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Successful Stabilization of Graphene Oxide in Electrolyte Solutions: Enhancement of Bio-functionalization and Cellular Uptake

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Procedures for calculation the C/O ratio of the graphene oxide. Elemental analysis (EA) was performed by Atlantic Microlabs (Norcross, GA) on lyophilized aliquots. Each sample was vacuum-dried at 80° C for 5 h to remove adsorbed water prior to being subjected to combustion (CHN) or pyrolysis (O) analysis. Karl Fischer titration was performed using a C20 Compact Karl Fischer Coulometer (Mettler Toledo, Columbus, OH). A lyophilized aliquot of the exfoliated, purified graphene oxide was vacuum-dried at 80° C for 5 h, then immediately weighed, dispersed in dry methanol, and analyzed. A pure aliquot of dry methanol was also analyzed to account for the contribution of solvent water content to the measured water concentration. The water content of the graphene oxide is then used to eliminate the contribution of water to the EA-determined C/O ratio.

Table S1. Elemental Analysis of Samples

Samples	C (wt%)	H (wt%)	N (wt%)	O (wt%)
As-prepared graphene oxide	49.5	2.0	0.0	38.0
Doubly oxidized graphene oxide	42.1	2.8	0.0	53.9

Table S2. Water Content of Samples as Determined by Karl Fischer Titration

Samples	Water content (wt %)
As-prepared graphene oxide	4.6 ± 0.7
Doubly oxidized graphene oxide	1.5 ± 0.6

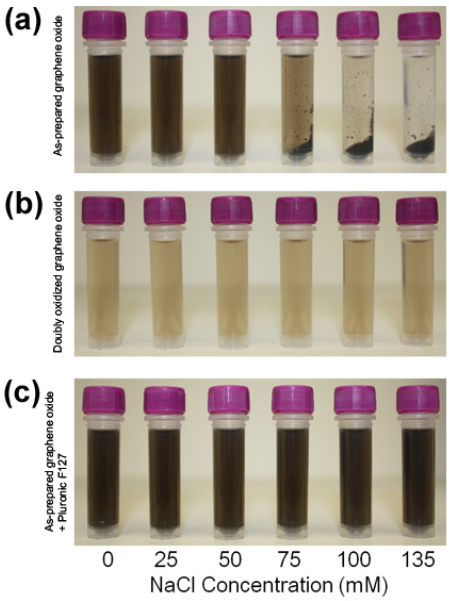

Figure S1. Digital images of solutions of: (a) as-prepared graphene oxide (0.1 mg mL^{-1}) in the presence of NaCl, (b) doubly oxidized graphene oxide (0.1 mg mL^{-1}) in the presence of NaCl, and (c) as-prepared graphene oxide (0.1 mg mL^{-1}) in the presence of Pluronic F127 (1.0 mM) and NaCl. Images were recorded 5 min after the graphene oxide formulations were mixed with aqueous solutions containing increasing NaCl concentrations (from left to right: 0, 25, 50, 75, 100, and 135 mM final salt concentrations). Samples were briefly centrifuged (~30 sec) to accentuate the extent of aggregation of the nanosheets aggregate masses at the bottom of the tube, if any occurred.

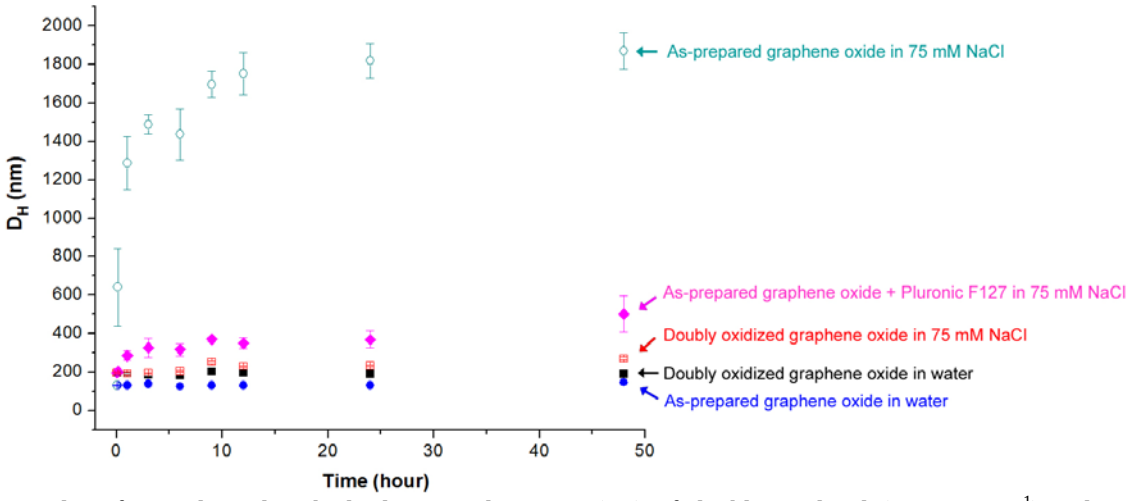

Figure S2. Plot of time-dependent hydrodynamic diameters (D_H) of doubly oxidized (0.1 mg mL^{-1}) and as-prepared graphene oxide nanosheets (0.1 mg mL^{-1}) in water and electrolyte solutions, measured by dynamic light scattering (DLS). Filled square (■), doubly oxidized graphene oxide in water; open square (□), doubly oxidized graphene oxide in the presence of NaCl (75 mM); filled circle (●), as-prepared graphene oxide in water; open circle (○), as-prepared graphene oxide in the presence of NaCl (75 mM); filled diamond (♦), as-prepared graphene in the presence of Pluronic F127 (1 mM) and NaCl (75 mM).

