Supporting Information for

A Graphene-based High-efficiency SERS-active Platform for Sensitive and Multiplex DNA Detection

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Experimental Section

Figure S1 to S4

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EXPERIMENTAL SECTION

The surface morphology of graphene film on SiO_2/Si substrate was examined by Optical microscopy and tapping mode atomic force microscopy (AFM) (Veeco Dimension-Ico System, Veeco, USA). The Raman maps for the graphene film was obtained by plotting peak width of the 2D-band (2600-2800 cm^{-1}) as the map height using a NT-MDT confocal Raman microscopic system (laser wavelength 473 nm and laser spot size is 0.4 μm).

To prepare the substrate which only has gold nanoparticles (Au NPs) on SiO_2/Si substrate, a droplet (10 μL) of mixture solution of different diameter Au NPs (15 nm and 50 nm, 0.8 nM and 0.2 nM respectively) was added onto the surface of the SiO_2/Si substrate. After the evaporation of water, the resulting substrate was referred to Au-SiO_2/Si substrate (Figure S2).

The measurement of the contact angle (CA) of water droplet on graphene film has been carried out under ambient conditions using a Theta Lite optical tensiometer (BiolinScientific, Sweden). A de-ionized water (Milli-Q water) droplet (4 μL) had been released onto the graphene film on SiO_2/Si (300 nm thick SiO_2) substrate from a syringe needle. The image of the liquid droplet was obtained in real time by using a CCD camera (Figure S4). A tangent line has been drawn onto the droplet from the droplet-graphene interface in the image using software coupled within the instrument, and the angle between the tangent line and the baseline indicates the contact angle of the solid and liquid interface. Three different spots on the same graphene film

substrate were measured and three piece of graphene film substrate were used in the experiment. All the CA data was shown in Table S2.

Enhancement factor: We estimated the enhancement factor (EF) of the Au-G-SiO_2/Si substrate with respect to the SiO_2/Si substrate through the following expression:[1,2]

$$EF = (I_{SERS} \times N_{Si})/(I_{Si} \times N_{SERS})$$

Where I_{SERS} is the peak intensity of Cy3 labeled DNA at 1480 cm^{-1} band on the Au-G-SiO_2/Si substrate and I_{Si} is noise intensity which is the maximum possible signal from the Cy3 labeled DNA attached onto the SiO_2/Si substrate. N_{Si} is the number of molecules on SiO_2/Si substrate, N_{SERS} is the number of molecules on Au-G-SiO_2/Si substrate.

For our Raman setup, the illumination focus has a diameter of $d = (\lambda/N.A) \times 1.22 = 1.03$ μm (the 1.22 factor accounts for the deviation of the laser beam from a perfect Gaussian profile), assuming the excitation volume as a cylinder and the laser illuminated area is 0.83×10^6 nm^2. Assuming that Cy3 labeled DNA molecules were adsorbed as a monolayer on the surface of SiO_2/Si substrate and each molecule occupies ~3 nm^2 (Cy3 is about 1 nm and 15 bp single strand DNA is about 3 nm). So N_{Si} is about 3×10^5.

The average diameter of Au NPs on the graphene film is about 30 nm and there are about 10 Au NPs in the area of 0.25×10^6 nm^2 (Figure 1b). There are about 100 strands of DNA on one Au NPs in solution at the similar condition (diameter of Au NP:30 nm, spacer: A10, Salt Concentration: 0.3 M NaCl).[3] Assuming that the

efficiency of DNA immobile on the Au-G-SiO_2/Si substrate is about 50% and 80% of these DNA formed the sandwich complex, there are about 40 Cy3 labeled DNA on one Au NP. Therefore, the calculated N_{SERS} is about 1.3×10^3. The ratio of I_{SERS} and I_{Si} is about 80 at the concentration of 100 nM. Finally, the EF of the Au-G-SiO_2/Si substrate was calculated to be 1.8×10^4.

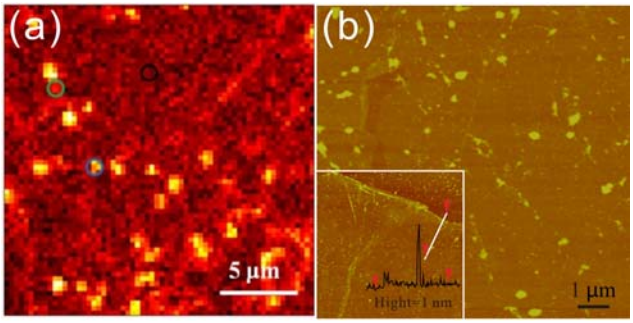

Figure S1. (a) Raman map of the graphene film. This map is constructed by plotting peak width of the 2D-band (2600–2800 cm^{-1}) as the map height. Colored circles represent the different layer of graphene corresponding to the color in figure 1b (b) AFM image of the transferred graphene taken at the edge of the SiO_2 to measure the graphene height.

