Electronic Supplementary Material (ESI) for Analyst

Luminol-Labeled Gold Nanoparticles for Ultrasensitive Chemiluminescence-based Chemical Analysis

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Supplementary Information Figures:

FIGURE S1:

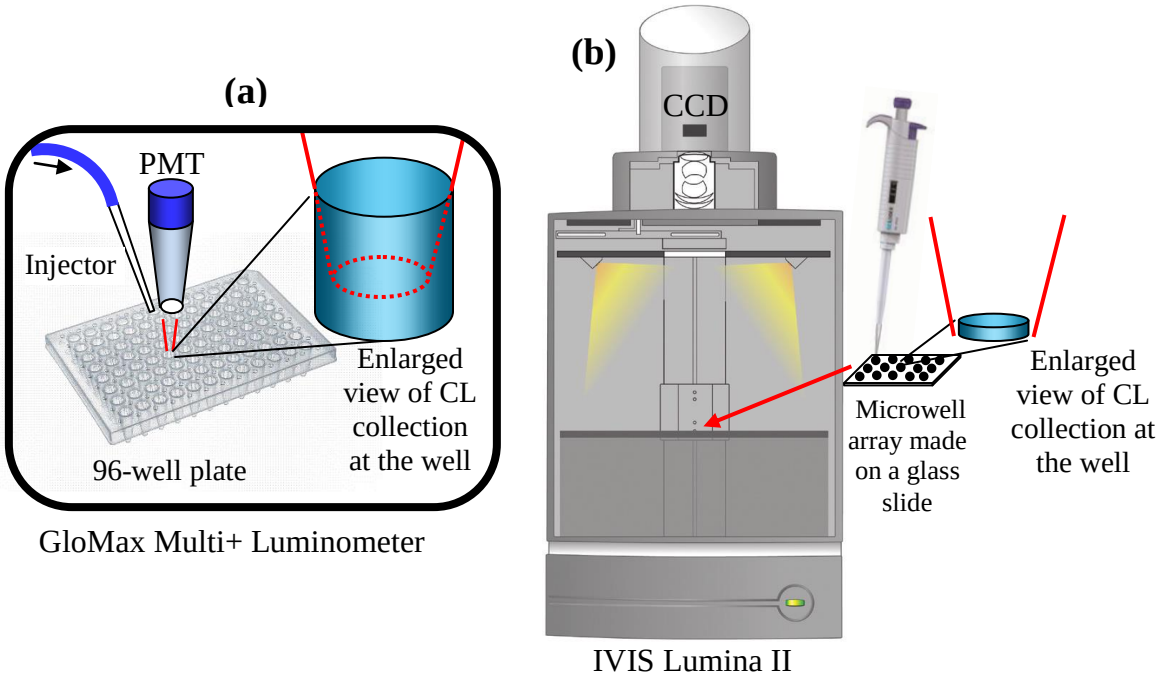

Fig. S1 Schematics of the experimental configurations of chemiluminescence (CL) measurements using (a) GloMax Multi+ system made by Promega Corporation and (b) IVIS Lumina II system made by Caliper Life Sciences. The GloMax Multi+ system uses a photomultiplier tube (PMT) detector for the single-channel measurement of the kinetic signal from individual wells sequentially in a standard white polystyrene 96-well plate with 300 μL per well (~6.5 mm in dia and ~11 mm in depth). The IVIS Lumina II system uses a cooled Charge Coupled Device (CCD) camera to record videos of the luminescence signals from an array of microwells (1.5 mm deep oval-shaped wells with 3 and 4 mm widths) that were cut in a PDMS slab laid on a glass slide. The volume of each well is ~12 μL.