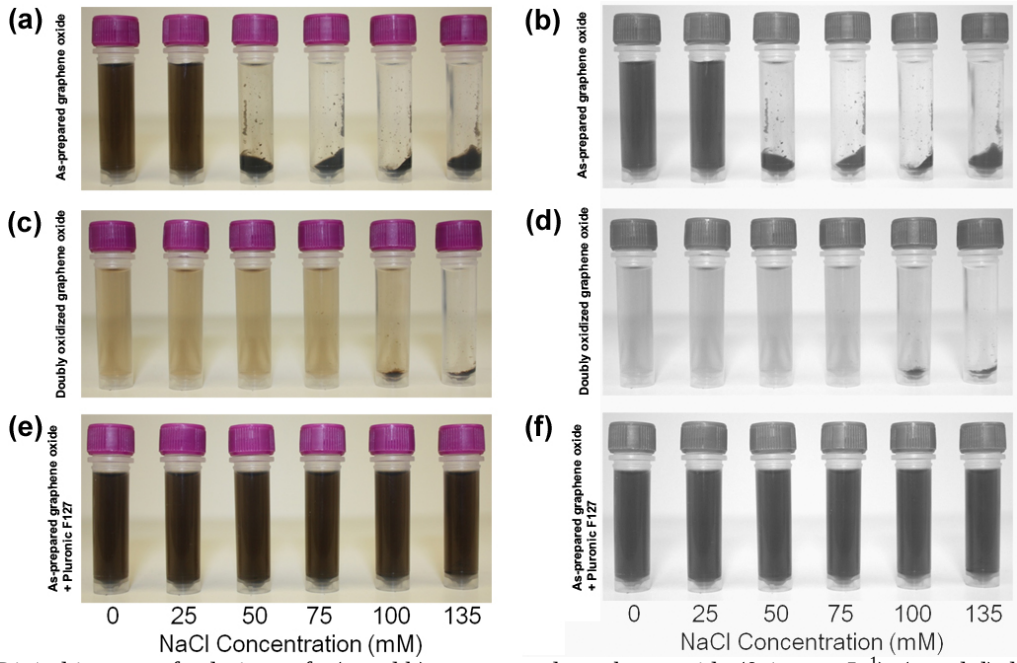

Figure S3. Digital images of solutions of: (a and b) as-prepared graphene oxide (0.1 mg mL^{-1}), (c and d) doubly oxidized graphene oxide (0.1 mg mL^{-1}), and (e and f) as-prepared graphene oxide (0.1 mg mL^{-1}) in the presence of Pluronic F127 (1.0 mM) and NaCl. The images in the right column are grayscale reproductions of the color images in the left column to better enhance contrast. Images were recorded after 2 h of incubation in aqueous solutions containing increasing NaCl concentrations (from left to right: 0, 25, 50, 75, 100, and 135 mM). Samples were briefly centrifuged (~30 sec) to accentuate the extent of aggregation of the nanosheets aggregate masses at the bottom of the tube, if any occurred.

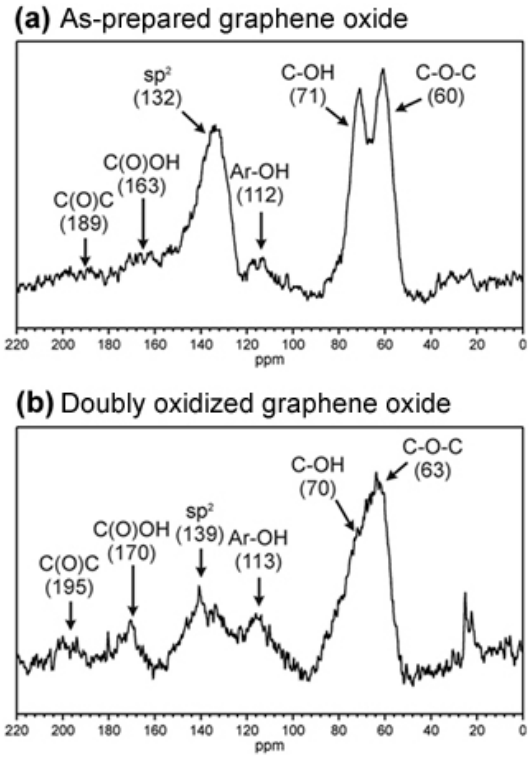

Figure S4. Solid state ^{13}C NMR spectra of: (a) as-prepared and (b) doubly oxidized graphene oxide illustrating the significant loss of graphitic sp^2 signal after the second cycle of oxidation. All discernable signals from oxygen-containing functional groups are labeled.

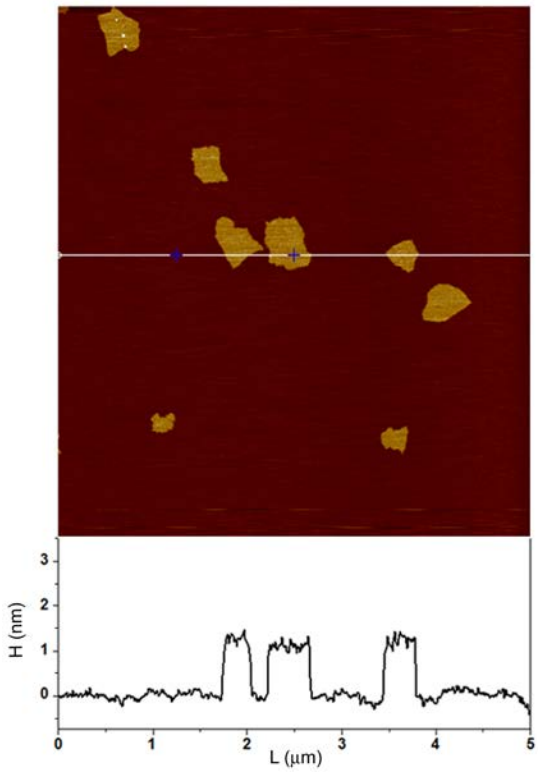

Figure S5. Top: AFM image of doubly oxidized graphene oxide sheets that have been deposited on a freshly cleaved mica plate showing similar sizes as the as-prepared grapheme oxide (Figure 4 in the main text). Bottom: A plot of the height profile for the white line in the top image.

