Supporting Information

Facile Synthesis of Robust and Biocompatible Gold Nanoparticles

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Materials and Methods

Materials.

Hydrogen tetrachloroaurate(III) hydrate was purchased from Kojima Chemicals Co.(Sayama, Saitama, Japan). Ammonium hydroxide (NH_3, 28~30% in H_2O), DTT (DL-dithiothreitol, 99%) and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Dextran (from *leuconostoc ssp.*, Mr~ 15000-25000) was purchased from Fluka Chemical Corp. (Milwaukee, WI, USA). Epichlorohydrin (99%) was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Sodium hydroxide and trisodium citrate dihydrate were purchased from Junsei Chemical Co. (Tokyo, Japan). SPDP (N-Succinimidyl-3-(2-Pyridyldithio) Propionate) was purchased from Pierce (Rockford, IL, USA). 10x PBS (Phosphate-Buffered Salline. 10x), DMEM (Dulbecco's Modified Eagle's Medium) and FBS (fetal bovine serum) were purchased from WelGENE Inc. (Seoul, Korea). Amicon Ultra centrifuge filter devices (cutoff:100 kDa) were purchased from Millipore (Billerica, MA, U.S.A.). Minisart RC 25 syringe filters (0.20μm and 0.45μm pore size) were purchased from Sartorius stedim biotech (Goettingen, Germany)
All chemicals and devices were used as received.

Synthesis of citrate stabilized AuNPs, dex-AuNP, cl-dex-AuNP, and N-cl-dex-AuNP.

Synthesis of citrate stabilized AuNPs

50 mL of 0.25 mM hydrogen tetrachloroaurate(III) solution was heated until boil, then added 200 μL of 34 mM trisodium citrate dihydrate solution. The reaction mixture was boiled for 20 min until the color changed to violet, then was cooled to room temperature.

Synthesis of dex-AuNPs

Dextran (12.0g) was dissolved in distilled water (160 mL) to prepare a 7.5 wt% solution. The solution was heated until boil, then added hydrogen tetrachloroaurate(III) hydrate stock solution (432 μL, 0.1g/mL stock solution) to the hot dextran solution. The reaction mixture was boiled for 15min until it turned reddish-violet. The reaction mixture was then cooled to room temperature. The product was rinsed with distilled water for 4 times using Amicon filter (cutoff:100kDa). Finally, dex-AuNPs were re-dispersed in distilled water (4mL) and stored at 4℃.

Synthesis of cl-dex-AuNPs

200uL of 1M NaOH solution was added to the synthesized 4mL of dex-AuNPs. The reaction mixture was vortexed vigorously for 15sec, then added 60μL of epichlorohydrin to the mixture. Mixed solution was allowed to incubate while shaking at 600rpm at room temperature, overnight. Purification of cl-dex-AuNPs was done by centrifugation at 3000 rpm for 20 min using 100kDa Amicon Ultra centrifuge filter. The product was rinsed with distilled water for 4 times, then re-dispersed in 4 mL of distilled water and stored at 4℃.

Synthesis of N-cl-dex-AuNPs

Amination was achieved by addition of 3% ammonium hydroxide to the aqueous cl-dex-AuNPs solution. Mixed solution was allowed to incubate at room temperature, 24hrs while shaking at 600 rpm. The purification was done by centrifugation at 3000 rpm for 20min with 100 kDa Amicon Ultra centrifuge filter. The product was rinsed with distilled water for 4 times, and finally re-dispersed in distilled water. The AuNPs remained stable more than 3 months at 4 ^{o}C.

Characterization of the AuNPs.

Field emission SEM S-4800 (Hitachi, Japan) was used to take images of AuNPs. Particle size distribution was determined by a particle size analyzer Plus90 (Brookhaven). SpectraMax Plus^{384} (Molecular Devices, USA) was used to obtain UV-Vis absorbance. Zeta potential measurement was carried out by a zeta sizer Nano ZS90 (Malvern, USA).

Conjugation of fluorescence dye and cell penetrating peptide to N-cl-dex-AuNPs

Preparation of Cy5.5 conjugated dex-AuNPs

To conjugate Cy5.5 fluorescence dye to 3% N-cl-dex-AuNPs, 50 nmol of Cy5.5 was dissolved in anhydrous DMSO and added to 5 nmol of 3% N-cl-dex-AuNPs in 1xPBS on ice bath. Mixed solution was allowed to incubate for 24hrs at room temperature whith shaking at 600 rpm. The AuNPs were purified by centrifugation at 3000 rpm using Amicon Ultra centrifuge filter (cutoff: 100 kDa). The product was rinsed 4 times with distilled water, and re-dispersed in distilled water.

Conjugation of CPP to Cy5.5 conjugated dex-AuNPs

To conjugate CPP, 1 μmol of SPDP was dissolved in anhydrous DMSO and added to 4 nmol of Cy5.5-dex-AuNPs. Mixed solution was allowed to incubate for 3hrs at room temperature with shaking at 600 rpm. The purification was done by size exclusion column (Sephadex G25) with 1xPBS buffer as a running buffer. Then, CPP in 1xPBS was added to the SPDP linker conjugated Cy5.5-dex-AuNPs. After shaking for 24hrs at 600 rpm, the reaction mixture was purified by centrifugation at 3000 rpm with Amicon Ultra centrifuge filter (cutoff:100kDa). The final AuNPs were rinsed 4 times with 1xPBS and re-dispersed in 1xPBS for cellular uptake study.

Cell viability assay (MTT assay)

Preparation of MTT stock solution

MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) powder was dissolved in 1x PBS at 5mg/mL concentration, and filtered to sterilize through a 0.2 μm syringe filter. The stock solution was stored in 4 ^{o}C, covered with aluminum foil.

