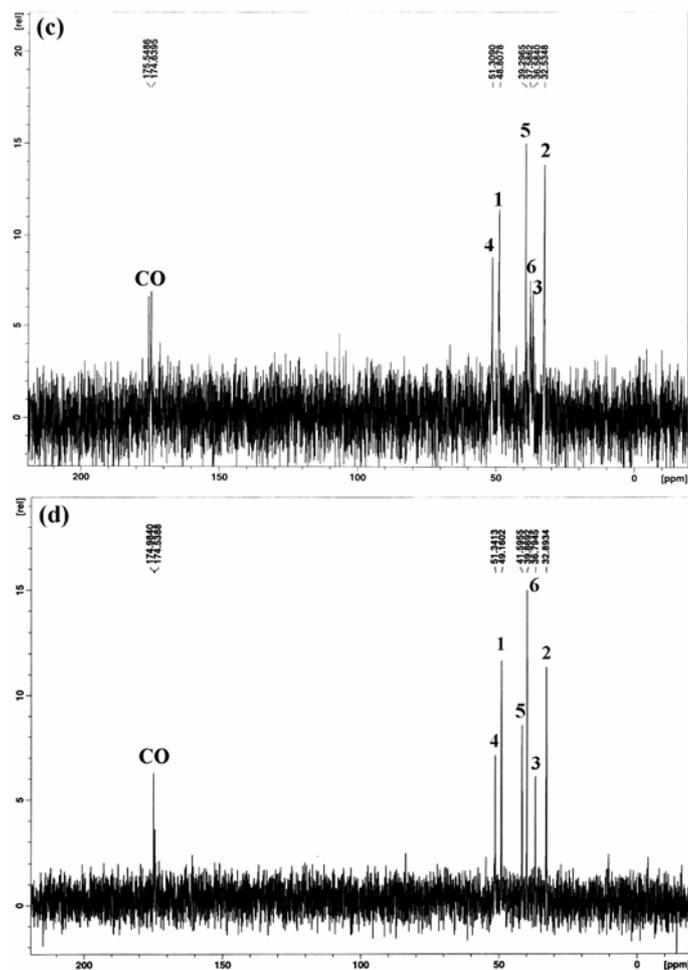
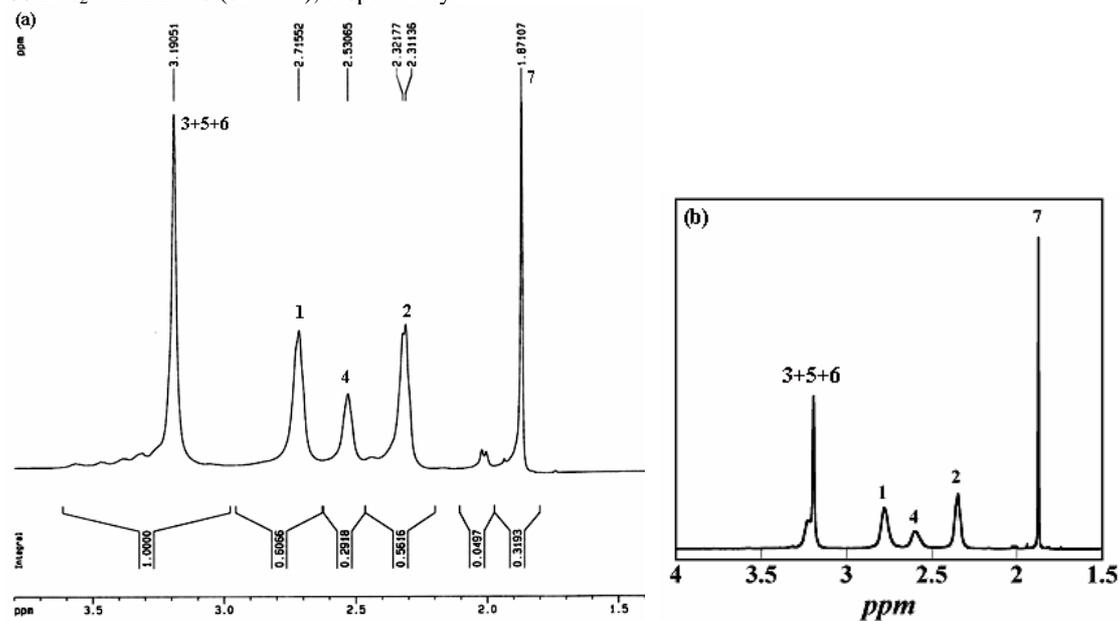


Figure S1. ^1H NMR (a and b) and ^{13}C NMR spectra (c and d) of $\{(\text{Au}^0)_{51.2}\text{-G5.NH}_2\}$ DENPs (a and c) and G5.NH₂ dendrimers (b and d), respectively.



