Supporting Information

Nano graphene oxide wrapped gold nanostars as ultrasensitive and stable SERS nanoprobes

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List of contents:

4. **Figure S3:** TEM images of (A) Au NSt-nGO, (B) Au NSp-nGO showing that nGO flakes do not wrap around the particles if no cysteamine is used.
5. **Figure S4.** Time dependent UV-Vis spectra of Au NSt@nGO colloids prepared by mixing with (A) 12 μM cysteamine and (B) 15 μM cysteamine.
6. **Figure S5.** UV-Vis spectra of Au NSt, nGO, cysteamine-nGO and Au NSt-nGO colloids.
7. **Figure S6:** (A) Raman spectra of Au NSt@nGO at different concentrations of cysteamine. (B) Raman spectra of Au NSt@RhB@nGO as a function of cysteamine concentration.
8. **Figure S7:** Raman spectra of Au NSt@RhB incubated for two hours with variable concentrations of RhB.
9. **Figure S8.** Raman spectra of Au NSt@RhB@nGO measured at different incubation times with nGO solution.
10. **Figure S9.** Raman spectra of (A) Au NSt@nGO, (B) Au NSt@RhB@nGO and (C) Au NSt@RhB nanoprobes incubatd in PBS (0.1 M, pH=7.4) at 37°C over a period of 7 days.
11. **Figure S10.** Time dependent UV-Vis spectra of (A) Au NSt@RhB and (B) Au NSt@RhB@nGO colloids.
12. **Figure S11.** Raman intensity of peak at 1618cm^{-1} Au NSt@CV and 1361cm^{-1} for Au NSt@R6G colloids incubated with different amounts of dyes for 2 hrs. Error bars represent standard deviation among three different batches (N=3). These results were collected in order to determine the optimal amount of CV and R6G to use in our experiments
13. **Table S1.** Names and composition of samples studied in this work.

14. **Table S2.** Atomic % of different elements obtained from XPS survey scans of Au NSp and Au NSp@nGO samples. The errors represent standard deviation among three different points.

Experimental section

Materials

Gold(III) chloride trihydrate (> 49% Au basis), 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, purity >99.5%), sodium hydroxide, sodium citrate tribasic dihydrate, rhodamine b isothiocyanate (RhB), crystal violet (CV, dye content >90%), rhodamine 6G (R6G, dye content ~95%), and cysteamine hydrochloride were purchased from Sigma Aldrich. Nano graphene oxide solution (purity >99%) was purchased from Graphene Supermarket, US. Ultrapure DI water was used in all experiments.

Synthesis of Au NSt

Highly branched Au NSt were synthesized using a previously reported one step method.[1] Briefly, 100mM solution of HEPES was prepared in DI water and pH was adjusted to 7.4 by using 1M NaOH. Next, 2 ml of HEPES solution were mixed with 3 ml of DI water and 50 µl of 20mM solution of gold(III) chloride hydrate solution in a glass vial. The vial was kept at room temperature for 60 minutes without shaking. The colour of the mixture changed from light yellow to colorless to light pink and finally dark blue over a period of 20 minutes. The reaction was allowed to complete for one hour.

Synthesis of Au NSp

Spherical Au NP were synthesized using a previously reported method with some modification.[2] Briefly a 50.0 ml solution of 0.01% w/v gold(III) chloride hydrate was mixed with 0.75ml of 1.0% solution of sodium citrate tribasic dihydrate in a glass vial and stirred thoroughly for 2

minutes. The vial was incubated in an oven at 95°C for 60 minutes. After the completion of reaction, the suspension was cooled to room temperature and stored under dark conditions.

Synthesis of nGO wrapped Au NP (Au NSt@nGO, Au NSp@nGO)

To prepare Au NSt@nGO, at first the nGO solution (1mg/ml) was ultra sonicated for 3 hrs in a bath sonicator (Cole-Parmer Inc. US). Next, 3 ml of as prepared Au NSt (0.000019w/v%) were mixed with cysteamine and nGO solutions to achieve final concentrations of 12 μM (cysteamine) and 0.01mg/ml (nGO). The mixture was left for one hour under mild stirring at room temperature. The same procedure was used to synthesize Au NSp@nGO except that the concentration of Au NSp was 0.01w/v%.