Cell Culture

HeLa (human cervical cancer) cells were cultured and used for cytotoxicity assay in the study. Growth media was Dulbecco's Modified Eagle's Medium (DMEM) containing 4.5g/L D-glucose and supplemented with 10% FBS (fetal bovine serum), 100 units/mL penicillin, and 100 ug/mL streptomycin. The cells were grown in a humidified 5% CO_2 incubator at 37℃.

MTT assay for Viability test

HeLa cells were seeded at 10,000 cells per well of a 96-well culture plate with 100 μL of growth media (about 50-70 % confluency for the experiments). The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used to quantify the viability of cell line. After dex-AuNPs and N-cl-dex-AuNPs at concentrations ranging from 2~35 pM (0.006~0.1 mg/mL) in distilled water were added and incubated for 4 hrs at 37 °C. Following AuNPs treatment, cells were rinsed with 1X PBS and incubated another 24 hrs with growth media. A 20 μL MTT reagent dissolved in PBS at 5 mg/mL concentration was added to detect the metabolically active cells in each well. The cells were incubated for 2-4 hrs until purple color develops indicating that MTT was to be metabolized. The media were discarded, and 200μL DMSO was added to each well to make insoluble formazan salt solublized. Then the optical densities of each well in the plates were read at 560nm. Mean and standard deviation for the triplicate wells were recorded by UV-vis spectrophotometer.

LIVE/DEAD cell stain for Biocompatibility Test

The biocompatibility of AuNPs was tested by examining the growth of HeLa cells using the LIVE/DEAD Viability/Cytotoxicity Assay Kit (from Molecular Probes Invitrogen). HeLa cells were seeded at $1X10^4$ cells per well of a 96-well cell culture plate (about 50-70 % confluency for the experiments). The cells were incubated with AuNPs at concentrations of 2~35 pM (0.006~0.1 mg/mL) in serum-free media for 4 h, then replaced with fresh-media and cultured with serum-containing fresh media for 24 h. Following incubation, 50 μL of the combined live-dead cell staining solution (2 μM calcein AM and 4μM EthD-1 in D-PBS) was added to each well and incubated with cells for 30~45 min. Images were obtained using a BX51M optical microscope (Olympus Co., Japan) equipped with fluorescence light source and filters.

Data

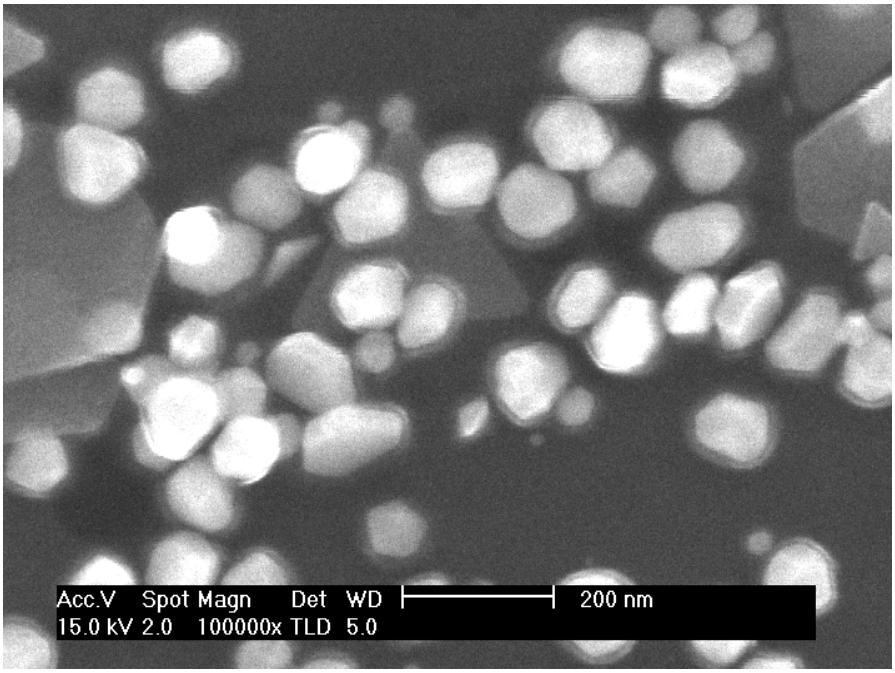

Figure S1. A SEM image of synthezied N-cl-dex-AuNPs.