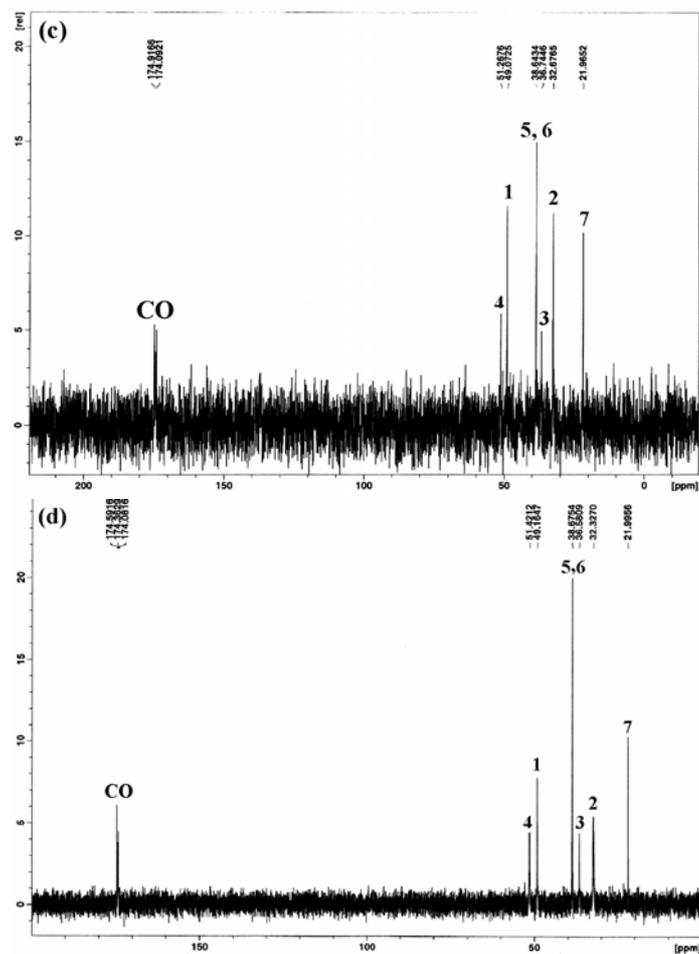
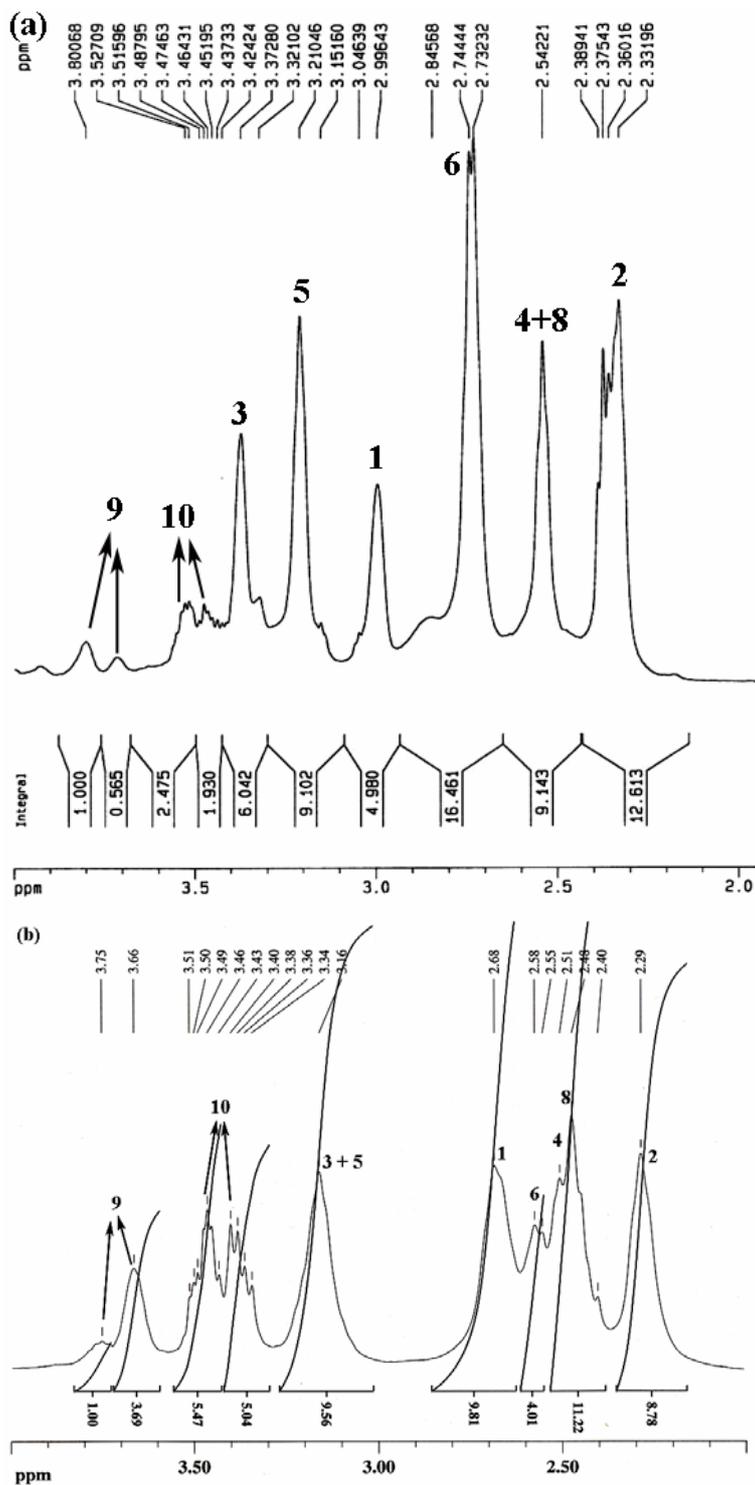


