Supplementary Material (ESI) for Chemical Communications
This journal is (c) The Royal Society of Chemistry 20XX

The photoluminescent graphene oxide serves as an acceptor rather than a donor in the fluorescence resonance energy transfer pair of Cy3.5-graphene oxide

Yunxian Piao, Fei Liu and Tae Seok Seo*

Experimental Section

Graphite powders, sodium chloride (NaCl), anhydrous dimethyl sulfoxide (DMSO), potassium permanganate ($KMnO_4$) were purchased from Sigma-Aldrich (USA). Hydrochloric acid (HCl, 35%), sulfuric acid (H_2SO_4, 95%), hydrogen peroxide (H_2O_2) were purchased from JUNSEI (Japan). Tris (hydroxymethyl)-aminomethane was obtained from BIO-RAD (USA). Cy3.5 *N*-hydroxy succinimidyl (NHS) ester was obtained from KDR Biotech (Korea). PD-10 column was purchased from GE Healthcare. Cellulose acetate membrane filter was purchased from ADVANTEC (USA). All water used was purified using a millipore water purification system. All the DNA oligonucleotides were synthesized and purified by Bioneer corporation (Korea), and the sequences are in Table S1.

The fluorescence and absorbance spectra were obtained by using a spectrofluorophotometer (RF-5301, Shimadzu, Japan) and an UV-vis spectrophotometer (UV-2450, Shimadzu, Japan), respectively. AFM (atomic force microscopy) images were collected in a tapping mode using the Veeco D3100 (Veeco Instruments Inc., USA), and analyzed by a Nanoscope (R) III software (Veeco Instruments Inc., USA). The samples for AFM images were prepared by spin-coating the aqueous graphene oxide solution (0.25 mg/ml). The oxygen content in the GO was evaluated by energy dispersive X-ray spectroscopy (EDX; SEM:Nova230, FEI). The hybridization was performed using a DNA engine (Bio-Rad ,USA).

Table S1. Sequence information for double stranded DNAs.

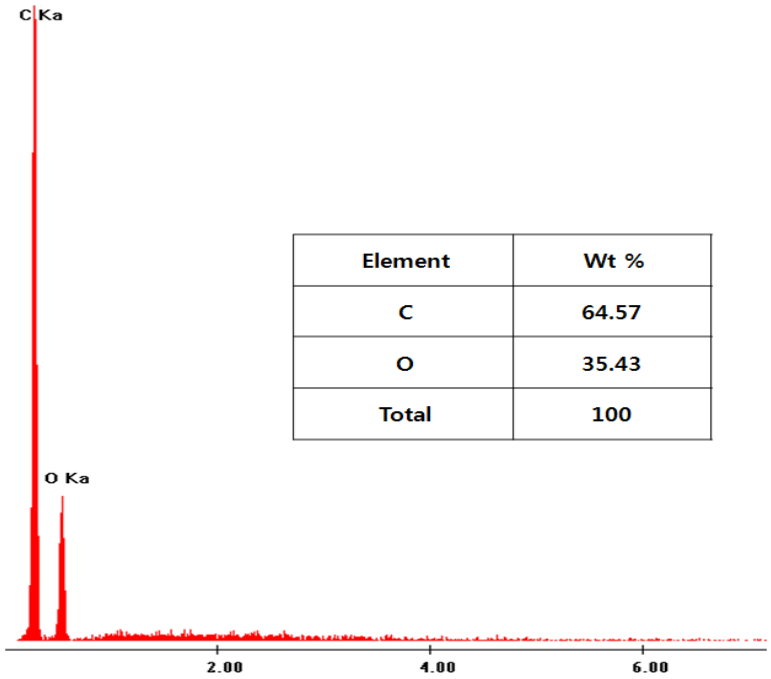
Cy3.5 labeled dsDNA	Sequences	Tm (^{o}C)	Length (nm)
5 base	5'-Cy3.5-CGC AC-3' 5'-AAAAA-GTG CG-3'	18.0	1.7
7 base	5'- Cy3.5-GGC GCA C -3' 5'-AAAAA-GTG CGC C-3'	26.0	2.4
10 base	5'- Cy3.5- GCT GGC GCA C-3' 5'-AAAAA-GTG CGC CAG C-3'	36.0	3.4
13 base	5'- Cy3.5- CGC GCT GGC GCA C-3' 5'-AAAAA-GTG CGC CAG CTC G-3'	48.0	4.4
15 base	5'- Cy3.5- GCC GCG CTG GCG CAC-3' 5'-AAAAA-GTG CGC CAG CTC GGC-3'	55.6	5.1
18 base	5'- Cy3.5-GGT GCC GAG CTG GCG CAC -3' 5'-AAAAA-GTG CGC CAC TCG GCA CC-3'	59.4	6.1

