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## ARTICLE

# Investigating the interaction of dye molecules with graphene oxide by using surface plasmon resonance technique

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Surface plasmon resonance technique was used to systematically study the interaction of two dye molecules with graphene oxide (GO) and electrochemically reduced GO (EC-rGO) substrates. EC-rGO shows higher binding ability with both dyes than GO, possibly due to the molecule doping or  $\pi$ - $\pi$  stacking. The results of SPR sensing are in agreement with the conclusions of fluorescence quenching experiments. This work may be valuable for graphene-related research works on optoelectronics and biosensors.

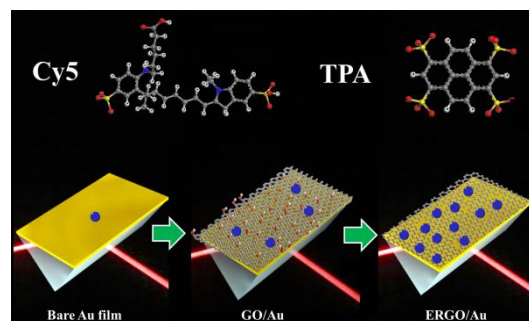
## 1. Introduction

The interaction of dye molecules with graphene-based materials are of key importance in energy storage,<sup>1,2</sup> molecular imaging,<sup>3</sup> biological and chemical sensing,<sup>4</sup> and drug delivery.<sup>5</sup> Graphene is an extended conjugated system, providing a perfect environment for the incorporation of a number of organic and inorganic moieties.<sup>6,7</sup> Numerous studies have been exploited that graphene can strongly interact with a variety of organic dyes and has a superior quenching efficiency for various fluorophores.<sup>8,9</sup> The most popular technique for investigating this interaction is the indirect method based on fluorescence resonance energy transfer (FRET) or photoinduced electron transfer (PET) mechanism.<sup>10,11</sup> However, development of direct, simple and sensitive method to investigate the interaction between dyes and graphene-based substrates (GBS) remains a challenge.

Surface plasmon resonance (SPR) technique is sensitive optical techniques for monitoring biomolecular binding events via detection refractive index changes on a metal sensor surface.<sup>12-17</sup> Our recent work showed that graphene-coated SPR interfaces have several advantages over gold-based SPR interfaces, in which graphene oxide (GO) has been proven to be beneficial for efficient adsorption of DNA biomolecules while electrochemically reduced GO (EC-rGO) cannot.<sup>18,19</sup>

In this study, we report the use of SPR technique to systematically study the different interaction abilities of Cy5 and tetrasodium 1, 3, 6, 8-pyrenetetrakisulfonic acid (TPA) with GO and EC-rGO. The reason that we choose Cy5 and TPA is based on: 1, they are normally used as typical molecular probes; 2, they have different molecular structures, which is good to serve as a model for investigating the difference of their binding ability to graphene oxide. The molecular configuration of Cy5 is complex with a long chain at one orientation, while TPA is simple and symmetric (as shown in Scheme 1). All the detection process was completed on the surface of sensor chip. The results show that EC-rGO presents much higher binding ability with both dyes than GO, which is opposed to the situation of binding with DNA.<sup>18</sup> The inner binding mechanism

between dye molecules and graphene-based substrates is quite different from that between DNA and GBS.  $\pi$ - $\pi$  stacking or molecular doping may play the key role for the interaction between dyes and GBS, while hydrogen binding is dominant for DNA-GBS interaction.<sup>4,20,21</sup> This work might pave the way for modulation of graphene-based materials and be valuable for organic molecule sensing and solar cell.



