

Supporting Information

Fast, ultrasensitive detection of reactive oxygen species using a carbon nanotube based-electrocatalytic intracellular sensor

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Surface stability using cyclic voltammetry

Two hundred consecutive cyclic voltammograms were performed with ITO-SWCNT-Osbpy electrodes in 50 mM PBS prior to performing any cell studies to ensure a stable surface was obtained. Typical voltammograms obtained can be seen in Fig 1S. There is a significant change in the peak current from the 1st and 50th scan. By the 200th scan the peak current is relatively stable.

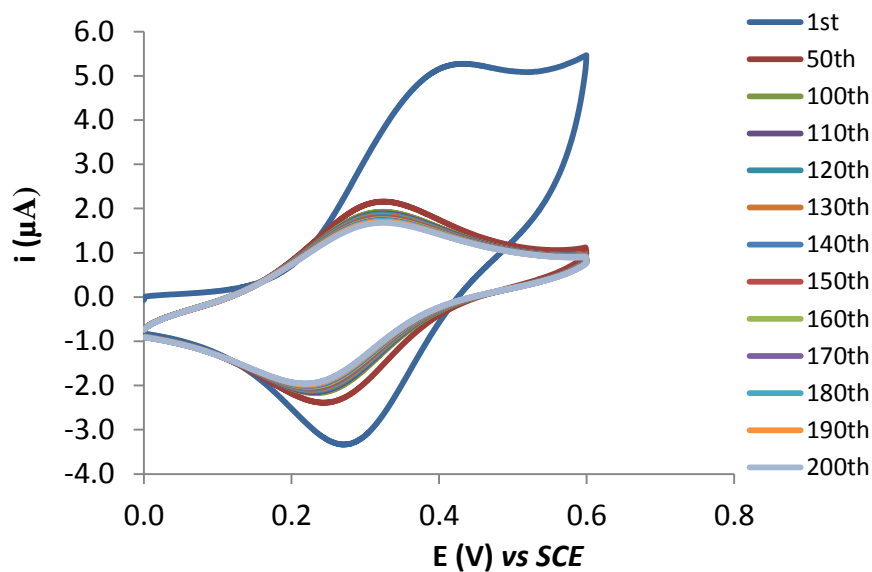


Fig 1S. Typical cyclic voltammograms obtained for 200 consecutive voltammograms in PBS at an ITO-SWCNT-Osbpy electrode at a scan rate of 100 mV s⁻¹.

Cyclic voltammetry on control surfaces: ITO-SWCNT modified electrodes in the presence and absence of H₂O₂

It was important to confirm the H₂O₂ disproportionation was not occurring due to catalyst contamination used in growing the SWCNTs. Therefore, a cyclic voltammogram was performed in the presence (red curve Fig 2S) and absence of 1 mM H₂O₂ (blue curve Fig 2S). No faradaic response was observed when a voltammogram was performed in the presence of H₂O₂ confirming that over the potential range studied, the bare SWCNTs are not catalysing the disproportionation of H₂O₂.

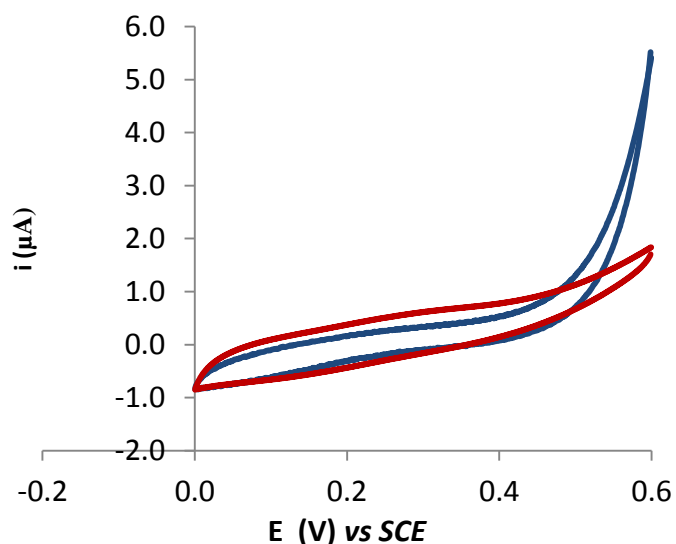


Fig 2S. Typical cyclic voltammograms obtained for solution of 50 mM PBS in the presence (red) and absence (blue) of 1mM H₂O₂ at a scan rate of 5 mV s⁻¹ at ITO-SWCNT electrodes.

