Supporting Information

Refreshable Nanobiosensor based on Organosilica Encapsulation of Biorecognition Elements

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Materials and Methods

Materials: Ascorbic acid (AA, ≥99.0%), gold(III) chloride trihydrate ($HAuCl_4.3H_2O$, ≥99.9%), sodium borohydride ($NaBH_4$, 98%), silver nitrate (>99%), cetyltrimethylammonium bromide (CTAB, ≥99%), cetyltrimethylammonium chloride (CTAC, ≥98%), 3-(mercaptopropyl)trimethoxysilane (MPTES), trimethoxy(propyl)silane (TMPS), (3-aminopropyl)trimethoxysilane (APTMS), sodium dodecyl sulfate (>99%) (SDS), albumin from human serum (Mw = 65 kDa), sodium hydroxide, phosphoric acid, glycine, 1 M hydrochloric acid and protease from streptomyces griseus were obtained from Sigma-Aldrich. Rabbit IgG, Goat anti-Rabbit IgG (Mw = 150 kDa), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were purchased from Thermo scientific. Thiol PEF COOH (Mw = 5000 g/mol), methoxy PEG thiol (Mw = 5000 g/mol) and methoxy PEG silane (Mw = 5000 g/mol) was purchased from Jenkem Technology. Neutrophil gelatinase-associated lipocalin (NGAL) and anti-NGAL were purchased from R&D Systems. The phosphate buffer saline (PBS) (10X) buffer was obtained from Thermofisher. Surine (Synthetic urine) was obtained from Dyna Tech Industries. All chemicals were used as received without further modifications.

Synthesis of Gold nanorods (AuNRs): Gold nanorods were synthesized using a seed-mediated approach. Briefly, first the seed solution was prepared by mixing 9.75 mL of aqueous CTAB solution (0.1 M) and 0.25 mL of $HAuCl_4$ (1 mM) in a 20 mL scintillation vial, followed by the rapid addition of 0.6 mL of ice-cold $NaBH_4$ (10 mM) under vigorous stirring (800rpm) to yield a brown colored seed solution. Next, the growth solution was

prepared by mixing 38 mL of CTAB (0.1 M), 2 mL of $HAuCl_4$ (10 mM), 0.5 mL of silver nitrate (10 mM), and 0.22 mL of ascorbic acid (0.1 M) in the given sequence. The solution was homogenized by gentle stirring which resulted in colorless solution. To the thus formed colorless solution, 0.1 mL of freshly prepared seed solution was added and set aside in dark and static environment for 14 h. Prior to use, the AuNR solution was centrifuged at 10000 rpm for 30 min to remove excess CTAB and re-dispersed in nanopure water (18.2 MΩ.cm).

Synthesis of Gold Nanorattles (AuNRT): First Au nanospheres were synthesized by mixing, in following order, 7 ml of aqueous CTAB solution (0.1 M), 5.25 mL of ascorbic acid (0.1 M), 0.175 ml of freshly synthesized seed solution and 7 ml of $HAuCl_4$ (1 mM) under constant stirring for 1 h. The resulting solution was then centrifuged at 13400 rpm for 30 min. The size of thus formed Au nanospheres was 10 nm. Next 5 ml of the 10 nm nanosphere particle solution was mixed with 45 ml of CTAC solution (20 mM) under stirring at 60 °C for 20 min. Subsequently, 5 mL of $AgNO_3$ (2 mM), 12.5 mL of CTAC (20 mM), and 2.5 mL of ascorbic acid (100 mM) were added under stirring at 60 °C for 4 h. After 4 h, the as-synthesized Au@Ag nanocubes were centrifuged (10000 rpm for 15 min) and redispersed into a 15 mL aqueous solution of CTAC (50 mM). Then to synthesize AuNRT, $HAuCl_4$ aqueous solution (0.5 mM) was injected dropwise into the Au@Ag nanocube solution at a rate of 0.5 mL/min (controlled using automated syringe pump) under stirring at 60 °C until the solution turned to blue color or, more precisely, until the LSPR wavelength shifted to ~665 nm. The AuNRT solution was then centrifuged at 10,000 rpm for 15 min and dispersed in nanopure water for further use.

