Electronic Supplementary Material (ESI) for Materials Horizons.

Supplementary Information:

Spatially isolated redox processes enabled by ambipolar charge transport in multi-walled carbon nanotube mats

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Experimental Section

Materials: All materials were purchased from Sigma-Aldrich (Australia) and used as is unless otherwise specified. All buffers were made using Milli-Q water (18.2 MΩ cm).

MWCNT mat embedded PDMS fabrication: A stock solution of MWCNTs (Cheap Tubes Inc, USA) suspended in chloroform (0.2 mg mL^{-1}) was sonicated for 10 to 20 minutes in a bath style ultrasonic cleaner (ELS030H TECHSPAN® Group, operating frequency 37 Hz, maximum power of 320 W). Then, either 2.5 mL, 5 mL or 10 mL of the MWCNT stock solution was diluted to 25 mL with chloroform and ultrasonicated for an additional 10-20 min. Each MWCNT suspension (25 mL) was then vacuum filtered through a poly(propylene) (PP) filter membrane mask (0.45 µm, 10 mm^2) onto a poly(vinylidene difluoride) (PVDF) filter membrane (0.2 µm pore size), followed by removal of the PP mask to reveal the MWCNT mat (10 mm^2) on the PVDF. Sylgard 184 (10:1, base:curing agent) (Dowsil, USA) was then poured on top of the MWCNT mat (or a gold foil in the case of gold foil bridged devices) on the PVDF. The Sylgard 184 was then cured at 80 ^oC for 1 h and the PVDF removed to form a PDMS embedded MWCNT mat. Each MWCNT suspension (25 mL) then corresponded to MWCNT mat loadings of 5 µg mm^{-2}, 10 µg mm^{-2} and 20 µg mm^{-2}, respectively.

Device fabrication: Two microwells (each 12 mm in diameter and 1.25 mm apart) were fabricated by placing 3D printed plastic moulds (12 mm in diameter, 1 cm high) inside a 5 cm diameter ring on top of a silicon wafer and Sylgard 184 (6:1) poured onto the top. Overflow of the Sylgard 184 was prevented by the ring. The Sylgard 184 was then partially cured at 60 ^oC for

25 min and then removed from the silicon wafer to produce a free-standing partially cured PDMS layer. The partially cured PDMS should be firm but slightly sticky when removed from the silicon wafer. The partially cured PDMS was then placed onto the PDMS embedded MWCNT mat (from above), ensuring the microwells overlapped the MWCNT mat to produce a device. An identical process was used for the fabrication of the control, gold foil bridge devices. The device was then placed in an oven at 60 °C for a 1 h (Fig. S1).

