Supporting Information

Electronic Detection of Bacteria using Holey Reduced Graphene Oxide

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Experimental Details:

Materials. Antimicrobial peptide Magainin I was procured from AnaSpec (Fremont, CA, USA). The stock solution of AMP was prepared by dispersing the lyophilized product in 10 mM phosphate buffer saline (PBS). Heat-killed bacteria cells of *E. coli* O157:H7, *Salmonella typhimurium*, and *Listeria* were obtained from KPL (Gaithersburg, MD, USA). The heat-killed bacterial cells were rehydrated in 50% glycerol and diluted by PBS according to the manufacturer recommendations. Pristine and oxidized single-walled carbon nanotubes (P2- and P3-SWNTs, respectively) were procured from Carbon Solution, Inc. (Riverside, CA, USA). P2-SWNTs were purified by Carbon Solution, Inc. from as prepared SWNTs (AP-SWNT) by air oxidation and subsequently treated to remove the catalyst, leaving low functionality and low chemical doping. P3-SWNTs were purified with nitric acid and left in highly functionalized form and contains1.1 ± 0.1 mol% carboxylic acid groups, which can be derivatized with a variety of functional groups (specifications were provided by the manufacturer). Both P2 and P3 were dispersed into dimethylformamide (DMF) from their as-received powder form *via* ultrasonication.

Holey reduced graphene oxide (hRGO) was prepared following a published procedure.[12] Briefly, a sample of graphene oxide was subjected to 8 days of HRP/H_2O_2 oxidation in phosphate buffer (0.1 M, pH 7.0) to produce holey graphene oxide. Next, to reduce the oxidized carbon nanomaterial, a mixture containing 5.0 mL of 0.125 wt% holey graphene oxide, 4.8 mL of nanopure water, 200 μL of hydrazine hydrate (50 wt%) and 35 μL of NH_4OH (28 wt%) was stirred for 5 min and heated at 95 °C for 1 h. The suspension containing hRGO was subsequently dialyzed against distilled water with 0.5% NH_4OH to remove the hydrazine. Reduced graphene oxide (RGO) was prepared by reducing graphene oxide before HRP/H_2O_2 digestion and subsequently dialyzed against distilled water with 0.5% NH_4OH to remove hydrazine.

FET Measurements. Metal interdigitated devices (Au/Ti, 100 nm/30 nm) with an interelectrode spacing of 10 μm were patterned on a Si/SiO_2 substrate using photolithography. hRGO was deposited between microelectrodes using an ac dielectrophoresis (DEP) method from an approximately 0.01 mg/mL suspension in water (Angilent 33250A waveform generator, with an applied ac frequency (300 kHz), bias voltage (10 V_{pp}), and bias duration (60 s)). P3-SWNTs were deposited by the DEP method from a 0.01 mg/mL suspension in DMF (Agilent 33250A waveform generator, with an applied ac frequency (10 MHz), bias voltage (8 V_{pp}), and bias duration (60 s)).

The electrical performance of each device was investigated employing an electrolyte-gated FET device configuration. A 1 mM solution of phosphate buffered saline (PBS) was used as the electrolyte and was placed in a small fluid chamber over the FET device. Two Keithley 2400 source meters were utilized for the FET measurements. A Ag/AgCl wire was used to apply liquid gate potential (+0.6 V to –0.6 V, with respect to the grounded drain electrode unless otherwise noted). The source-drain current was measured at a constant source-drain voltage (*i.e.* 50 mV). Transfer characteristics of each device were measured (conductance (G) *versus* gate voltage (V_g)).

The transfer characteristics of bare FETs were first measured before further functionalization. AMP was then covalently functionalized to the carbon nanomaterial. The oxygen-containing functional groups on the deposited hRGO or P3-SWNTs were first activated using a solution of 100 nmol EDC and 25 nmol NHS in buffer containing 50 mM MES (pH 5.5) for 30 minutes. The FET devices were then soaked overnight with 1 mL of 2 μM AMP solution in PBS (pH 7). After the overnight incubation with AMP solution, the devices were thoroughly rinsed with PBS to remove any non-specifically bound AMP, and their transfer characteristics were measured. Non-specific binding was prevented by incubating devices for 1 h with Tween 20 (0.1%), then rinsing thoroughly with PBS to remove excess Tween. Transfer characteristics were measured again after this treatment. The

bacterial cells solutions were prepared according to the dilution guidelines provided by the manufacturer. The devices were incubated with bacterial cell solutions for 1 h before additional measurements were conducted.