Figure S2. ^1H NMR (a and b) and ^{13}C NMR (c and d) spectra of $\{(\text{Au}^0)_{51.2}\text{-G5.NHAc}\}$ DENPs (a and c) and G5.NHAc dendrimers (b and d), respectively.



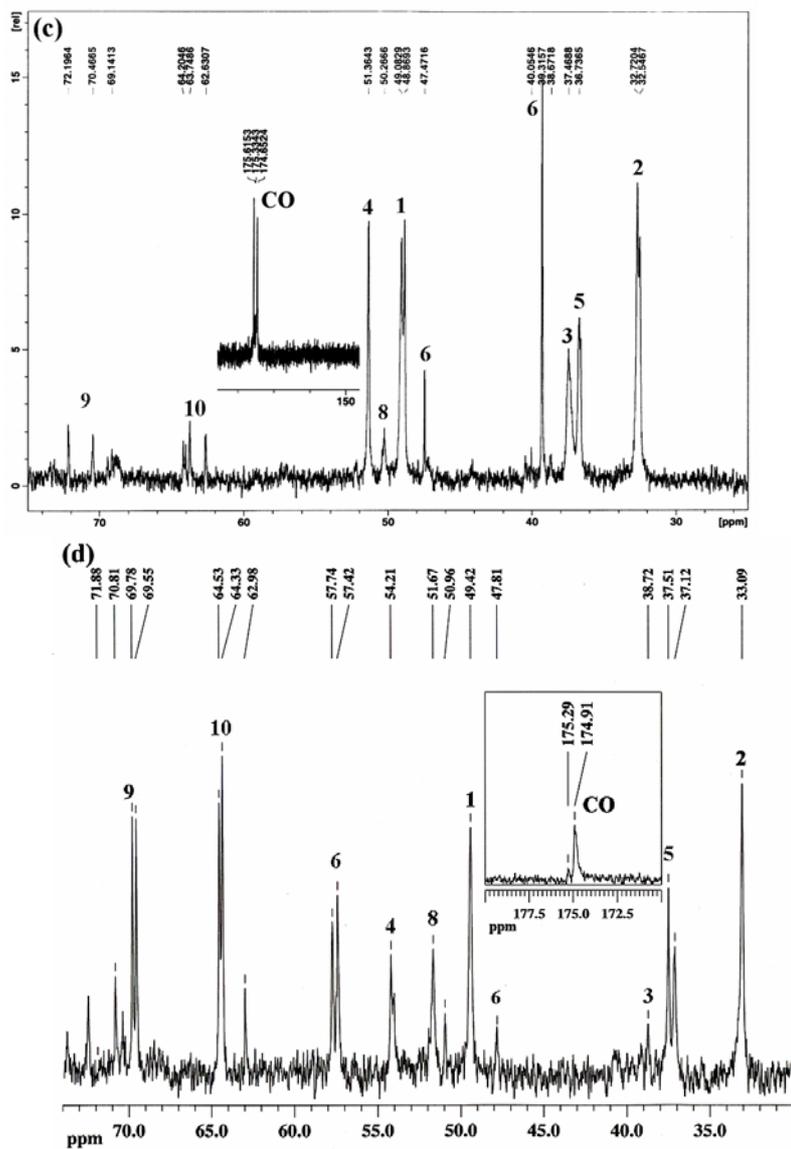


Figure S3. ^1H NMR (a and b) and ^{13}C NMR (c and d) spectra of $\{(\text{Au}^0)_{51.2}\text{-G5.NGlyOH}\}$ DENPs (a and c) and G5.NGlyOH dendrimers (b and d), respectively.

