Supporting Information

From Mono-PEGylation toward Anti-Nonspecific Protein Interaction: Comparison of Dihydrolipoic Acid versus Glutathione-Capped Fluorescent Gold Nanoclusters Using Gel Electrophoresis

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SUPPORTING MATERIALS AND METHODS

Synthesis of glutathione-functionalized fluorescent AuNCs (AuNC@GSH). The synthesis of fluorescent AuNCs (AuNC@GSH) was adopted from Xie's protocol, using glutathione (GSH) as a reducing and a capping agent[1]. 2.5 mL of $HAuCl_4$ (4 mM) and freshly prepared equal volumes of GSH (3, 6, or 12 mM) were rapidly mixed at 600 rpm for 1 minute. The reaction solution was then gently heated to 70 ˚C and stirred for 24 hours in a dark environment. The as-prepared AuNC exhibits reddish-orange fluorescence under UV excitation (365 nm), which could be observed using the naked eye (Fig. S1). A molar ratio of $HAuCl_4$ to GSH (2:3) produced the strongest fluorescent intensity (Fig. S1). The samples passed through a 30 kDa MWCO centrifuge filter (Millipore) to eliminate unwanted particles and were then concentrated using a 10 kDa MWCO centrifuge filter. The concentration step was repeated three times to produce gold nanoclusters (AuNC@GSH) of around 10~30 kDa and 250 μl of AuNC@GSH. The fluorescence and colloidal stability of AuNC@GSH remained stable for 6 months at 4°C.

Synthesis of fluorescent dihydrolipoic acid protected gold nanoclusters (AuNC@DHLA). The synthesis of fluorescent AuNC@DHLA used Lin's protocol via a nanoparticle etching method[2]. 100 mM of DDAB or decanoic acid in toluene was prepared as stock solution. 25 mM of gold (III) chloride ($AuCl_3$) in DDAB stock solution was prepared as a gold precursor solution. 1 ml of freshly prepared TBAB solution (100 mM in DDAB

stock solution) was then added into the vigorously stirred decanoic acid solution (0.625 ml). 0.8 ml of the gold precursor solution was then immediately added and a dark red solution of 6 nm gold nanoparticles was obtained. The gold precursor solution was then added drop-wise into the as-prepared 6 nm gold nanoparticles until the colour changed to a yellowish hue, which signifies the formation of 3 nm DDAB-stabilized gold nanoclusters (AuNC@DDAB). AuNP@DDAB was then added to reduced lipoic acid of which the premixing molar ratio of lipoic acid to TBAB (50 mM) was 4 to 1 to produce sufficient DHLA for ligand exchange. The resulting solution was exposed to 365 nm UV-light for 20 - 30 min and agglomerates of fluorescent gold nanoclusters were then obtained by ligand-exchange. Discarding the supernatants, the agglomerates was re-dissolved in methanol. All solvents were then evaporated under reduced pressure on a rotary evaporator (Laborota 4000, Heidolph) and then re-dissolved in sodium borate buffer (SBB, pH 9). Fluorescent AuNC@DHLA was purified by passing it through the membrane of a 100 kDa MWCO centrifugal filter (Millipore) to remove unwanted particles, followed by the collection of using another 30 kDa MWCO centrifugal filter. The purified AuNC@DHLA were adjusted to 40 μM for testing. It is assumed that the extinction coefficient of AuNC@DHLA is 297000 $M^{-1}cm^{-1}$ at 420 nm.