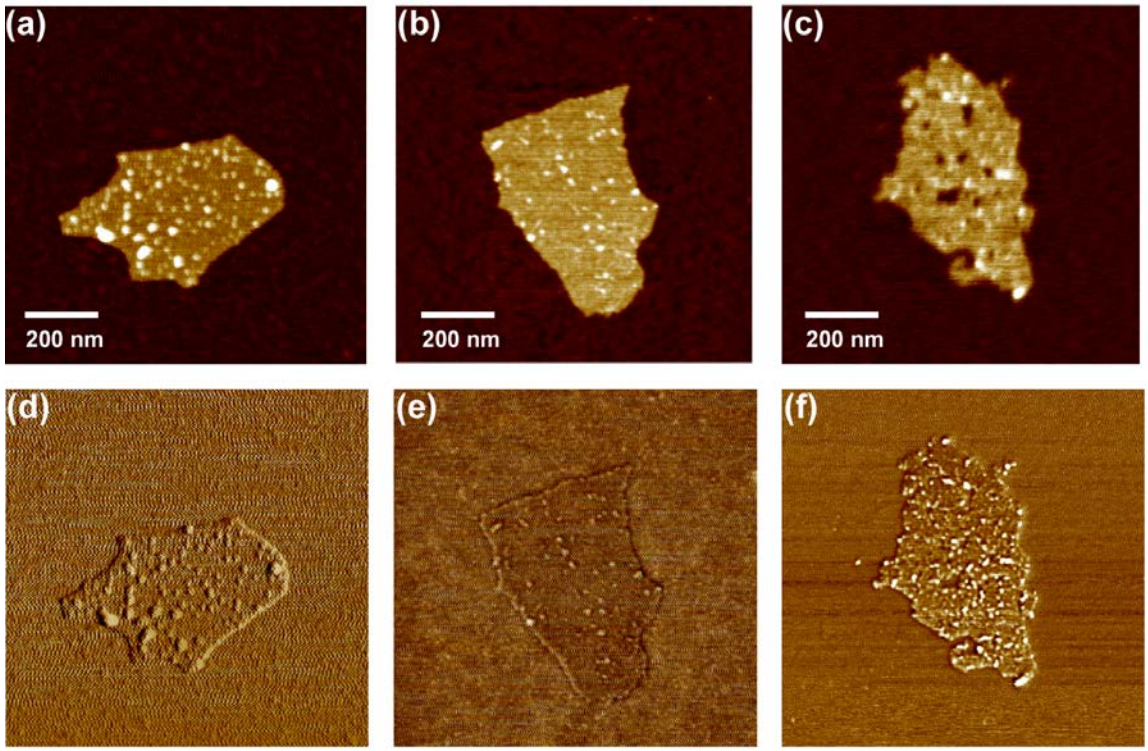

Figure S6. AFM images of as-prepared graphene oxide sheets coated with Pluronic 127 deposited on a freshly cleaved mica plate: (a-c) AFM height images and (d-f) AFM phase images corresponding to the height images in a-c. All three images are from the same batch of materials.

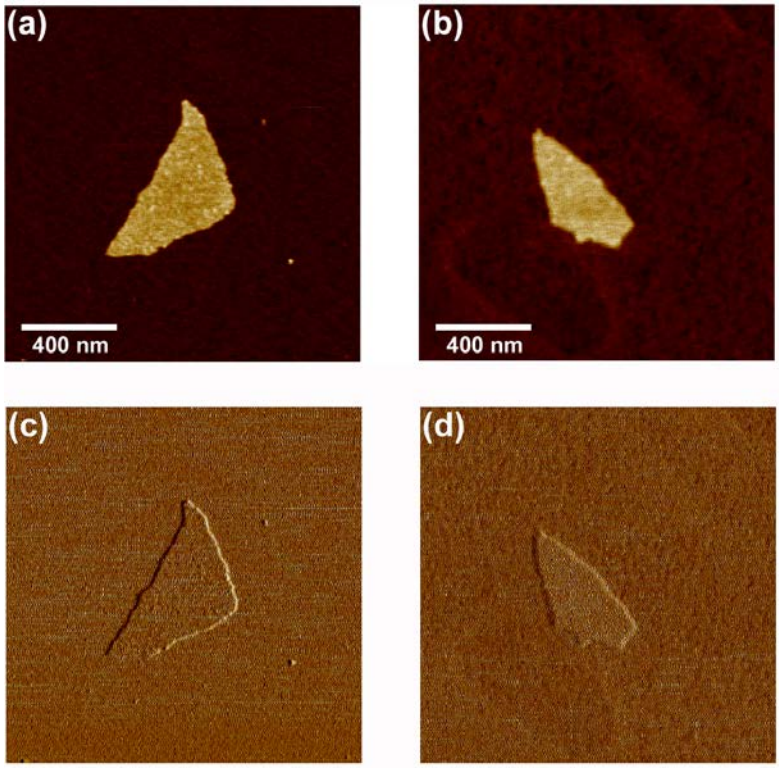

Figure S7. AFM images of as-prepared graphene oxide sheets deposited on a freshly cleaved mica plate: (a-b) AFM height images and (c-d) AFM phase images corresponding to the height images in a-b. Both images are from the same batch of materials.