FIGURE S2:

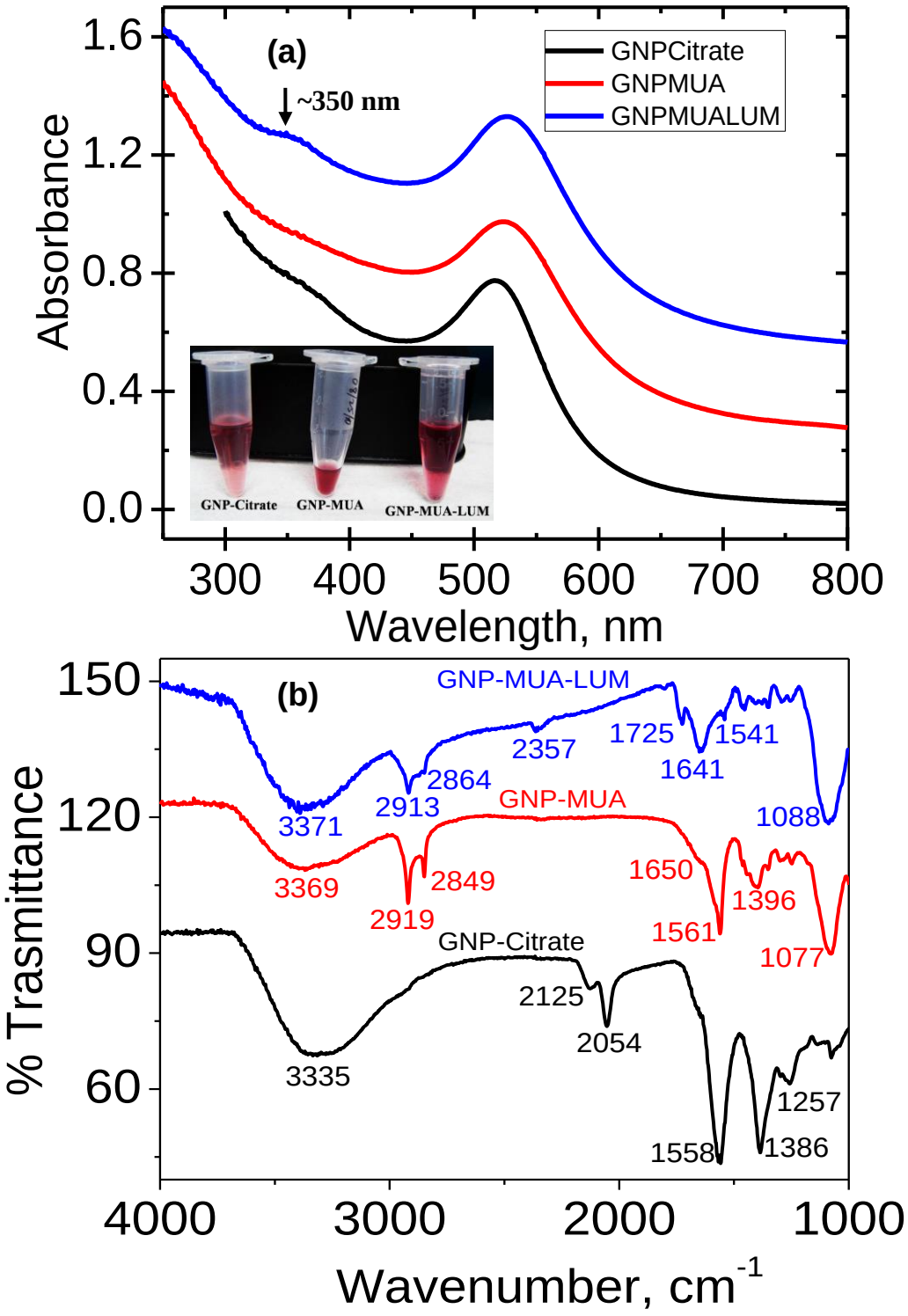

Fig. S2 (a) UV-visible and (b) FT-IR spectra of citrate-stabilized GNPs (GNP-citrate, black curve), MUA modified GNPs after replacing citrate (GNP-MUA, red curve), and LUM attached

GNPs (GNP-MUA-LUM, blue curve). The concentrations are about (1.0 to 1.2) x 10^{12} GNPs/mL. The characteristic absorbance peaks at ~520 nm in all three curves in (a) are attributed to the surface plasmon resonance (SPR) of 10-nm GNPs, which is consistent with red color of all three samples shown in the inset. The small peak at ~350 nm in the blue curve of panel (a) corresponds to an absorption peak of luminol. The GNP-MUA (red) and GNP-MUA-LUM (blue) curves in panel (a) were translated upward by 0.2 and 0.5 units, respectively, along the y-axis for better view. The GNP-MUA and GNP-MUA-LUM curves in panel (b) were translated upward by 20 and 50 units, respectively, for the same reason.

