Electronic supplementary information:

Graphene laminated gold bipyramids as sensitive detection platforms of antibiotic molecules

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1. Experimental Methods

1.1. Synthesis of Gold Bipyramids

Gold bipyramids were synthesized using the standard solution synthetic procedure. Anisotropic Au bipyramids (Au BPs) were synthesized by specific protocol reported earlier.^{S1,S2} For each synthesis, solutions were freshly prepared with deionized (DI) water, except for the hydrogen tetrachloroaurate (III), which was prepared in a 25 mM stock solution and kept in the dark. First, gold nanoparticles were prepared as seeds for further growth by stabilization in sodium citrate. Generally, a 20 mL solution including 0.125 mM hydrogen tetrachloroaurate (III) and 0.25 mM sodium citrate were prepared. Then, fresh and ice-cold aqueous 0.3 mL of 10 mM NaBH₄ solution was added and mixed gently. The seed solution was kept at room temperature for ~2 h to complete the reaction. Next, 0.5 mL of 10 mM hydrogen tetrachloroaurate (III) and 0.1 mL of 10 mM silver nitrate were added to 10 mL of 100 mM cetyltrimethyl ammonium bromide (CTAB) for the preparation of growth solution. Next, 0.2 mL of 1.0 M hydrochloric acid and 0.08 mL of 100 mM L-ascorbic acid were added to the solution sequentially. Finally, the seed solution was added to the growth solution. 40 μ L of this seed solution was used for gold bipyramids.

1.2. PEGylation of Gold Bipyramids

1 mL of gold bipyramid solution and 0.1 mL of 2 mM potassium carbonate was centrifuged at 7000 rpm for 30 min to form a pellet. The suspended solution was decanted, and the pellet was resuspended in 1 mL of DI water and 0.01 mL of 1 mM thiol terminated methoxypoly (ethylene glycol) (mPEG-SH, 5000 MW) and the solution was shaken briefly in a vortex mixer. The resulting solution was then left overnight to displace the CTAB. Thereafter, the solution was centrifuged again under the same condition. The gold bipyramids were then taken through at least one more centrifugation/decant cycle, resuspending each time in deionized water, to further reduce the CTAB concentration.

1.3. Fabrication of Gold Bipyramid Film

Glass slides were cleaned with piranha solution ($3:1 H_2SO_4/30 \% H_2O_2$), thoroughly rinsed with deionized water, and dried. Next, the slides were then immersed in an ethanolic solution of 10 % aminopropyltriethoxysilane (APTES) for at least 12 hrs, rinsed with water, and dried with nitrogen. The APTES coated slides were then immersed in PEGylated gold bipyramid solution overnight. Once rinsed and dried, a uniform film of the gold bipyramids remained on the surface and was ready for use. To remove the mPEG layer and other contaminants, the substrate was cleaned with oxygen plasma cleaner (Branson Plasma Asher).

1.4. Laser Shock Lamination

Chemical vapour deposition (CVD) coated Graphene on copper sheet (Graphene supermarket) were spin coated (~100 nm thin) with PMMA at 3000 rpm and cured at 180° C for 2 minutes. The copper sheet under graphene was then etched away in aqueous solution of FeCl₃. PMMA/graphene stack was then completely washed with DI water several times till the water became colorless even with graphene samples on it for 30 minutes and then the stack was transferred on top of Au bipyramids coated glass substrates. After adequate drying, the PMMA/graphene stacks transferred on Au BP were soaked in acetone for 30 minutes to dissolve PMMA and consequently cleaned by Isopropyl Alcohol (IPA) and deionized (DI) water and dried further.

Atomic sheets of graphene were laminated on to the Au nanostructures by laser shock to tightly integrate and wrap these nanostructures. As shown in Fig 2(a), a Q-switch Nd-YAG laser (Continuum Surelite III) with 5 ns pulse duration was used for achieving the laser shock lamination. Ultrahigh laser shock pressure directed downwards was achieved by laser ablation of aerosol graphite painting (Asbury Carbons, U.S.A.) coated on 4 µm aluminium film (Lebow Company Inc., Bellevue, WA). Graphite layer absorbs the laser energy, and is vaporized and ionized. The ablation plasma when confined, generates a very high laser shock pressure. The laser intensity was 0.3 GW/cm². A 200 µm thick rubber cushion layer placed below the aluminium foil and above the graphene layer prevents excessive shock loading which would otherwise deform the gold bipyramids. It should be noted that features at lower dimensions are more vulnerable to deformations. A motorized XYZ stage was employed to conveniently adjust the laser focused area.

1.5. Characterization and Measurement

Gold nanostructures were imaged by transmission electron microscope (FEI Tecnai) and the images were collected by scanning electron microscope (FEI Nova) and atomic force microscopy (NanoscopeIIIa). Laser shock induced graphene wrapped gold nanobipyramid substrates were then immersed into tetracycline solution (IBI Scientific Inc.) prepared with DI water for 3 hours. The substrate was then rinsed with DI water and dried with nitrogen gas. The resulting samples were then analyzed by a Raman spectrometer (Bruker Senterra) equipped with an infrared laser ($\lambda = 785$ nm) and the signal collection optics.