Synthesis of RhB/CV/R6G-tagged, nGO wrapped Au NSt (Au NSt@RhB@nGO, Au NSt@CV@nGO and Au NSt@R6G@nGO)

First, RhB was adsorbed on the surface of Au NSt through electrostatic interactions between negatively charged Au NSt and positively charged RhB molecules. In a typical experiment 3 ml of as synthesized Au NSt were mixed with variable concentrations of RhB (15-60nM) and the mixture was mildly stirred for two hours at room temperature. This was followed by the addition of cysteamine and nGO as described in the previous step. A similar procedure was used to prepare Au NSt@CV@nGO and Au NSt@R6G@nGO nanoprobes, using concentrations of CV and R6G of 50 nM and 35 nM respectively. These concentrations were chosen based on the results plotted in Figure S10.

Synthesis of control samples

To prepare dye tagged control samples (Au NSt@RhB, Au NSt@CV and Au NSt@R6G) Au NP were mixed with Raman dyes and stirred mildly for 2 hrs. To prepare Au NSt-nGO and Au NSp-nGO samples, Au NP were mixed with nGO for 2 hrs under mild stirring. To pepare dye tagged control samples without cysteamine modification (Au NSt@RhB-nGO, Au NSt@CV-nGO and Au NSt@R6G-nGO), Au NSt were mixed with dyes for 2 hrs under mild stirring. The samples were mixed with nGO and stirred for an additional hour. The conentrations of Au NSt, RhB, CV, R6G, cysteamine and nGO were kept at 0.000019w/v%, 30nM, 50nM, 35nM, 12μM and 0.01 mg/ml respectively.

Ultraviolet-Visible (UV-Vis) and Raman spectroscopy

The optical properties of all samples were characterised using UV-Vis spectroscopy. The samples were centrifuged, washed with DI water to remove residual reactants and unbound nGO before taking UV-Vis spectra. The samples were then diluted 5 folds with DI water and absorption spectra were collected using UV-Carry 5000 NIR-UV-Vis spectrometer (Varians Inc. US).

All of the Raman measurements were taken using a SENTERRA confocal Ramanscope (Bruker, US) equipped with a 785 nm laser. This laser was chosen to avoid fluorescence caused by the Raman dyes, as shown in previous work by Vo-Dinh et al.[11,12] The samples were centrifuged, washed with DI water to remove residual reactants and unbound nGO before taking Raman spectra. The samples were loaded into clear glass capillaries (Micro-Hematocrit capillary tubes, nonheparinized, sodalime glass, ID=1.1mm, length=75mm, Kimble-Chase, NJ, US).[3] The samples were focused using 20X objective and Raman spectra were collected for 15 seconds

using 50mW power. All spectra were baseline corrected and background substracted using Thermo Scientic GRAMS/AI software before plotting.

TEM imaging

To prepare the samples for TEM analysis, small aliquots of Au NSt, Au NSp, Au NSt@nGO, Au NSt@RhB@nGO, Au NSp@nGO, Au NSp@RhB@nGO, Au NSt-nGO and Au NSp-nGO were deposited on carbon coated copper TEM grids and dried in air. Philips Technai G2 20 TEM (FEI, US) equipped with a Gatan imaging filter was used to characterise the samples for size and shape analysis.

X-ray Photoelectron Spectroscopy

As synthesized Au NSt, Au NSt@nGo, Au NSp and Au NSp@nGO were washed with DI water 3 times, deposited on aluminum foil and dried in vaccuum. Survey and high resolution scans were collected using monochromatic X-ray photoelectron spectometer K Alpha (Thermo Scientifc) equipped with Al K-α X-ray source (1486.6 eV) and ultrahigh vaccuum chamber. All samples were scanned at 3 points with spot size of 400µm each. The peak energies were calibrated using C-C peak at 284.8 eV as a standard. The peak fitting was performed using Thermo Avantage software.