Electrochemical characteristics of the osmium redox couple

In the absence of H₂O₂, peak currents were directly proportional to scan rate (Fig 3S (A)) and is well known to be indicative of a surface bound redox process. If the currents were under the control of diffusion then it would be expected that in a peak current versus scan rate plot the current would be proportional to the square root of scan rate. Investigations into the kinetics of the reaction were performed to provide further insight into the Osbpy catalysed degradation of H₂O₂ using a cyclic voltammetric scan rate study in the presence of H₂O₂. A plot of mean peak current versus scan rate obtained for the osmium redox couple in the absence and presence of H₂O₂ can be seen in Fig 3S (A) and (B), respectively. Interestingly, in the presence of H₂O₂ (Fig 3S (B)) there is a deviation from the proportionality with scan rate. If it were a simple case of the peak currents being controlled by the diffusion of H₂O₂ then a current function plot¹ would yield a line with no gradient. However, on the contrary, a positive slope (Fig 3S (C)) was observed and therefore indicates that the rate limiting step controlling the rate of H₂O₂ disproportionation is the interaction and electron transfer of the H₂O₂ with the Osbpy.²

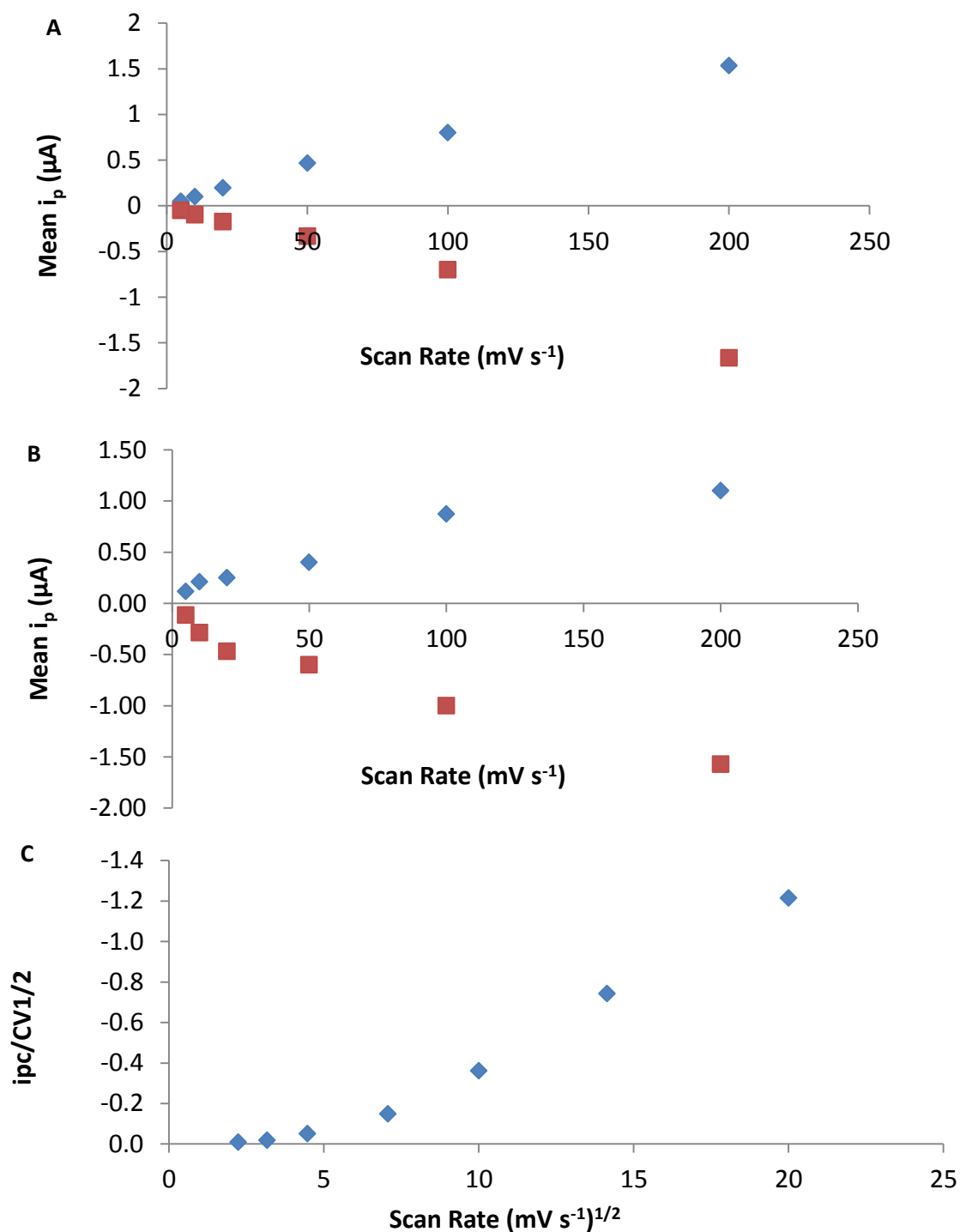


Fig 3S. Plots of peak current versus the scan rate obtained from cyclic voltammograms performed in solutions of PBS (A) and in the presence of 100 mM H₂O₂ (B). (C) Current function plot of the cathodic peak current in the presence of H₂O₂. Currents were obtained from cyclic voltammograms (Fig 1 main document) by extrapolating the baseline and measuring the peak height.

Fixed potential amperometry studies using standard additions of H₂O₂

For cellular studies, it was important to show the effect of fixed potential amperometry so that the mechanism for any change in current could be elucidated. Therefore fixed potential amperograms were performed in both PBS (Fig 4S) and culture medium (Fig 5S). To help quantitate the detection ability of the electrode a calibration graph was generated by adding standard additions of H₂O₂ whilst performing fixed potential amperograms in PBS at 3 electrodes and a typical amperogram can be seen in Fig 4SA. Current was measured after 30 seconds of adding an aliquot of H₂O₂ and a plot of this versus concentration can be seen in Fig 4SB. A sensitivity value of 59.4 $\mu\text{M nA}^{-1}$ was obtained. A lower limit of detection was calculated by dividing twice the standard deviation of the noise by the sensitivity yielding a theoretical lower limit of detection of 61 $\mu\text{M H}_2\text{O}_2$. When an amperogram was performed on electrodes exposed to culture medium the background current was no longer cathodic but displayed anodic currents (Fig 5S). This can be attributed to the fact there must be species in the culture medium that undergo oxidation events.

