Supporting Information

Scalable Production of Highly-Sensitive Nanosensors Based on Graphene Functionalized with a Designed G Protein-Coupled Receptor

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1 Raman Spectrum of Graphene Field Effect Transistor (GFET) Channel Region

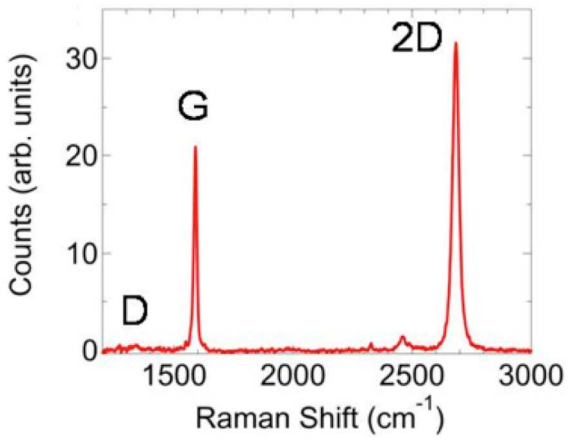

Figure S1. Raman spectrum from the channel region of a GFET device shows small D/G ratio, G/2D ratio of ~1.5, and the full width at half maximum of the 2D peak of ~30 cm^{-1}, all indicative of high quality monolayer graphene.

2 Protein Functionalization Control Experiment

To confirm the efficacy of the functionalization procedure, we performed a control measurement of the mu receptor protein (MUR) attachment density for a sample that was *not* incubated in diazonium but received the subsequent attachment chemistry. Without the diazonium anchor, the remaining chemistry proved ineffective at immobilizing MUR. As shown in Supplementary Figure 2, the density of protein attachment on the graphene sheet (3 proteins over 12 μm^2 = 0.25 proteins/μm^2) was comparable to the density seen on SiO_2 for the functionalized sample (0.55 proteins/μm^2) and significantly less than the density of proteins for the functionalized sample (4.7 proteins/μm^2). This experiment provided compelling evidence that the proteins are bound to the graphene by the covalent attachment chemistry and not simply through non-specific adsorption.

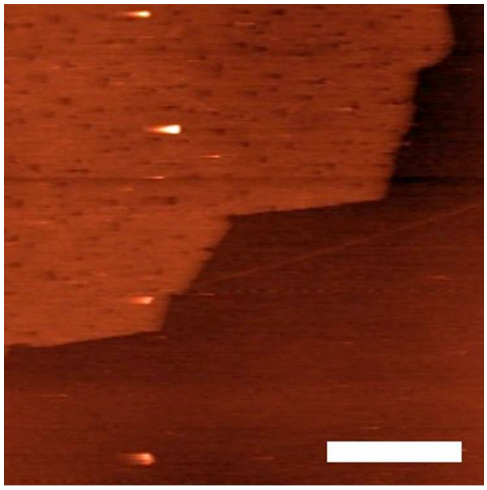

Figure S1: AFM image of graphene surface (top left) and bare SiO_2 (bottom right) after performing the protein attachment chemistry with the diazonium functionalization step omitted. The density of adsorbed proteins (3 proteins over 12 μm^2) is comparable to the density of proteins adsorbed to the SiO_2 on the functionalized sample. Scale bar is 2 µm. Vertical scale is 10 nm

3 Use of Raman Spectroscopy for Sensor Readout

Along with the electronic readout discussed in the main text, we also found that Raman spectroscopy could be used to monitor the sensors, and we observed Raman shifts that were sensitive to the presence and concentration of naltrexone. The Raman spectrum was collected from GFET channel regions after each step of the protein-functionalization process (Supp. Fig. 4). Enhancement of the D-band and a reduction of the 2D/G ratio were both observed after the diazonium incubation step, consistent with the formation of sp3 bonded sites[1]. Exposure to naltrexone led to a shift in the position of the G peak as well as the 2D peak, which are indicative of an increase in the number of charged dopants present[2, 3]. The Raman shifts varied with naltrexone concentration, and they were consistent with the shifts in the location of the Dirac voltage discussed in the main text.