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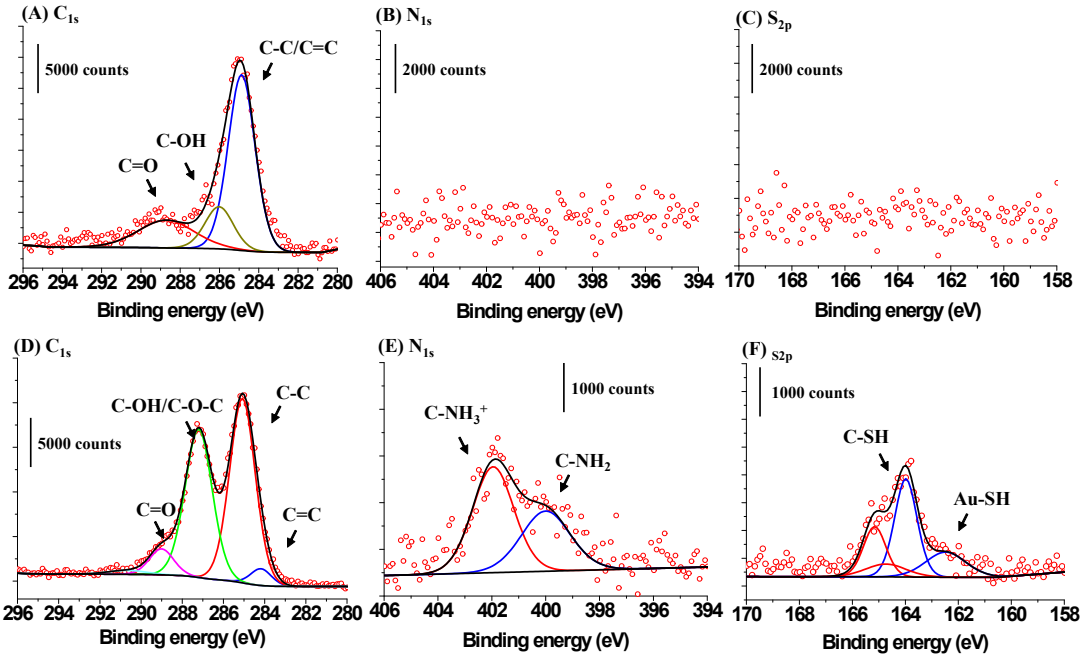

Figure S1: High resolution XPS spectra of (A, D) C_{1s}, (B, E) N_{1s} and (C, F) S_{2p} on (A-C) Au NSp and (D-F) Au NSp@nGO. C_{1s} spectrum of as synthesized Au NSp shows three peaks at 284.8, 286 and 288.9 eV corresponding to C-C/C=C, C-OH and C=O respectively. These are related to the citrate ions capping the Au NSp.[4] N_{1s} and S_{2p} spectra do not show any peaks, as expected since Au NSp are synthesized with citrate ions only. The C_{1s} spectrum of Au NSp@nGO can be deconvoluted in four main peaks at 284.1, 284.9, 287.1 and 289.1 eV, which correspond to C=C, C-C, C-OH/C-O-C and C=O of nGO respectively.[4-6] The N_{1s} spectrum of Au NSp@nGO shows two peaks at 399.9 and 402 eV, which can be attributed to the C-NH_2 groups and protonated amino groups of cysteamine, respectively.[7] The S_{2p} spectrum shows two doublets at 162.3 eV ($S_{2p3/2}$) and 163.5 eV($S_{2p1/2}$), corresponding to Au-S coupling and 164 eV ($S_{2p3/2}$) and 165.2 eV ($S_{2p1/2}$) corresponding to C-S from free cysteamine molecules entrapped between Au NSp and nGO coating.[8-10] These results confirm the successful modification of Au NSp with cysteamine and wrapping with nGO.