Imaging. Bright field and fluorescence images were collected with an Olympus (Center Valley, PA, USA) IX81 inverted fluorescent microscope. Cells were first stained by incubating with a 1 μM solution of propidium iodide (PI) in 10 mM PBS for at least 1 h. Fluorescence images were collected through a red fluorescent protein (RFP) filter (excitation 590-650 nm, emission 663-737 nm) with an exposure time of 200 ms. Scanning electron microscopy (SEM) and EDX spectra were performed on a Philips (Andover, MA, USA) XL30 FEG microscope at an accelerating voltage of 10 keV to monitor the attachment of bacterial cells to the device surfaces. Before imaging, FET devices were sputter coated with Pd to increase image contrast. Atomic force microscope (AFM) images were obtained using a Veeco (Plainview, NY, USA) Nanoscope II scanning probe microscope in tapping mode configuration using AppNano (Mountain View, CA, USA) N-type Si tips (<10 nm radius). hRGO was spin-coated onto a substrate consisting of a freshly cleaved sheet of mica that was first treated with poly-L-lysine. The image of bare hRGO was taken after 45 min of drying in ambient conditions. AMP functionalization and *E.coli* O157:H7 incubation was completed following the procedure described for FET functionalization. After incubating with bacterial cells, the mica surface was washed three times with PBS and allowed to dry under ambient conditions for 45 min before the image was obtained.

EDX Spectra were obtained using a JEOL (Peabody, MA) JSM-6510 SEM equipped with a large area SDD EDX detector. 3 spectra at different sample locations at 10,000x magnification were collected and averaged.

XPS Spectra were obtained using a Thermo Scientific (Pittsburgh, PA, USA) K-Alpha using monochromated Al Kα x-rays (1486.6 eV) as the source, with a spot size of 400 μm and pass energy of 50 eV. Samples of hRGO and RGO were first dialyzed versus nanopure water to remove any excess hydrazine and ammonium hydroxide from the reduction step, and then dropcasted onto an aluminum substrate heated to 120 ^{o}C. XPS binding energies were corrected using the C1s peak at 285 eV as an internal standard. Spectra were fitted after background subtraction of a Shirley type baseline. Peak shapes were optimized by using a Gaussian:Lorentzian ratio of 50:50 with a full width half maximum of 0.5-1.6 eV.

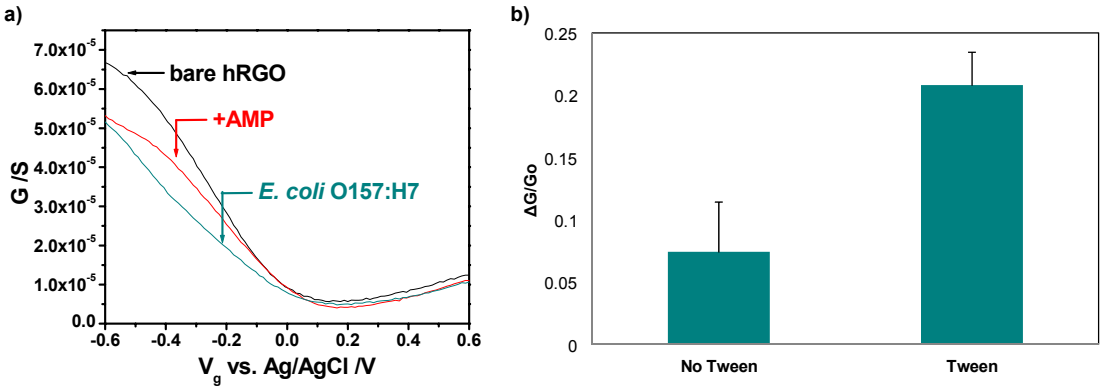

Figure S1. Effect of Tween 20 on device performance. a) Conductance (G) vs. gate voltage (V_g, swept from +0.6 V to –0.6 V) of bare hRGO FET device, after incubation with AMP, and after incubation with 10^7 cfu/mL *E. coli* O157:H7. b) Comparison between response of a device at –0.5 V_g to *E. coli* with and without usage of Tween 20 as a blocking buffer. Results are average between 4 devices and error bars represent one standard deviation.

