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CASE STUDY

TRANSMEMBRANE ELECTRON TRANSPORT USING CARBON NANOTUBE PORINS

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Transmembrane Electron Transport Using Carbon Nanotube Porins

TEM-Cryogenic case study

CELLULAR HOMEOSTASIS AND CARBON NANOTUBES

- Cells modulate their homeostasis through the control of redox reactions *via* transmembrane electron transport systems. These are mediated by oxidoreductase enzymes.
- The use of oxidoreductase enzymes in biology has been linked to a host of systems including reprogramming for energy requirements in cancer.
- Consequently, the ability to modulate membrane redox systems may give rise to opportunities to modulate underlying biology.
- A novel approach is the develop a wireless bipolar electrochemical systems to form on-demand electron transfer across biological membranes.
- Membrane inserted ***carbon nanotube porins (CNTPs)*** can act as bipolar nanoelectrodes, one can control electron flow with externally applied electric fields across membranes.

CHARACTERISATION

- In order to investigate these materials, we need a technique capable of generate high-quality imaging of fine details of CNTPs within the membrane

- It is noted that the technique must permit the manipulation and control, under cooled temperatures

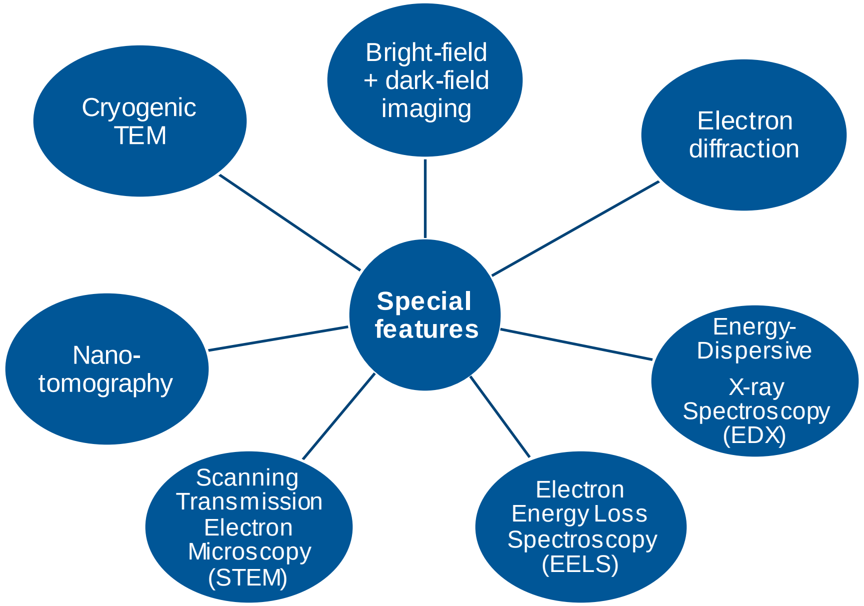
- Here, we use the technique of **cryogenic transmission electron microscopy (Cryo-TEM)** to appraise the CNTPs under controlled temperature (-160 °C) during analysis.

CRYOGENIC-TEM

- Range of specialised sample holders including **heating and cryogenic stages** for variable temperature work and an analytical stage for tomographic and high contrast chemical analysis

The versatile JEOL 2100+ TEM for nanostructural characterisation at the Nanoscale and Microscale Research Centre (nmRC), University of Nottingham

- MEMS Heating holder (DENSsolutions Wildfire S3) capable of analyses up to 1300°C with millisecond heat and quench speed, and nanoscale sample drift with step changes of hundreds of degrees. Enables **EELS and EDS mapping at elevated temperatures**.