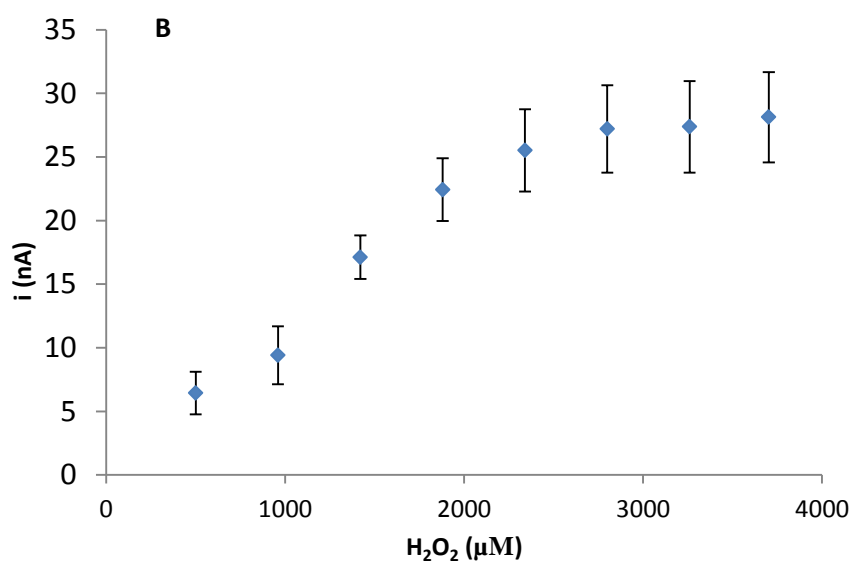
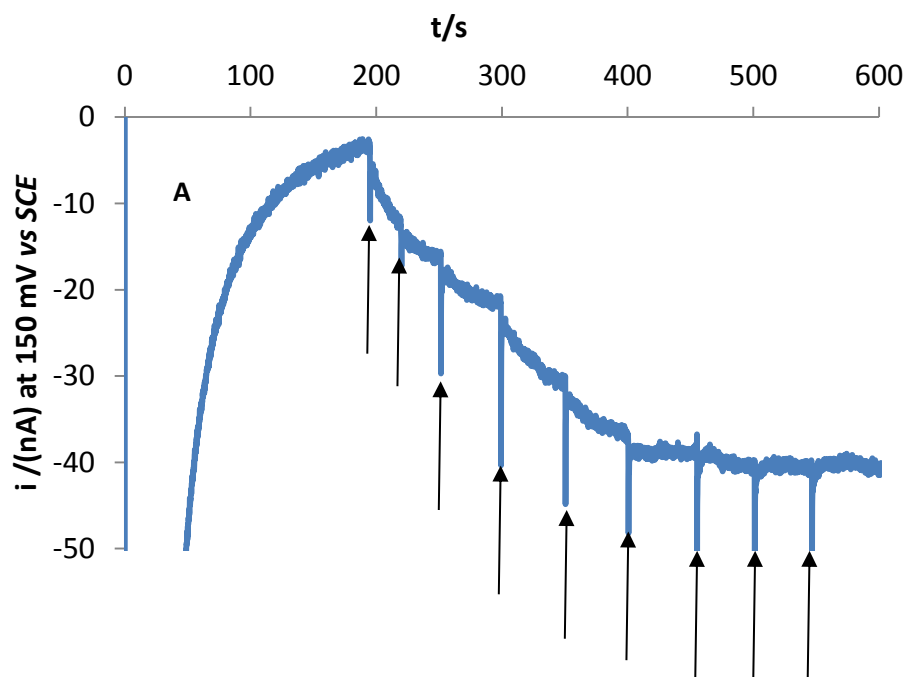


Fig 4S. (A) Typical amperogram obtained at an applied potential of 150 mV with standard additions of H_2O_2 . (B) Calibration graph of mean current sampled after 30 seconds of the H_2O_2 addition. ($n=3$ ITO-SWCNT-Osbpy electrodes. error bars represent $\pm 1SD$ of the mean)