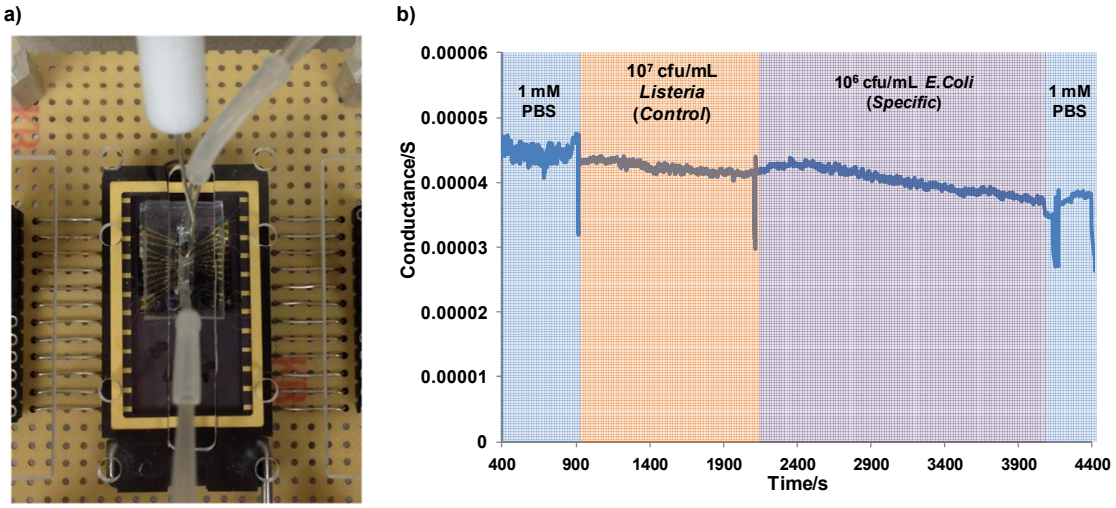

Figure S2. Flow cell experiment to determine length of incubation. a) Photograph of flow cell setup. A PDMS channel is placed on top of a 6-device chip with inlet/outlet tubing and Pt wire gate electrode inserted into the channel. PBS or desired analyte is pumped through the channel using syringe pumps at a rate of 0.05 mL/min. b) Conductance vs. time trace of a functionalized hRGO device at a constant gate voltage (–0.5 V_g). Exposure to 10^6 cfu/mL *E. coli* yields 90% response in less than 25 minutes. Bacteria were allowed to incubate for 1 hr in static solution to ensure full response from bacteria were obtained at each concentration.