**Scheme 1.** Schematic illustration of the interactions between three substrates and Cy5 or TPA molecules.

## 2. Experimental Section

### 2.1 Materials

The Cy5 and TPA were purchased from Sigma-Aldrich. Graphene oxide was prepared and characterized by previously described method.<sup>19</sup>

### 2.2 SPR Measurement

SPR spectrometry was carried out using a TR2005 spectrometer (RES-TEC resonant sensor technology, Germany). The set-up was based on the conventional Kretschmann configuration included a He-Ne laser of wavelength  $\lambda=632.8$  nm which was coupled to the system via a high refractive index prism, LAFSN9 glass  $\epsilon=3.40$ .<sup>22</sup> The Gold-coated sensor chip mounted with a homemade electrochemical flow cell was

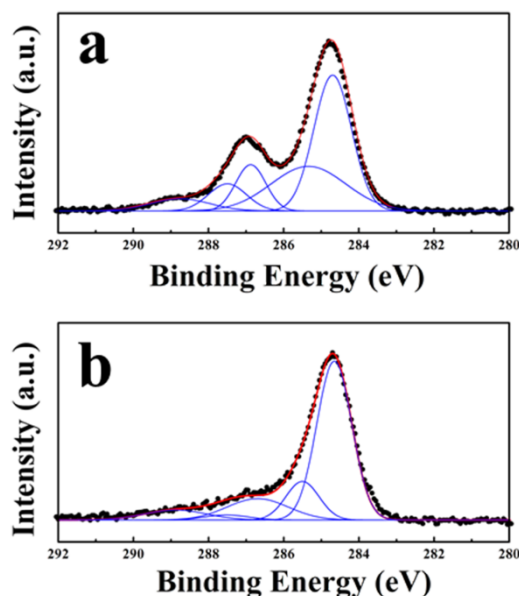
attached to the prism base with high index matching oil. The reflected light was detected by a photodiode.<sup>23</sup>

GO modified sensor chip surface was prepared by injecting GO aqueous solution (1 mg/mL) and incubating for 2 hours over gold surface. The sensor chip surface was then rinsed with water and dried under N<sub>2</sub> flow. GO is electrochemically reduced by cyclic voltammetry with a potential range from -1.6 to -0.0 V in PBS (pH=7.4) with a CHI650D electrochemical workstation (Shanghai, Chenhua Co., China).<sup>24, 25</sup> A three-electrode cell was used with the Gold film as the working electrode, a AgCl electrode as the reference electrode, and a platinum electrode as the counter electrode. All experiments were done at room temperature.

### 2.3 Instruments

X-ray photoelectron spectroscopy (XPS) is acquired with an ESCALAB-250 instrument (performed with a monochromatic Al K $\alpha$  (1486.6 eV) radiation source and a hemisphere detector with an energy resolution of 0.1 eV). The Raman spectrum was detected with a Renishaw 1000 microspectrometer connected to a Leica microscope with an objective lens of 50 (NA=0.5) using excitation wavelength of 514 nm. The fluorescence spectrum of the mixed solution was measured by using a Perkin-Elmer LS 55 spectrometer.

## 3. Results and discussion



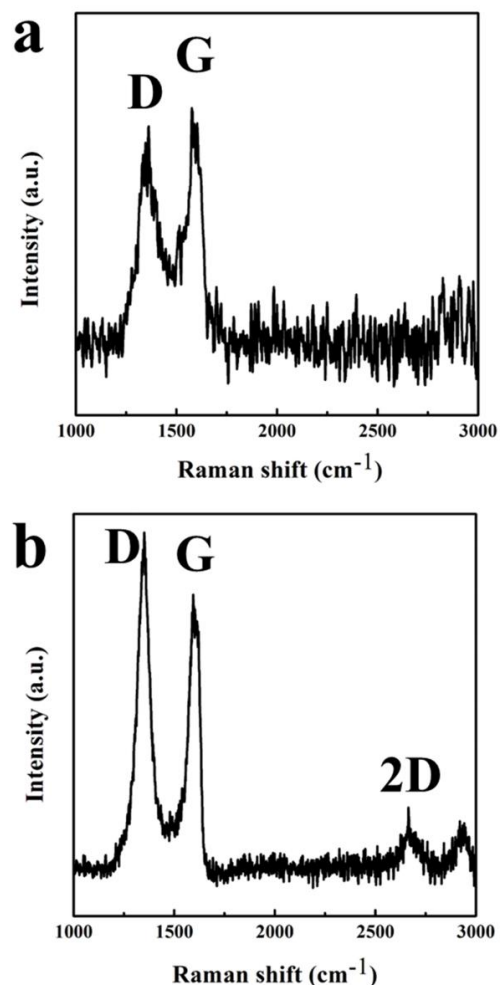
**Figure 1.** XPS spectra of GO (a) and EC-rGO (b) on a Au sensor chip.