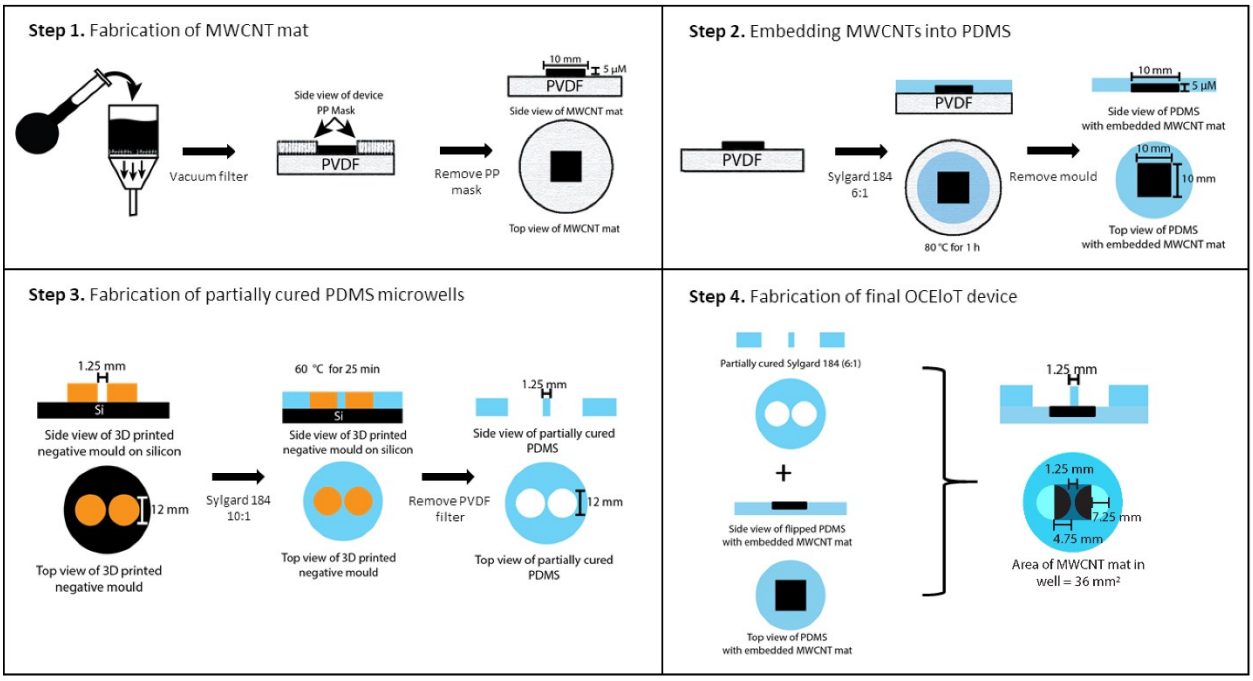

Fig. S1. General schematic for the fabrication of a two-well microwell device. Step 1. A suspension of MWCNTs in chloroform (80 µg mL^{-1} in 25 mL) was vacuum filtered through a polypropylene (PP) filter membrane mask (0.45 µm, 10 mm^2) onto a polyvinylidene difluoride (PVDF) filter membrane (0.2 µm pore size), followed by removal of the PP mask to reveal the MWCNT mat (10 mm^2) on the PVDF. Step 2. Sylgard 184 (10:1, base:curing agent) was then poured on top of the MWCNT mat on the PVDF. The Sylgard 184 was then cured at 80 °C for 1 h and the PVDF removed to form a PDMS (blue) embedded MWCNT mat (black). Step 3. Two

microwells (each 12 mm in diameter and 1.25 mm apart) were fabricated by placing 3D printed plastic moulds (orange) inside a 5 cm diameter ring on top of a silicon wafer and Sylgard 184 (6:1) poured onto the top. Overflow of the Sylgard 184 was prevented by the ring. The Sylgard 184 was then partially cured at 60 °C for 25 min and the cured PDMS removed from the silicon wafer to produce a free-standing partially cured PDMS layer. Step 4. The partially cured PDMS layer was placed on top of the PDMS embedded MWCNT mat (from Step 2) and the entire device was cured at 80 °C for 1 h, resulting in a two-microwell device.

Preparation of cytochrome C and sodium dithionite solutions: First, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES) buffer (0.1 M, 12 mL, pH = 6) was added to cytochrome C (bovine heart) (10 mg). A second solution of sodium dithionite (11.5 mM) was prepared in HEPES buffer. The sodium dithionite solution was used immediately after preparation.

Preparation of 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) solutions: TMB (10 mg) was dissolved in dry dimethyl sulfoxide (DMSO, 1 mL) and stored at 4 ^{o}C in the dark. A 1:100 dilution of dissolved TMB was made up with citrate/acetate buffer (0.1 M sodium acetate titrated to pH 6.0 with 0.1 M citric acid). A second solution of H_2O_2 (800 µM) was prepared by diluting 30% v/v H_2O_2 (1 µL) into Milli-Q water (10 mL). The H_2O_2 solution was used immediately after preparation.

Leak testing of the device: First, fluorescein (1.5 mM in Milli-Q water) was added to the analyte well and Milli-Q water was added to the detection well and the device was left for 24 h in a humidity chamber (minimising evaporation). After 24 h the solutions from both wells were placed

into a RotoGene 6-plex real-time PCR device and the fluorescence was measured using the blue excitation (450 nm) and green (520 nm) detection channel at Gain 10.

In a separate device, a $CuCl_2$ solution (100 mM in Milli-Q water) was added to the analyte well and Milli-Q water was added to the detection well. After 24 h the solutions from each well were removed and the device analysed under SEM with EDX spectroscopy.

Colorimetric sensing: The cytochrome C reduction analysis was performed by adding dithionite (11.5 mM, 300 µL) to the analyte well and cytochrome C (25 µM, 300 µL) to the detection well the device. The entire device was then placed in the dark at room temperature for 16 h and the solutions were then extracted from each well and analyzed using UV-Visible (UV-Vis) spectroscopy. The TMB oxidation analysis was performed by adding TMB (400 µM, 300 µL) to the detection well and H_2O_2 (800 µM, 300 µL) to the analyte well. The entire device was then placed in the dark at room temperature for 2.5 h and the solutions were then extracted from each well and analysed using UV-Vis spectroscopy.