AuNR-IgG and AuNRT-NGAL antibody bioconjugates preparation: First, EDC and NHS were added to a solution of SH-PEG-COOH in water (37.5 µl, 20 µM), with the same molar ratio as EDC and NHS, followed by shaking for 1 h. Next, the pH of the above solution was adjusted to 7.4 by adding concentrated phosphate buffered saline (10X PBS). Subsequently, rabbit IgG (10 µl, 75 µM) was added to the solution and the resulting solution was then incubated on shaker for 2 h. Then the mixture was filtered to remove any byproduct during the reaction using centrifuge tube with a 50 kDa filter. The final SH-PEG-IgG conjugate solution (0.75 µM) was obtained after washing with PBS buffer (pH 7.4) twice through the filter. AuNR-IgG bioconjugates were prepared by adding 8 µl of the SH-PEG-IgG (concentration ~1.3 mM in water), 2 µl at a time to a 1 ml solution of twice centrifuged gold nanorods (AuNRs). The amount of SH-PEG-IgG was optimized to obtain maximum coverage of IgG on the AuNR surface by monitoring the red sift in the LSPR. The solution was left for 1 hour on a shaker to complete the conjugation. A similar procedure was employed to prepare AuNRT-NGAL antibody bioconjugates where SH-PEG-NGAL antibody bioconjugates were prepared using NGAL antibody instead of IgG and AuNRT were used instead of AuNRs.

Adsorption of AuNR-IgG and AuNRT-NGAL antibody on glass surface: First, 1×2 cm rectangular slides of glass were cleaned with piranha solution (1:3 (v/v) mixture of 30% H_2O_2 and H_2SO_4) followed by extensive rinsing with nanopure water. Please note: Piranha solution is extremely dangerous and thus proper care must be taken while handling and disposing the solution. The cleaned glass slides were then modified with

MPTES, to render thiol functionality, by immersing the glass substrate into 1% (w/v) MPTES solution in ethanol for 1 h followed by immersion in ethanol for 30 min and thoroughly rinsing with nanopure water and ethanol. AuNR-IgG conjugates were immobilized onto MPTES-functionalized glass substrates by exposing the glass substrates to AuNR-IgG conjugates solution for 3 h. The modified substrates were rinsed with water and drying under the stream of nitrogen to remove the loosely bound AuNR-IgG bioconjugates. To make sure that the amount of IgG conjugated on the AuNR is consistent for each batch, we used the same amount and concentration of IgG solution (8 µL, 1.3 mM) and AuNR solution (1 mL, optical density of 2.0). Also, we used the LSPR shift to monitor bioconjugation of each batch to ensure similar LSPR red shift (8 nm). Moreover, by controlling the absorption time (3 hours) and optical density of the substrates after incubation (0.8), we make sure that the same amount of AuNRs deposited on the glass substrates. Similar procedure was employed to immobilize AuNRT-NGAL antibody bioconjugates on the glass substrates.

Polymer encapsulation and PEGylation: Glass substrates with AuNR-IgG bioconjugates were immersed in 4 mL of 1x PBS (pH 7.4) containing different concentration of TMPS and APTMS for 10 min, followed by rinsing with water and drying under a stream of nitrogen. Subsequently, to avoid nonspecific binding, the substrates were PEGylated by immersing the glass slide in 2 mg/ml methoxy-PEG-thiol (AuNR-IgG glass substrates) and methoxy-PEG-silane (polymer encapsulated AuNR-IgG glass substrates) solution for 2 hours and then rinsed with water and dried under nitrogen stream.

SDS treatment: After polymerization and PEGylation the substrates were exposed to different concentration of Anti-IgG or NGAL in 1x PBS. To release analyte (anti-IgG or NGAL) the substrates were washed by immersion in 3 mL of 0.4 wt.% aqueous solution of SDS in 1x PBS for 5 min. Prior to recapture of analyte on bioconjugates by exposure to different concentration of Anti-IgG, the substrates were rinsed with water and dried in nitrogen.