### 3.1 Assembly and in situ electrochemical reduction of GO on Au sensor chip

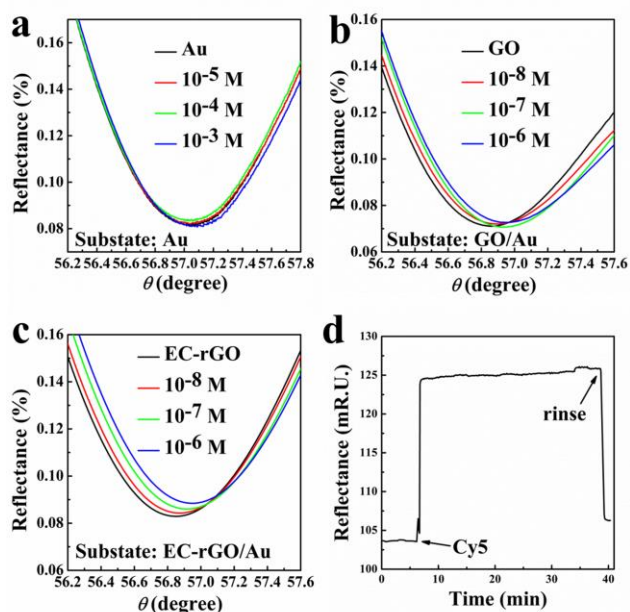
Considering the strong metal-carbon coupling between GO and the Au surface, two dimensional GO nanosheets were expected to self-assemble on a flat Au surface.<sup>19</sup> Cyclic voltammetry technique was then used to electrochemically reduce the assembled GO nanosheets on the Au surface. As shown in Figure S1, GO is reduced at a starting potential of -0.8 V with a current peak at -1.4 V, which is ascribed to the reduction of oxygen groups of -OH, -COOH, and epoxides.<sup>24</sup> The reduction current begins to drop exceptionally in the second cyclic voltammogram scanning cycle and continues

decreasing until it is disappeared. This electrochemical behaviour is similar to a previous report of the reduction of GO sheets on glass carbon electrodes.<sup>26</sup> The reduction of GO was characterized by XPS (as shown in Figure 1).<sup>18, 27</sup> The C 1s XPS spectrum of GO clearly indicates a considerable oxidation with four components that correspond to carbon atoms in functional groups of: non-oxygenated ring C (sp<sup>2</sup> 284.6 and sp<sup>3</sup> 285.4 eV), C in C-O bonds (286.8 eV), carbonyl C (C=O, 287.6 eV), and carboxylate carbon (O-C=O, 288.9 eV). These assignments are in agreement with previous results.<sup>28</sup> The C-O, C=O and O-C=O peaks in the C1s spectra area of EC-rGO are accounted for 7.1%, 2.4% and 5.9%, instead of 12.6%, 9% and 6% in the case of GO.

The GO and EC-rGO substrates are further characterized by Raman spectroscopy, as shown in Figure 2. G peak at ~1596 cm<sup>-1</sup> is usually assigned to the E<sub>2g</sub> phonon of C sp<sup>2</sup> atoms, D peak at ~1346 cm<sup>-1</sup> is a breathing mode of  $\kappa$ -point phonons of A<sub>1g</sub> symmetry, while 2D-band at ~2700 cm<sup>-1</sup> is due to a double resonance process.<sup>29</sup> The intensity ratio of the D to G band is a measure of the disorder. The D/G intensity ratio (I<sub>D</sub>/I<sub>G</sub>) of GO and EC-rGO is 0.84 and 1.20, respectively. The change can be explained by a decrease in the average size of the sp<sup>2</sup> domains and an increase in the number of new graphitic domains upon reduction of the GO. Further, a single sharp 2D-band at 2677 cm<sup>-1</sup> confirms that graphene employed in our work has only a few layers.