UV-Vis spectroscopy: Samples for UV-Vis spectroscopy were prepared by extracting the solutions from the microwells and placing them into a plastic microcuvette (1 mL). The microcuvettes were placed into a Perkin-Elmer Lambda 950 UV-Vis Spectrometer and the absorbance recorded (300-600 nm). In order to calculate the concentration of cytochrome C the absorbance at 540 nm was subtracted from the absorbance at 550 nm. The concentration of cytochrome C was then calculated using Beers law, where $\text{concentration} = \Delta A/\varepsilon$, with $\varepsilon = 24.6\ mM^{-1}$. The TMB^+ concentration after HCl addition was also calculated using Beers Law, where $\varepsilon = 59.0\ mM^{-1}$.

Resistance measurements of MWCNT mats: The resistance measurements of the 5 μg mm^{-2}, 10 μg mm^{-2} and 20 μg mm^{-2} MWCNT mats embedded in PDMS were taken using a Digitech (QM1323) multimeter using the resistance setting. The probes of the multimeter were placed onto the MWCNT mat embedded in the PDMS at various distances (denoted as probe-to-probe distance) and the most stable resistance value recorded. The probe-to-probe distance of 8.0 mm, 6.0 mm, 5.0 mm, 4.0 mm, and 2.0 mm were measured in triplicate on three separate MWCNT mats (total n = 9 for each distance) for each of the three loadings. The average resistance (with error) of each measurement was calculated using Microsoft Excel. The average resistance was plotted and fit with a linear function (Fig. S2).

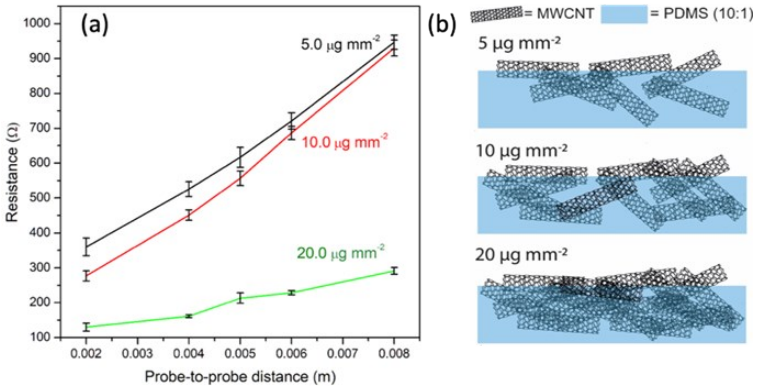

Fig. S2. (a) The resistance (Ω) versus probe-to-probe distance (m) for MWCNT mats of 5 μg mm^{-2}, 10 μg mm^{-2}, and 20 μg mm^{-2}. (b) Schematic of a MWCNT mats (black) at different loadings embedded into PDMS (blue), illustrating how the junction density of the MWCNTs increases with MWCNT loading in the PDMS.

Helium ion microscopy (HIM): Samples for HIM were prepared by cold-fracturing the device in liquid nitrogen to present a pristine surface, these samples were then mounted on conventional scanning electron microscopy stubs with carbon tape. HIM was performed using a Zeiss ORION

NanoFab system running a modified version of the Zeiss ZEN software suite. Images were obtained using a 30 keV accelerating voltage with a beam current of approximately 3.5 pA. Due to the resistive nature of the PDMS, for cross-sectional images the 'Flood Gun' was used in line mode providing a secondary electron source was used for imaging. Sample images were obtained with tilt angles between 5° and 45° to orient the samples optimally with the detector.

Raman Spectroscopy: Raman spectroscopy was performed using a Renishaw InVia spectrophotometer using an 1800 lines mm^{-1} grating and a 532 nm laser. Measurements were performed of pristine, filtered MWCNTs on the PVDF filter paper, and after exposure to H_2O_2 or TMB reaction conditions for 3 hours.

Electrochemical Reactions: A 5 mM Ferricyanide solution was made up in PBS buffer and placed in the detection well, PBS buffer only was placed in the analyte well. A platinum mesh working and counter electrode were placed in the detection and analyte wells respectively, and750 mV was applied between the electrodes in opposing wells for 1 hr. The color change was quantified by UV-Vis spectroscopy.