Characterization: Transmission electron microscopy (TEM) micrographs were recorded on a JEM-2100F (JEOL) field emission instrument operating at an accelerating voltage of 200 kV. Samples were prepared by drying a drop of the solution on a carbon-coated grid, which had been previously made hydrophilic by glow discharge. Atomic force microscopy (AFM) images were obtained using Dimension 3000 (Digital instruments) AFM in light tapping mode.

Extinction Spectra and Raman Spectra Measurements: A Shimadzu UV-1800 spectrophotometer was employed for collecting UV−vis extinction spectra from solution and glass substrates. Raman spectra were obtained using a Renishaw inVia confocal Raman spectrometer mounted on a Leica microscope with 20x objective (NA = 0.4) and 785 nm wavelength diode laser (0.5 mW). The spectra were obtained in the range of 600−1800 cm^{-1} with three accumulations and 10 s exposure time.

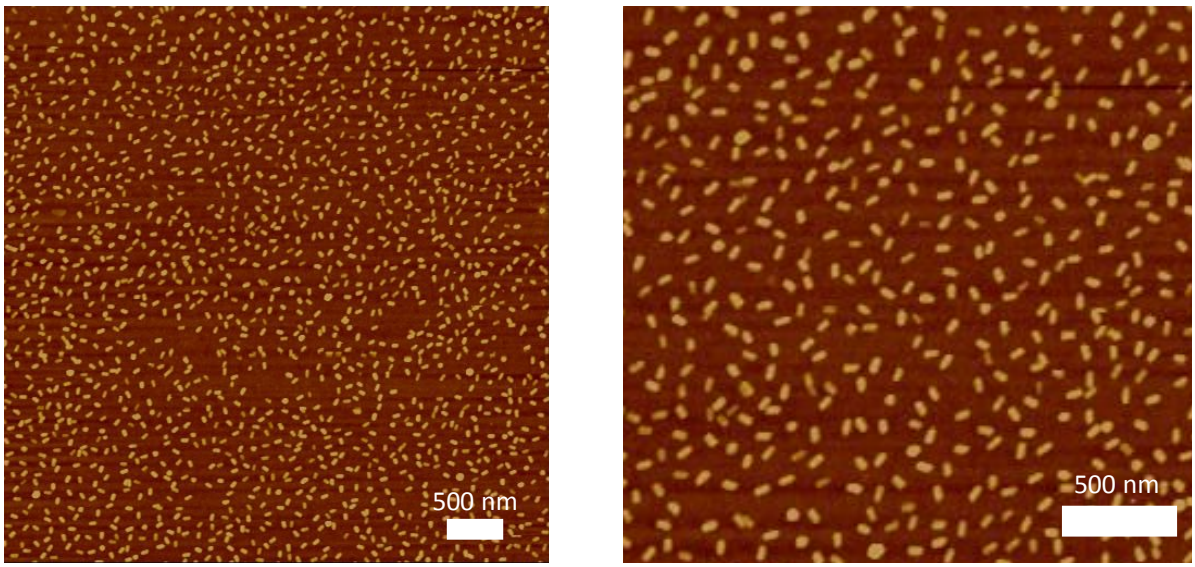

Figure S1. AFM images of AuNR-IgG bioconjugates uniformly adsorbed on a glass substrate with no signs of aggregation or patchiness.