3. UV-vis spectra of $\{(Au^0)_{51.2}\text{-G5.NH}_2\}$, $\{(Au^0)_{51.2}\text{-G5.NHAc}\}$, and $\{(Au^0)_{51.2}\text{-G5.NGlyOH}\}$ DENPs at different conditions.

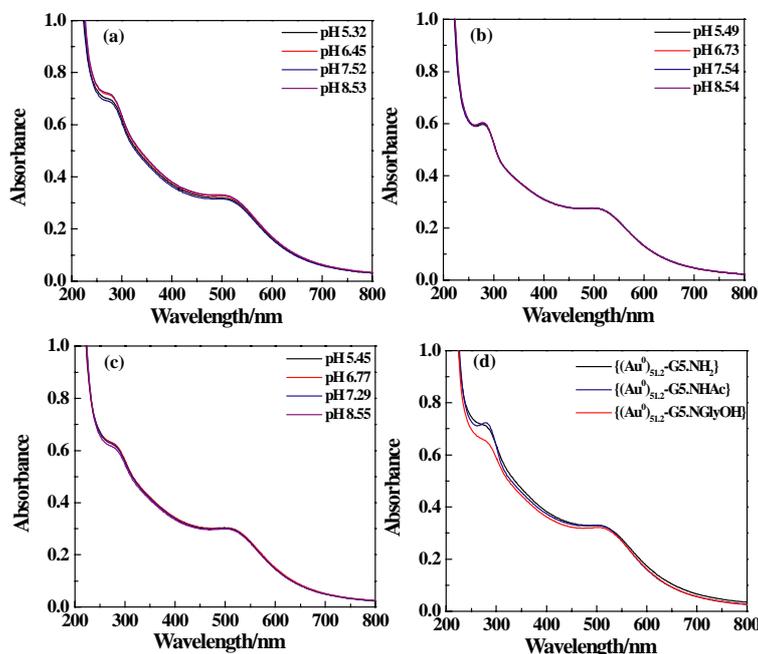


Figure S4 UV-vis spectra of $\{(Au^0)_{51.2}\text{-G5.NH}_2\}$ (a), $\{(Au^0)_{51.2}\text{-G5.NHAc}\}$ (b), and $\{(Au^0)_{51.2}\text{-G5.NGlyOH}\}$ (c) DENPs at different pH conditions. UV-vis spectra of $\{(Au^0)_{51.2}\text{-G5.NH}_2\}$, $\{(Au^0)_{51.2}\text{-G5.NHAc}\}$, and $\{(Au^0)_{51.2}\text{-G5.NGlyOH}\}$ DENPs dissolved in PBS buffer are shown in (d).

4. UV-vis spectra of G5.NH₂, G5.NHAc, and G5.NGlyOH dendrimer derivatives. The dendrimers were dissolved in water at a concentration of 1 mg/mL.

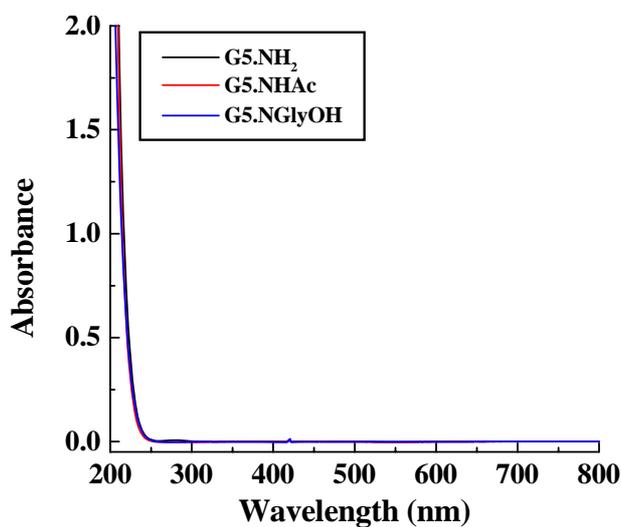


Figure S5. UV-vis spectra of G5.NH₂, G5.NHAc, and G5.NGlyOH dendrimers.