FIGURE S3:

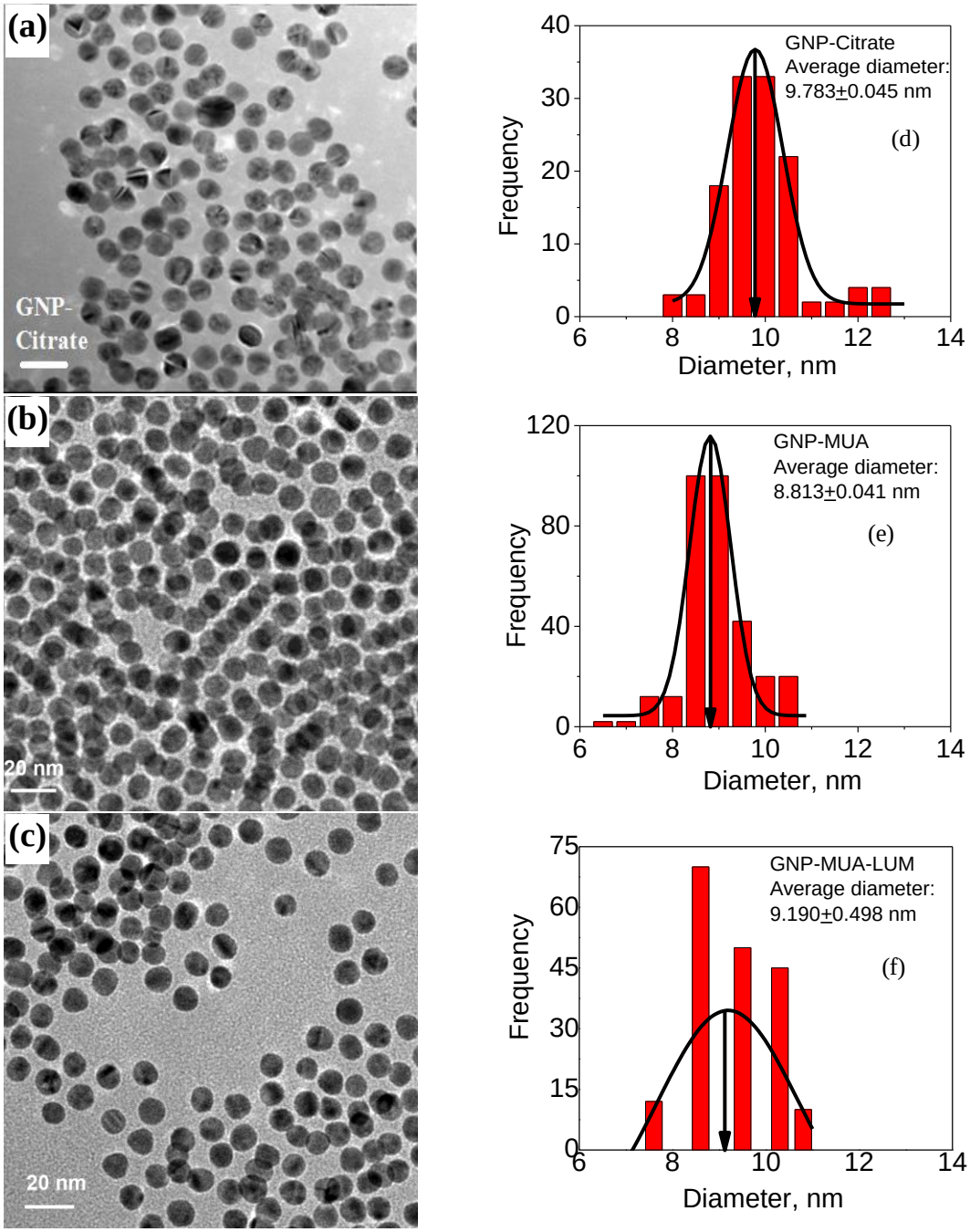

Fig. S3 TEM images of gold nanoparticles (a) with the original citrate protection (GNP-citrate), (b) after ligand exchanging with mercaptoundecanoic acid (GNP-MUA), and (c) after luminol functionalization (GNP-MUA-LUM). The scale bars are 20 nm. (d)–(f) show the size distribution of GNPs from TEM images. The average diameters are ~9.8, ~8.8, and ~9.2 nm for GNP-citrate, GNP-MUA, and GNP-MUA-LUM, respectively.