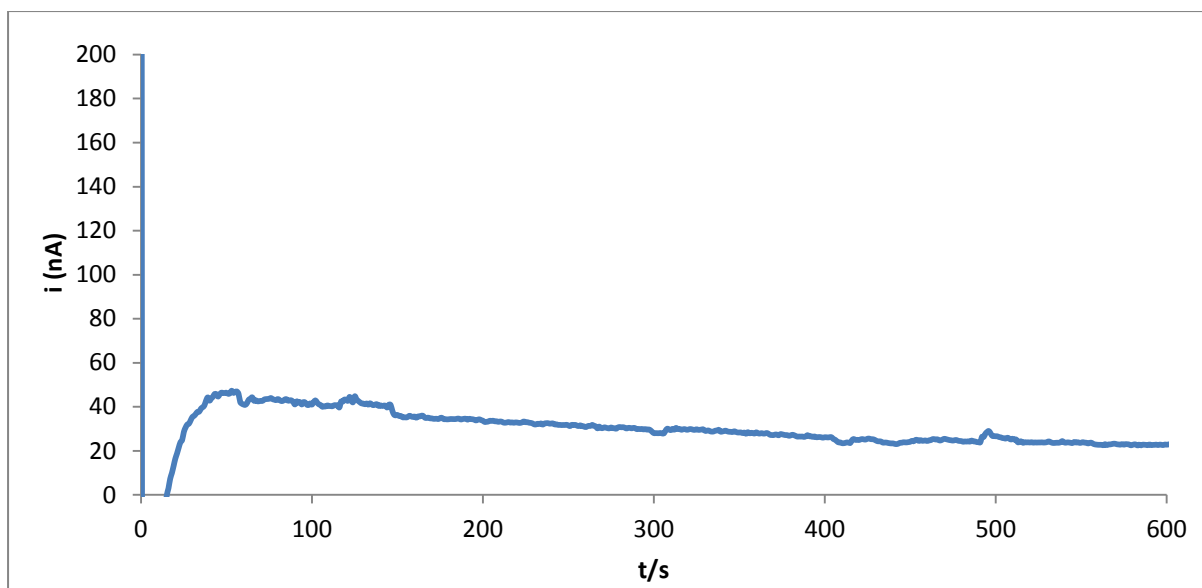


Fig 5S. Typical amperogram obtained for ITO-SWCNT-Osbpy electrode exposed to culture medium for 60 minutes prior to performing fixed potential amperometry using an applied potential of 150 mV in PBS.

Evidence that SWCNT- Osbpy structures are positioned inside the cell

Evidence that centrifugation of ITO-SWCNT-Osbpy structures enables them to be positioned within the cells is obtained by observing the characteristic methylene blue redox peaks at approximately -0.225 V and -0.12 V. This behaviour is similar to that observed for insertion of SWCNTs DNA modified nanostructures which were shown to be up taken naturally³. The control experiment did not display any redox peaks (Figure 6S red curve), providing further support that the voltammetric peaks observed with the ITO-SWCNT-Osbpy electrodes were due to the reduction and oxidation of methylene blue within the cells.

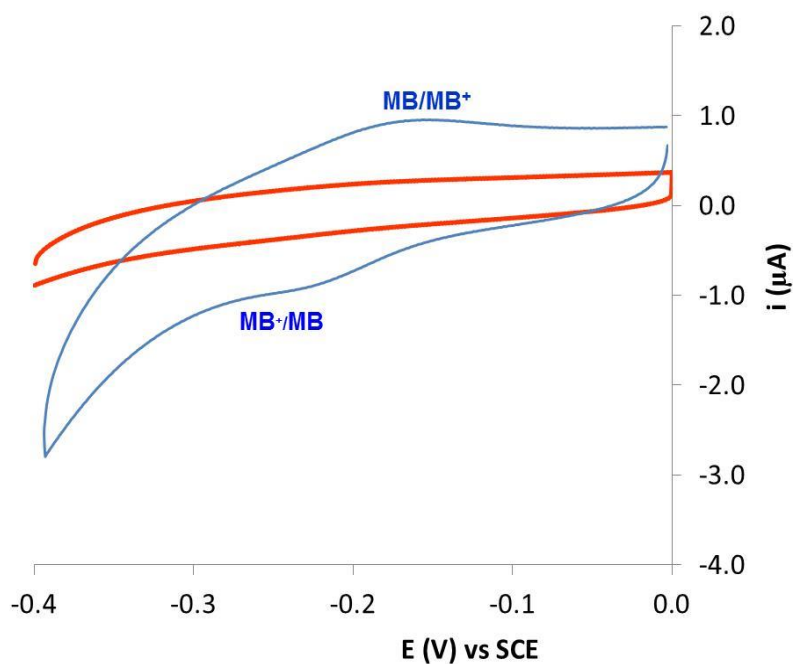


Fig 6S. Typical cyclic voltammograms of methylene blue stained cells drop coated (red curve) and centrifuged (blue curve) onto ITO-SWCNT-Osbpy.

Calculations on the average of H₂O₂ produced per cell

In order to calculate the average of H₂O₂ produced per cell, we performed a cell density study in which cells were centrifuged onto the electrodes and then the average density was calculated from microscopy images giving an average of 529 cells/mm² (± 35 cells which is ± 1 SE of the mean n=18). If we then calculate the electroactive area defined by a 4 mm diameter O-ring, using πr^2 , this yields a geometric area of 12.57 mm². We can then estimate the number of cells found within the electroactive area by multiplying the mean cell density by the electroactive area which gives a total number of 6650 cells. The mean change in charge observed after addition of the LPS was 34.125 pC. Assuming a 2 electron reduction process for H₂O₂, this equates to a total of 0.1768 fmoles of H₂O₂ being sensed on stimulation with 1 μ g/mL of LPS. If we divide the number of moles of H₂O₂ produced by the number of cells covering the defined electrode area (6650), we estimate that a single cell produces 26.59 zmoles of H₂O₂ in response to LPS stimulation.