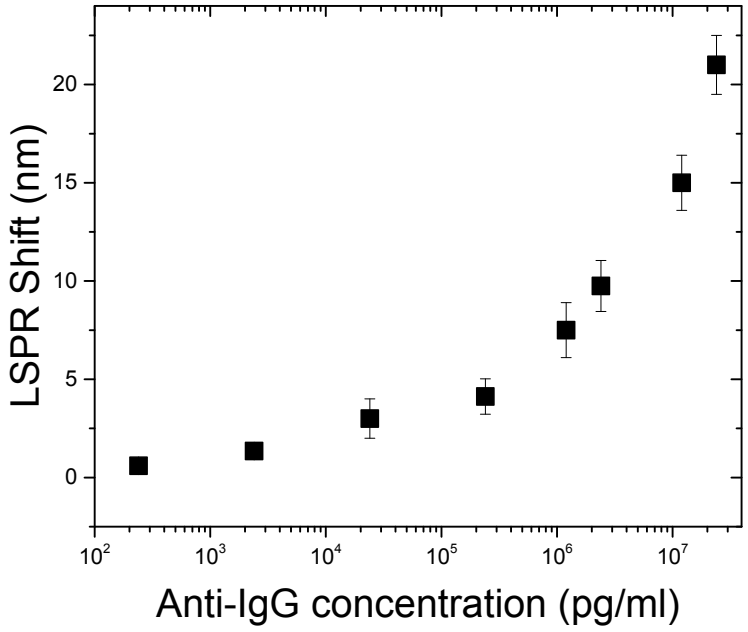

Figure S2. LSPR shift of AuNR-IgG bioconjugates on glass substrates upon exposure to various concentrations of anti-IgG showing monotonic increase in the LSPR shift with concentration. Error bars represent standard deviations from three different samples.

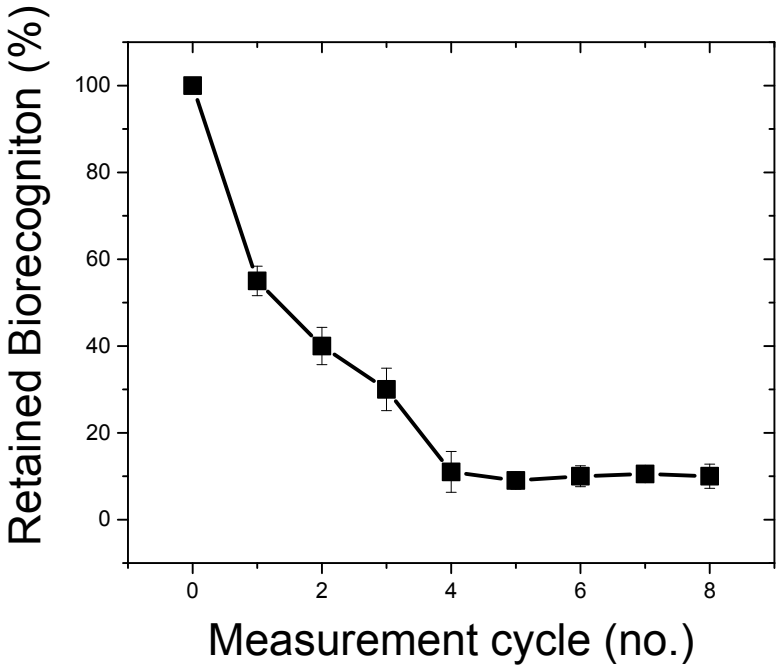

Figure S3. (%) Retained biorecognition capability of AuNR-IgG bioconjugates measured over multiple anti-IgG capture/release cycles. Error bars represent standard deviations from three different samples.

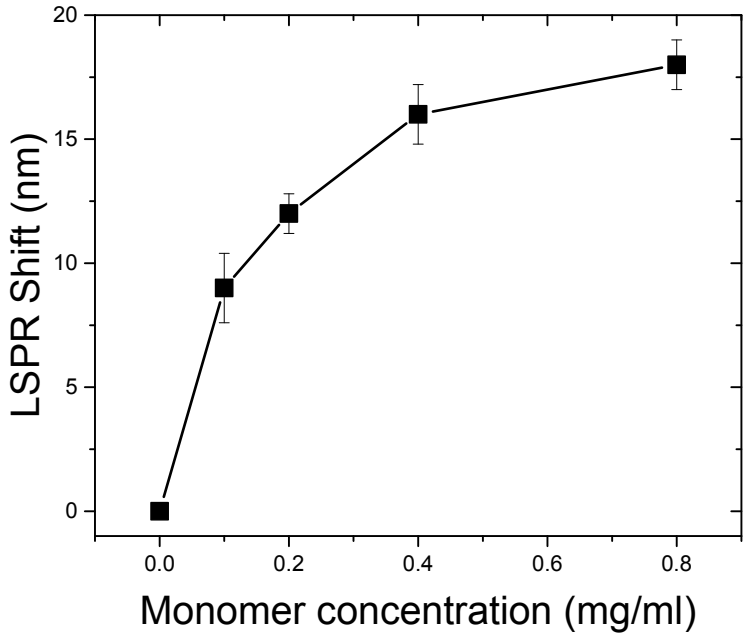

Figure S4. LSPR wavelength shift after exposure of AuNR-IgG bioconjugates to different concentrations of APTMS and TMPS monomers to achieve polymer encapsulation. Error bars represent standard deviations from three different samples.