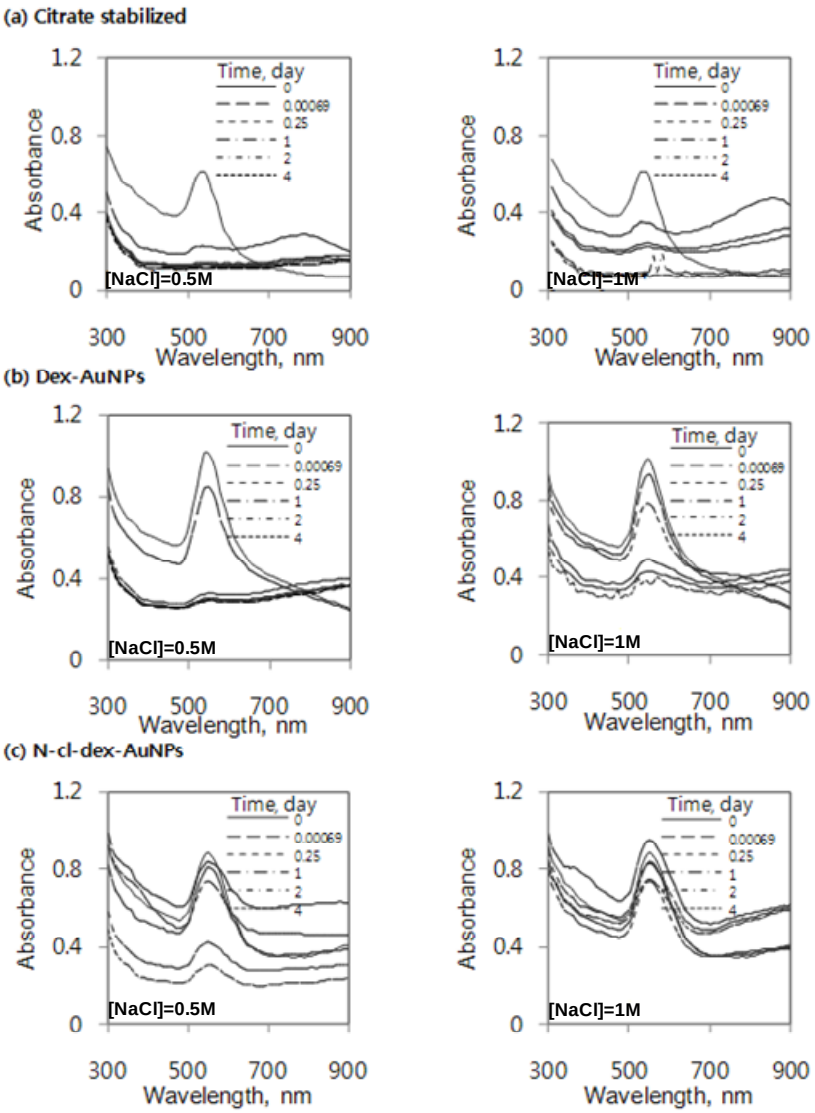

Figure S2. UV-vis absorption spectra of (a) AuNPs stabilized by citrate, (b) dex-AuNPs, (c) N-cl-AuNPs (3 % aminated) in 0.5M and 1M sodium chloride solution

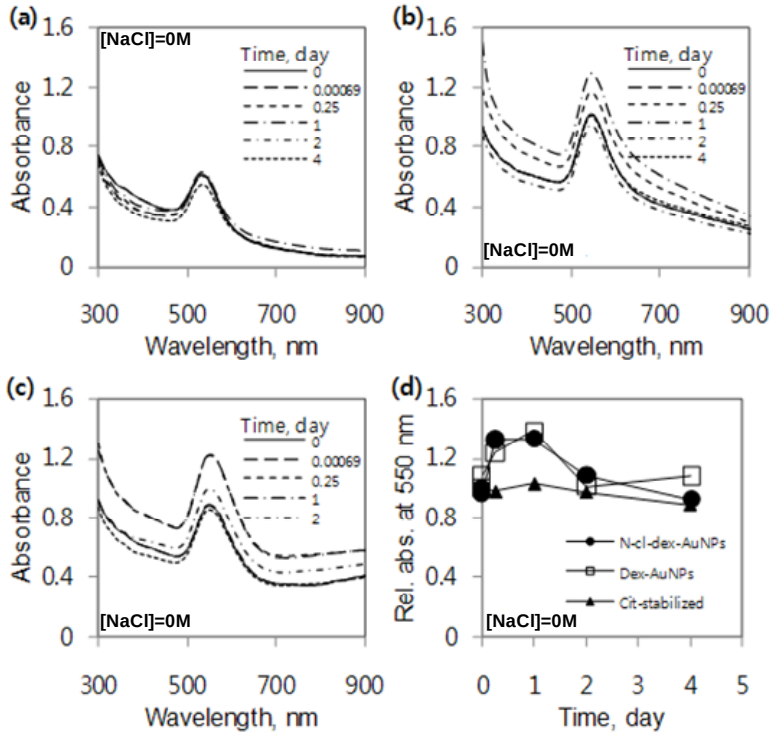

Figure S3. UV-vis absorption spectra of (a) AuNPs stabilized by citrate, (b) dex-AuNPs, (c) N-cl-AuNPs (3 % aminated) in 0M sodium chloride solution. (d) A plot of relative absorbance of AuNPs at 550nm versus time.

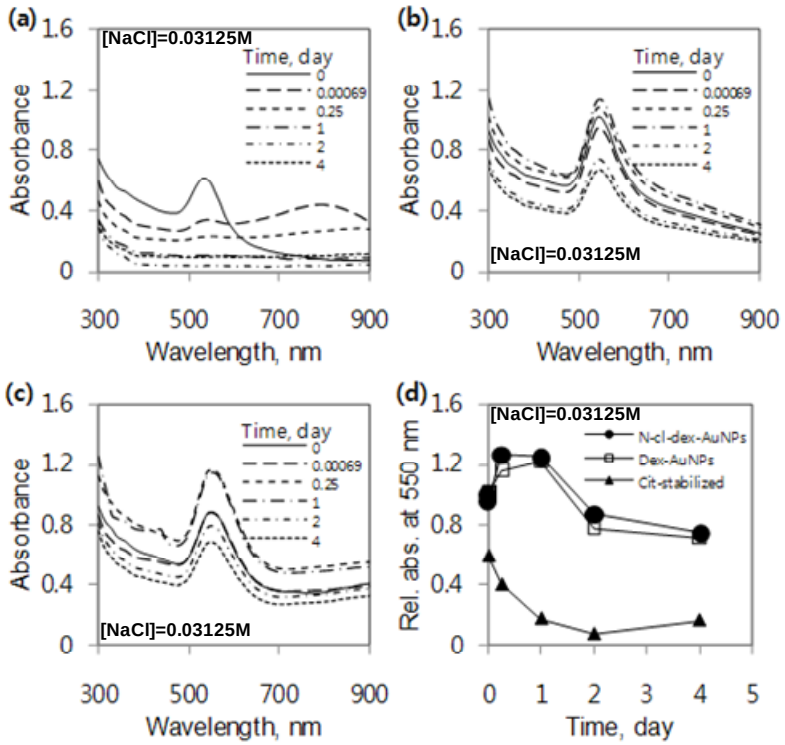

Figure S4. UV-vis absorption spectra of (a) AuNPs stabilized by citrate, (b) dex-AuNPs, (c) N-cl-AuNPs (3 % aminated) in 0.03 M sodium chloride solution. (d) A plot of relative absorbance of AuNPs at 550nm versus time.