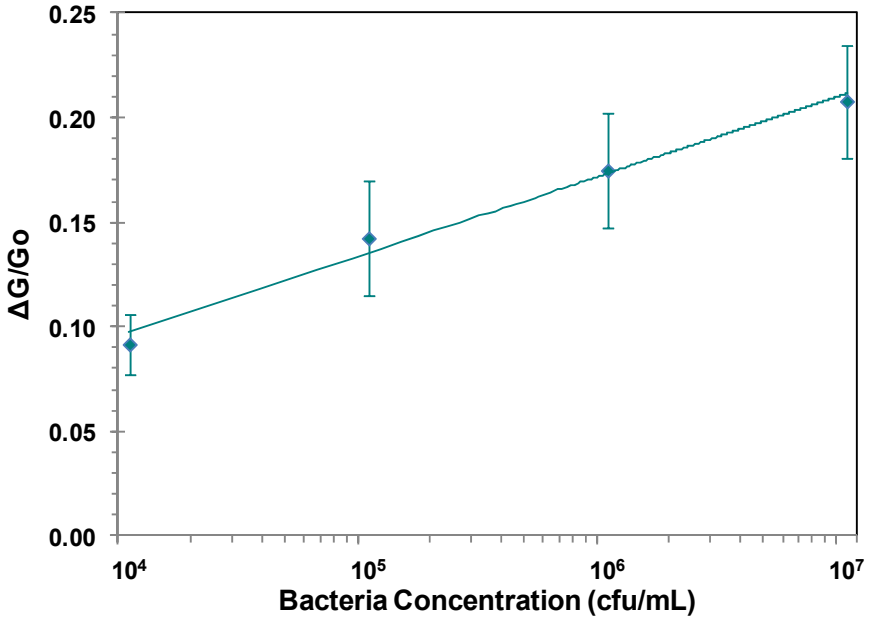

Figure S3. Calibration plot to determine limit of detection. Relative response at V_g = –0.5 V across the tested concentration region. Limit of detection, c_m, was calculated to be 803 cfu/mL using the relationship

$$c_m = 10\text{^}\left(\frac{ks_b}{m}\right)$$

Where m is the line slope, 0.0381, s_b is the standard deviation from the rinse experiment (blanks) of 0.0369, and k has a value of 3.

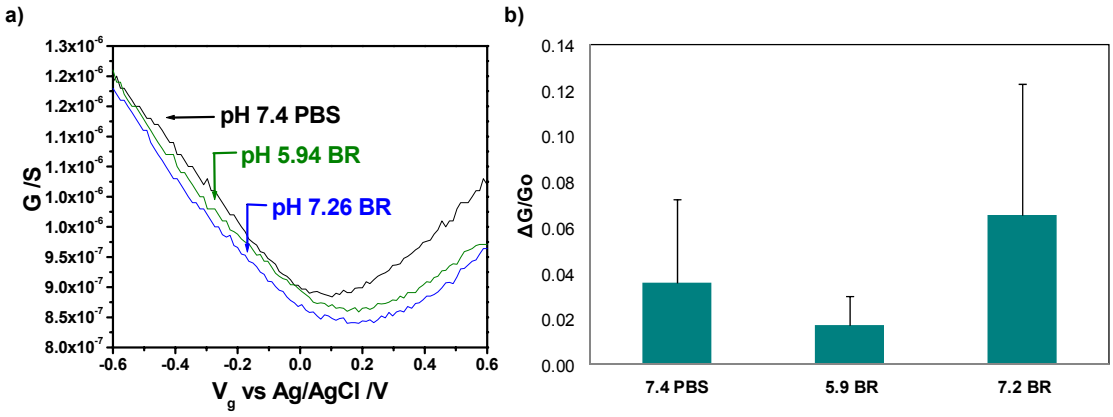

Figure S4. Changes in device characteristics due to pH and ionic strength a) Conductance (G) vs. gate voltage (V_g, swept from +0.6 V to –0.6 V) of a functionalized hRGO FET device in pH 7.4 PBS, pH 7.26 Britton Robinson (BR) buffer , and pH 5.94BR buffer. b) Relative response to varying buffer conditions. Error bars indicate one standard deviation with n = 6 for pH 7.4 PBS, and n = 2 for BR buffers.