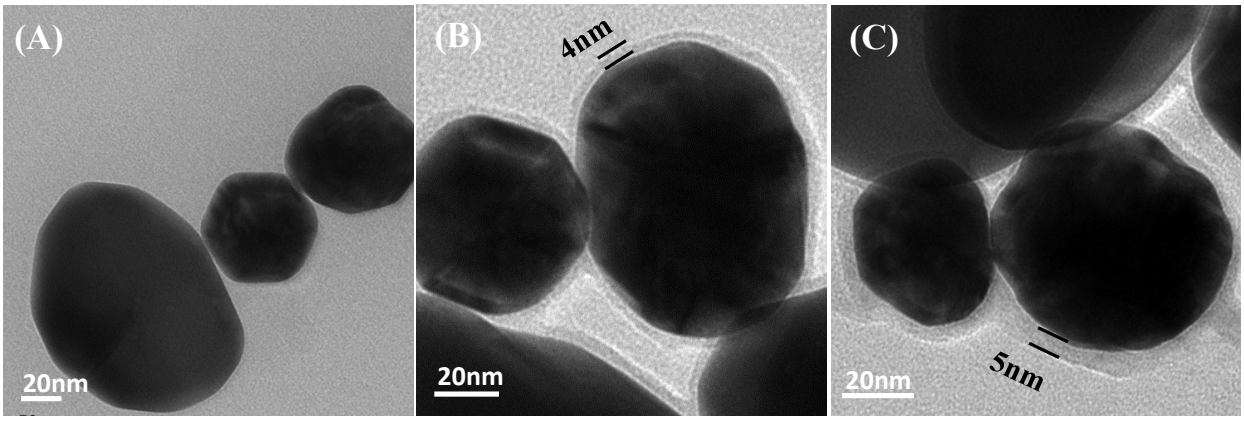

Figure S2: TEM images of (A) Au NSp, (B) Au NSp@nGO and (C) Au NSp@RhB@nGO showing that the average diameter of as synthesized Au NSp is 78.6±12.3 nm and a 4-5 nm thick shell of nGO around is produced both in the presence and absence of RhB.

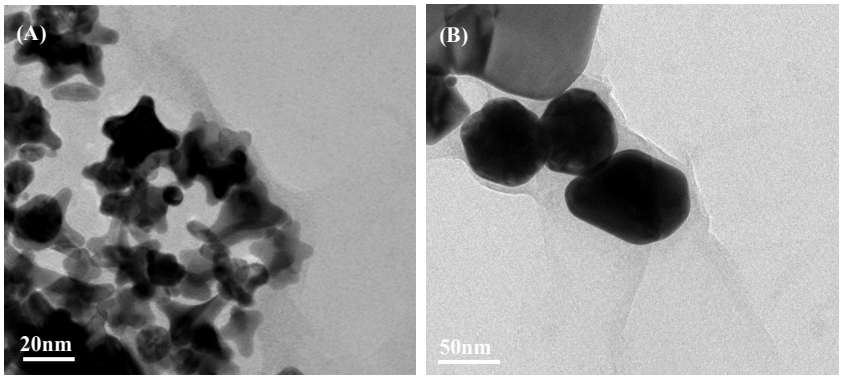

Figure S3: TEM images of (A) Au NSt-nGO, (B) Au NSp-nGO showing that nGO flakes do not wrap around the particles if no cysteamine is used.