Synthesis of Nano-sized Graphene Oxide (GO)

1 g of graphite powder was ground with 50 g of NaCl for 10 min. Subsequently, the mixture was dissolved in distilled water, followed by filtration to remove the excess salt. 23 ml of H_2SO_4 was added to the remaining ground graphite powder and then stirred more than 8 h. Next, 3 g of $KMnO_4$ was slowly added at 0 ^{o}C and then mixed for 30 min at 40 ^{o}C. The mixture was additionally heated at 80 ^{o}C for 45 min. Then, 46 ml of distilled water was added and stirred at 100 ^{o}C for 30 min. The reaction was stopped by sequentially addition of 140 ml of distilled water and 10 ml of H_2O_2 (30%). Finally, the mixture was washed with 700 ml of 5 % HCl solution and then filtered by a cellulose acetate membrane filter with distilled water. The graphite oxide powders were excessively washed by distilled water followed by centrifugation until the solution became a neutral pH 7. As-prepared graphite oxide powder solution was sonicated for 2 h, and followed by centrifugation at 16000 rpm for 10 min. Finally, the monolayered small size GO was harvested from the supernatant. The oxidation status on the GO was confirmed by the energy dispersive X-ray spectroscopy as shown in Fig. S1.

Synthesis of Cy3.5-dsDNA-GO Conjugates

Cy3.5-labeled ssDNA (0.1 nmol) was hybridized with polyA tailed complementary



Element	Wt %
C	64.57
O	35.43
Total	100

Fig. S1 Energy dispersive X-ray spectroscopy (EDX) data shows the content of carbon (64.57 %) and oxygen (35.43 %) on the nano-sized GO sheets.

ssDNA (0.1 nmol) in 100 µl of hybridization buffer (50 mM Tris-HCl, 50 mM NaCl, pH 8.3). Hybridization was performed by heating the solution to 95 ^{o}C and slowly cooling to 16 ^{o}C with 10 cycles. For the polyA tailed dsDNA to be immobilized on the GO sheets, 30 µg of GO solution (0.25 mg/ml) was suspended homogeneously in a hybridization buffer (264 µl), and then Cy3.5-labeled polyA tailed dsDNA (0.1 nmol) was added. The mixture was incubated at room temperature for 1 h to induce the formation of Cy3.5-dsDNA-GO conjugates. For the fluorescence measurement, 60 µg/mL of the GO and Cy3.5-dsDNA-GO conjugate solution were used.

Quantum Yield (Φ) of GO

We determined the quantum yields (Φ = ratio of the number of photons emitted to the number of photons absorbed by the fluorophore) of GO in comparison with a diluted fluorescent standard of known quantum yield ($\Phi_{fluorescein}$ = 0.95 in 0.1 N NaOH). The quantum yield of the GO (Φ_{GO}) was determined according to the following equation:#

$$\Phi_x = \Phi_{ST}\left(\frac{Grad_x}{Grad_{ST}}\right)\left(\frac{\eta_x}{\eta_{ST}}\right)^2 \qquad (S1)$$

Φ_x is the quantum yield of a sample, and Grad is the gradient from the plot of integrated fluorescence intensity *vs* absorbance. η is the refractive index of the solvent (η_{water} = 1.33; $\eta_{1N\ NaOH}$ = 1.333). A UV-vis absorption spectrum and fluorescence emission spectrum of the sample were recorded by gradually increasing the concentrations from 0 to 0.06 mg/mL. The integrated fluorescence intensity was calculated by summing up the area of the GO emission spectrum, and a linear line was obtained by plotting the integrated fluorescence intensity *vs* absorbance. Fig. S2 showed the straight line of GO together with fluorescein (standard). The gradient of each sample (2769 for GO and 11962 for fluorescein) is proportional to the sample's fluorescence quantum yield. From the slope values, the absolute quantum yield of GO (Φ_{GO}) was calculated to be 21.9 % by using the equation S1.