2. Control experiments for shape, size and concentration:

1. **Shape analysis**: We have extensively worked on various Au nanostructures viz. Au spheres, Au nanocapsules and Au bipyramids. Au Bipyramids (Au BPs) with their sharp tips exhibited the maximum electric field enhancement.

2. **Size of Au BPs**: While carrying out optimization experiments, we found that Au BPs in the length range 90-100 nm and tips sharper than 10 nm works was found to be the best.

3. Concentration of Au BPs on the substrate: Apart from the shape and size, uniformity of thickness (monolayer is the best) and optimized coverage density, Au BPs provided the desired coverage density. Experiments indicate that moderate coverage density works best. Higher density results in clustering and non-uniform formation of BPs on the film, which effectively

reduces the density of hot spots (where the plasmonic field is maximum) and hence Raman intensity is greatly reduced. Below a lower density limit, no measurable Raman signal was observed. Thus there is a window of particle concentration (100 nM-10 μ M) for AuBPs where one can obtain a significant signal enhancement. It should be noted that plasmonic field distribution in 2D exhibits a maxima not only at sharp tips, but rather midway between the tips as well. Distribution of Au BPs is thus the most crucial in SERS experiments and provides the motivation for our current investigations where the concentration of Au BPs is the principal experimental parameter.

3. Control Raman spectroscopy measurements:

Figure S1 (a) reveals the Raman spectrum of mPEG-SH on bipyramids before plasma cleaning. With the number of plasma cleaning steps (Ar:O₂=1:1 ratio with 100 mW RF power), the Raman signal for mPEG-SH is dramatically reduced after the second time of plasma cleaning, due to removal of mPEG-SH. The broad peak between 1300 and 1500 cm⁻¹ is due to fluorescence from the glass substrate. Prior to Raman fingerprinting of tetracycline using the laser shock integrated graphene onto gold bipyamids, Raman spectra of mPEG-SH powder and tetracycline as well as the drop-and-dry solutions on plain gold substrates were collected to differentiate the real signals of tetracycline on the hybrid SERS-active substrate. The plain gold substrate were cleaned by piranha cleaning for 30 secs, rinsed with DI water, and dried with N₂ gas. The normalized Raman spectra of mPEG-SH and tetracycline shown in Figure S1 (b) were obtained to identify clear signals depending on their form such as pure powder and drop-and-dried sample of the solution and to assess whether the assignment of the prominent Raman peaks overlap. Figure S1 (b), (i) and (ii) displays notable peaks of C-O stretching and CH₂ rocking (1060 and 1141 cm⁻¹), CH₂ twisting (1230 and 1279 cm⁻¹), and CH₂-CH₂ symmetric bending mode (1479 cm⁻¹)^{S3,S4}, which are also observed with enhanced intensity at slightly shifted positions in Figure S1 (a) (i). Raman spectra of pure tetracycline in Figure S1 (b) (iii) and (iv) show prominent peaks denoted with stars such as 1061, 1140, 1248, 1278, 1314, 1565 and 1624 cm⁻¹ which are consistent with the standard peak assignment of tetracycline reported previously.^{S5,S6} Among these, Raman peaks at 1248 and 1565 cm⁻¹ overlaps with the D (1234 cm⁻¹) and G peak (1568 cm⁻¹) of the laser shocked graphene in Fig. 3.



Figure S1. (a) Raman spectra of PEGylated Au BPs substrate for (i) pristine sample, after (ii) 1st and (iii) 2nd plasma cleaning steps. (b) Normalized Raman spectra of (i) drop and dried mPEG-SH solution, (ii) mPEG-SH powder, (iii) drop and dried tetracycline solution, and (iv) tetracycline powder.









Figure S5. SERS spectra of 100 nM Tetracycline.

at 1060 cm ⁻¹				
	10µM	5μΜ	500nM	100nM
Average	1142.4	461.0	374.1	199.5
Std. Dev.	346.4	116.9	97.2	80.6
at 1130 cm ⁻¹				
	10µM	5μΜ	500nM	100nM
Average	2208.9	1279.5	772.3	513.8
Std. Dev.	572.5	164.4	112.0	21.6
at 1276 cm ⁻¹				
	10µM	5μΜ	500nM	100nM
Average	2934.1	928.4	291.4	216.5
Std. Dev.	2724.5	341.7	113.0	81.4
at 1309 cm ⁻¹				
	10µM	5μΜ	500nM	100nM
Average	1905.2	1078.6	981.4	491.5
Std. Dev.	367.4	342.0	181.3	85.4

Table S1. Averages and standard deviations of the spectra (Figure S2 to Figure S5)

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