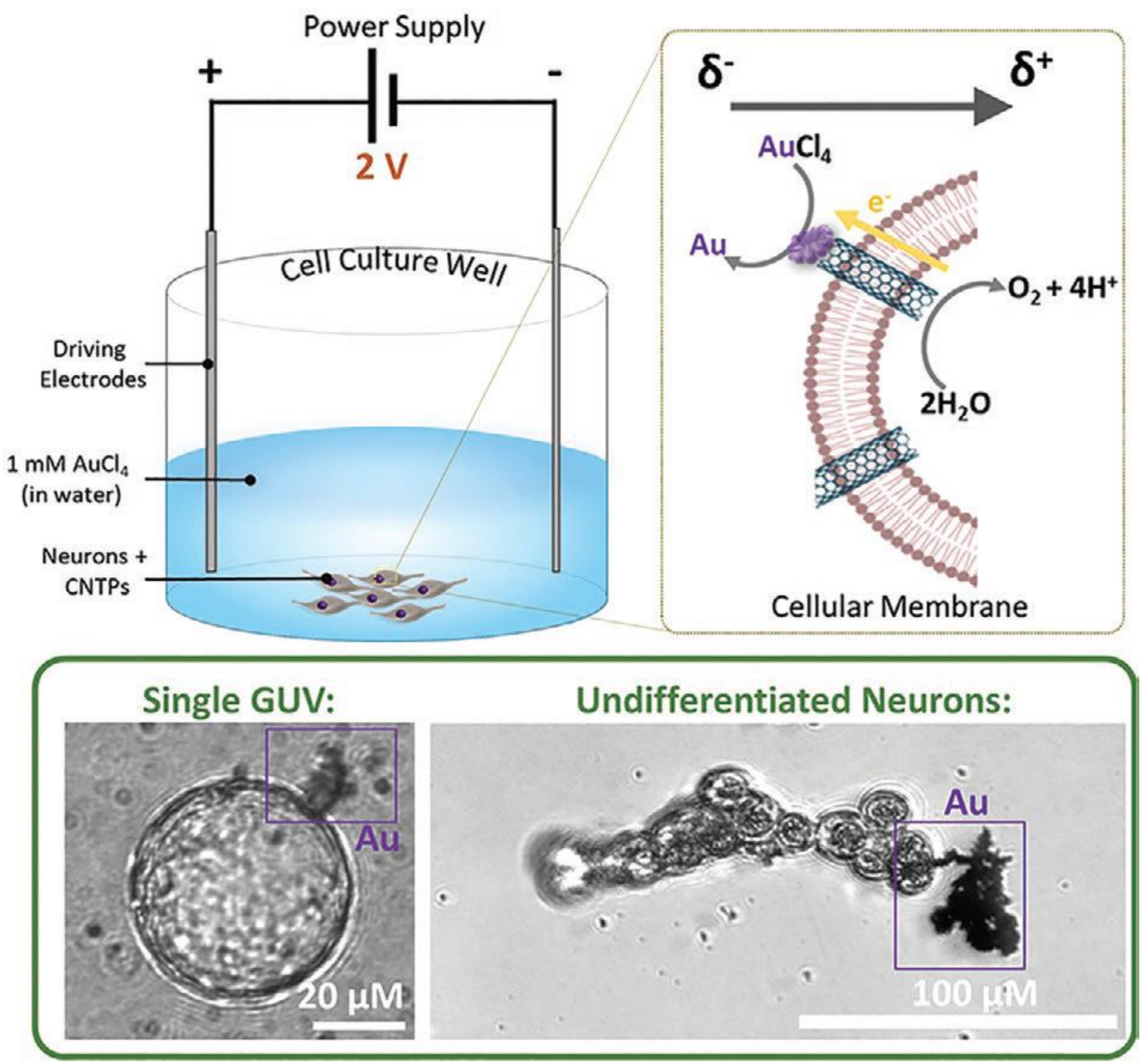
EXPERIMENTAL

To investigate the use of CNTPs as wireless bipolar electrodes and artificial voltage-dependent anion-selective channels (switchable porins within the membrane) within biological systems, CNTPs were self-inserted into ***giant unilamellar vesicles***.