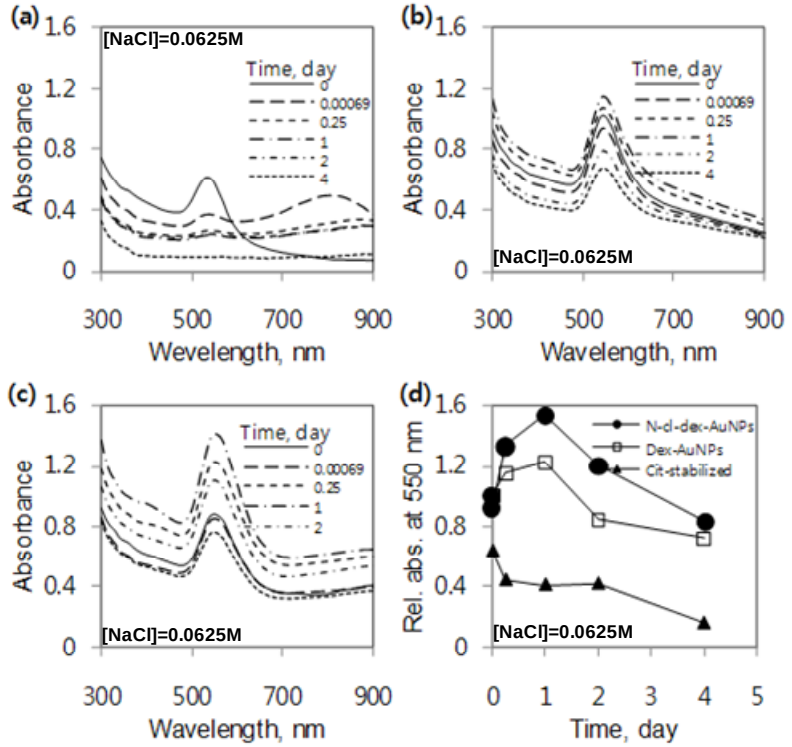

Figure S5. UV-vis absorption spectra of (a) AuNPs stabilized by citrate, (b) dex-AuNPs, (c) N-cl-AuNPs (3 % aminated) in 0.06 M sodium chloride solution. (d) A plot of relative absorbance of AuNPs at 550nm versus time.

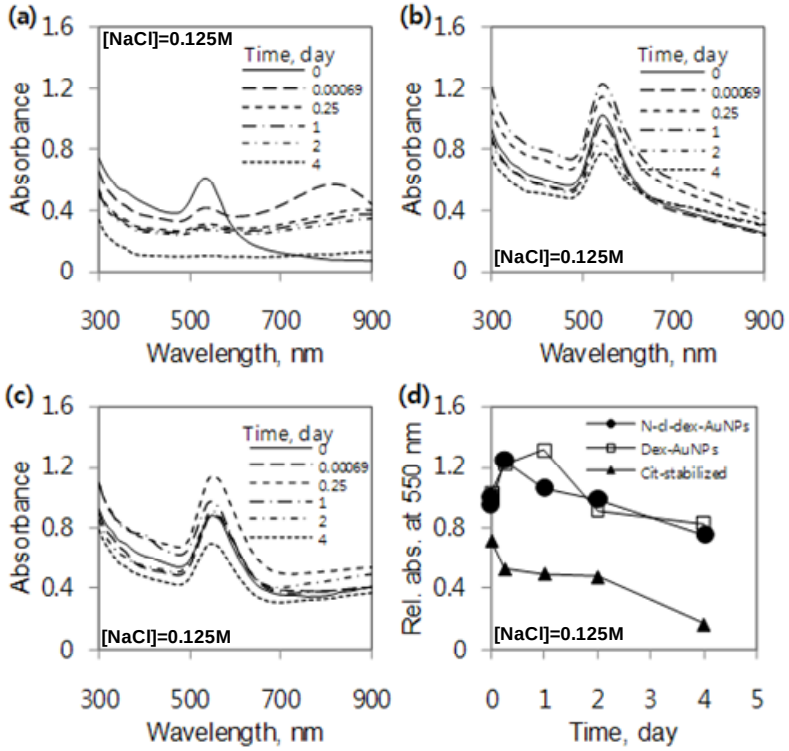

Figure S6. UV-vis absorption spectra of (a) AuNPs stabilized by citrate, (b) dex-AuNPs, (c) N-cl-AuNPs (3 % aminated) in 0.1 M sodium chloride solution. (d) A plot of relative absorbance of AuNPs at 550nm versus time.