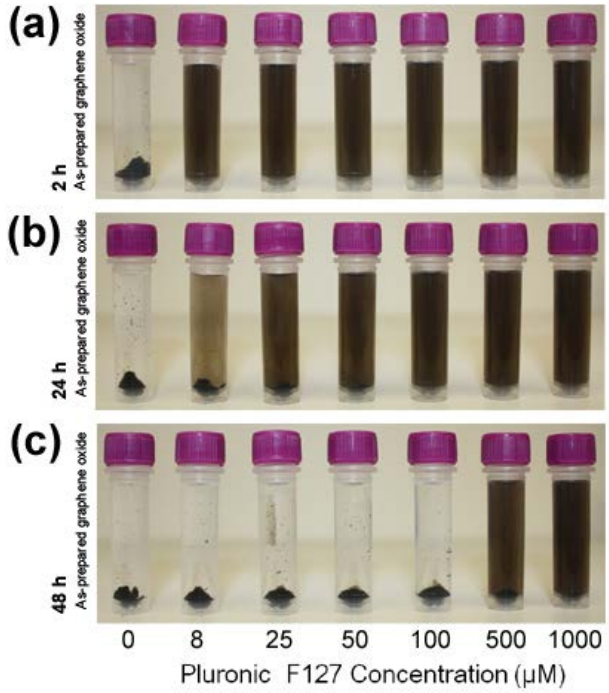

Figure S8. This is the color image of Figure 5 in the main text. Digital images of solutions of as-prepared graphene oxide dispersions (0.1 mg mL^{-1}) taken after addition of Pluronic F127 and incubation for (a) 2 h, (b) 24 h, and (c) 48 h in aqueous solutions of NaCl (75 mM). The concentrations of Pluronic F127 in the mixture are increased from left to right (0, 8, 25, 50, 100, 500, and 1000 μM) and are listed below the image. Samples were briefly centrifuged (~30 sec) to accentuate the extent of aggregation of the nanosheets into concentrated masses at the bottom of the tube, if any occurred.

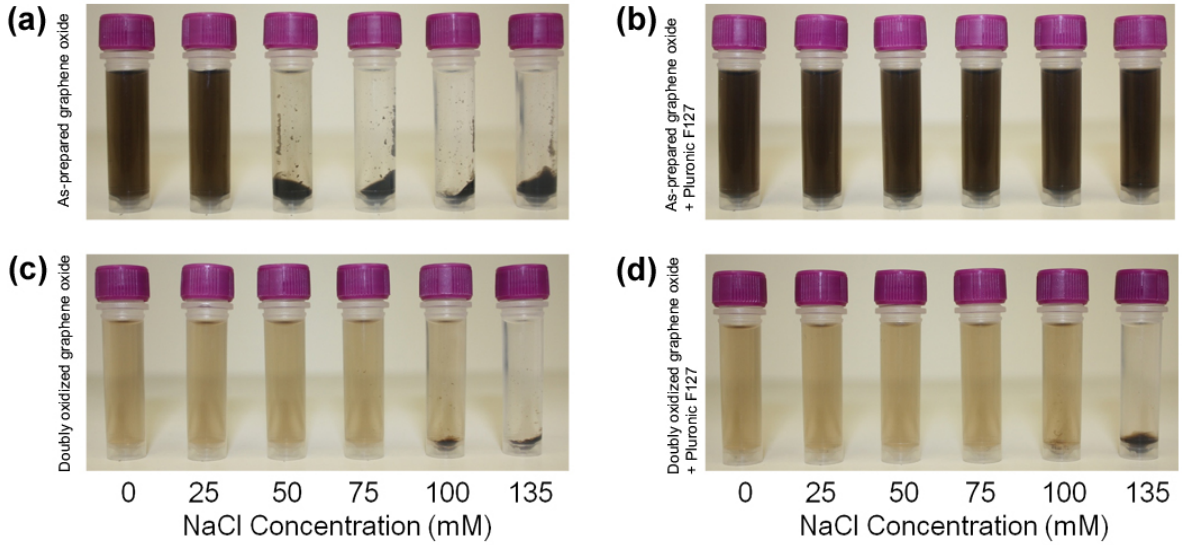

Figure S9. Digital images of solutions of: (a) as-prepared graphene oxide (0.1 mg mL^{-1}) in the presence of NaCl, (b) as-prepared graphene oxide (0.1 mg mL^{-1}) in the presence of Pluronic F127 (1.0 mM) and NaCl, (c) doubly oxidized graphene oxide (0.1 mg mL^{-1}) in the presence of NaCl, and (d) doubly oxidized graphene oxide (0.1 mg mL^{-1}) in the presence of Pluronic F127 (1.0 mM) and NaCl. Images were recorded after incubation for 2 h in aqueous solutions containing increasing NaCl concentrations (from left to right: 0, 25, 50, 75, 100, and 135 mM). Samples were briefly centrifuged (~30 sec) to accentuate the extent of aggregation of the nanosheets into concentrated masses at the bottom of the tube, if any occurred.