Selectivity of the sensor

The selectivity of the sensor was established by investigating the effect of *in situ* generation of other common reactive moieties including, $O_2^{\bullet-}$, and reactive nitrogen species (RNS) including peroxyxynitrite ($OONO^-$), NO (nitric oxide) and nitrite anion (NO_2^-) (Figure 7S). Peroxyxynitrite (Cayman chemical company) was stored at -80°C . $ONOO^-$ was defrosted and stored on ice until required. The concentration of the stock was confirmed by measuring at $OD_{302\text{nm}}$ using a Cecil CE1020 UV spectrometer before every experiment. Stock solutions of $ONOO^-$ were then diluted in 0.3 M NaOH and stored at -20°C for the length of the experiment. An aliquot of $ONOO^-$ was added to an electrochemical cell comprising PBS operating at an applied potential of 150 mV giving assay concentration of 50 μM . For generation of $O_2^{\bullet-}$, an aliquot of KO_2 (Sigma Aldrich) was added to the electrochemical cell to give a final concentration of 50 μM and current was sampled at an applied potential of 150 mV. In order to generate NO and NO_2^- , a stock solution of DEANONOate (Sigma) was diluted in 0.1 M NaOH and stored on ice throughout the experiment. An aliquot was added to an electrochemical cell comprising PBS operating at an applied potential of 150 mV giving assay concentration of 50 μM .