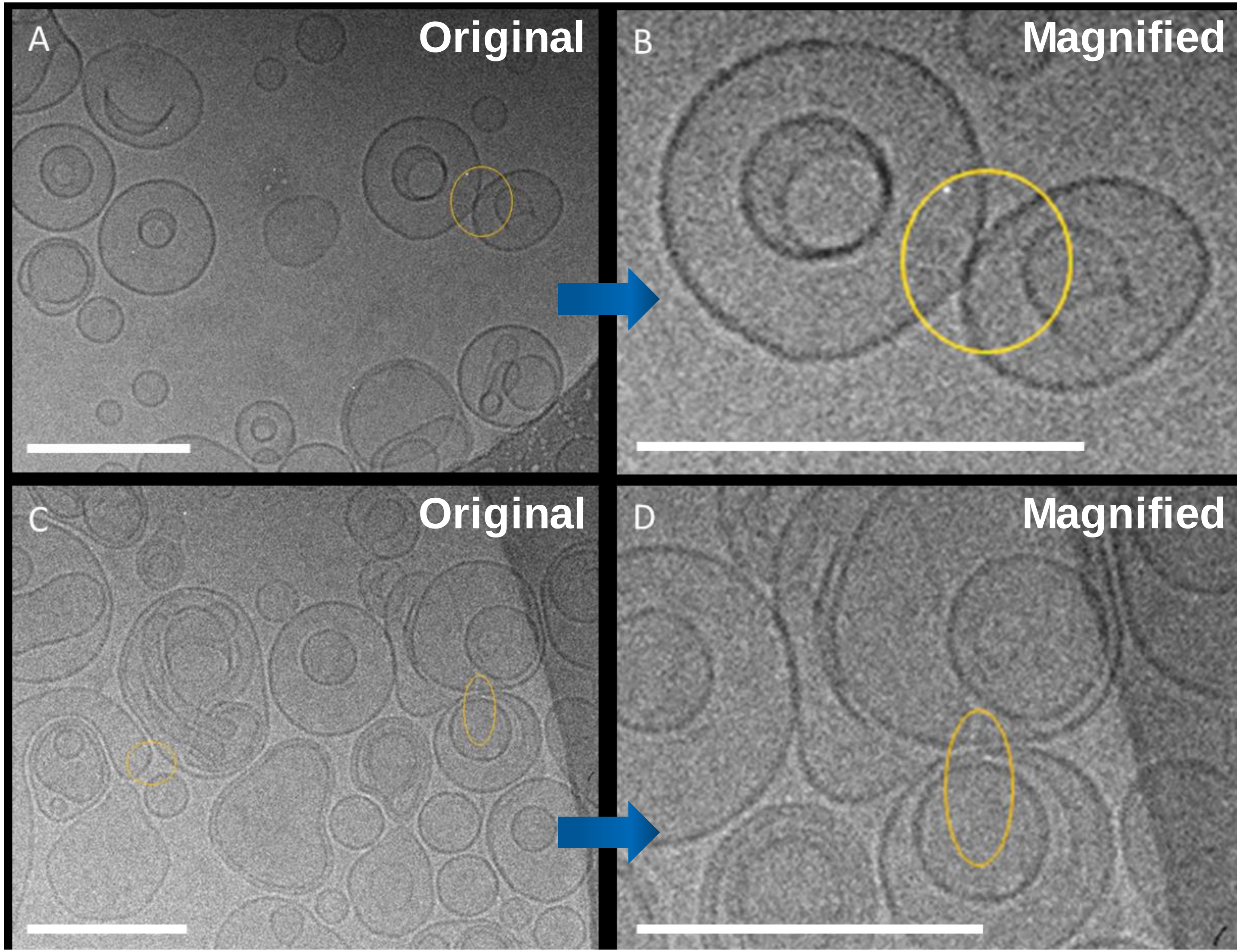
Experimental setup within a cell culture well of the driving electrodes and their placement on either side of the growth of undifferentiated neurons.

CNTPs within neuronal membranes were polarized triggering the reduction of gold chloride to solid gold deposits on the CNTP terminus.

Bright field images show this deposition on both a giant unilamellar vesicle and the neurons.