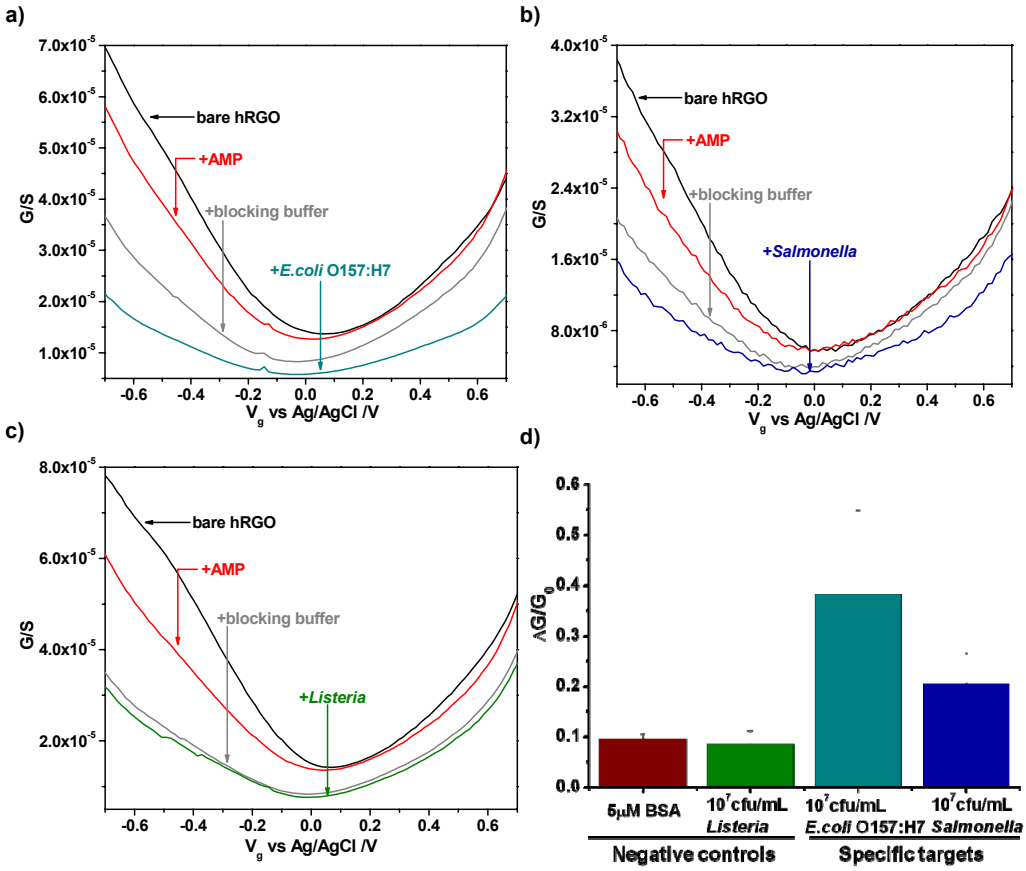

Figure S5. Additional experiment including *Salmonella*, a gram-negative bacterium also specific to Magainin I. Conductance (G) vs. gate voltage (V_g, swept from –0.75 V to +0.75 V) of bare hRGO FET device, after incubation with AMP, after incubation with blocking buffer and after incubation with (a) 10^7 cfu/mL *E.coli* O157:H7, (b) 10^7 cfu/mL *Salmonella typhimurium*, and (c) *Listeria*. (d) Comparison of the normalized responses at V_g = –0.5 V of Magainin I functionalized hRGO FET devices to controls: *Listeria* and 5 μM bovine serum albumin (BSA, a non-specific protein, raw data not shown); and specifics: *E.coli* O157:H7 and *Salmonella*. (Averaged from 4 devices; error bars indicate one standard deviation).

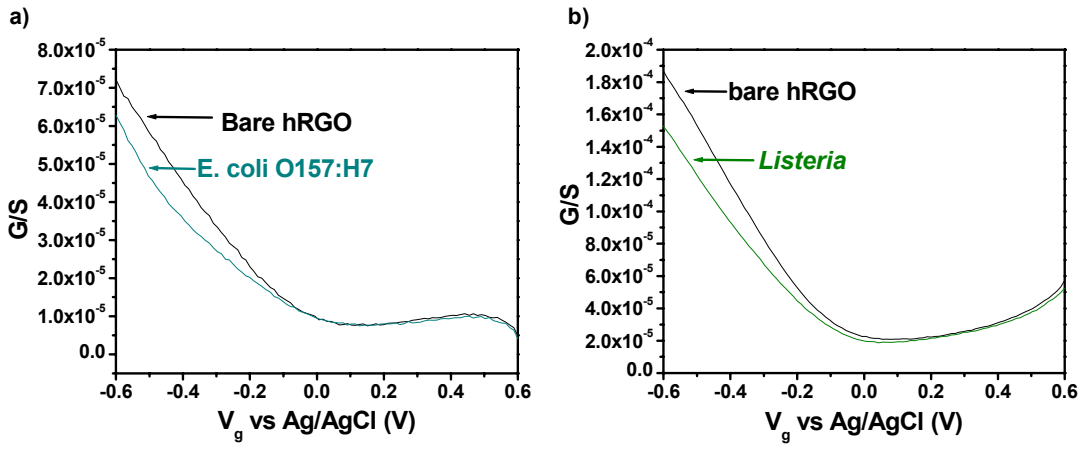

Figure S6. Response of bare hRGO to bacteria. Conductance (G) vs. gate voltage (V_g) of bare hRGO FET device before and after exposure to (a) 10^7 cfu/mL *E. coli* O157:H7 and (b) 10^7 cfu/mL *Listeria.*

