Supporting Information

Spectroscopy and Fluorescence Lifetime Imaging Microscopy to Probe the interaction of Bovine Serum Albumin with Graphene Oxide

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(1) Measurement of the lifetime in FLIM:

The FLIM systems use a combination of multidimensional time-correlated single photon counting (TCSPC) process with confocal scanning. A pulsed laser is used to excite the sample, which is mounted on a stage of a laser-scanning confocal microscope. A confocal microscope provides better depth resolution than a standard microscope because it has a pinhole in the beam path which blocks all out-of-focus light. The fluorescence passes back through the objective lens before it is spectrally separated from the excitation light using a dichroic beam splitter (mirror) and is detected using a photomultiplier tube. The emitted fluorescence photons are detected using TCSPC, which measures the time between the excitation pulse and the arrival of each individual fluorescence photon. A histogram is built up to show the number of fluorescence photons arriving within a given time interval. This curve is fitted to exponential decay equations to obtain the fluorescence lifetime. The fluorescence lifetime provides the contrast of the image, which is the basis of fluorescence lifetime imaging. In FLIM, parallel and perpendicular polarization is not use simultaneously. The signals are detected at magic angle (54.47°). The fluorescence lifetime of an isolated fluorophore from a single excited state is given by:

$$I(t) = I_0 e^{-t/\tau_f}$$

where $I_0$ is the fluorescence intensity at time $t=0$ and $\tau_f$ is the fluorescence lifetime. FLIM measures the fluorescence decay in each pixel of an image. Once $\tau_f$ is obtained for each pixel of the specimen, a FLIM image is created by assigning a color scale to the lifetime.
Figure S1. Fitted correlation curve measures for R6G in water along with the residual.

Figure S2. UV-Visible Absorption Spectra of GO
Figure S3. FTIR Spectra of GO

Figure S4: Tapping modes AFM image of (a) GO and (b) GO/BSA conjugate deposited on a freshly cleaved mica surface and height profile diagram of GO flakes