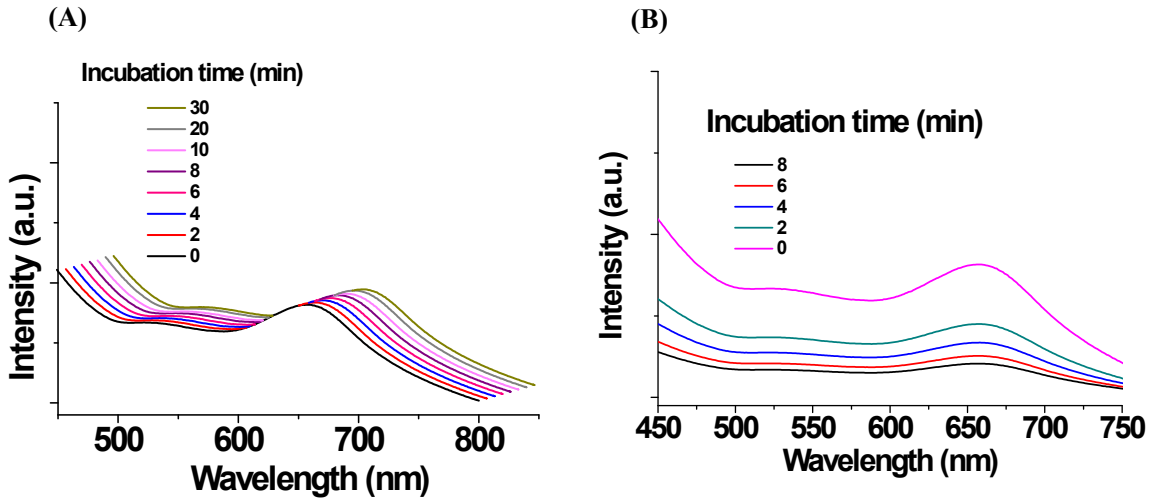

Figure S4. Time dependent UV-Vis spectra of Au NSt@nGO colloids prepared by mixing with (A) 12 μM cysteamine and (B) 15 μM cysteamine.

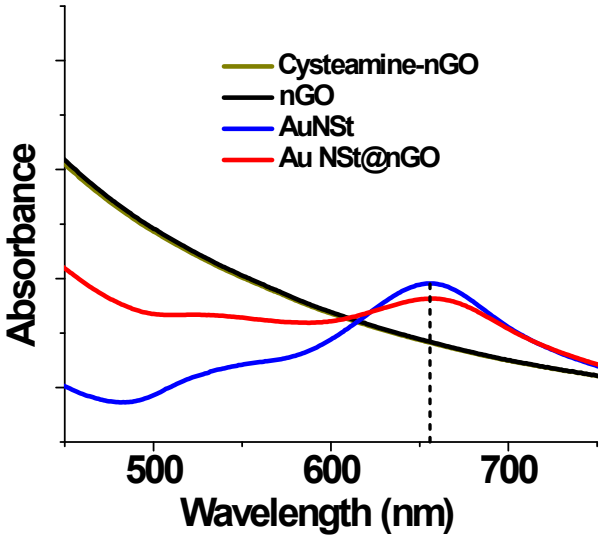

Figure S5. UV-Vis spectra of Au NSt, nGO, cysteamine-nGO and Au NSt-nGO colloids.

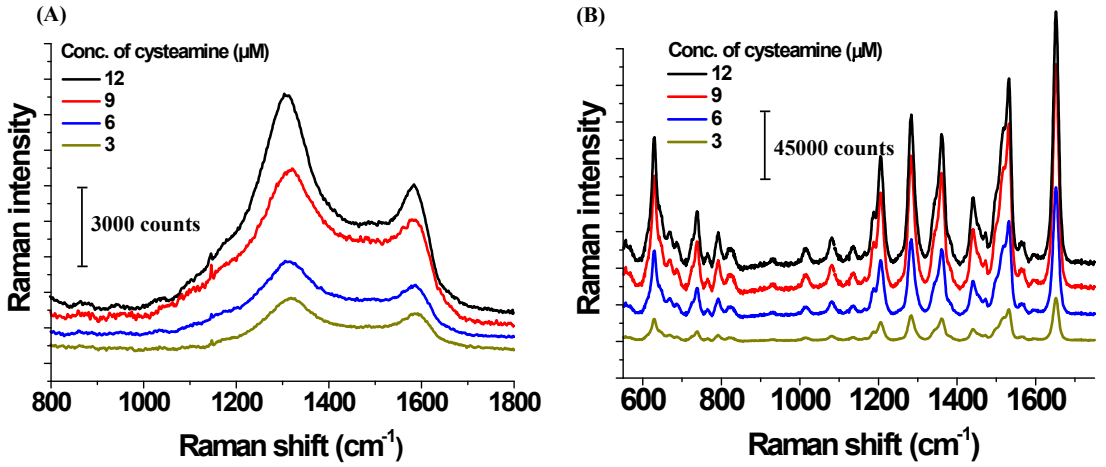

Figure S6: (A) Raman spectra of Au NSt@nGO at different concentrations of cysteamine. (B) Raman spectra of Au NSt@RhB@nGO as a function of cysteamine concentration.