Scanning electron microscopy (SEM)/Energy-dispersive X-Ray (EDX) spectroscopy: Samples for EDX/SEM were prepared by mounting the $CuCl_2$ leak tested wells on a conventional scanning electron microscopy stub with carbon tape and analysis was performed on a Hitachi FlexSEM system. Images and EDX spectra were obtained with an accelerating voltage of 20 kV. EDX spectra were recorded using a Bruker EDX detector. Mapping was performed over user defined regions of interest and allowed to accumulate for >5 min such that adequate signal was obtained for analysis (Fig. S3).

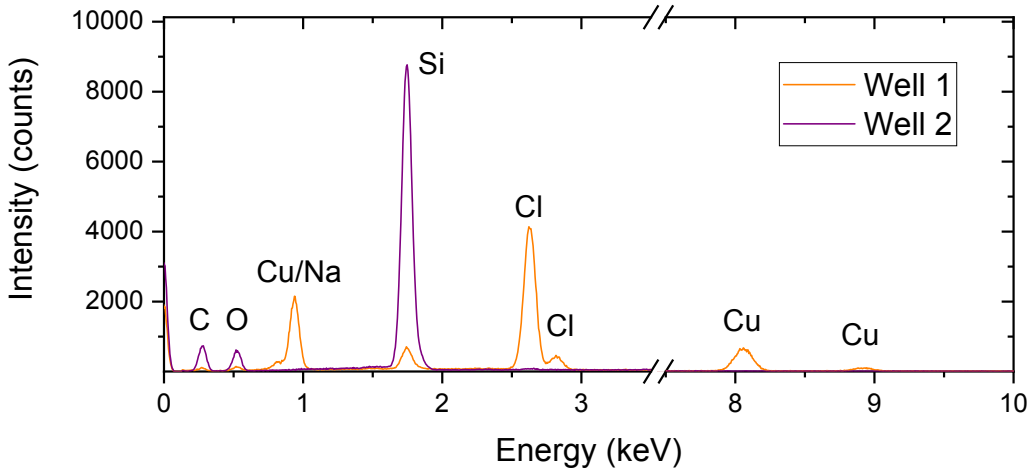

Fig. S3. EDX spectra of leak testing of $CuCl_2$ in MWCNT bridge wells, with Well 1 initially containing $CuCl_2$ solution and Well 2 containing only water.

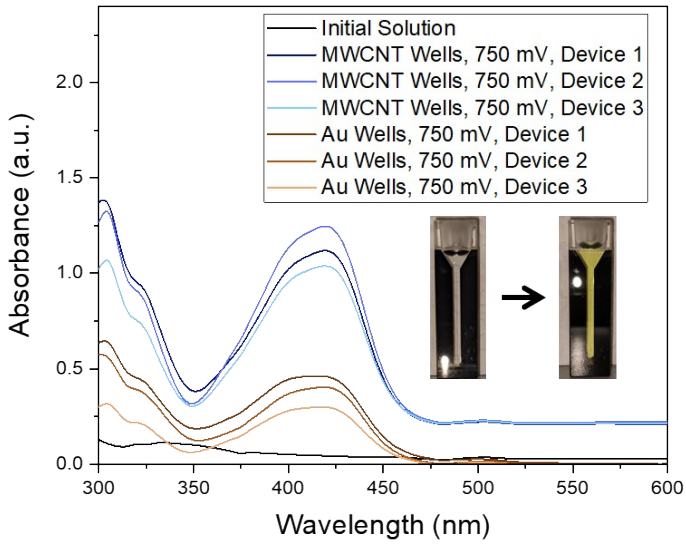

Fig. S4. UV-Vis spectra of electrochemical switching of Fe^{2+} to Fe^{3+} through a gold foil bridged device or a MWCNT bridge dual-well device (inset, photo of; left) the initial clear solution; and right) the post oxidised yellow solution).

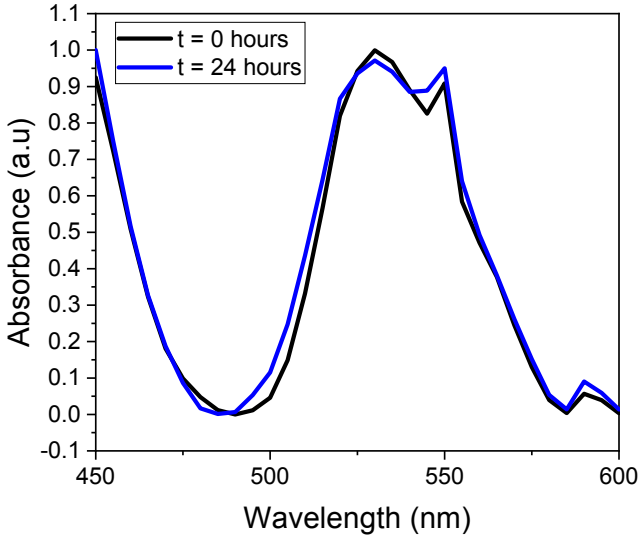

Fig. S5. Control experiment where H_2O_2 was placed in the analyte well and cytochrome c in the detection well, after 24 h the cytochrome C remained in the Fe^{2+} oxidation state.