CNTPS CRYO-TEM IMAGES

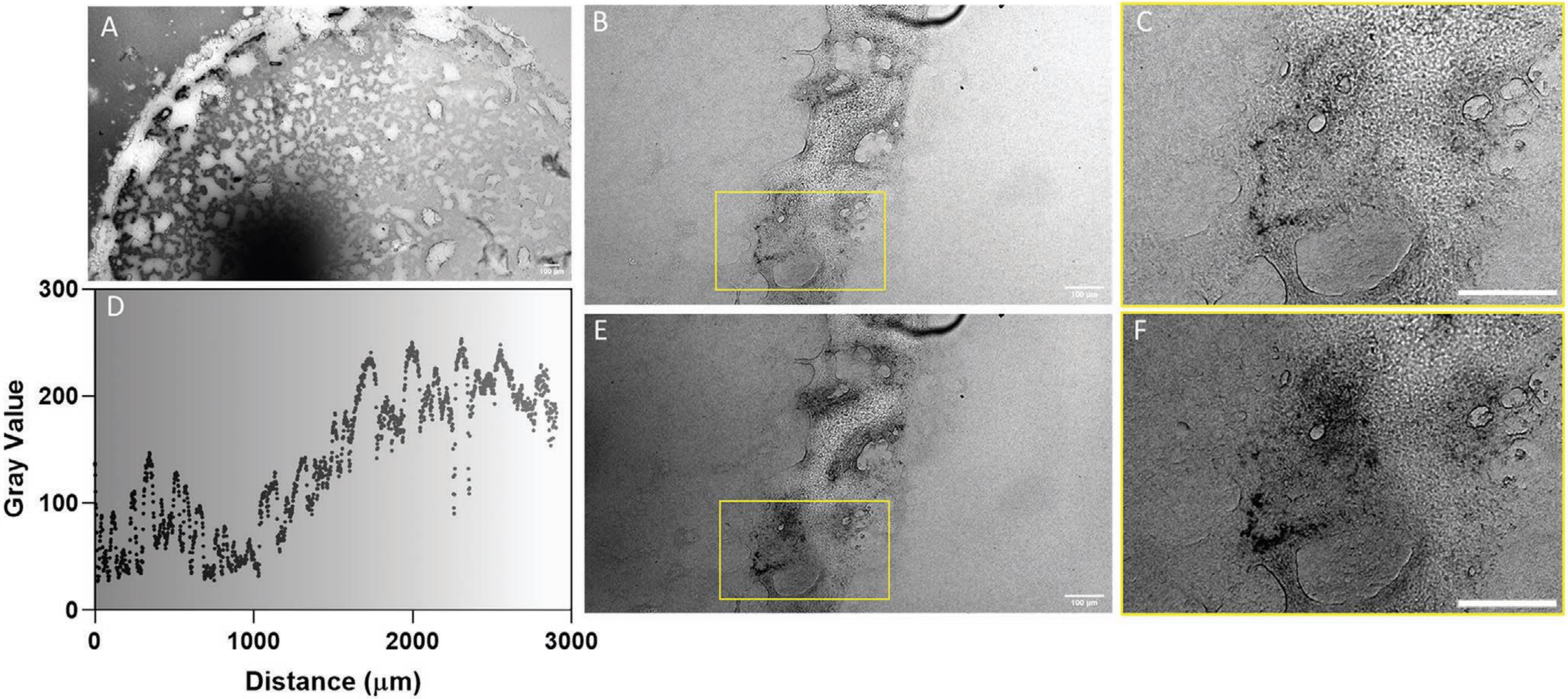


Cryo-TEM images of giant unilamellar vesicles with CNTPs within the membrane.

CNTs visible within the membrane are circled.