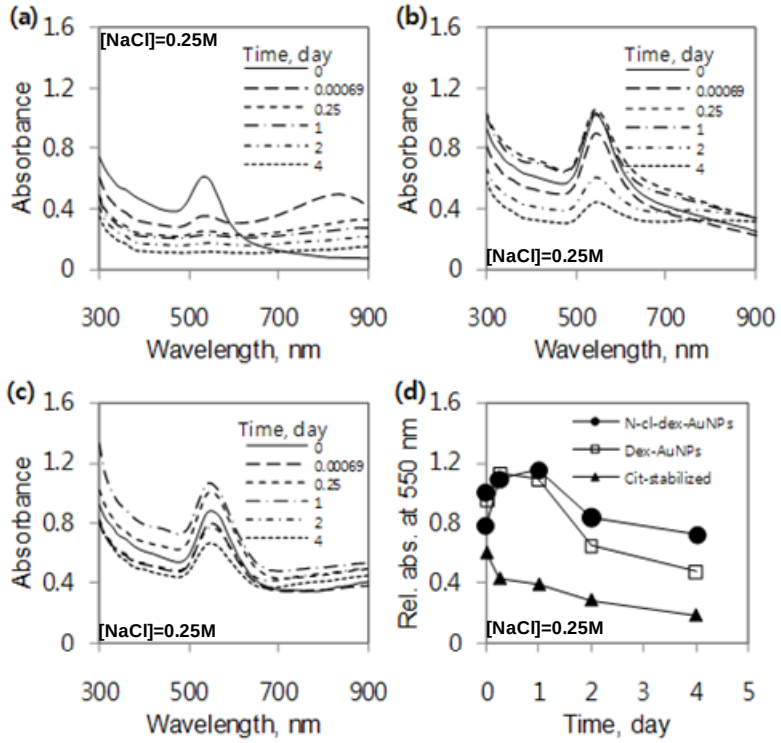

Figure S7. UV-vis absorption spectra of (a) AuNPs stabilized by citrate, (b) dex-AuNPs, (c) N-cl-AuNPs (3 % aminated) in 0.25 M sodium chloride solution. (d) A plot of relative absorbance of AuNPs at 550nm versus time.

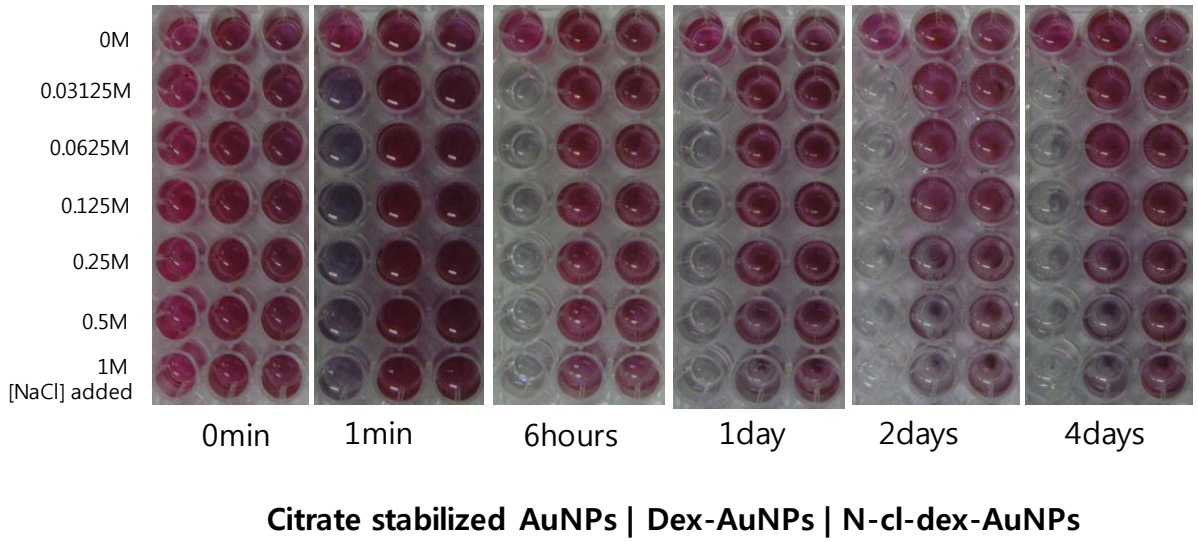

Figure S8. Pictures of AuNPs in various salt concentrations taken over 4 days. First, second, and third columns correspond to AuNPs stabilized by citrate, dex-AuNPs and N-cl-dex-AuNP, respectively.