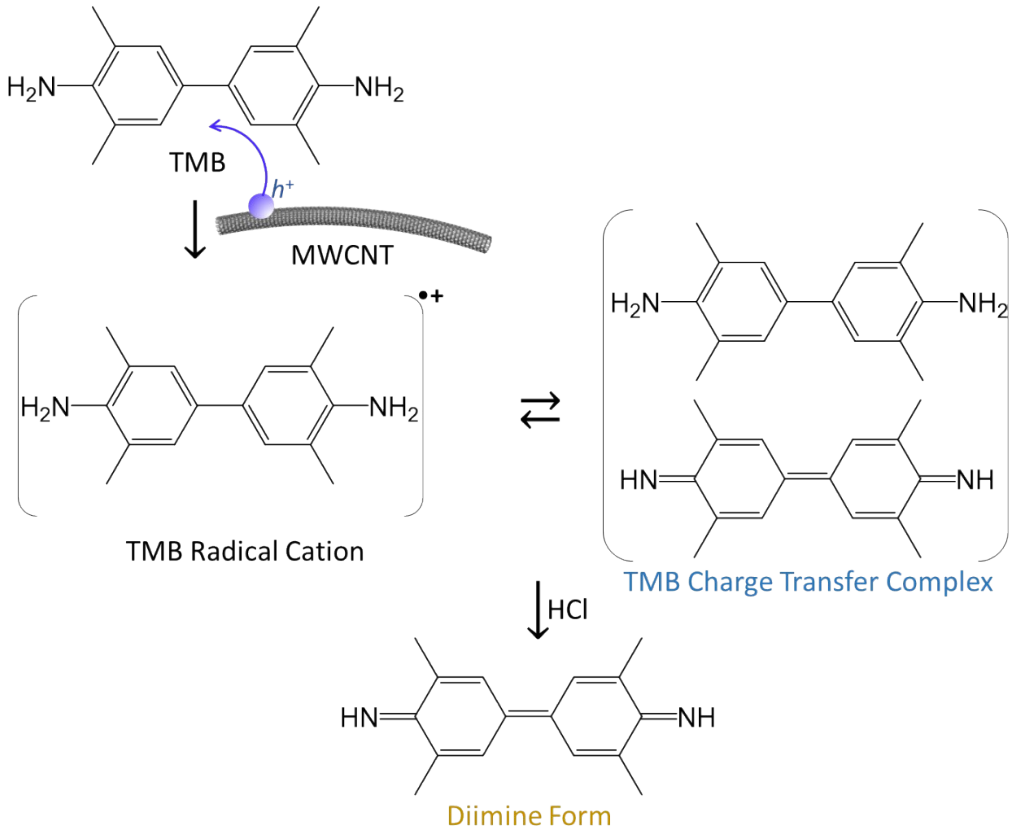

Fig. S6. Schematic of the oxidation of TMB. The holes in the MWCNTs extracts an electron from a local TMB molecule, this results in the formation of the meta-stable TMB radical cation, which rapidly decays to the blue charge transfer complex. The addition of an acid completes the oxidation to the bright yellow diimine form of TMB.[1]