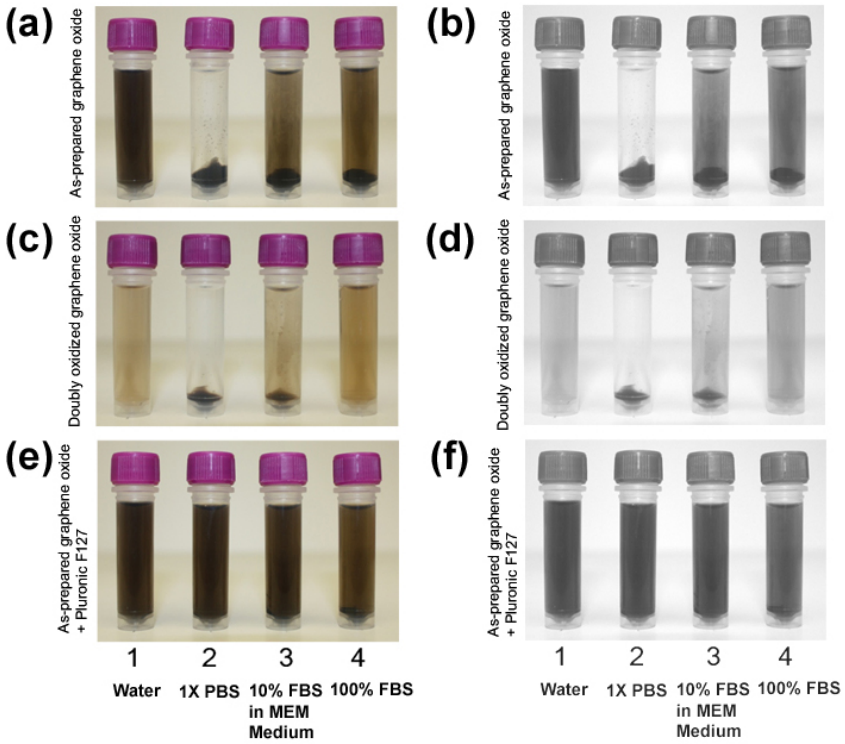

Figure S10. Digital images of solutions of: (a and b) as-prepared graphene oxide (0.1 mg mL^{-1}) in different media, (c and d) doubly oxidized graphene oxide (0.1 mg mL^{-1}) in different media, and (e and f) as-prepared graphene oxide (0.1 mg mL^{-1}) in the presence of Pluronic F127 (1.0 mM) and different media. The images in the right column are grayscale reproductions of the color images in the left column to better enhance contrast. Images were recorded after incubation for 24 h in the following media: (1) ultrapure deionized water, (2) 1× PBS, (3) 10% FBS in MEM, and (4) Pure FBS. Samples were briefly centrifuged (~30 sec) to accentuate the extent of aggregation of the nanosheets into concentrated masses at the bottom of the tube, if any occurred.

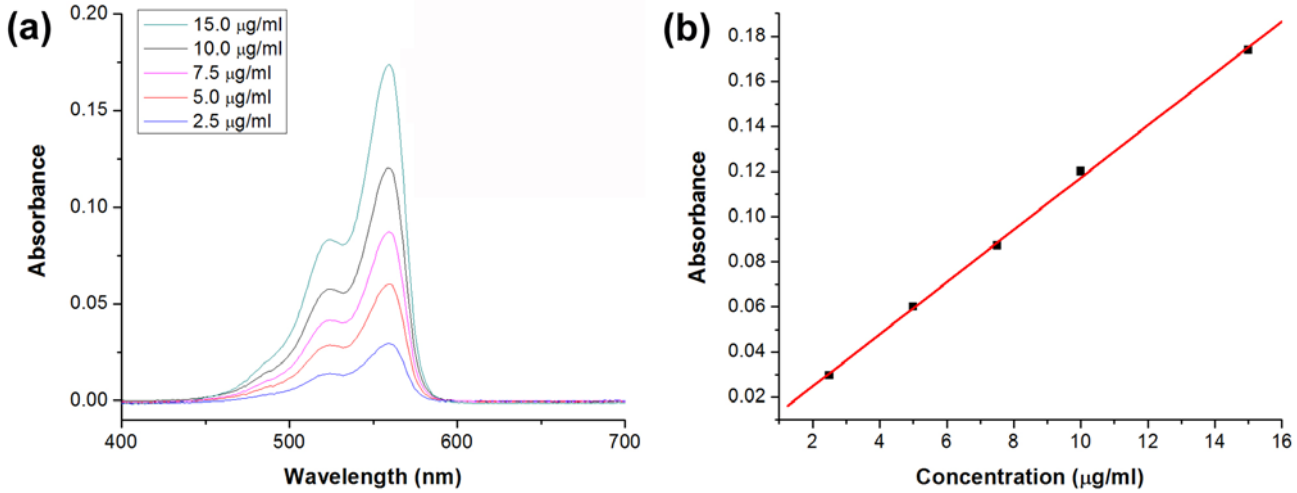

Figure S11. (a) The UV-vis spectra of Cy3-labeled streptavidins at different concentrations. (b) The calibration curve used to calculate the concentration of Cy3-labeled streptavidins coupled to graphene oxide nanosheets. The absorption maximum value at 559 nm for Cy3 was used for these calculations.

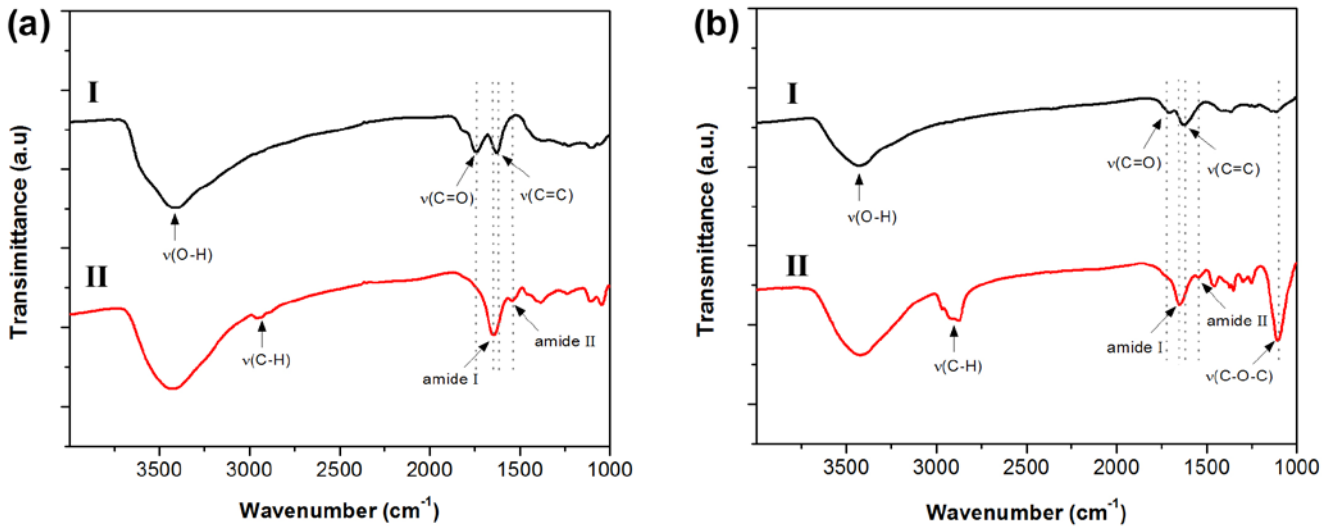

Figure S12. FTIR spectra of (a) doubly oxidized graphene oxide and (b) as-prepared graphene oxide (I) before and (II) after coupling with Cy3-labeled streptavidins. Pluronic F127 (1 mM) was used to improve dispersity of as-prepared graphene oxide during this coupling reaction. After combination with Cy3-labeled streptavidins both graphene oxide sheets show unique amide I and II vibrational peaks. The amount of streptavidins loaded onto graphene oxide samples was 12-16 wt%, which was calculated by UV-vis measurement.