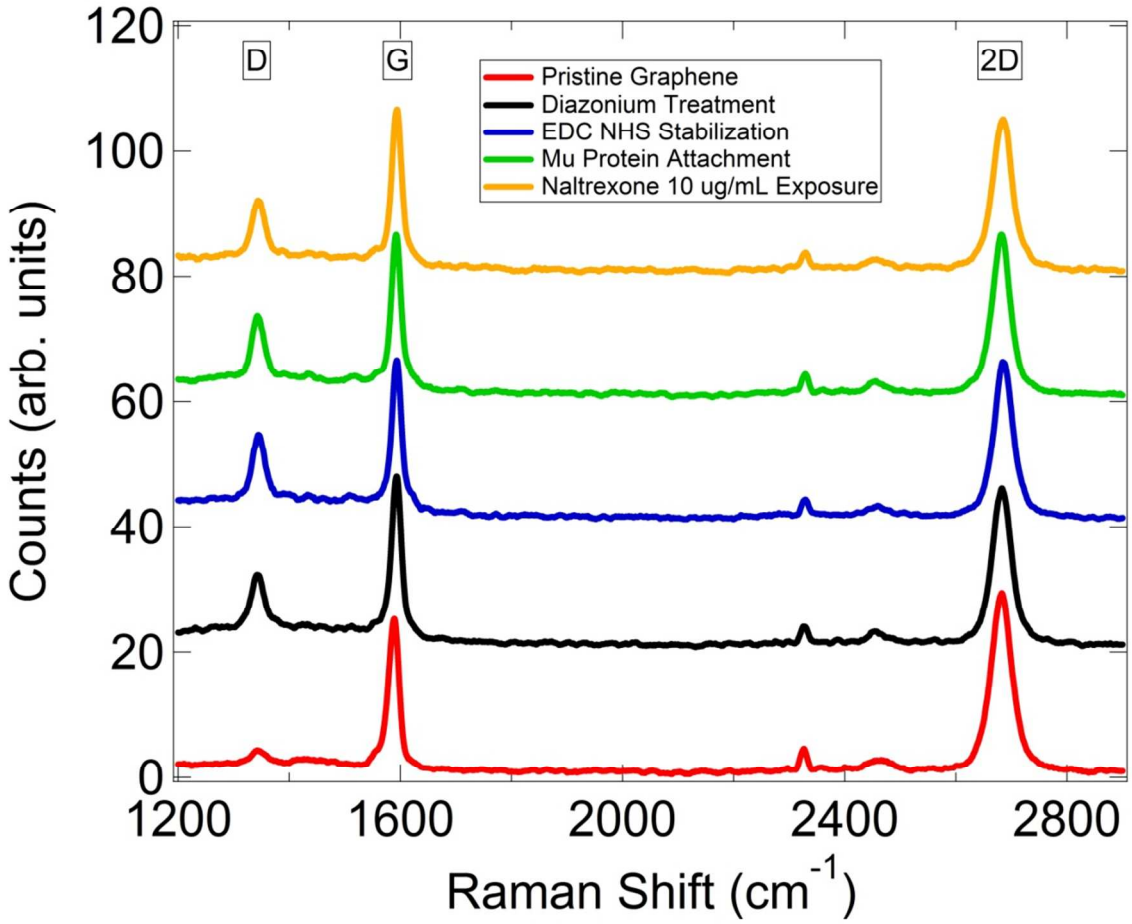

Figure S2. Raman spectra taken on GFET channel regions after successive functionalization steps. As discussed in the main text, the D/G ratio increased after diazonium treatment due to the formation of sp^3 hybridized defect sites. An additional signature of defect formation/doping was the decrease in the 2D/G ratio from 1.5 to 0.95. There was little change between diazonium treatment and mu protein attachment. Upon exposure to Naltrexone, there were significant shifts in the G-peak and 2D peak positions which were concentration dependent (Supp. Fig 4).