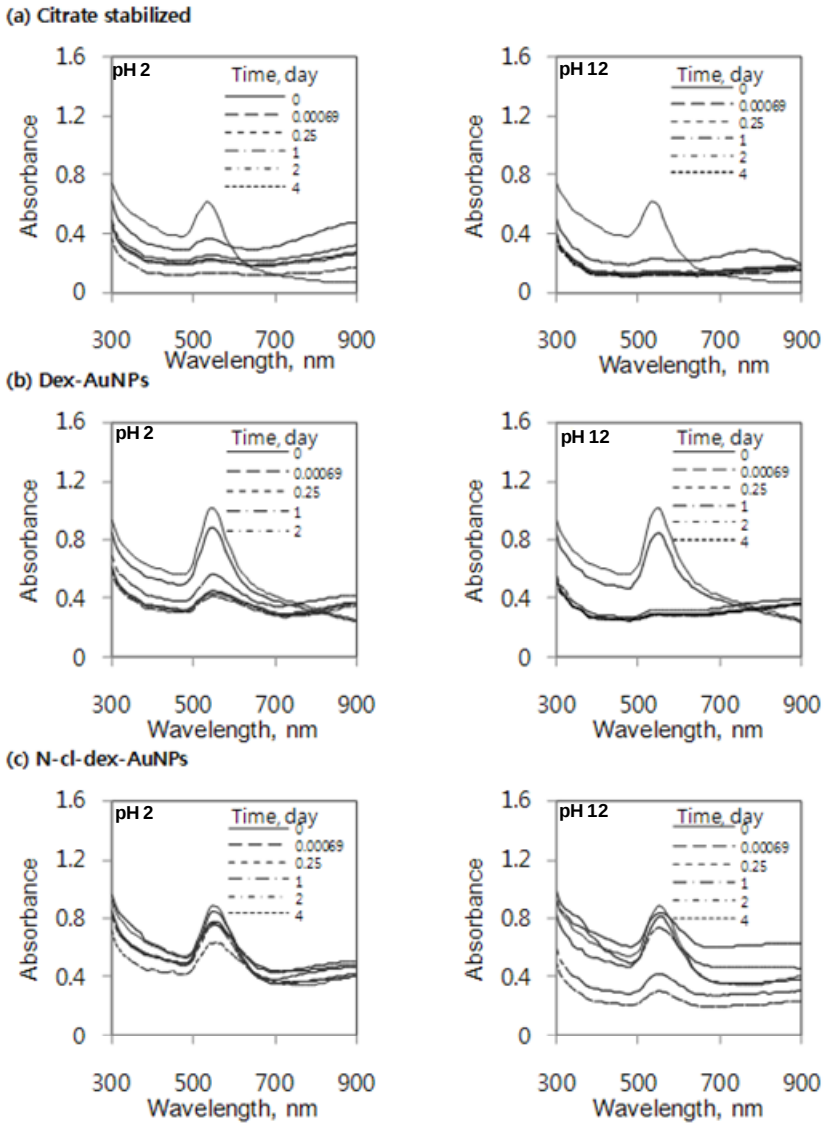

Figure S9. UV-vis absorbance spectra of (a) citrate stabilized AuNPs which synthesized by Turkevitch's method, (b) dex-AuNPs, (c) N-cl-AuNPs (3 % aminated) in pH 2 and pH 12 solution.

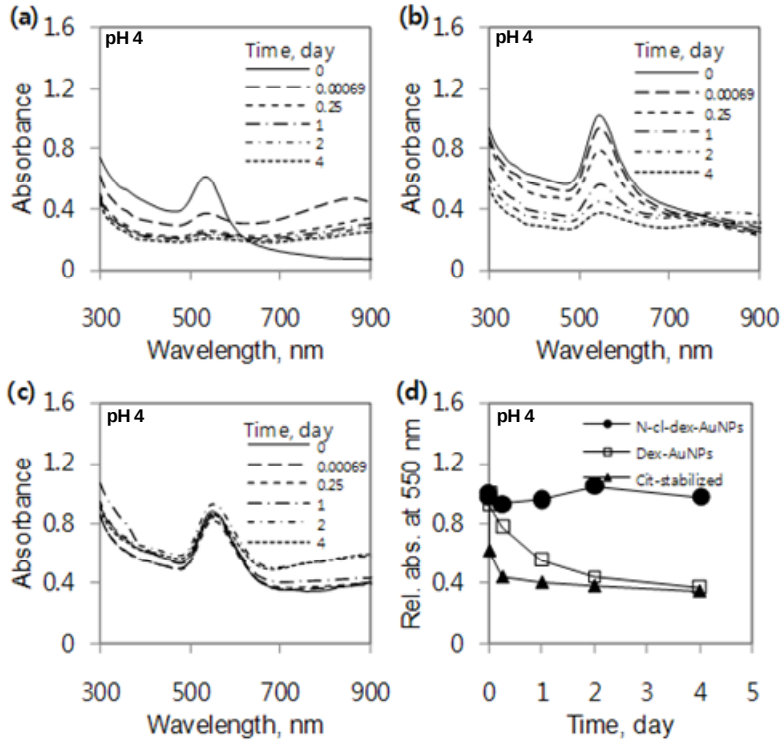

Figure S10. UV-vis absorbance spectra of (a) citrate stabilized AuNPs which synthesized by Turkevitch's method, (b) dex-AuNPs, (c) N-cl-AuNPs (3 % aminated) in pH 4 solution. (d) a plot of relative absorbance of AuNPs at 550nm versus time.

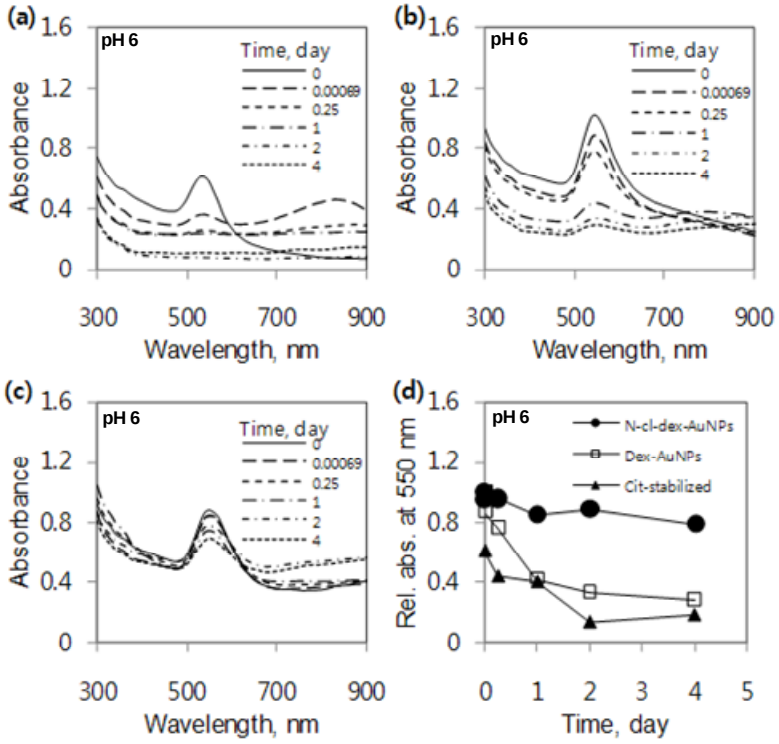

Figure S11. UV-vis absorbance spectra of (a) citrate stabilized AuNPs which synthesized by Turkevitch's method, (b) dex-AuNPs, (c) N-cl-AuNPs (3 % aminated) in pH 6 solution. (d) a plot of relative absorbance of AuNPs at 550nm versus time.