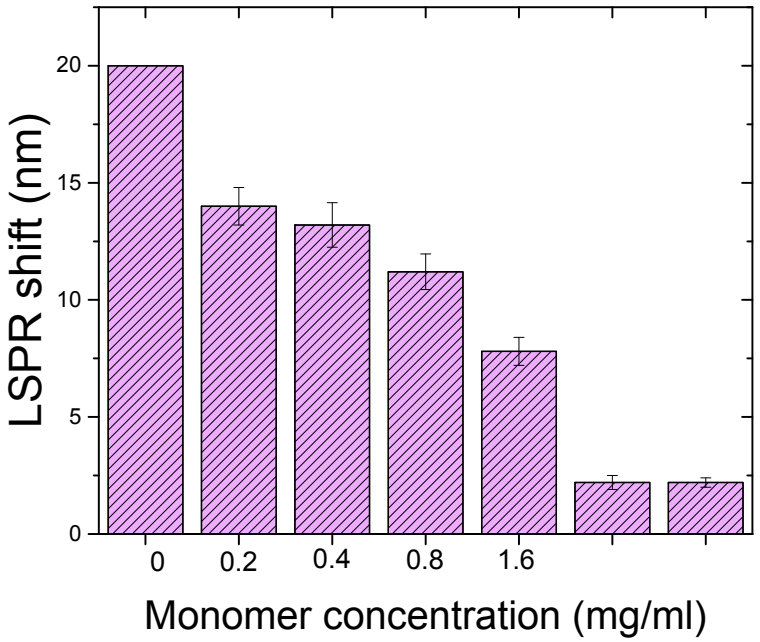

Figure S5. Maximum LSPR shift obtained upon exposure of the AuNR-IgG biosensors, encapsulated with different polymerization conditions, to anti-IgG.