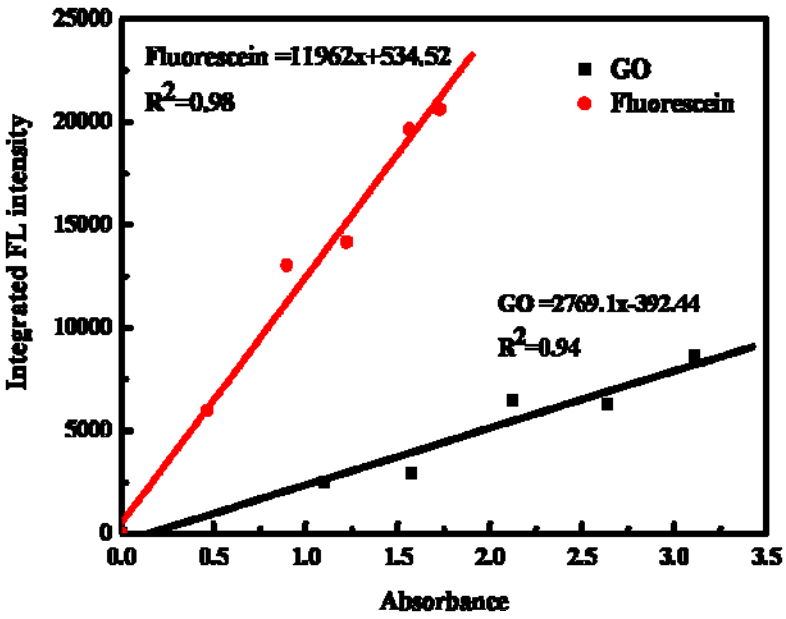


Fig. S2. Linear plot of FL intensity *vs* absorbance for GO and fluorescein.

Quantum Yield (Φ) and Quenching Efficiency (Q) of the GO in the Cy3.5-dsDNA-GO Conjugate

Based on the calculated Φ_{GO}, the quantum yield (Φ) and quenching efficiency (Q) in the Cy3.5-dsDNA-GO was measured. The emission spectra were obtained under the conditions of the sampling interval of 1 nm, the emission wavelength (λ_{Em}) range from 490 to 700 nm with the excitation wavelength of 400 nm (λ_{Ex}). Slits were set to 10 nm for both excitation and emission. And the quantum yield was determined according to

the following equation:#

$$\Phi_{DA} = \Phi_{Re}\left(\frac{A_{Re}}{A_{DA}}\right)\left(\frac{a_{DA}}{a_{Re}}\right)\left(\frac{\eta_x}{\eta_{Re}}\right)^2 \qquad (S2)$$

Φ_{DA} is the quantum yield of the individual chromophores in energy transfer (ET) pair of a donor (D; GO) and an acceptor (A; Cy3.5). And Φ_{Re} is the quantum yield of a reference sample of GO taken from the equation S1 (Φ_{GO}=0.219). A_{DA} is the donor absorbance in the ET pair and and A_{Re} is the reference sample absorbance. a_{DA} is the fluorescence peak area of individual chromophores in ET pair, and a_{Re} is the fluorescence peak area of the reference sample. η is the refractive index of the solvent. (η_{water} = 1.33, $\eta_{(50\ mM\ Tris\text{-}HCl + 50\ mM\ NaOH)}$ = 1.333). The quantum yield of GO (Φ_{GO}) and Cy3.5 ($\Phi_{Cy3.5}$) in the ET pair were shown in Table S2. Based on the obtained quantum yield, the donor (GO) quenching efficiencies (Q) in Cy3.5-dsDNA-GO conjugate were calculated according to the following equation.

$$Q_D = \left(\frac{\Phi_{Re} - \Phi_x}{\Phi_{Re}}\right) \qquad (S3)$$

Φ_{Re} is the quantum yield of a reference sample taken from equation S1 (GO, 0.219). Φ_x is the donor quantum yield (Φ_{GO}) in the ET pairs.

Table S2. Quantum yield (Φ) and donor quenching efficiency (Q_D,%) of GO in the Cy3.5-dsDNA-GO conjugates with excitation of 400 nm.

GO/Cy3.5 conjugates	Φ_{GO}	$\Phi_{Cy3.5}$	Q_D %
GO	0.219	-	-
5 base	0.192	0.092	12.48
7 base	0.198	0.085	9.94
10 base	0.210	0.103	4.46
13 base	0.211	0.110	3.53
15 base	0.210	0.110	4.23
18 base	0.217	9.976	0.98