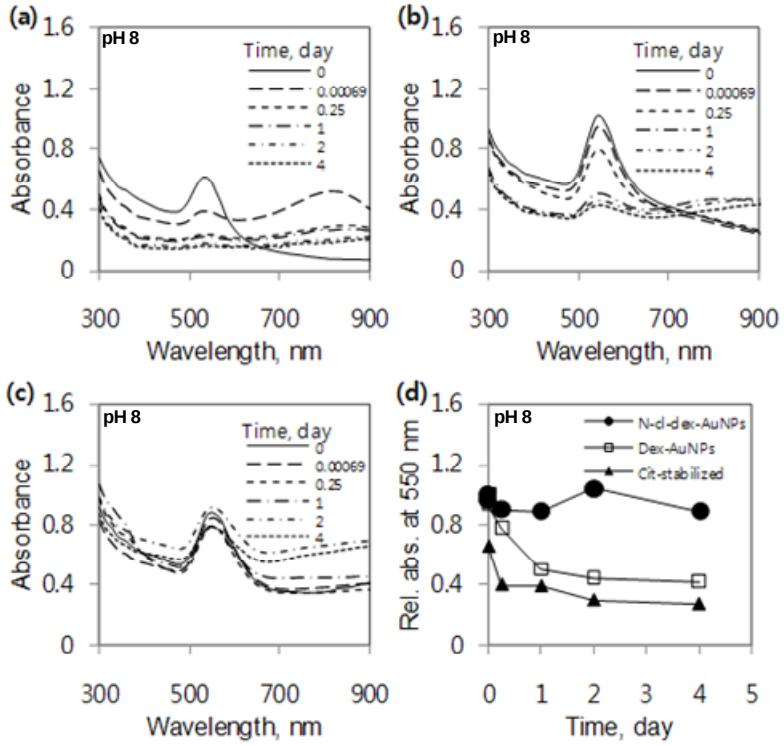

Figure S12. UV-vis absorbance spectra of (a) citrate stabilized AuNPs which synthesized by Turkevitch's method, (b) dex-AuNPs, (c) N-cl-AuNPs (3 % aminated) in pH 8 solution. (d) a plot of relative absorbance of AuNPs at 550nm versus time.

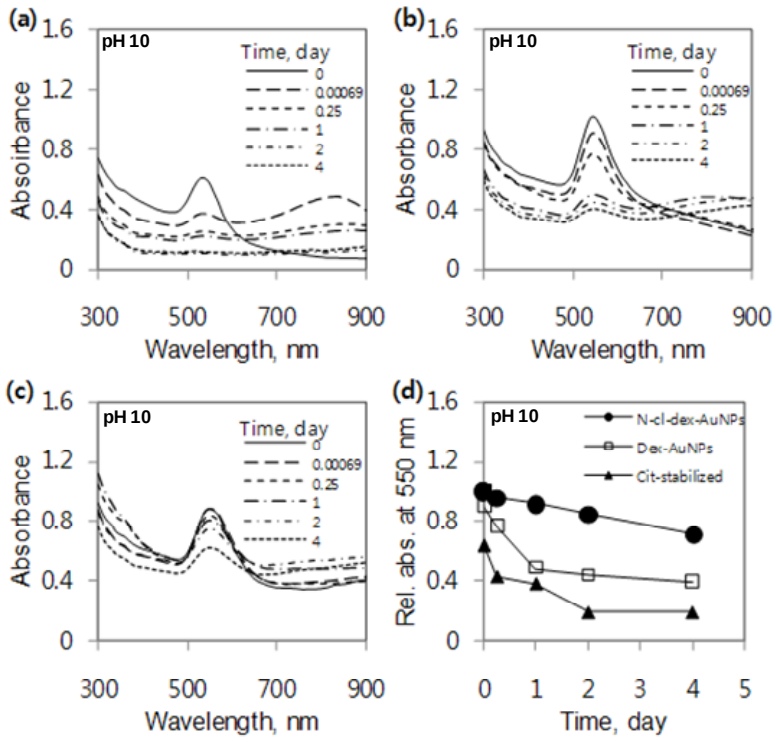

Figure S13. UV-vis absorbance spectra of (a) citrate stabilized AuNPs which synthesized by Turkevitch's method, (b) dex-AuNPs, (c) N-cl-AuNPs (3 % aminated) in pH 10 solution. (d) a plot of relative absorbance of AuNPs at 550nm versus time.

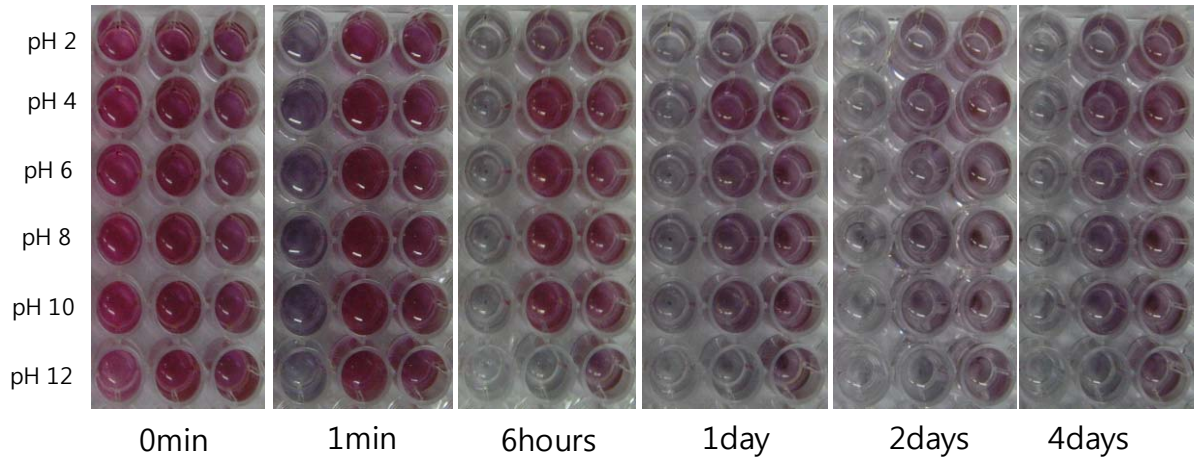

Figure S14. Pictures of AuNPs in various pH solutions taken over 4 days. First, second, and third columns correspond to AuNPs stabilized by citrate, dex-AuNPs and N-cl-dex-AuNP, respectively.