SUPPORTING FIGURES

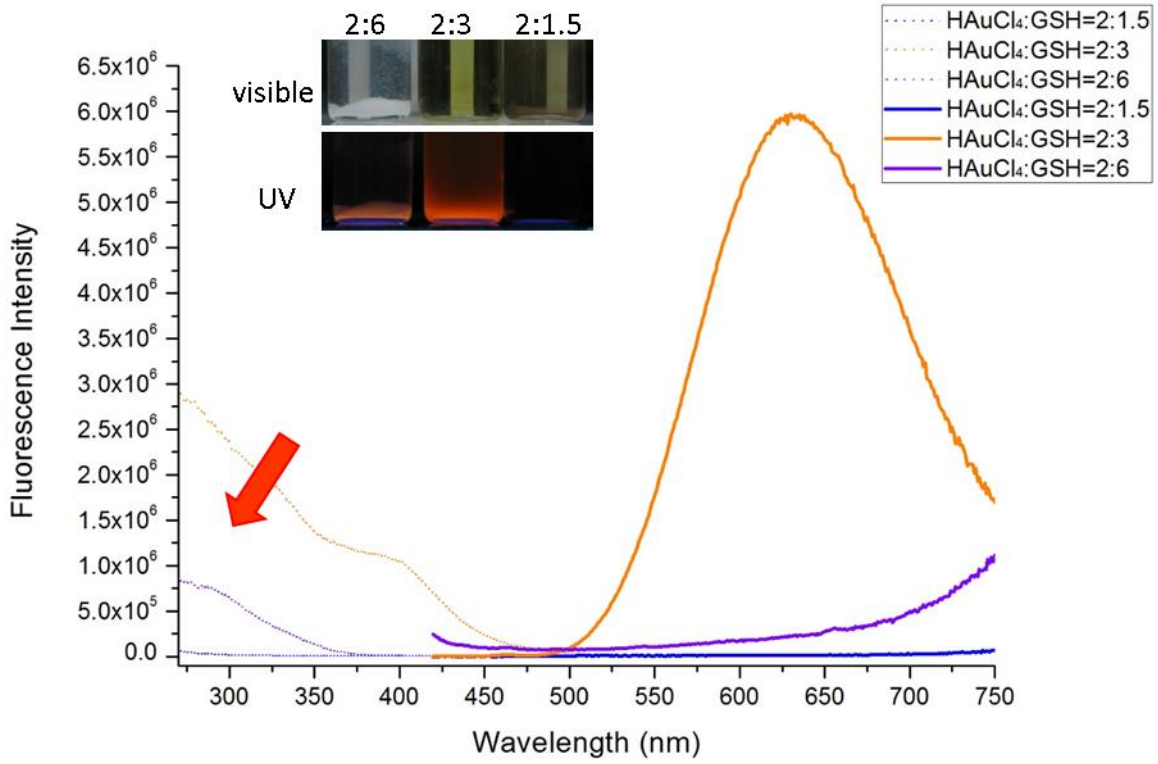

Fig. S1 Photoluminescence (PL), PL excitation spectra and photos of the as-synthesized gold nanoclusters that are produced by mixing $HAuCl_4$ and GSH in different stoichiometric ratios: the UV irradiation and photoluminescence spectra show that the best volumetric ratio of $HAuCl_4$ (6 mM) to GSH (6 mM) is 2:3 (solid orange line), which ratio gives the brightest red-orange fluorescence with an emission peak around 622 nm. The excitation spectra for AuNC@GSH used a fixed emission at 622 nm. There is an excitation window below 450 nm. An excessive glutathione ratio of **2:6** produces white precipitates, which are attributed to polymerized Au(I)-glutathione complexation. An insufficient ratio of glutathione of **2:1.5** results in a non-fluorescent gold nanocluster and gold precipitation is absorbed on the vial wall.