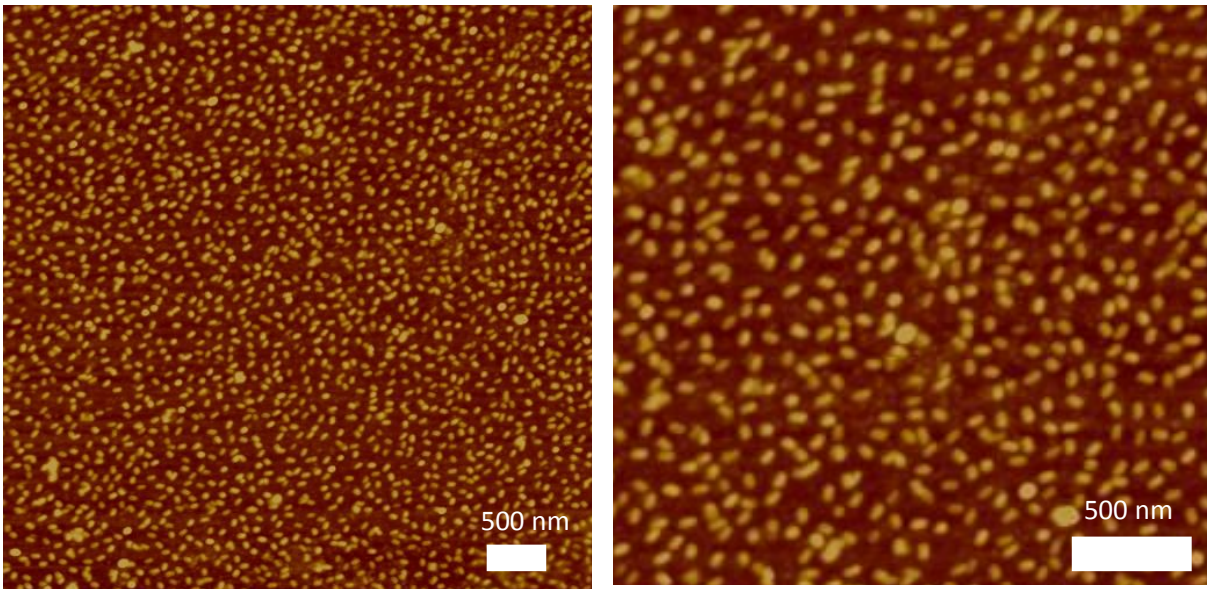

Figure S6. AFM image of polymer encapsulated AuNR-IgG bioconjugates on glass substrates.

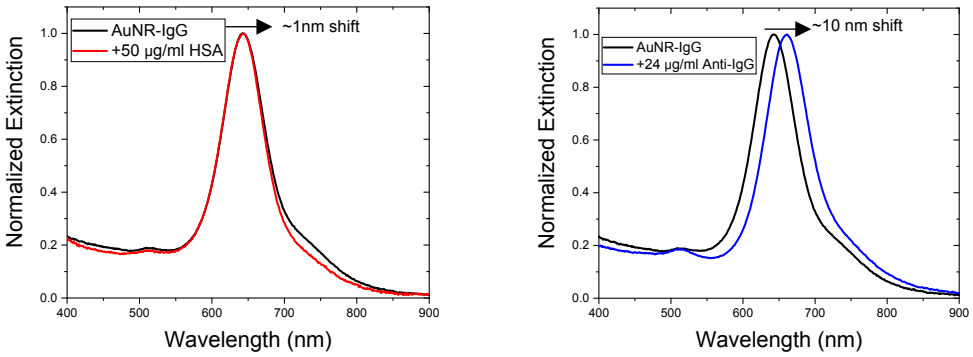

Figure S7. Normalized extinction spectra of PEGylated AuNR-IgG bioconjugates upon exposure to human serum albumin (HSA) (left) and anti-IgG (right) depicting significantly low non-specific binding of HSA even at high concentration.

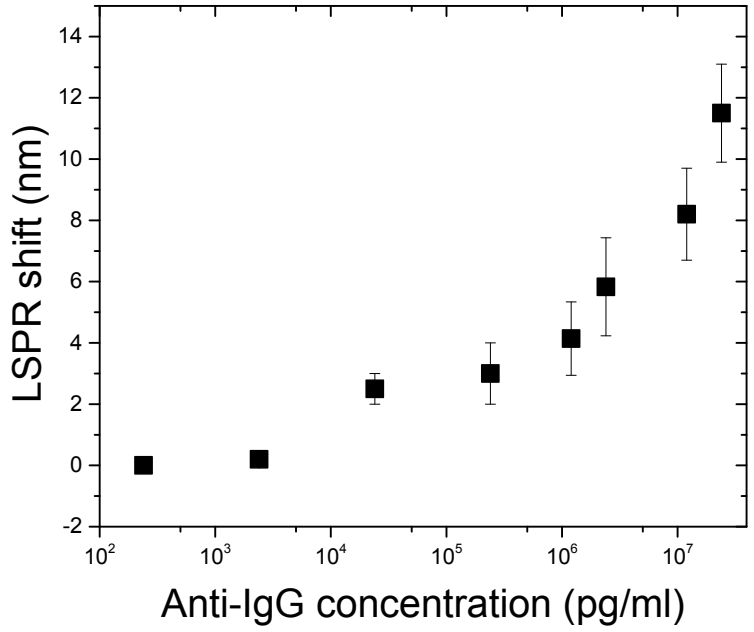

Figure S8. LSPR shift of polymer encapsulated AuNR-IgG bioconjugates upon exposure to various concentrations of anti-IgG showing monotonic increase in the LSPR shift with concentration. Error bars represent standard deviations from three different samples.