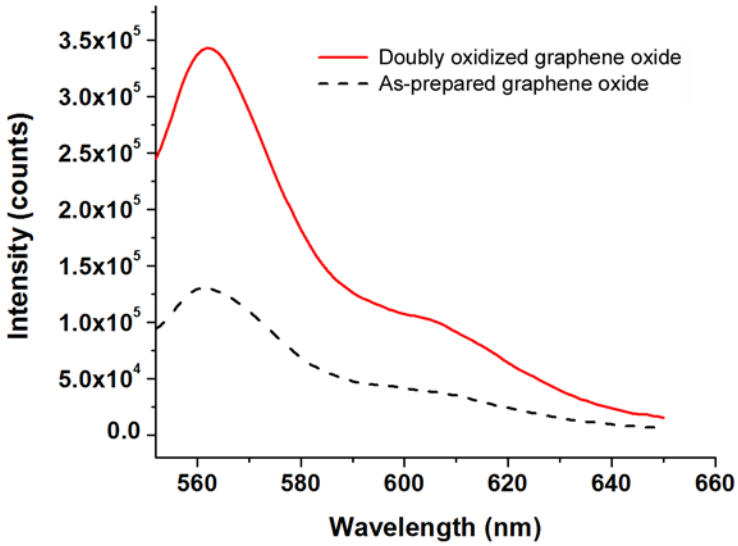

Figure S13. Fluorescence spectra of as-prepared (black dashed line) and doubly oxidized graphene oxide (red solid line) that have been coupled with Cy3-labeled, single-stranded oligodeoxyribonucleotides (ssODNs) in ultrapure deionized water. The concentration of graphene oxide is 30 μg mL^{-1} and the amounts of the ssODNs loaded on the graphene oxide are 480 and 420 pmol each for as-prepared graphene oxide and doubly oxidized graphene oxide. The excitation wavelength is 546 nm.

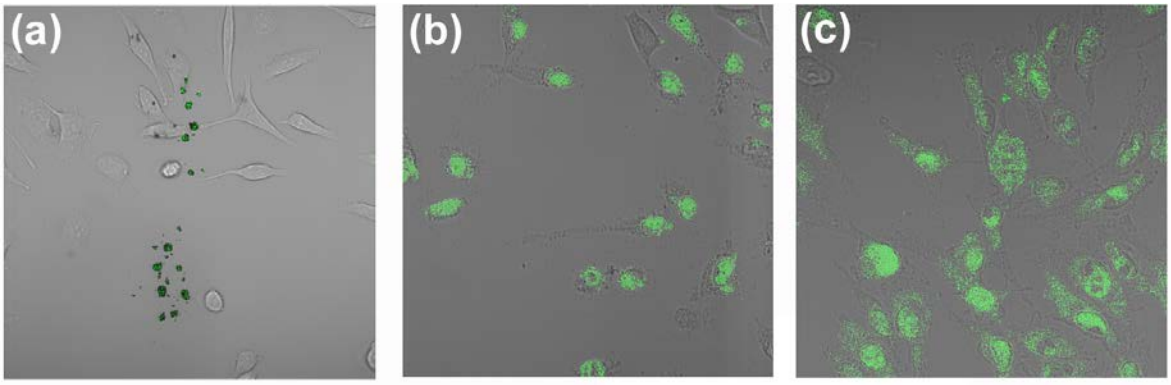

Figure S14. Confocal laser scanning fluorescent microscopy (CLSM) images, centered on the fluorescence of Cy3, of HeLa cells in fresh MEM after 2 h exposure to Cy3-labeled, ssODNs-coupled graphene oxide formulations: (a) as-prepared graphene oxide (1.7 μg mL^{-1}), (b) doubly oxidized graphene oxide (1.7 μg mL^{-1}), (c) as-prepared graphene oxide (1.7 μg mL^{-1}) in the presence of Pluronic F127 (1 mM). Image (a) clearly shows the clustering of the Cy3-labeled, ssODNs-coupled as-prepared graphene oxide as dark fluorescent spots outside the cells.