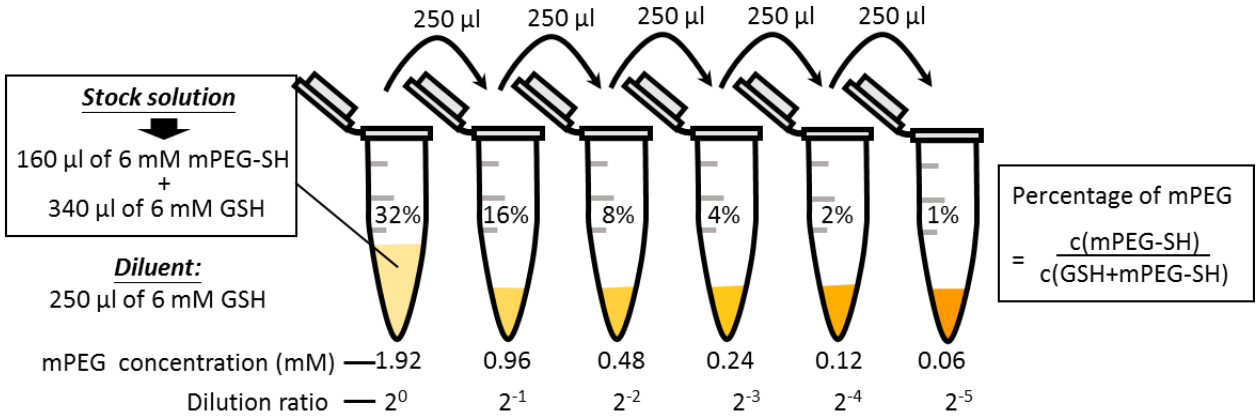

Fig. S2 Preparation of the two-fold serial dilutions from mPEG-SH stock solution (1.92 mM, or 32%)

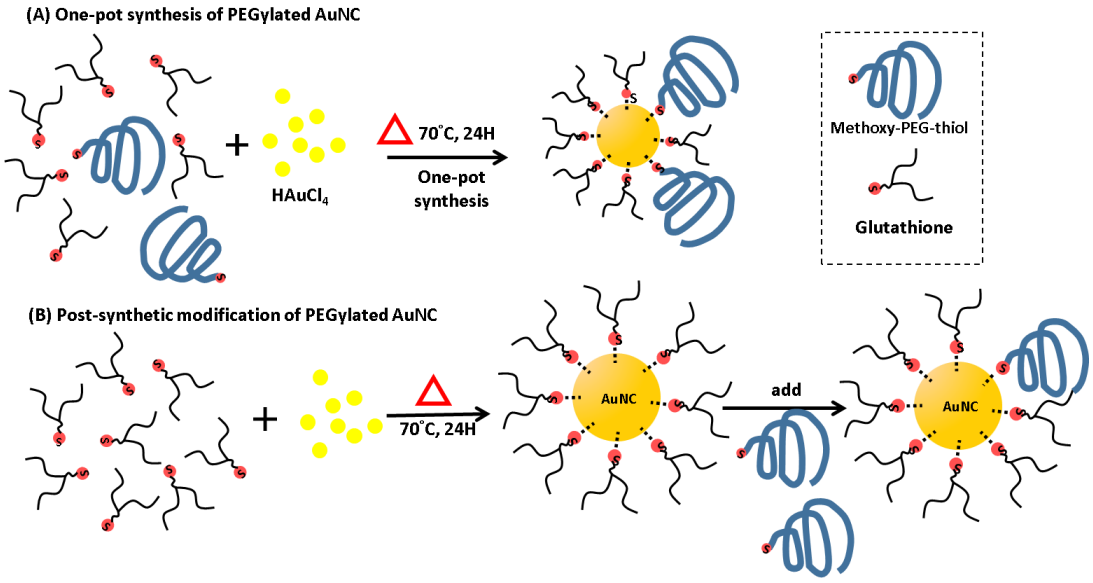

Fig. S3 Schematic representation of PEGylated glutathione gold nanoclusters (AuNC@GSH) that are produced using one-pot synthesis or post-synthetic modification: (A) schematic diagram showing the one-pot synthesis of PEGylated AuNC, followed by sequential mixing of glutathione, methoxy-PEG-thiol, and Gold(III) chloride trihydrate at 70 ˚C for 24 hours; (B) schematic diagram showing the post-synthetic modification of PEGylated AuNC by adding methoxy-PEG-thiol to purified gold nanoclusters AuNC@GSH, which are pre-synthesized by mixing an appropriate ratio of glutathione and Gold(III) chloride trihydrate at 70 ˚C for 24 hours.