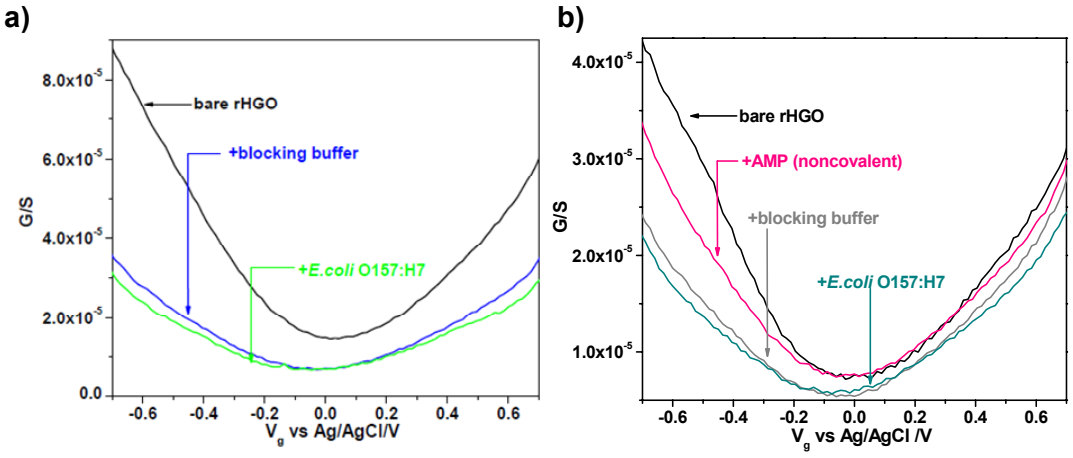

Figure S7. Raw FET data for effect of covalent attachment. Conductance (G) vs. gate voltage (V_g, swept from –0.75 V to +0.75 V) of bare hRGO FET device (a) after both incubation with blocking buffer and attachment of 10^7 cfu/mL *E. coli* O157:H7 and (b) after incubation with AMP without the carboxylic group activation step, incubation with blocking buffer, and incubation with 10^7 cfu/mL *E. coli* O157:H7.