5. A photograph of the aqueous solutions of $\{(Au^0)_{51.2}\text{-G5.NH}_2\}$, $\{(Au^0)_{51.2}\text{-G5.NHAc}\}$, and $\{(Au^0)_{51.2}\text{-G5.NGlyOH}\}$ DENPs (from left to right).



Figure S6. A photograph of the aqueous solutions of $\{(Au^0)_{51.2}\text{-G5.NH}_2\}$, $\{(Au^0)_{51.2}\text{-G5.NHAc}\}$, and $\{(Au^0)_{51.2}\text{-G5.NGlyOH}\}$ DENPs (from left to right).

6. Magnified high-resolution TEM images of $\{(Au^0)_{51.2}\text{-G5.NH}_2\}$, $\{(Au^0)_{51.2}\text{-G5.NHAc}\}$, and $\{(Au^0)_{51.2}\text{-G5.NGlyOH}\}$ DENPs.

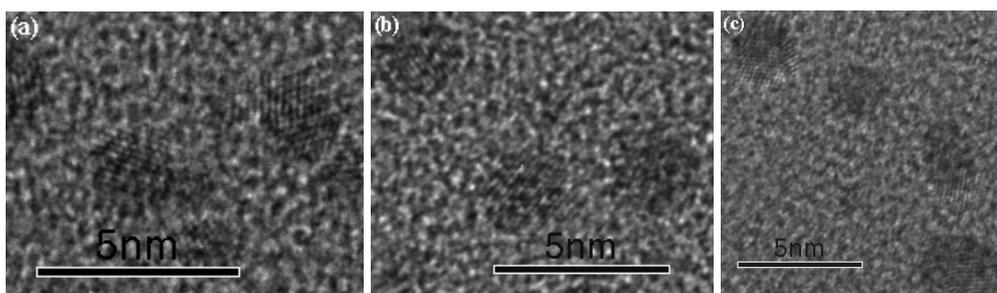


Figure S7. A magnified high-resolution TEM image of (a) $\{(Au^0)_{51.2}\text{-G5.NH}_2\}$, (b) $\{(Au^0)_{51.2}\text{-G5.NHAc}\}$, and (c) $\{(Au^0)_{51.2}\text{-G5.NGlyOH}\}$ DENPs.

7. An MTT assay of KB cell viability after treatment with G5.NH₂, G5.NHAc, and G5.NGlyOH dendrimers for 24 hours. The data are expressed as mean ± S. D.

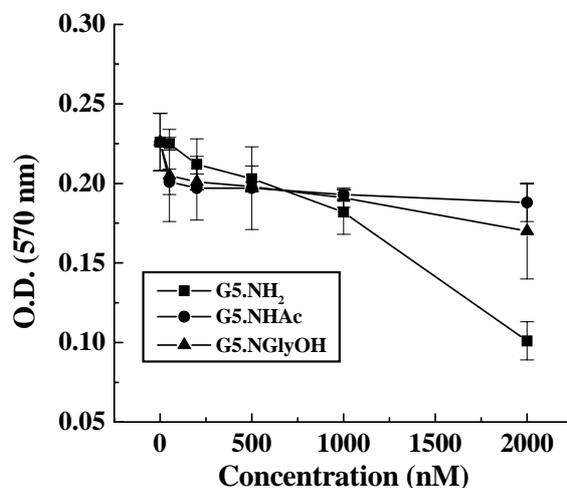


Figure S8. An MTT assay of KB cell viability after treatment with G5.NH₂, G5.NHAc, and G5.NGlyOH dendrimers for 24 hours. The data are expressed as mean ± S. D.

8. A photograph of the aqueous solutions of Au NPs synthesized using preformed G5.NHAc (1) and G5.NGlyOH (2) dendrimers as templates.

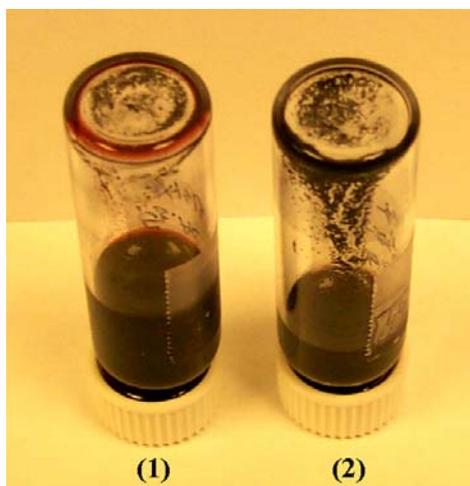


Figure S9. A photograph of the aqueous solutions of Au NPs synthesized using preformed G5.NHAc (1) and G5.NGlyOH (2) dendrimers as templates.