**Figure 2.** Raman spectra of GO (a) and EC-rGO (b) on a Au sensor chip.

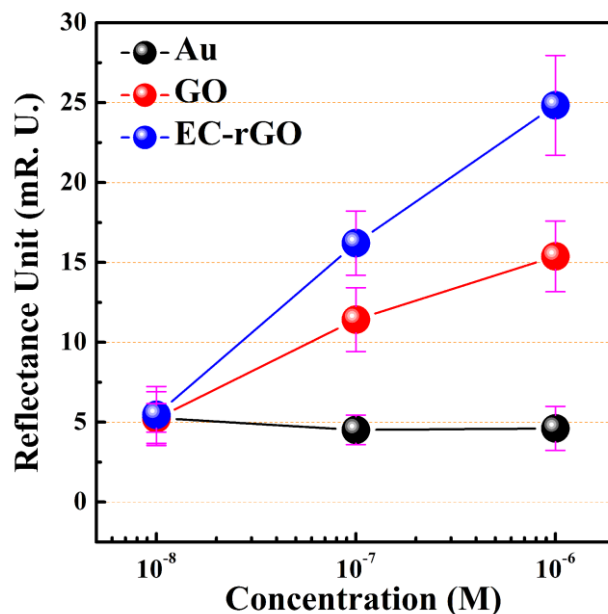


**Figure 3.** SPR spectra of Cy5 with different concentrations ( $10^{-8}$ – $10^{-6}$  M) adsorbed on a bared gold (a), GO (b), and EC-rGO substrates (c). The real-time SPR response for Cy5 adsorption onto the EC-rGO surface for 30 min (d). All measurements were performed in deionized water.

### 3.2. The interaction of Cy5 with GO, EC-rGO, and Au surfaces

The SPR angle reflectivity spectrum is very sensitive to small changes of the refractive index on the sensor chip surface. The SPR spectra of Cy5 on different GBS materials are shown in Figure 3. In the case of the EC-rGO substrate, the adsorption of Cy5 molecules results in an obvious right-shift of SPR angles. With the same process, but using Au films and GO as substrates, the right shifts of SPR angles are less than that on EC-rGO for the same series of concentrations. Figure 3d shows the real-time SPR response for Cy5 adsorption onto the EC-rGO surface. An injection of the Cy5 solution results in an immediate increase of the SPR signal, which indicates that fast binding occurs between Cy5 and EC-rGO surface.

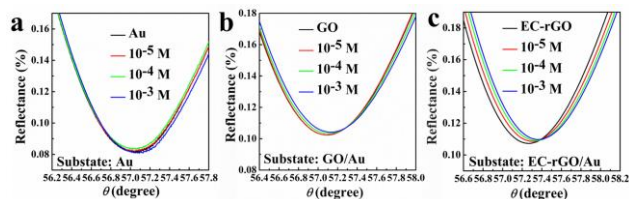
The different binding abilities of Cy5 to Au, GO, and EC-rGO surfaces are investigated by monitoring the changes of SPR response while injecting a series of concentrations of Cy5 on these substrates. The interaction between Cy5 and three substrates (Au, GO, EC-rGO) is clearly revealed as a function of concentration (Figure 4). It can be seen that the Cy5 molecules are adsorbed more efficiently on GO and EC-rGO surface compared to Au films. The binding tendency of EC-rGO to Cy5 is much higher than GO. For instance, the adsorption of  $10^{-6}$  M Cy5 onto EC-rGO surface results in an SPR reflectance change of 24 mRU, which is about two times higher than that onto the GO surface. These results directly show that the strong interaction between Cy5 and EC-rGO, possibly due to the molecule doping or  $\pi$ - $\pi$  stacking.