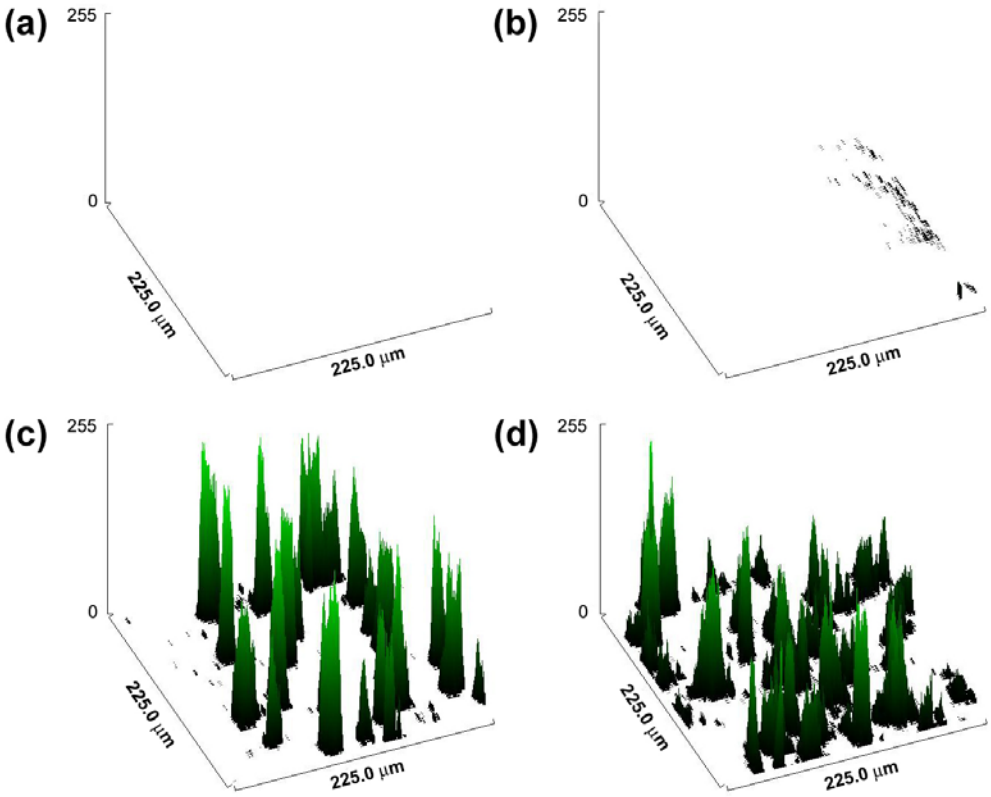

Figure S15. Fluorescence intensity distribution plots of the CLSM images in Figure 8 of the main text. (a) Fluorescence intensity distribution plot of HeLa cells shown as proof for the lack of background fluorescence in the blank sample. (b, c, and d) Fluorescence intensity distribution plots of HeLa cells in fresh cell culture media after 2 h exposure to Cy3-labeled, ssODNs-coupled graphene oxide formulations: (b) as-prepared graphene oxide (1.7 μg mL^{-1}), (c) doubly oxidized graphene oxide (1.7 μg mL^{-1}), and (d) as-prepared graphene oxide (1.7 μg mL^{-1}) in the presence of Pluronic F127 (1 mM).

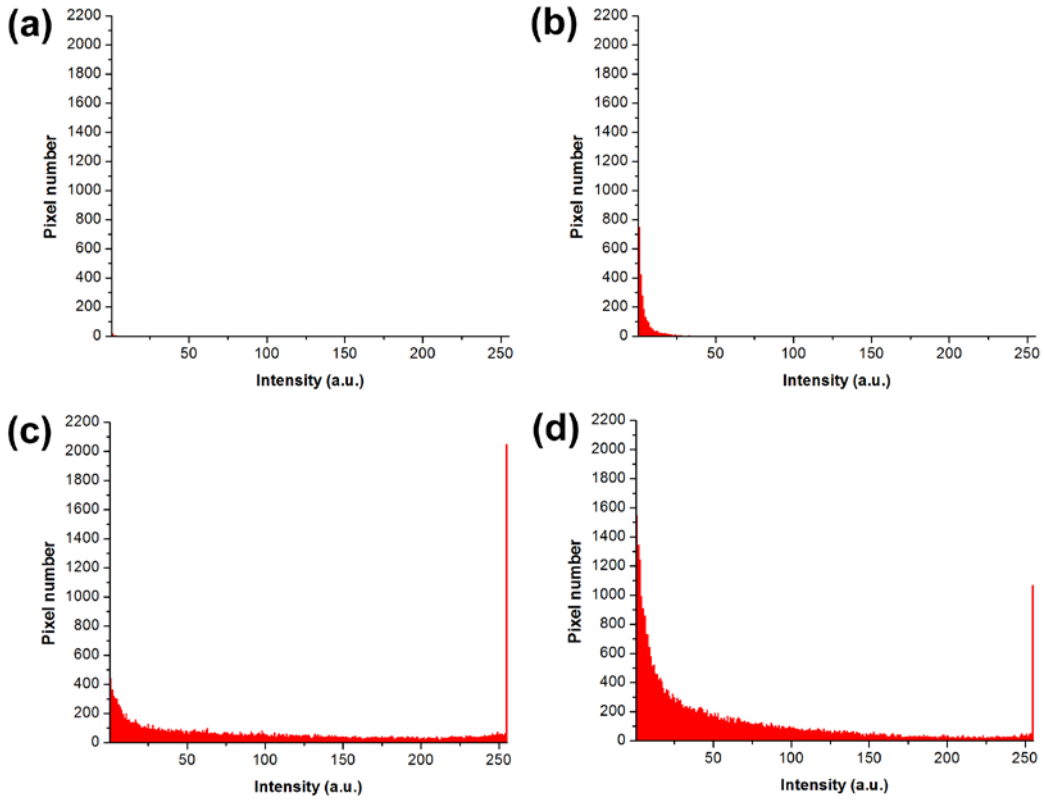

Figure S16. Fluorescence intensity histograms of the CLSM images in Figure 8 of the main text. (a) Fluorescence intensity histogram of HeLa cells shown as proof for the lack of background fluorescence in the blank sample. (b, c, and d) Fluorescence intensity histograms of HeLa cells in fresh cell culture media after 2 h exposure to Cy3-labeled, ssODNs-coupled graphene oxide formulations: (b) as-prepared graphene oxide (1.7 μg mL^{-1}), (c) doubly oxidized graphene oxide (1.7 μg mL^{-1}), and (d) as-prepared graphene oxide (1.7 μg mL^{-1}) in the presence of Pluronic F127 (1 mM).