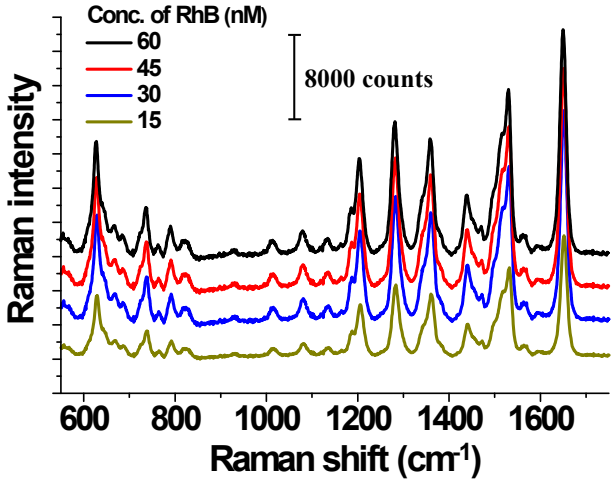

Figure S7: Raman spectra of Au NSt@RhB incubated for two hours with variable concentrations of RhB.

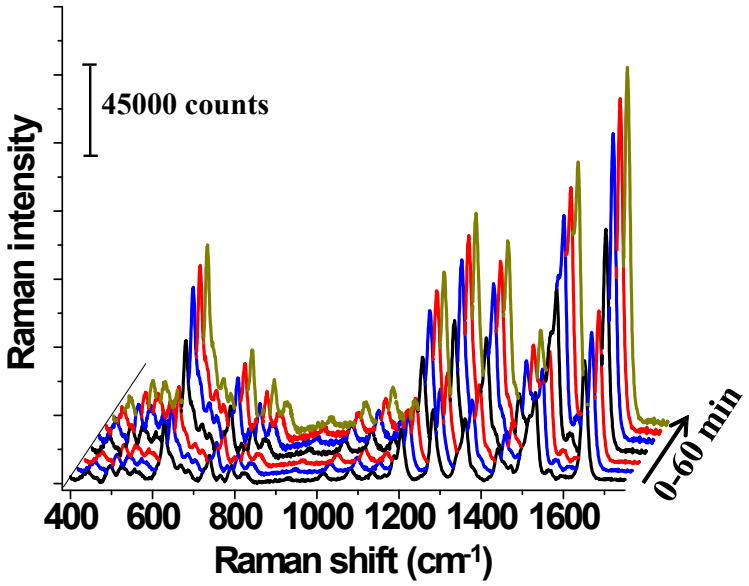

Figure S8. Raman spectra of Au NSt@RhB@nGO measured at different incubation times with nGO solution.