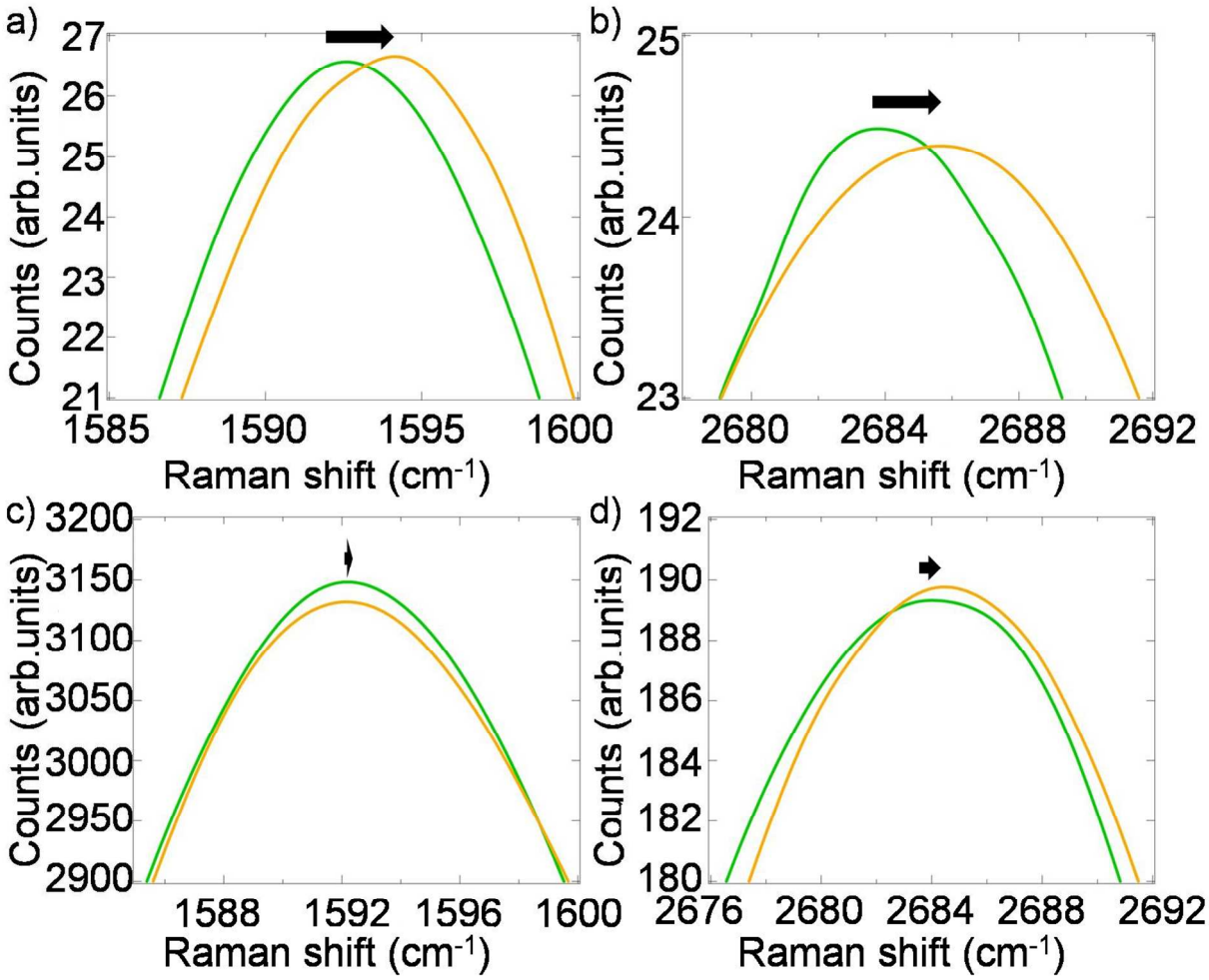

Figure S3. a) Mu protein-functionalized device showing Raman G peak shift of ~1.5 cm^{-1} before (green) and after (orange) Naltrexone exposure at 10 µg/mL. b) Same device as (a) showing a shift in the 2D peak position of ~2 cm^{-1}. c) Device from the same array treated analogously as sample from (a) and (b) but exposed to buffer not containing Naltrexone. G peak does not appreciably shift. d) The 2D peak position is only slightly affected by buffer exposure, shifting only 0.5 cm^{-1} for this device.

Table S1. Measured shift of the Raman G-peak with naltrexone concentration (7-10 GFET devices tested for each condition)

naltrexone concentration	Average G peak position after protein	Average G peak position after analyte	Average G peak shift	st error
0 (buffer control)	1592.06 ± 0.14	1592.17 ± 0.23	0.11	0.27
10 ng/mL	1590.45 ± 0.20	1591.61 ± 0.13	1.16	0.24
10 ug/mL	1592.86 ± 0.14	1594.39 ± 0.15	1.53	0.21

Table S2. Measured shift of the Raman 2D-peak with naltrexone concentration (7-10 GFET devices tested for each condition)

naltrexone concentration	Average 2D peak position after protein	Average 2D peak position after analyte	Average 2D peak shift	st error
0 (buffer control)	2683.96 ± 0.27	2684.38 ± 0.33	0.42	0.23
10 ng/mL	2682.10 ± 0.19	2683.19 ± 0.20	1.09	0.28
10 ug/mL	2683.86 ± 0.22	2685.83 ± 0.26	1.97	0.34

References

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3. Das, A.; Pisana, S.; Chakraborty, B.; Piscanec, S.; Saha, S. K.; Waghmare, U. V.; Novoselov, K. S.; Krishnamurthy, H. R.; Geim, A. K.; Ferrari, A. C.*, et al.*, Monitoring Dopants by Raman Scattering in an Electrochemically Top-Gated Graphene Transistor. *Nat. Nanotechnol.* **2008,** *3*, 210-215.