FIGURE S4:

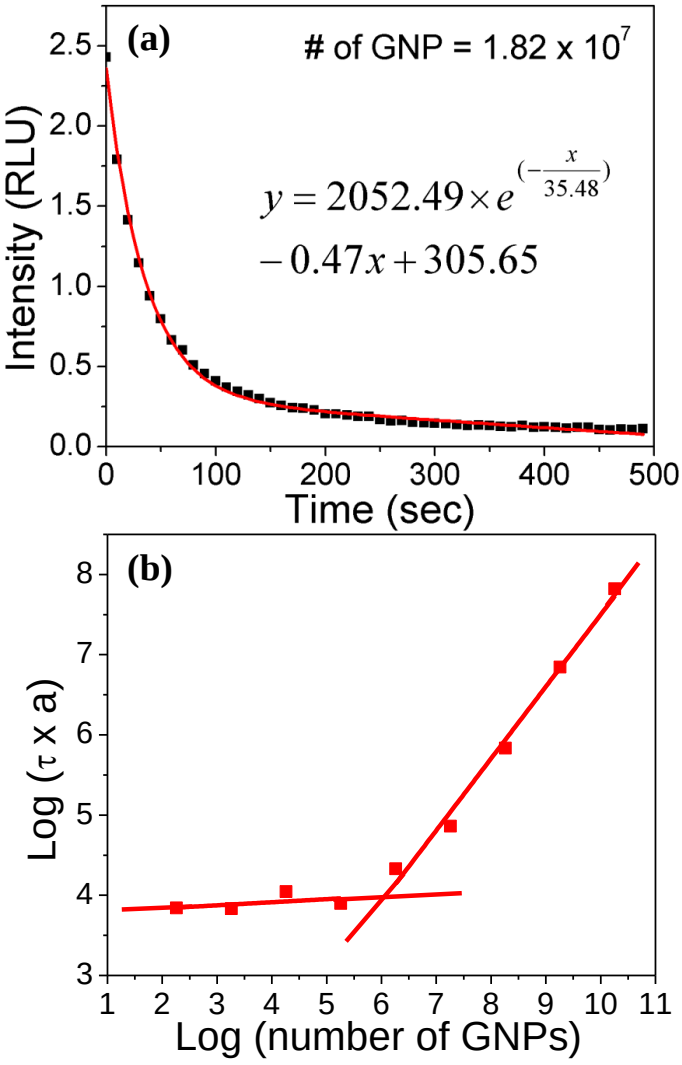

Fig. S4 (a) The fitting of a representative kinetic chemiluminescence curve of GNP-MUA-LUM solutions (as shown in Figure 1) recorded with GloMax Multi+ system. In this set of data, 1.82 x 10^7 GNP-MUA-LUM nanoparticles was used. The curve fits nicely with an exponential decay over a linear background. The decay time constant is 35 s. (b) The calibration curve using integrated CL signal (the initial intensity ***a*** multiplied by the decay time constant τ) instead of ΔI in the vertical axis. The slopes of the two linear curves are 0.90 (at high N) and 0.038 (at low N), nearly the same as those using ΔI (Fig. 3a).

FIGURE S5:

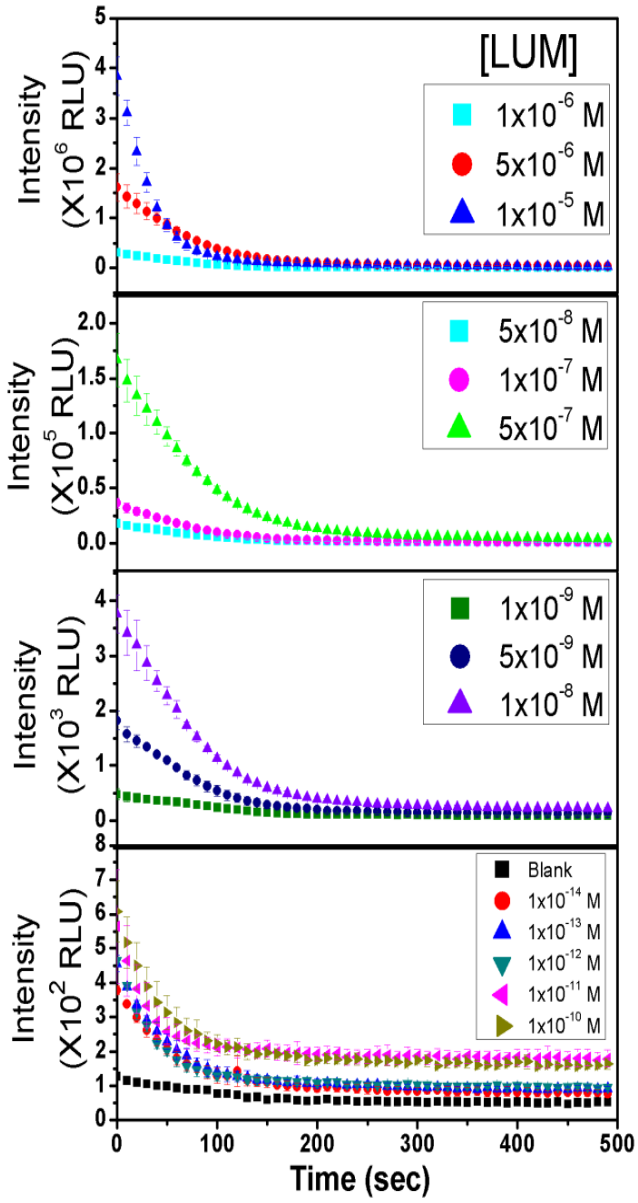

Fig. S5 The kinetic CL measurements with bulk aqueous luminol solution at varied concentrations. The experimental conditions were identical with those in Fig. S2 except the GNP-MUA-LUM solutions being replaced with bulk luminol solutions. About 25 µL of 0.10 M NaOH, 25 µL of 1.41 M H_2O_2 and 25 µL of 1.0 mM $K_3Fe(CN)_6$ solution were preloaded in a 96-well plate. Then 25 µL of aqueous luminol solution with varied concentration (1.0 x 10^{-14} to 1.0 x 10^{-5} M) was added by the micro-injector to initialize the CL reaction.

FIGURE S6:

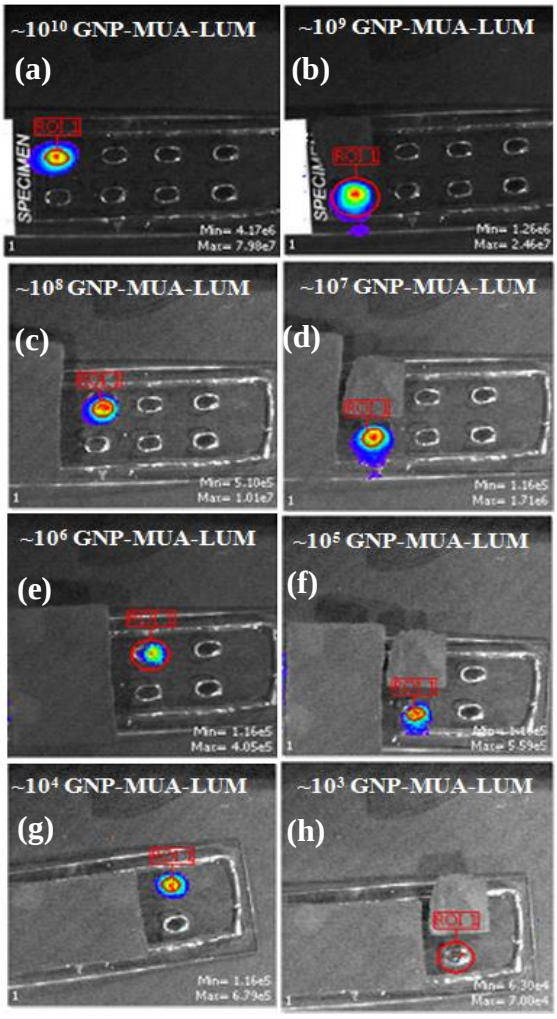

Fig. S6 Chemiluminescence signal recorded by a CCD camera in IVIS Lumina II at PDMS microwells loaded with ~1.0 x 10^{10} to 1.0 x 10^{3} luminol-labeled GNPs (GNP-MUA-LUM). The images are presented in pseudocolor to represent the chemiluminescence intensities. The number of GNP-MUA-LUM nanoparticles in the well is indicated on each image panel. All microwells measured earlier are covered with a piece of paper to cut down the cross-well background.