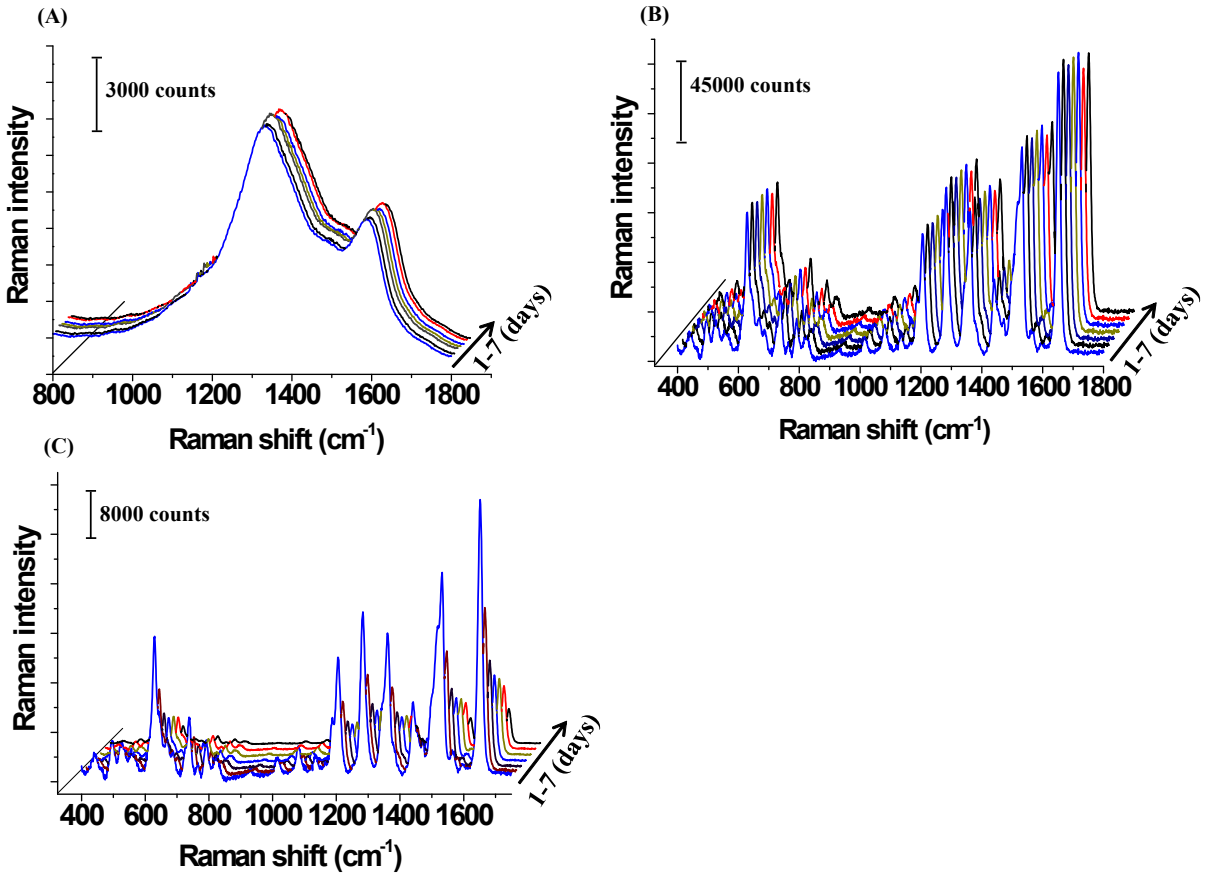

Figure S9. Raman spectra of (A) Au NSt@nGO, (B) Au NSt@RhB@nGO and (C) Au NSt@RhB nanoprobes incubatd in PBS (0.1 M, pH=7.4) at 37°C over a period of 7 days.