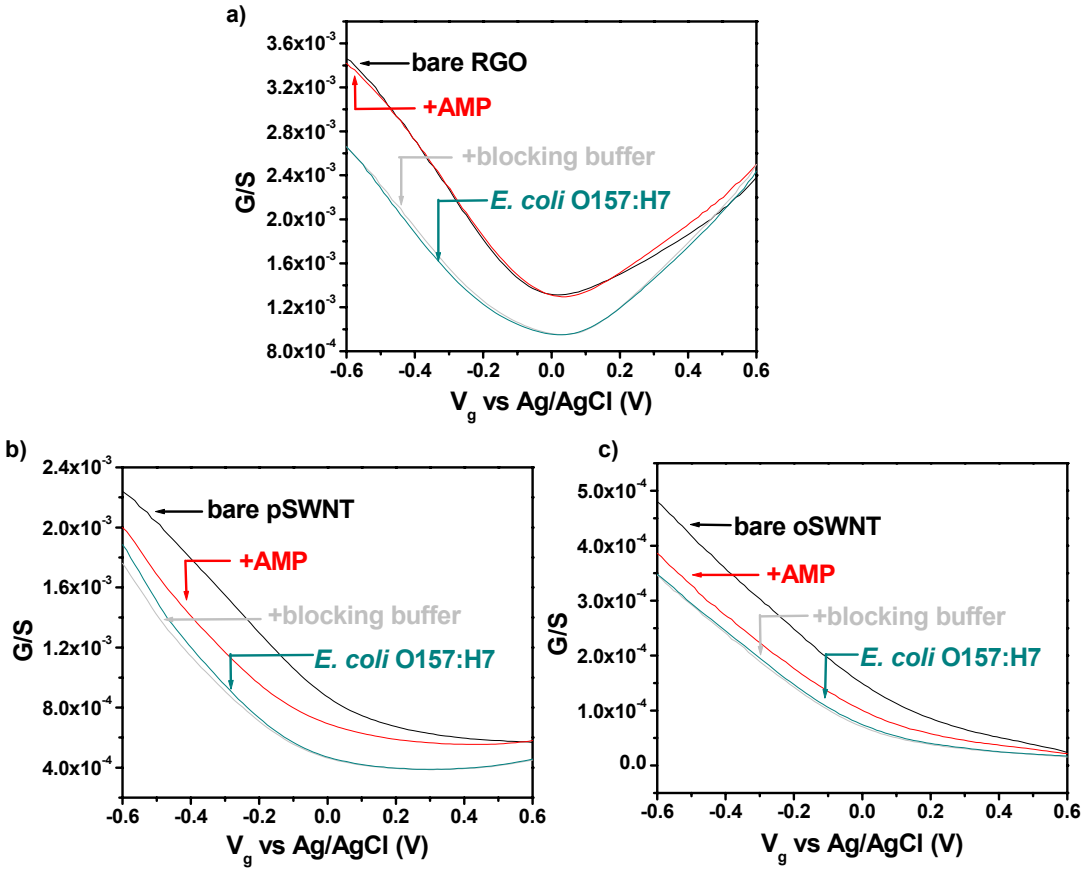

Figure S8. Comparison between carbon nanomaterials. FET curves taken before functionalizing (a) RGO, (b) pSWNT, and (c) oSWNT, after incubation with AMP, after blocking with blocking buffer, and after exposure to 10^7 cfu/mL *E. coli* O157:H7