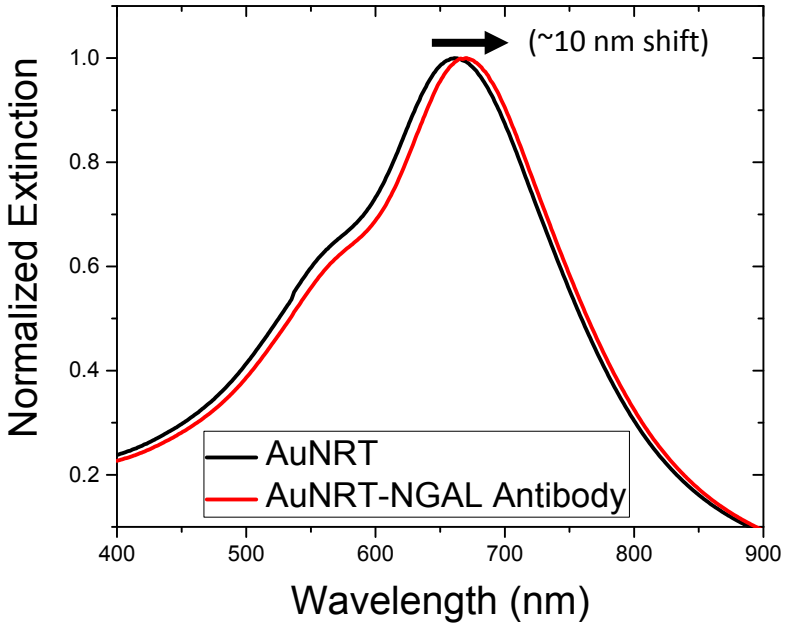

Figure S9. Normalized vis-NIR extinction spectra of AuNRT and AuNRT conjugated with NGAL antibody in solution depicting ~10 nm redshift in LSPR wavelength.

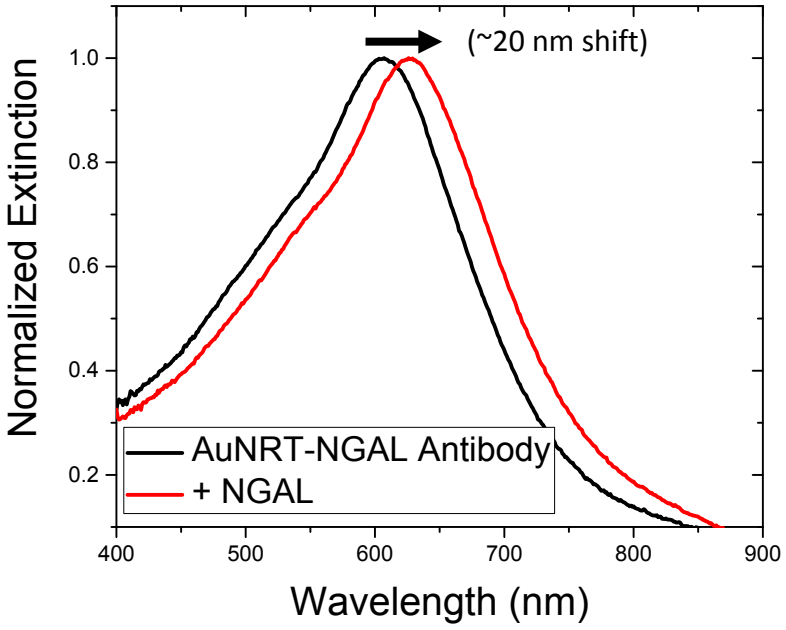

Figure S10. Normalized vis-NIR extinction spectra of AuNRT-NGAL antibody bioconjugates before and after exposure to 5 µg/ml NGAL depicting ~20 nm redshift in LSPR wavelength.