FIGURE S7:

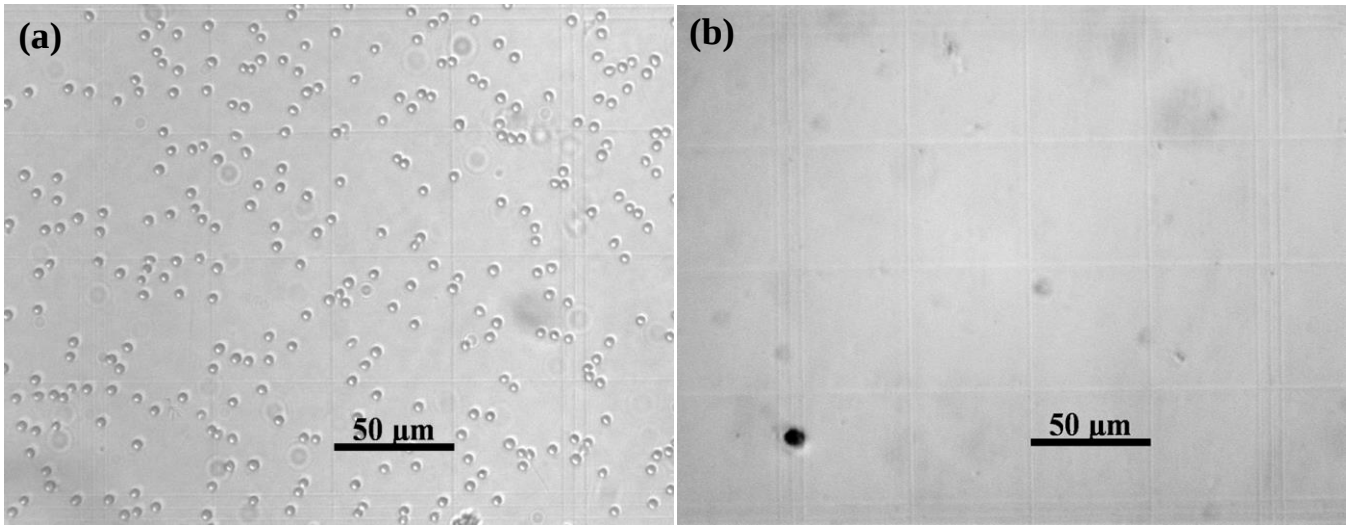

Fig. S7 The bright-field image of (a) the intact (unlysed) and (b) the lysed sheep red blood cells in a Petroff Hausser counting chamber under an optical microscope. The stock solution was found to contain 4.6 x 10^9 cells/mL with the size of the red blood cells of ~4–5 µm in diameter.

FIGURE S8:

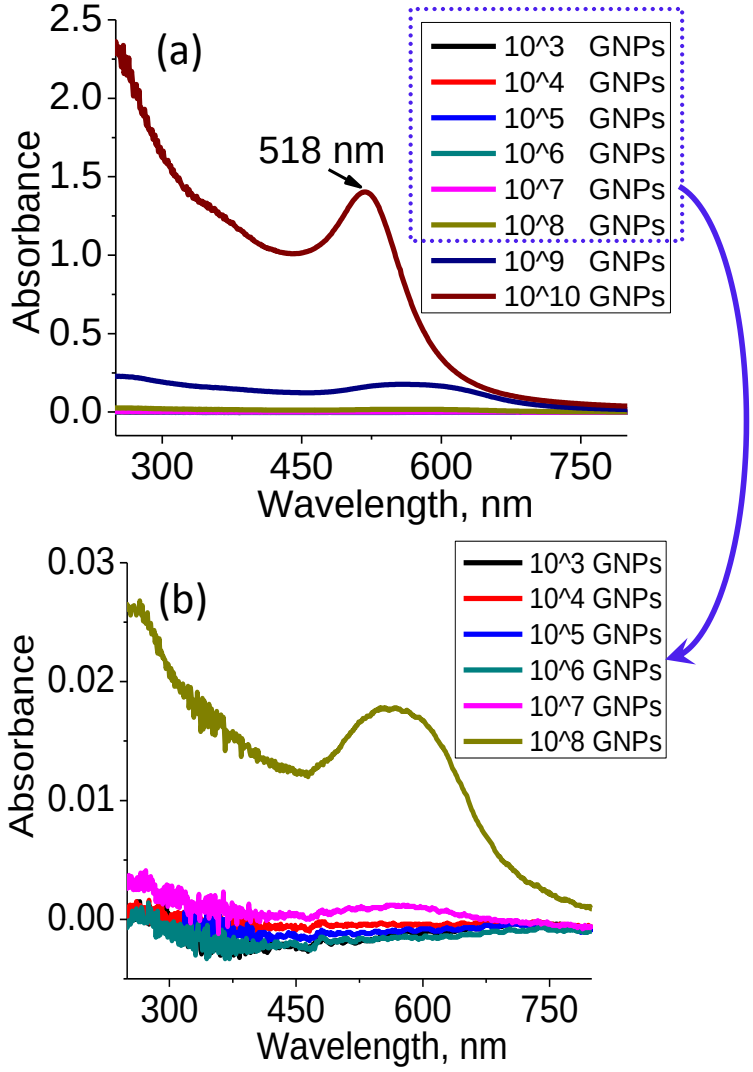

Fig. S8 (a) The UV-Visible absorption spectra measured with varying number of GNP-MUA-LUM in 350 μL solution in a microcuvette with an optical pathlength of 10.0 mm. (b) The enlarged view to show the absorption spectra of highly diluted GNP-MUA-LUM solutions.

FIGURE S9:

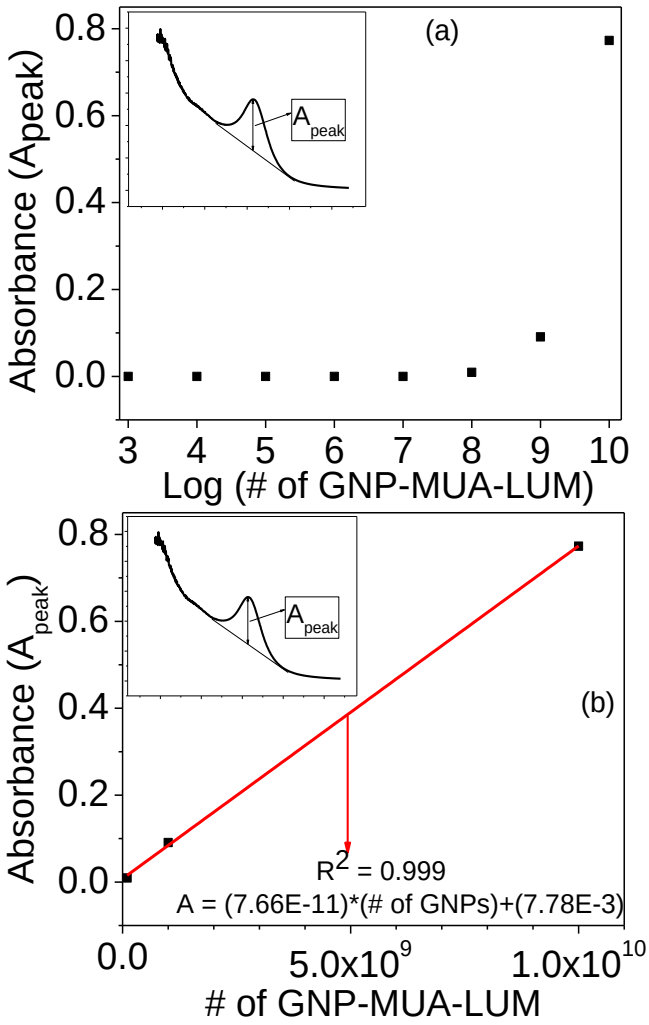

Fig. S9 The background subtracted peak absorption (A_{peak}) at ~520 nm derived from the UV-visible spectra shown in Figures S8a and S8b. (a) The value of A_{peak} vs. the logarithm of the number of luminol-labeled GNPs. (b) The linear plot of A_{peak} vs. number of luminol-labeled GNPs obtained with the sample containing ~1.0 x 10^{10}, 1.0 x 10^{9} and 1.0 x 10^{8} GNP-MUA-LUM. The solid line in (b) is the linear fitting curve. This indicates that the UV-visible signal linearly decreases till 10^{8} GNPs. The detection limit by UV-visible absorption is about 10^{7}–10^{8} GNPs in the 350 μL microcuvette.