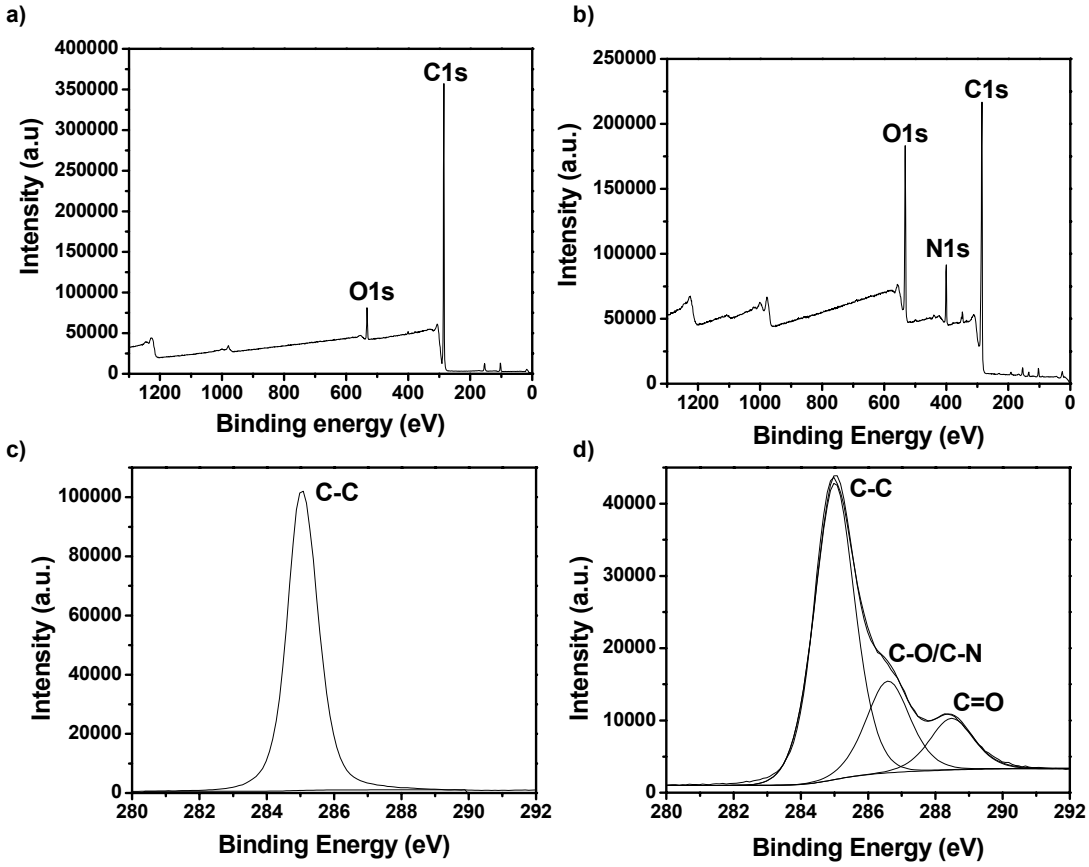

Figure S9. XPS spectra of RGO and hRGO. Survey scans of a) RGO and b) hRGO. High-res C1s scans of c) RGO and d) hRGO. RGO contains a total of 4.32 atomic% O, while hRGO contains 25.2 atomic% O. hRGO also contains 10.5% N as a result of the binding of NH_4^+ or hydrazine to carboxylic acid groups which was not fully removed by dialysis.

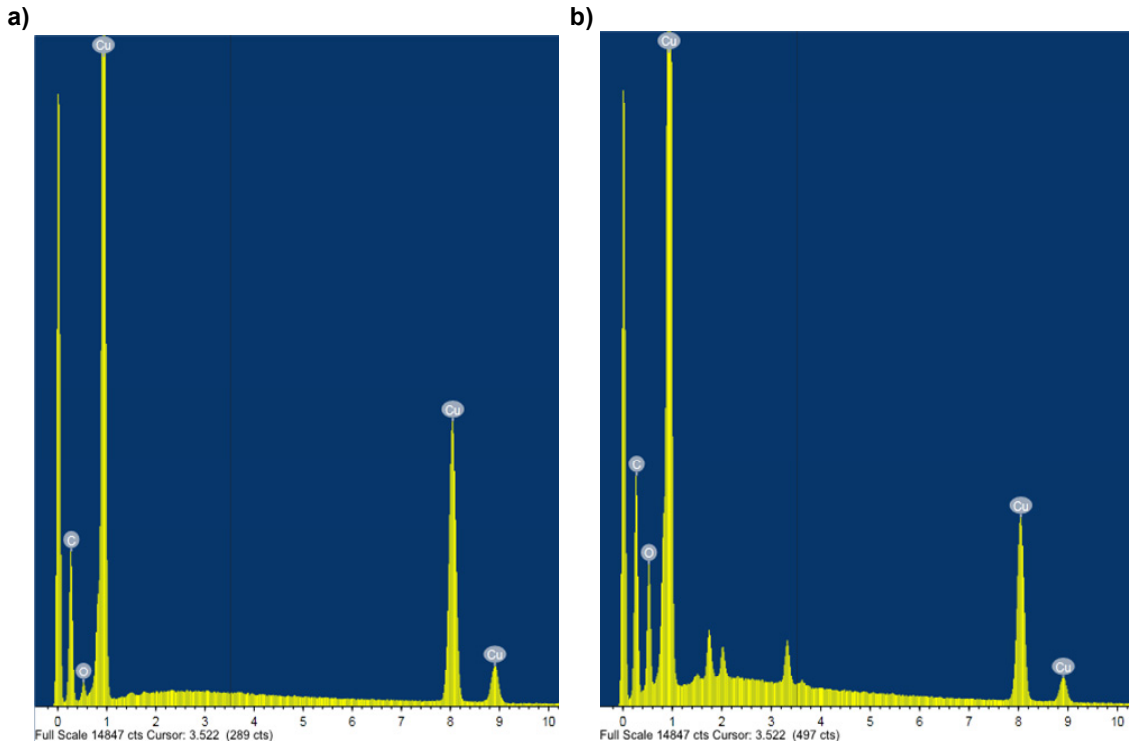

Figure S10. EDX spectra of a) RGO and b) hRGO. RGO contains 4.8 ± 0.5 at% O averaged from two samples, and hRGO contains 21 ± 3 at% O, averaged from three samples.