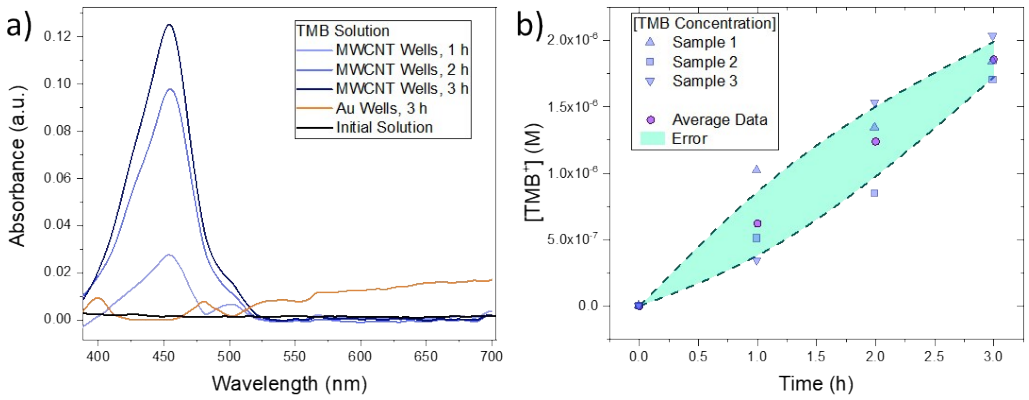

Fig. S7. a) UV-Vis of the TMB solutions after various time points using either the MWCNT bridge or the gold bridge device; and b) the rate of production of the blue TMB intermediate calculated from three individual MWCNT devices (all produced under identical conditions, Fig. S1). The rate of the TMB conversion was calculated using the slope of the line of best fit.

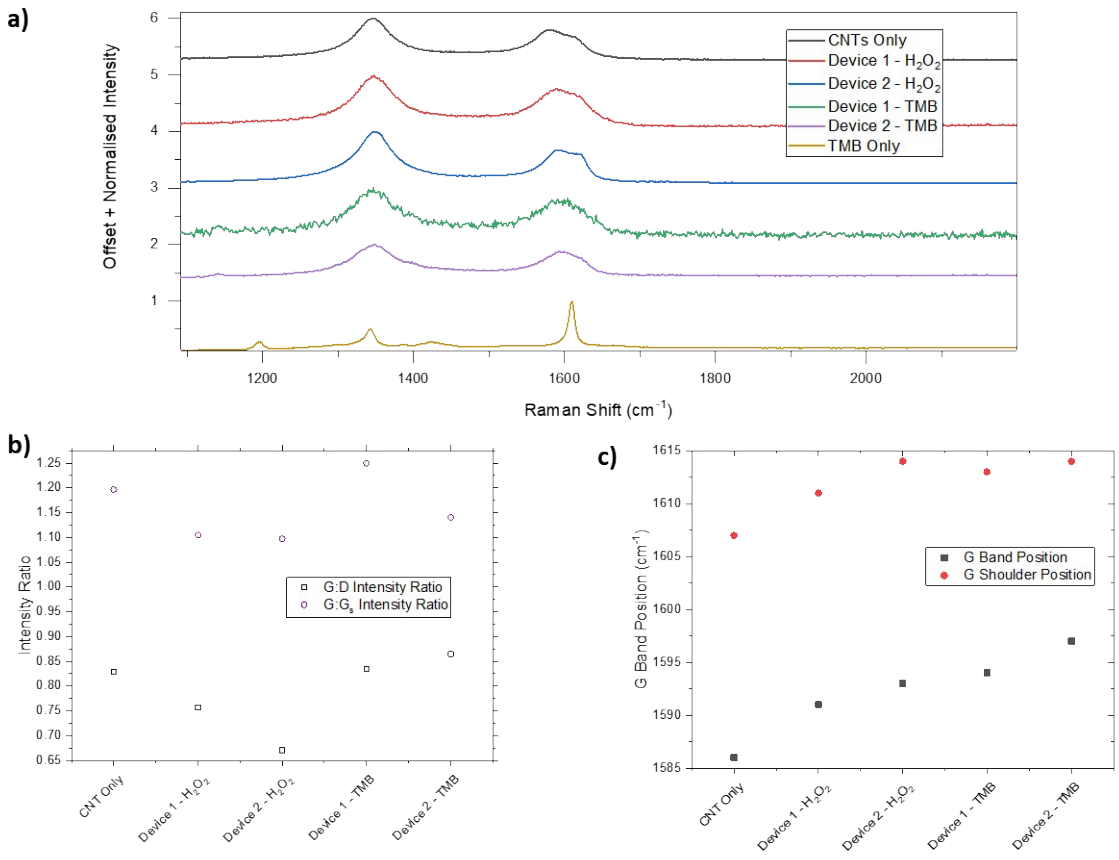

Fig. S8. Raman spectroscopy of MWCNT mats; a) Raman spectra; b) relative intensity ratios of sp^2:sp^3 carbons (G:D) and sp^2:functional group adjacent sp^2 carbons ($G:G_s$)for each device; and c) relative peak position for the G band and the G_s bands.

References:

1 P. D. Josephy, T. Eling, and R. P. Mason, The horseradish peroxidase-catalyzed oxidation of 3,5,3',5'-tetramethylbenzidine. Free radical and charge-transfer complex intermediates, *J. Biol. Chem.*, 1982, **257**, 3669–3675.