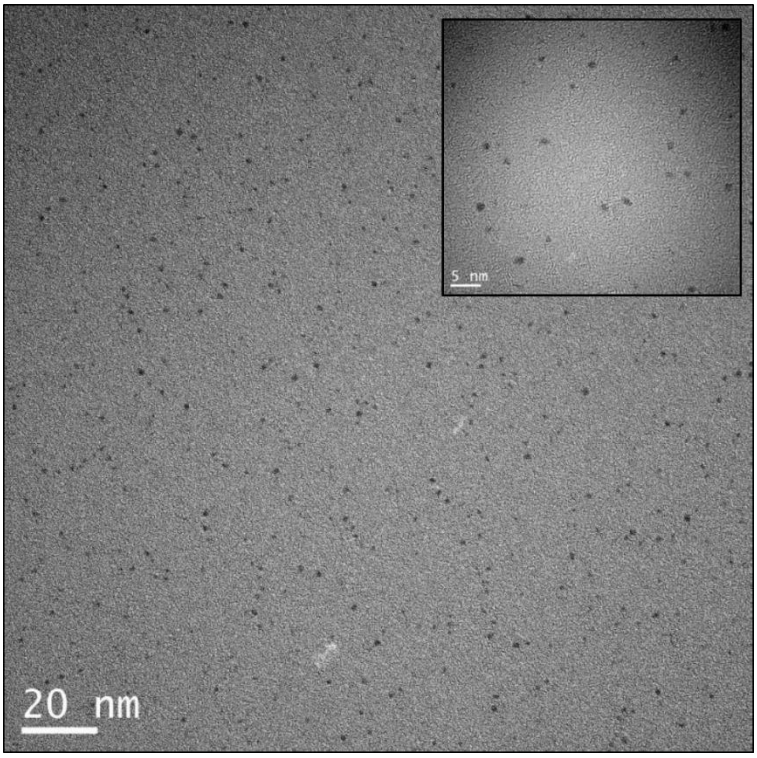

Fig. S4 TEM image of fluorescent AuNC@GSH that is prepared using a 2:3 volumetric ratio of $HAuCl_4$ and GSH. The gold nanoclusters are about 1.5 nm in the TEM images. Respective scale bars for the large scale and the inset TEM image are 20 nm and 5 nm.