**Figure 4.** Relationship between SPR signal change and Cy5 concentration on Au, GO, and EC-rGO substrates. Each point corresponds to the SPR response shift for the concentration of Cy5 molecules

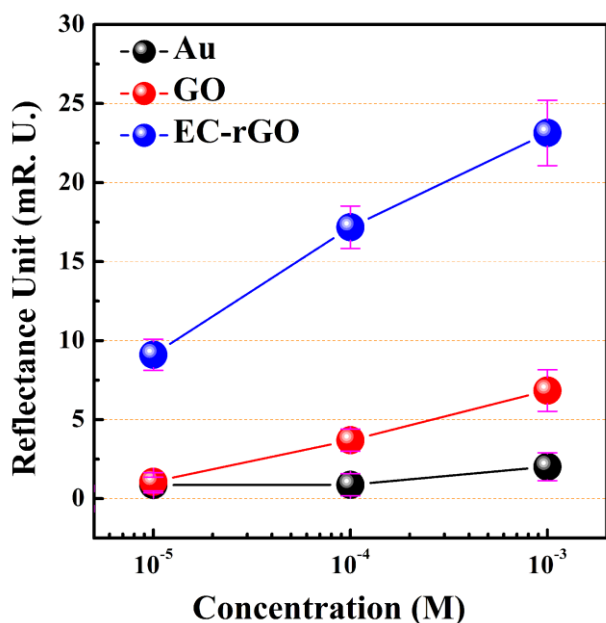
### 3.3. The interaction of TPA with GO, EC-rGO, and Au films.

We discuss next the study of the interaction of TPA with GO, EC-rGO, and Au films using SPR sensor. The TPA molecule is also a typical dye with simpler molecular structure as shown in Scheme 1. When the concentration of TPA molecule is increased from  $10^{-5}$  M to  $10^{-3}$  M, the SPR spectrum on EC-rGO surface is shifted to right, which is increased much more obviously than that on GO surface (Figure 5b,c). The binding of TPA molecules onto Au sensor surface is negligible with no observable changes in SPR spectra (Figure 5a). Figure 6 shows the shifts of SPR angles versus the concentration of TPA. It is clear that the binding tendency of TPA to EC-rGO is the highest among all three substrates. For instance, the adsorption of  $10^{-5}$  M TPA onto the EC-rGO surface results in an SPR response of 8 mRU, which is eight times higher than other two substrates. This suggests that EC-rGO shows the highest binding ability to TPA.



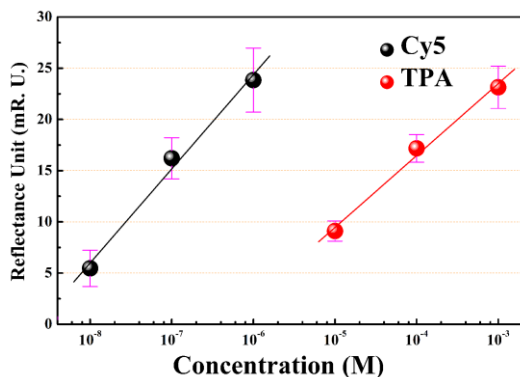
**Figure 5.** SPR spectra of TPA with different concentrations adsorbed on bared gold film (a), GO (b), and EC-rGO films (c). Each concentration of TPA solution was adsorbed onto substrates for 30 min



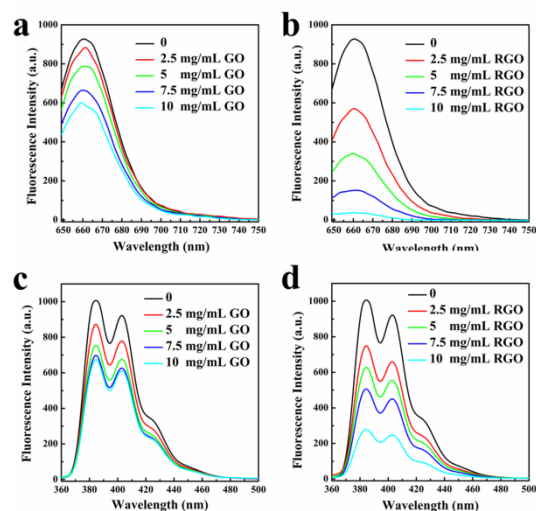


**Figure 6.** Relationship between SPR signal change and TPA concentration on Au, GO, and EC-rGO substrates. Each point corresponds to the SPR response shift for the concentration of TPA molecules.