The fluorescent intensity per cell (F_{cell}) in each CLSM image (Figure 8 in the main text) was calculated using the following formula:

$$F_{cell} = F_{tot}/N$$

F_{tot}: total fluorescence intensity in each histogram (Figure S16)
N: total number of cells in each CLSM image (Figure 8 in the main text)

The calculated F_{cell} values are 280, 79000, and 42000 for as-prepared graphene oxide, doubly oxidized graphene oxide, and Pluronic F127-stabilized as-prepared graphene oxide, respectively. Because the same Cy3-labeled, ssODNs-coupled as-prepared graphene oxide sample was used to make the corresponding Pluronic F127-stabilized materials, the F_{cell} values for as-prepared graphene oxide and Pluronic F127-stabilized as-prepared graphene oxide can be compared directly. In contrast, Cy3-labeled ssODNs coupled with doubly oxidized graphene oxide used in the CLSM study provides ~2.6 times higher fluorescence intensity at the same concentration of graphene oxide than that for as-prepared graphene oxide (see Figure S13) so a 2.6 normalization factor must be taken into account before the F_{cell} for the former material can be compared to that for the latter. Thus, the final relative F_{cell} values, a quantitative measurement of the amount of each type of graphene oxide being taken up by cells, are 1:110:150 (as-prepared graphene oxide/doubly oxidized graphene oxide/Pluronic F127-stabilized as-prepared graphene oxide), clearly showing a highly enhanced uptake for the latter two samples. In performing this calculation, we assumed that the local environment around the Cy3-labeled, ssODNs are not significantly different in the different samples.

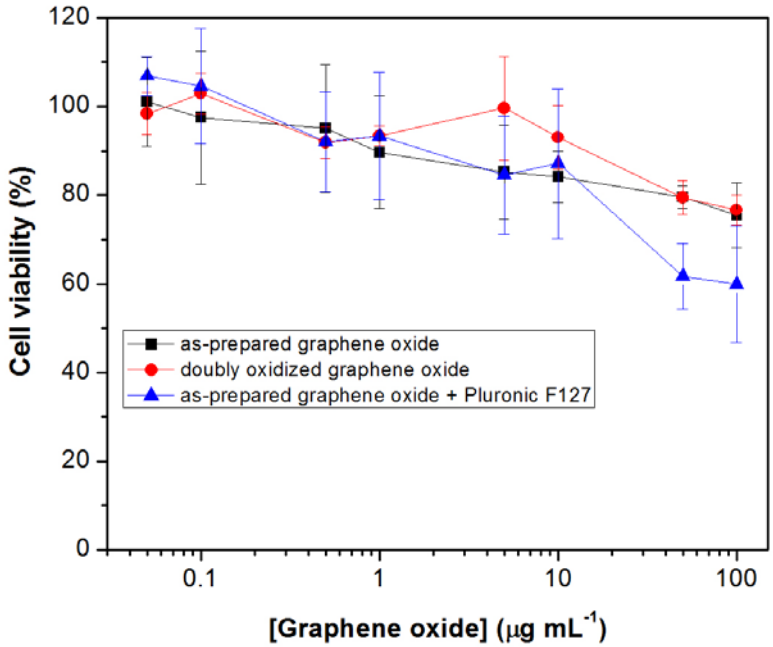

Figure S17. Cell-viability plots for HeLa cells that have been exposed to as-prepared graphene oxide (squares), doubly oxidized graphene oxide (circles), and as-prepared graphene oxide in the presence of Pluronic F127 (0.14 mg per 1.0 μg of graphene oxide) (triangles). After incubation for 48 h, cell viability was measured via an MTS cell-proliferation assay and the relative cell-survival percentages were normalized against the graphene oxide-free control before being were plotted against the graphene oxide concentration in logarithmic scale. The reported data and error bars represent three measurements from different experiments.

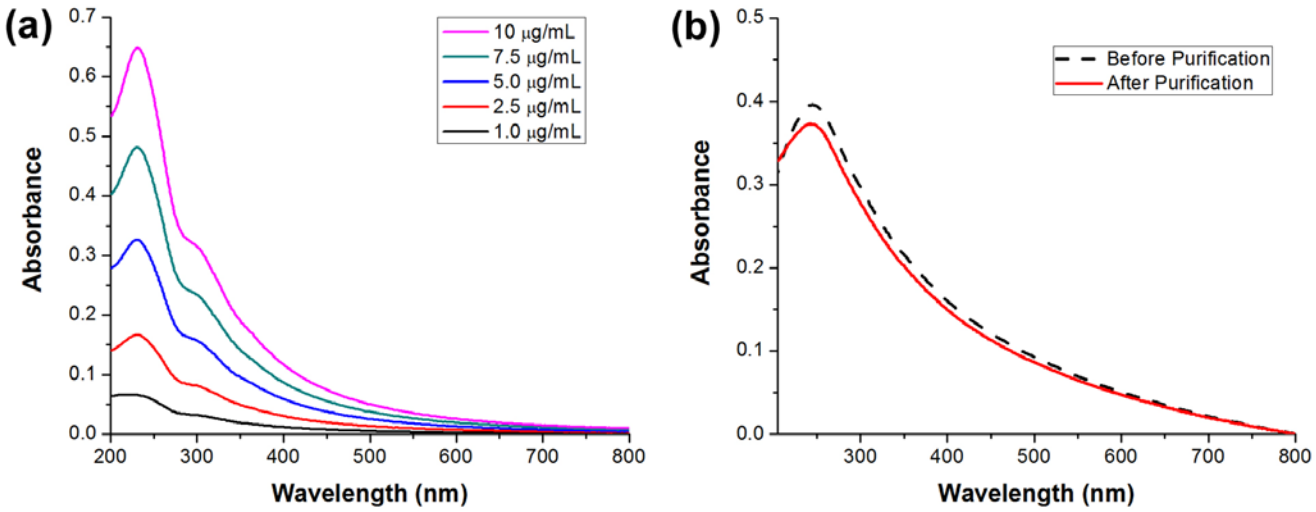

Figure S18. The UV-vis spectra of (a) as-prepared graphene oxide at different concentrations and (b) as-prepared graphene oxide before and after centrifugation filtering.