Analysis of FRET Quenching

A single FLG plate can be modeled as a very thin, flat cylinder of width (diameter) W and height h. The sample studied has an average diameter of 800 nm and an average of 4 layers. The interlayer spacing of graphitic carbon is approximately 0.34 nm, giving h a value of about 1.2 nm. The resulting volume is $6x10^5$ nm^3 per plate.

Fluorescence resonance energy transfer (FRET) is a mechanism of quenching that can affect dye molecules that are within a certain distance (δ) of the quencher. It has been shown that δ may have a value as large as 30 nm for graphene surfaces.[1] The volume of the zone where FRET has an impact can be calculated. It is the volume of the original FLG plate extended in every direction by the value of δ (see **Figure S1**), minus the volume of the FLG plate itself.

$$V_{FRET} = \left[(W + 2\delta)^2 \cdot \frac{\pi}{4} \cdot (h + 2\delta) \right] - \left[W^2 \cdot \frac{\pi}{4} \cdot h \right] \tag{1}$$

Using the values stated, each FLG plate has a V_{FRET} of $3.5x10^7$ nm^3. The ratio of V_{FRET} to the volume of the FLG plate is 58, i.e. the FRET zone of an FLG plate is 58 times larger than the volume occupied by the plate itself.

The highest concentration of FLG in our experiments used was 100 ppm, or 0.1 mg/ml. This weight to volume ratio can be converted to a volume per volume ratio of FLG within the aqueous solution using the density of graphite (2.2 g/ml):

$$\frac{0.1mg}{ml} x \frac{1ml}{2.2g} x \frac{1g}{1{,}000mg} = 4.5x10^{-5} \text{ v/v} \tag{2}$$

Multiplying this value by 58 (and assuming that the FLG is perfectly dispersed and therefore occupying the maximum possible volume in solution that it can) gives us the fraction of total volume contained within the FRET zone, which is $2.6x10^{-3}$, or less than 0.3% of the total liquid volume in the system. This percentage similarly corresponds to the percentage of dye molecules affected, and was not considered to be a significant contribution to the total signal loss measured experimentally.

If we consider a dynamic system, additional dye molecules may be quenched if they diffuse into the FRET zone from the bulk solution. In the interest of creating a conservative estimate, we will additionally assume that molecules starting within a distance of δ and diffusing away from the FLG sheet will remain quenched.

The diffusivity (D) of a DCF molecule has been reported to be 4.1 ± 0.07 $x10^{-6}$ cm^2/s,[2] and the excited state lifetime (τ) has been reported as 3 ± 1.8 ns.[3] Given the nature of the calculation, an overestimate of 5ns was used for τ.

The distance, ℓ, a DCF molecule can diffuse during ist excited lifetime is given by

$$\ell = \sqrt{D \cdot t} \tag{3}$$

Which, using the values stated, is equal to 1.4 nm.

If we now consider V_{FRET} to extend to include everything within the distance δ, as before, as well as anything a distance ℓ away that can diffuse to be within δ of the FLG during its excited lifetime:

$$V_{FRET,extended} = \left[\left(W + (2\delta + \ell)\right)^2 \cdot \frac{\pi}{4} \cdot \left(h + 2(\delta + \ell)\right)\right] - \left[W^2 \cdot \frac{\pi}{4} \cdot h\right] \tag{4}$$

The volume increases from 3.5×10^7 nm^3 to 3.8×10^7 nm^3. Continuing through the calculation, the ratio of the FRET zone to the volume of a single FLG increases from 58 to 61. This results in a slight increase in the percentage of dye molecules affected; however, this amount is still less than 0.3%.

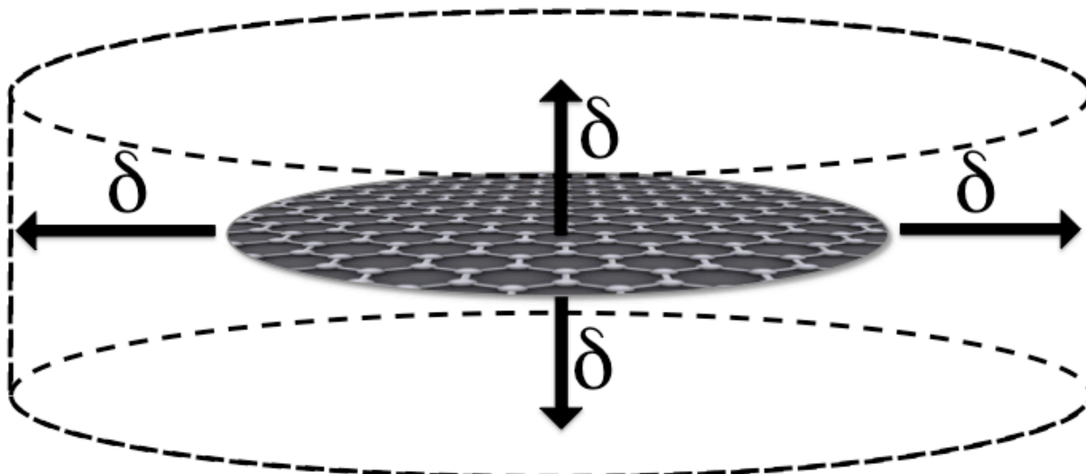

Figure S1. Illustration of the zone surrounding a single FLG plate where the FRET mechanism is active.