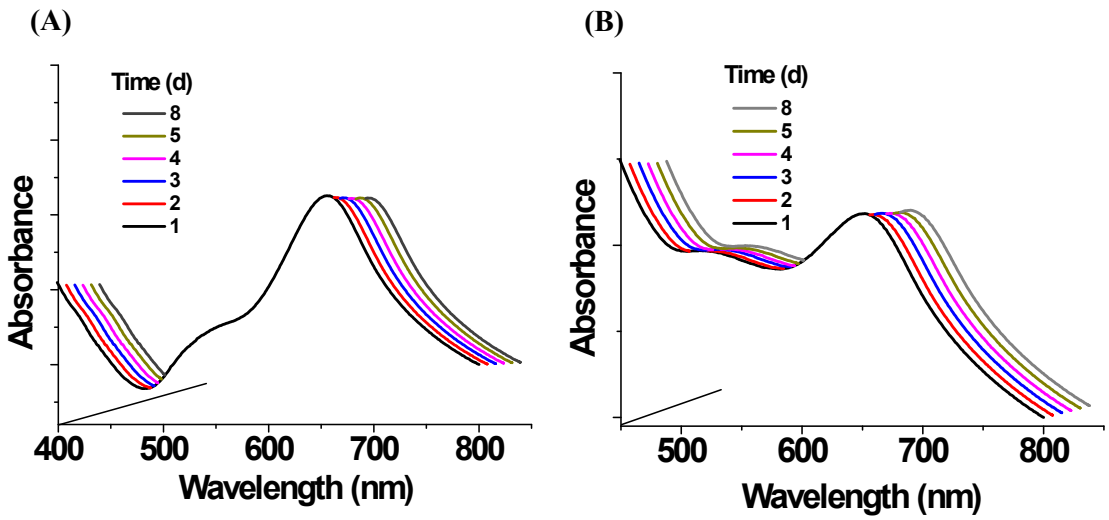

Figure S10. Time dependent UV-Vis spectra of (A) Au NSt@RhB and (B) Au NSt@RhB@nGO colloids.

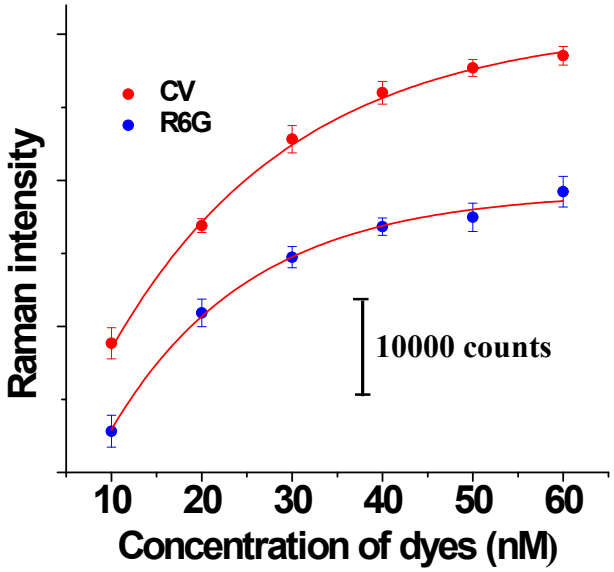

Figure S11. Raman intensity of peak at 1618cm^{-1} Au NSt@CV and 1361cm^{-1} for Au NSt@R6G colloids incubated with different amounts of dyes for 2 hrs. Error bars represent standard deviation among three different batches (N=3). These results were collected in order to determine the optimal amount of CV and R6G to use in our experiments.

Table S1. Names and composition of samples studied in this work

Samples	Cysteamine	Dye (RhB/CV/R6G)	nGO
Au NSt@nGO, Au NSp@nGO	Yes	No	Yes
Au NSt-nGO, Au NSp-nGO	No	No	Yes
Au NSt@RhB	No	Yes	No
Au NSt@RhB-nGO	No	Yes	Yes
Au NSt@RhB@nGO	Yes	Yes	Yes
Au NSt@CV	No	Yes	No
Au NSt@CV-nGO	No	Yes	Yes
Au NSt@CV@nGO	Yes	Yes	Yes
Au NSt@R6G	No	Yes	No
Au NSt@R6G-nGO	No	Yes	Yes
Au NSt@R6G@nGO	Yes	Yes	Yes
Cysteamine-nGO*	Yes	No	Yes

*these samples do not contain Au NSt.

Table S2. Atomic % of different elements obtained from XPS survey scans of Au NSp and Au NSp@nGO samples. The errors represent standard deviation among three different points.

Sample	Au	C	O	N	S
Au NSp	14.2±3.1	27.8±4.1	58.5±5.4	Not detected	Not detected
Au NSp@nGO	3.3±0.1	65.2±0.9	25.3±0.9	3.7±0.6	2.5±0.5

As-synthesized Au NSp samples show only three elements, namely Au, C and O. The amount of C is significantly increased in Au NSp@nGO, while Au and O are decreased because of the presence of nGO coating. The appearance of N and S in Au NSp@nGO samples confirms the presence of cysteamine on the Au NSp.