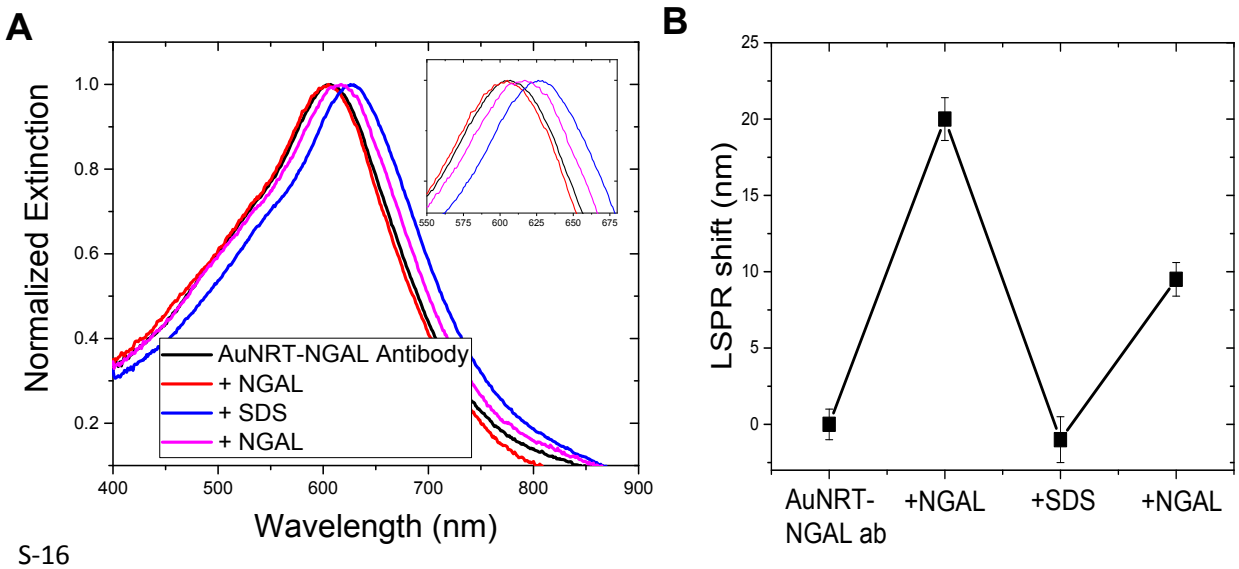

Figure S11: (A) Normalized UV-vis extinction spectra of AuNRT-NGAL antibody corresponding to each step involved: immobilization of AuNRT-NGAL antibody bioconjugates on glass substrates; capture of NGAL on PEGylated AuNRT-NGAL antibody bioconjugates; exposure to SDS solution to remove NGAL; recapture of NGAL. Inset shows zoomed in spectra highlighting the shifts in the LSPR wavelength. (B) LSPR shifts corresponding to biodetection and sensor refreshing.

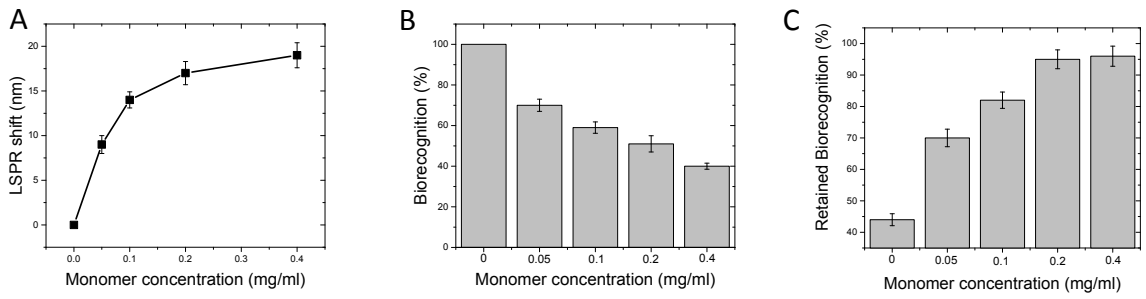

Figure S12: (A) LSPR wavelength shift after exposure of AuNRT-NGAL antibody bioconjugates to different concentrations of APTMS and TMPS monomers. (B) Retained biorecognition capability with increasing polymer thickness/monomer concentration. (C) Retained biorecognition capability of AuNRT-NGAL antibody bioconjugates with increasing monomer concentration, after SDS treatment. Error bars represent standard deviations from three different samples.