Analysis of Effects of Cell Uptake on Adsorptive Artifacts

Cells may internalize probe dyes, which typically causes a large increase in the dye concentration due to the small cell volume relative to the extracellular medium. The effect of increased intracellular dye concentration on the amount adsorbed by FLG is considered here to better understand how the main article results may translate to intracellular conditions.

At low concentrations of dye, the adsorption isotherm is approximately linear, described by Henry's law :

$$N = K \cdot C_f \tag{5}$$

Where N is the fraction of adsorbate on the surface of the adsorbent, K is the Henry's law constant, and C_f is the concentration of adsorbate remaining free in solution.

Applying a mass balance on the adsorbate yields the following equation:

$$N \cdot m + V \cdot C_f = C_{tot} \cdot V \tag{6}$$

Where m is the total mass of the adsorbent, V is the total volume of solution, and C_{tot} is the total concentration of adsorbent in the system (this could also be considered to be the initial concentration of adsorbent, before any adsorbs to the surface of the adsorbent). Substituting the Henry's law equation into the previous equation and rearranging leads to:

$$\frac{C_f}{C_{tot}} = \frac{1}{\left(K \cdot \frac{m}{V}\right) + 1} \tag{7}$$

In the Henry's law regime, as the total amount of adsorbate in the system ($\frac{m}{V}$) increases, the fraction that remains free in solution ($\frac{C_f}{C_{tot}}$), which is the amount that would be measured in a fluorescent or colorimetric assay, decreases, thus making the adsorptive artifacts more severe in the intracellular space.

As total amount of adsorbate in the system ($\frac{m}{V}$) increases further, at some point the adsorption isotherm will lose its linear behavior and the surface of the adsorbent can become saturated. Here the same mass balance written earlier still applies, but now N is a constant and can be denoted N_m:

$$\frac{N_m \cdot m}{V} + C_f = C_{tot} \tag{8}$$

Rearranging this expression leads to the following equation:

$$\frac{C_f}{C_{tot}} = 1 - \frac{N_m \cdot m}{C_{tot} \cdot V} \tag{9}$$

Wherein everything on the right side of the equation is a constant for a given system, meaning that $\frac{C_f}{C_{tot}}$ will reach a steady state value and remain unchanged.

This effect is illustrated in **Figure S2,** where an example isotherm is shown along with the concentration changes associated with cellular uptake of dye and adsorption on intracellular graphene. The fraction of dye adsorbed increases with dye concentration for all isotherms with a positive slope, as evidenced in the above calculations. Some of the

dye (N_{ext}) will adsorb to the surface of any extracellular graphene. As the cell takes up dye, the total concentration contained within the cell will increase to $C_{int,total}$, which will exceed the external concentration ($C_{ext,total}$). Inside the cell, DCF dye will continue to adsorb onto the surface of internalized FLG (N_{int}), leaving a smaller fraction of the total dye as the free molecule in the intracellular volume.

The effective, measurable concentration of dye is the free dye, either within the cell ($C_{int,free}$) or in the extracellular space ($C_{ext,\ free}$). As can be seen in Figure S1, these values are less than the total concentration of dye in the surrounding environment (denoted either $C_{int,tot}$ and $C_{ext,tot}$, respectively), leading to reduced fluorescent signals.

It is possible that the adsorption on graphene may approach saturation, meaning N_{int} or N_{ext} will cease to increase. Saturation thus limits the extent to which the material can cause adsorptive artifacts. While we do not see adsorptive saturation under our experimental conditions, it may occur in the intracellular space due to elevated dye concentrations.

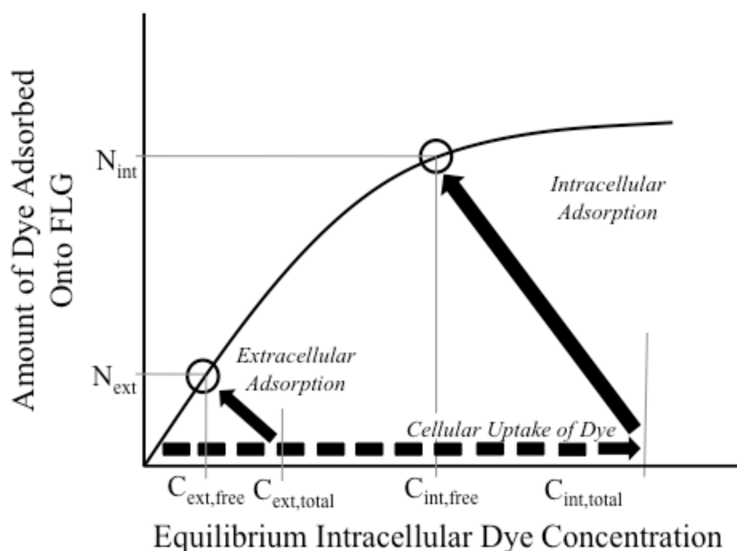

Figure S2. Adsorptive artifacts may be enhanced by cellular uptake. DCF-based dyes are often used to measure intracellular ROS, and the high intracellular concentrations of dye and graphene lead to enhanced adsorption and greatly attenuated fluorescent signals that can underreport ROS activity.

References

[1] R. S. Swathi, K. L. Sebastian, *J Chem Phys* **2009**, *130*, 086101.
[2] C. T. Culbertson, S. C. Jacobson, J. M. Ramsey, *Talanta* **2002**, *56*, 365.
[3] E. D. Bott, E. A. Riley, B. Kahr, P. J. Reid, *J Phys Chem A* **2010**, *114*, 7331.