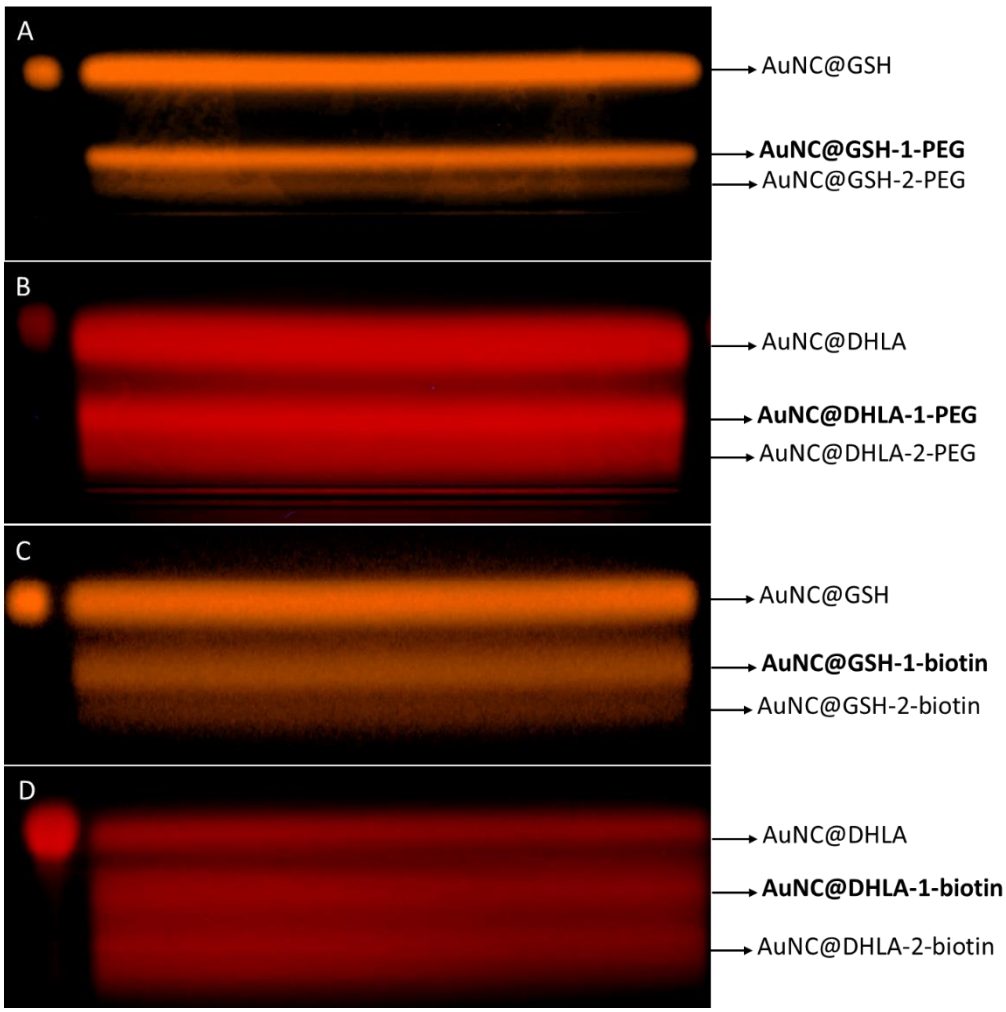

Fig. S5 Scaling up of mono-PEGylated or mono-biotinyated gold nanoclusters using gel electrophoresis. All the scaling-up procedures for the mono-biotinyated gold nanoclusters were run in 2% agarose gel at 10 V/cm for 40 min. ***Mono-functionalized AuNC@GSH****:* 30 µl of x-PEG-SH solution ($mPEG_{5k}$-SH or biotin-PEG_{2k}-SH, 1 mM , 6 mM) and 720 µl of GSH solution (6 mM) were premixed together and 750 µl of fresh $HAuCl_4$ solution (4 mM) was added and the mixture was stirred in a 70 ˚C water bath for 24 hours (dark room). The resulting sample was loaded into the wells of a 2% agarose gel and was subjected to gel electrophoresis (10 V/cm, 40 min). The results from AuNC@GSH are shown in (A) for AuNC@GSH-1-PEG and in (C) for AuNC@GSH-1-biotin. ***Mono-functionalized AuNC@DHLA****:* 500 µl of AuNC@DHLA (40 µM) and 500 µl of x-PEG-NH2 solution ($mPEG_{5k}$-NH_2 or biotin-PEG_{3k}-NH_2, 1 mM) were premixed together and 500 µl of fresh EDC crosslinking solution was added (8 mM). After 2 hours of reaction, the mixture was loaded into the wells of 2 % agarose gels and subjected to gel electrophoresis (10 V/cm, 40 minutes). The results from AuNC@DHLA are shown in (B) for the AuNC@DHLA-1-PEG and in (D) for AuNC@DHLA-1-biotin. For isolation, mono-functionalized gold nanoclusters were further cut and eluted by electrophoresis.