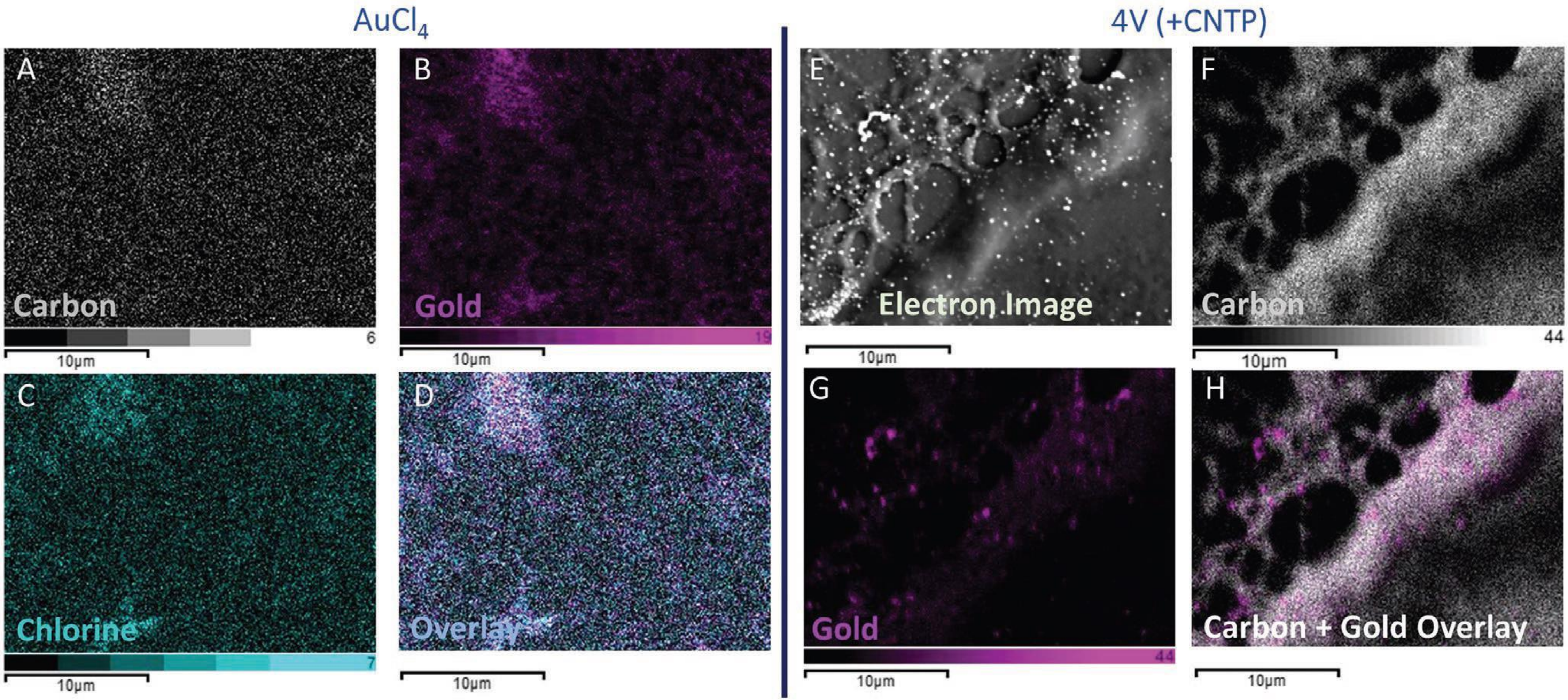
All scale bars are 200 nm.

BRIGHT-FIELD IMAGES – SEM



Bright-field images of giant unilamellar vesicles with CNTPs and $AuCL_4$ incorporated. A DC of 4 V was applied to investigate these materials as bipolar electrodes. A) Distribution of gold deposits across the giant unilamellar vesicles spot. Panels (B) and (C) are images taken 30 min after the beginning of stimulation. D) Gray scale values when a line is drawn across (A). E,F) The same sample area imaged 45 min after the beginning of stimulation. Right-hand panels are enlarged regions of the areas indicated in the left-hand panels. All scale bars are 100 μm.

EDS MAPPINGS



EDS mapping used to confirm the gold deposition of $AuCl_4$ solution (A-D) and giant unilamellar vesicles containing CNTPs, 4V (DC) applied for 1 hour in a solution of $AuCl_4$ (E-H).

Carbon (A,D), gold (magenta, B,G) and chlorine (cyan, E) elements were mapped and overlays plotted (D,H).

SUMMARY

- Cryo-TEM was useful to image fine-details of CNTPs within the membrane

- Complementary SEM images and EDS mapping provided morphological and chemical information important for the bipolar electrodes investigations

- This investigation showed that bipolar electrochemical reaction *via* gold reduction at the nanotubes can be modulated at low cell-friendly voltages, providing an opportunity to use bipolar electrodes to control electron flux across membranes.

- The results presented give rise to a new method using CNTPs to modulate cell behaviour via wireless control of membrane electron transfer.

REFERENCES / ACKNOWLEDGEMENTS

For more details on the work showcased in this case study see the following publications:

J.M. Hicks, Y.-C. Yao, S. Barber, N. Neate, J.A. Watts, A. Noy, F.J. Rawson, Electric Field Induced Biomimetic Transmembrane Electron Transport Using Carbon Nanotube Porins, Small 17(32) (2021) 2102517. https://doi.org/10.1002/smll.202102517.

The Cryo-TEM analysis documented here was performed at the Nanoscale and Microscale Research Centre (nmRC) at the University of Nottingham. www.nottingham.ac.uk/nmrc

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ADDITIONAL INFORMATION

- If you wish to get in touch with us to discuss the information provided, raise a query/concern or provide feedback then please feel free to get in touch via any of the methods listed below:

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