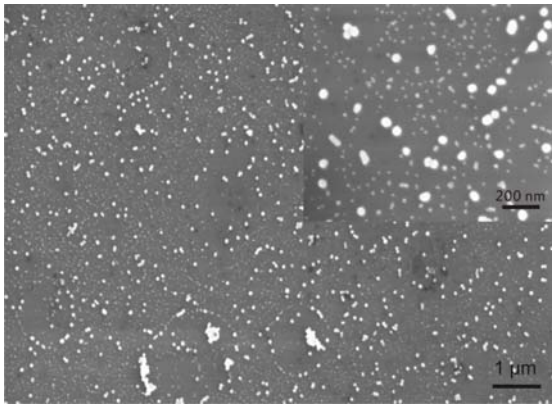

Figure S2. SEM image of the surface of Au-SiO_2/Si substrates.

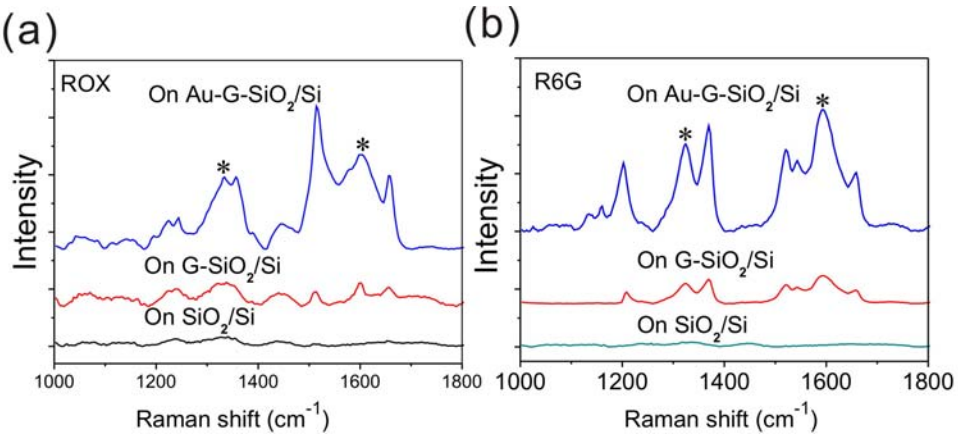

Figure S3. Raman spectra of 10 μM ROX (a) and R6G (b) on SiO_2/Si, G-SiO_2/Si, and Au-G-SiO_2/Si substrate. The peaks marked by the star (*) are the D-band and G-band of graphene.

Figure S4. A water droplet of 4 μL on graphene film which was on SiO_2/Si (300 nm thick SiO_2) substrate.

Table S1. The relative Raman intensity of three dyes on different substrates at the selected Raman peaks.*

ROX	The relative intensity on G-SiO_2/Si substrate	The relative intensity on Au -SiO_2/Si substrate	The relative intensity on Au-G-SiO_2/Si substrate
1514 cm^{-1}	7	-	69
1655 cm^{-1}	4	-	14
R6G			
1520 cm^{-1}	17	-	73
1658 cm^{-1}	5	-	23
Cy3			
1141 cm^{-1}	9	28	32
1218 cm^{-1}	16	68	73
1401 cm^{-1}	**7**	15	17
1449 cm^{-1}	13	37	41
1588 cm^{-1}	5	26	29

*The Raman signals of these dyes on the SiO_2/Si substrate are set to “1”.

Table S2. Average Contact Angle of Graphene film on SiO_2/Si substrate.

Number of the graphene film substrate	Contact angle (standard deviation)
NO.1	89.7 (3.1)
NO.2	91.4 (5.5)
NO.3	88.3 (2.3)

References

(1) Li, W. Y.; Camargo, P. H. C.; Lu, X. M.; Xia, Y. N. *Nano Lett.* **2009**, *9*, 485.

(2) Kang, T.; Yoo, S. M.; Yoon, I.; Lee, S. Y.; Kim, B. *Nano Lett.* **2010**, *10*, 1189.

(3) Hurst, S. J.; Lytton-Jean, A. K.; Mirkin, C. A. *Anal. Chem.* **2006**, *78*, 8313.