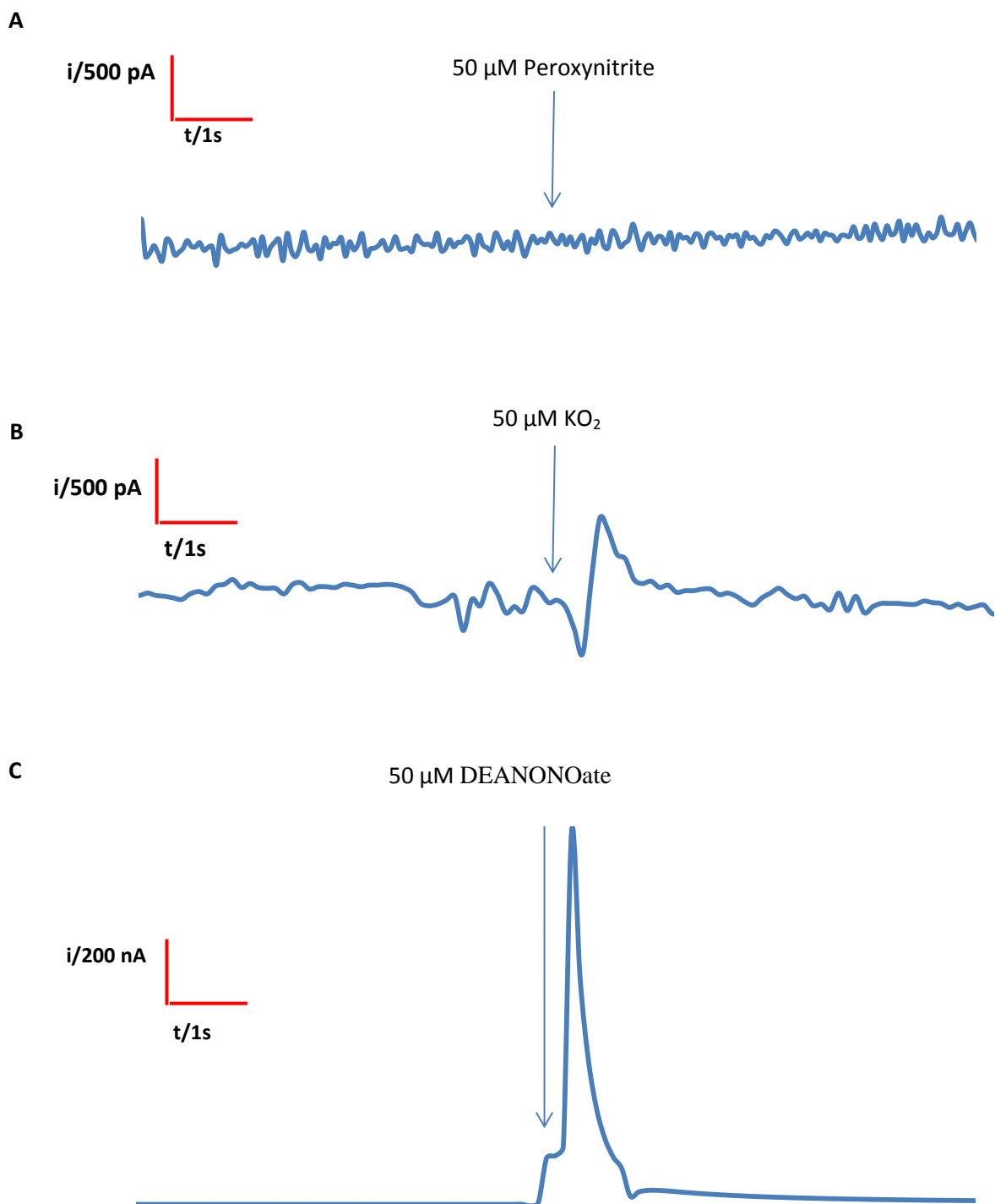


Fig 7S. Typical amperogram obtained for ITO-SWCNT-Osbpy electrode exposed to (A) a 50 μM aliquot of peroxynitrite, (B) 50 μM KO_2 and (C) 50 μM DEANONOate at an applied potential of 150 mV in PBS.

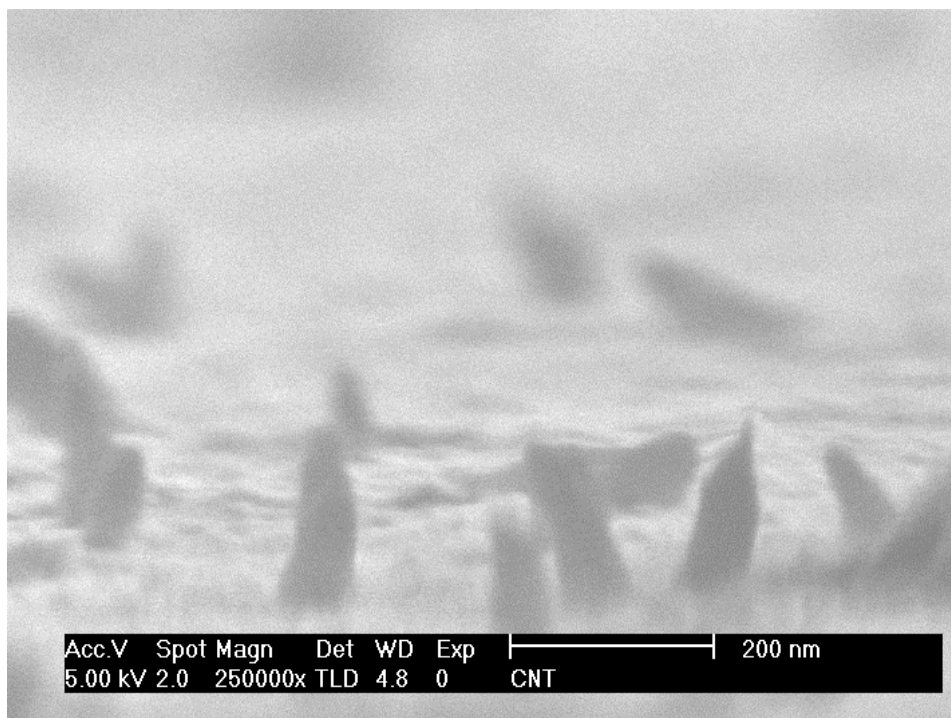


Fig 8S. Typical scanning electron micrograph of vertically assembled SWCNTs.

References

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- (2) PrévotEAU, A.; Mano, N. Oxygen Reduction on Redox Mediators May Affect Glucose Biosensors Based on “Wired” Enzymes. *Electrochim. Acta* 2012, *68*, 128-133.
- (3) Rawson, F. J.; Yeung, C. L.; Jackson, S. K.; Mendes, P. M. Tailoring 3D Single-Walled Carbon Nanotubes Anchored to Indium Tin Oxide for Natural Cellular Uptake and Intracellular Sensing. *Nano Lett.* 2013, *13*, 1-8.