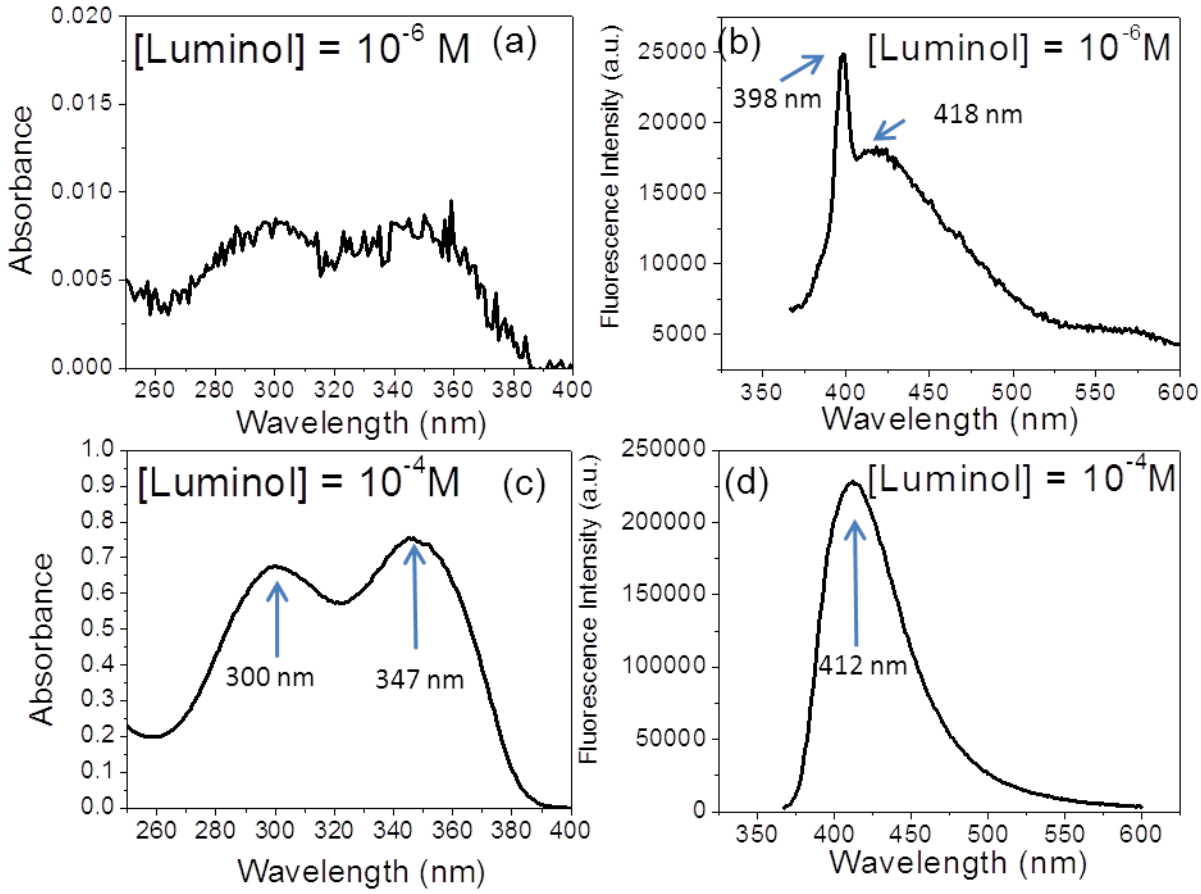

Figure S10. (a) & (c) The UV-visible absorption spectra of aqueous luminol solutions at concentration of 1.0 x 10^{-6} M and 1.0 x 10^{-4} M, respectively. (b) & (d) The corresponding fluorescence emission spectra of the two luminol solutions measured with the excitation wavelength of at 347 nm.