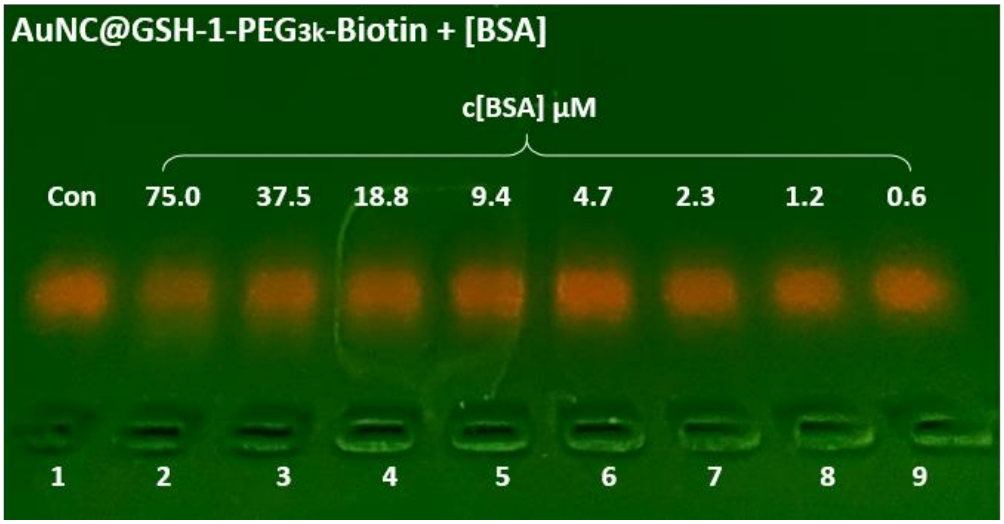

Fig. S6 Non-specific adsorption testing of BSA to AuNC@GSH-1-biotin. Lane 1: AuNC@GSH-1-biotin (i.e. one biotin-PEG_{3k}-SH attached AuNC@GSH); Lane 2 ~ Lane9: the purified AuNC@GSH-1-biotin mixing with a series of two-fold BSA dilution from 75 μM. All bands are observed after a 2% gel electrophoresis (7.5 V/cm) for 30 minutes but no additional retarded bands of AuNCs were found as similar to the results of AuNC@GSH-1-PEG_{5k} with BSA diluents. The gel image was taken under UV excitation and the blue background containing BSA auto-fluorescence was removed by Image J software.

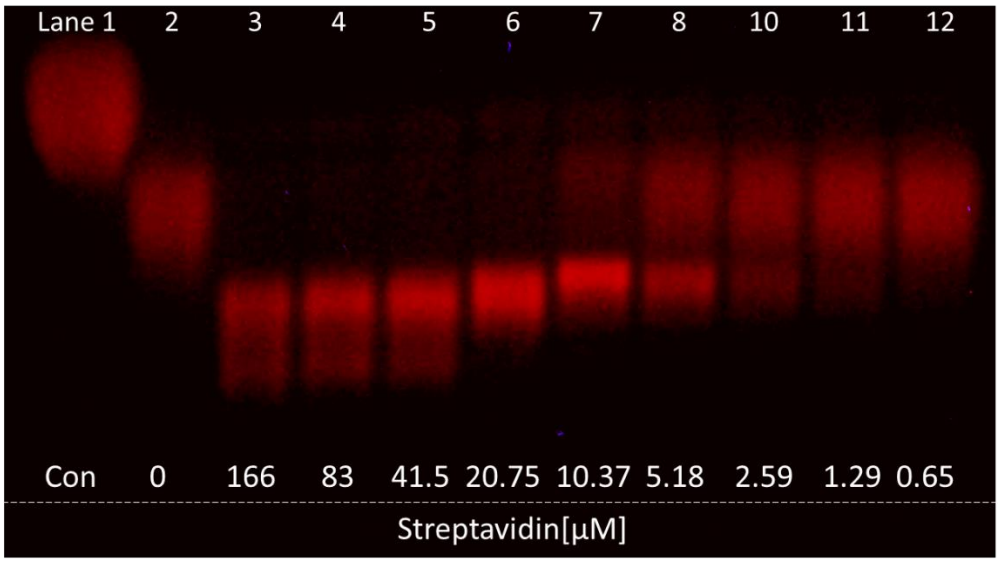

Fig. S7 AuNC-1-biotin assembly with streptavidin. Mono-biotinylated gold nanoclusters (AuNC@DHLA-1-biotin) were confirmed by the assembly of streptavidin. Lane 1: AuNC@DHLA; Lane 2: AuNC-1-biotin (i.e. one biotin-PEG_{3k}-NH_2 attached Au@DHLA); Lane 3 ~ Lane12 are AuNC-1-biotin which sequentially mixed with two-fold diluents of streptavidins of 166μM, 83μM, 41.5μM, 20.75μM, 10.37μM, 5.18μM, 2.5938μM, 1.29μM and 0.64μM. Each streptavidin biomolecule contains four biotin-binding sites, which induce the assembly of mono-biotinylated gold nanoclusters, so different discrete bands are observed after a 2% gel electrophoresis (7.5 V/cm) for 30 minutes. The dashed line marks the positions of gels where the samples are loaded.

References

1. Z. Luo, X. Yuan, Y. Yu, Q. Zhang, D. T. Leong, J. Y. Lee and J. Xie, *Journal of the American Chemical Society*, 2012, **134**, 16662-16670.
2. C. A. J. Lin, T. Y. Yang, C. H. Lee, S. H. Huang, R. A. Sperling, M. Zanella, J. K. Li, J. L. Shen, H. H. Wang, H. I. Yeh, W. J. Parak and W. H. Chang, *ACS Nano*, 2009, **3**, 395-401.