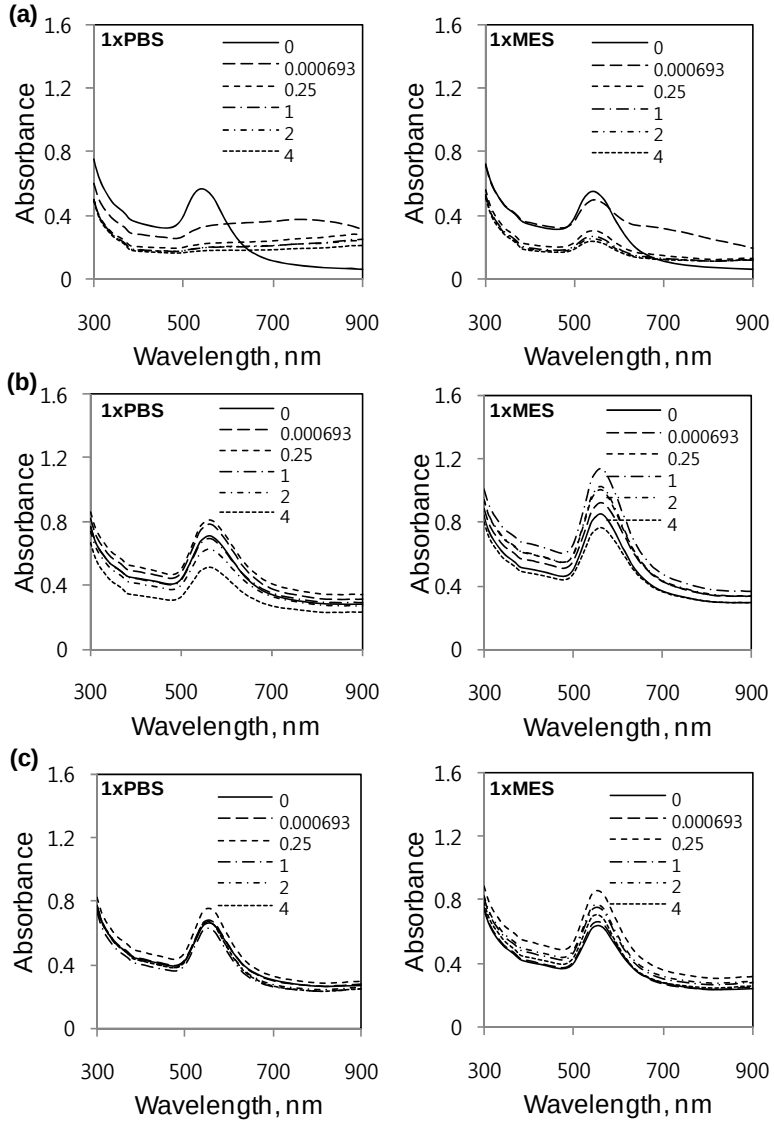

Figure S15. UV-vis absorbance spectra of (a) citrate stabilized AuNPs which synthesized by Turkevitch's method, (b) dex-AuNPs, (c) N-cl-AuNPs (3 % aminated) in 1x PBS and 1xMES solutions

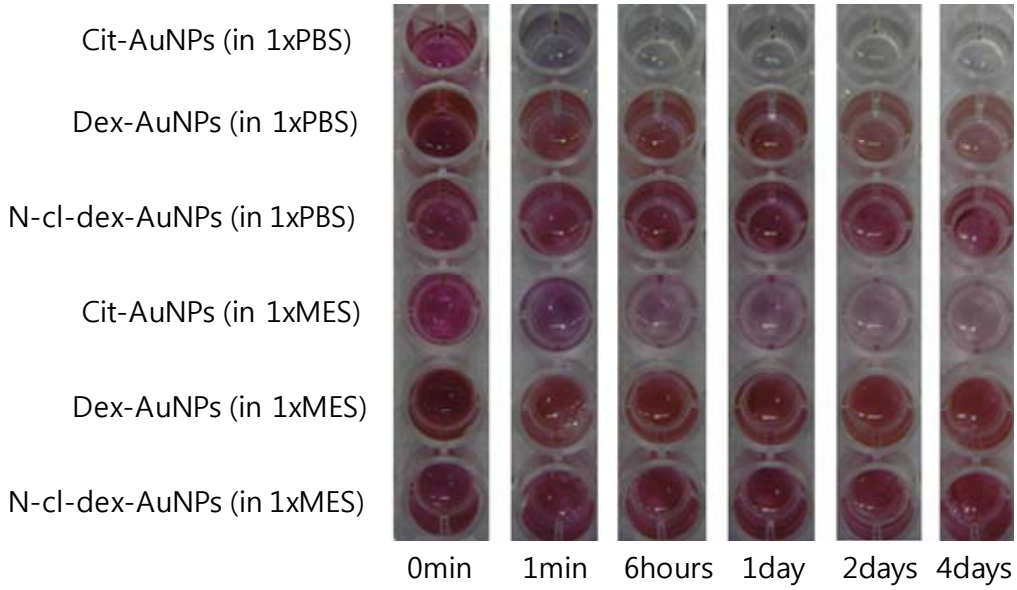

Figure S16. Pictures of AuNPs in 1xPBS and 1xMES biological buffer solutions taken over 4 days.