To further explore the interaction of dye molecules with EC-rGO, we compare SPR responses of Cy5 and TPA on the EC-rGO surface. It clearly shows that the concentration of the detection range of Cy5 is of  $10^{-8}$ – $10^{-6}$  M, which is three order of magnitude lower than that of TPA with the same SPR response (Figure 7). The dissociation constants (Kd) of the interaction between Cy5 and EC-rGO is calculated to be of  $5.45 \times 10^{-8}$  M, while the Kd of TPA-EC-rGO is of  $3.40 \times 10^{-5}$  M. This observation indicates that the interaction of Cy5 molecules with EC-rGO results in a large change of SPR response. Three key points should be taken into account to elucidate these phenomena: (1) dielectric constant of Cy5 molecule is larger than TPA molecule. In this case, the SPR response of Cy5 is obvious;<sup>19</sup> (2) the interaction of Cy5 and EC-rGO that caused by  $\pi$ - $\pi$  stacking is stronger;<sup>30</sup> (3) the doping of EC-rGO by Cy5 is higher than that by TPA, considering the fact that the dielectric constant of EC-rGO will be changed by a hole doping when dyes adsorb on EC-rGO.<sup>31</sup>



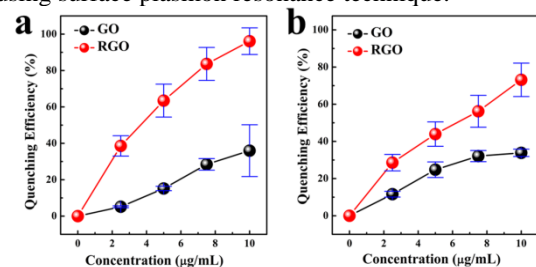
**Figure 7.** Relationship between SPR signal change and dye molecules concentrations (Cy5 and TPA) on EC-rGO substrates.



**Figure 8.** Fluorescence spectra of Cy5 (a and b) and TPA (c and d) in the presence of various concentration of GO (a and c) and RGO (b and d) solution.

### 3.4. The fluorescence quenching experiment.

Typical fluorescence quenching experiments were performed to validate our method (Figure 8).<sup>9</sup> The fluorescence emission of Cy5 and TPA are located at 660 nm and 385 nm, respectively. With the increase of GO and RGO concentration, the emission intensity of dyes gradually decreased. With 10 mg/mL RGO, more than 90% of fluorescence from Cy5 was quenched; while only 30% was quenched for GO (Figure 9a). Figure 9b shows that the quenching efficiency of RGO is much higher than GO for TPA solution. These results illustrate that fluorescence of dye can be efficiently quenched by RGO due to FRET between dye and RGO. These results support the conclusion that the study of interaction of dye with GO and EC-rGO using surface plasmon resonance technique.



**Figure 9.** Quenching efficiency as a function of concentrations of GO/RGO of Cy5 (a) and TPA (b).

## 4. Conclusions

The interaction of GBS materials with dye molecules has been studied with surface plasmon resonance technique. EC-rGO shows a much higher binding ability to dyes than GO. This result is opposed to the interaction between GBS and DNA, in which GO shows the higher binding ability than EC-rGO because that hydrogen bond is dominant for DNA-GBS interaction. However,  $\pi$ - $\pi$  stacking or molecular doping may play the key role for the interaction between dyes and GBS in this study. We developed a feasible method to in-depth understand the interaction between dye molecules and graphene oxide. These research results are beneficial for development of potential applications based on hybrid materials of dye

molecules and graphene oxide, such as energy storage, molecular imaging, biological and chemical sensing and solar cell.

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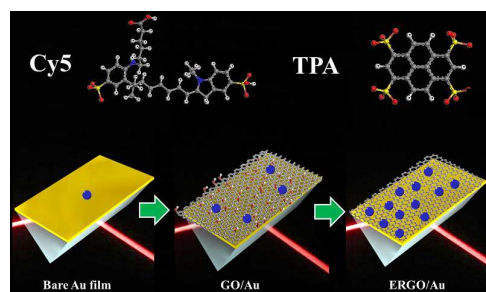
### Notes and references

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### Highlight

We developed a feasible method to in-depth understand the interaction between dye molecules and graphene oxide.