Supporting information

Electric Field Induced Biomimetic Transmembrane Electron Transport using Carbon Nanotube Porins

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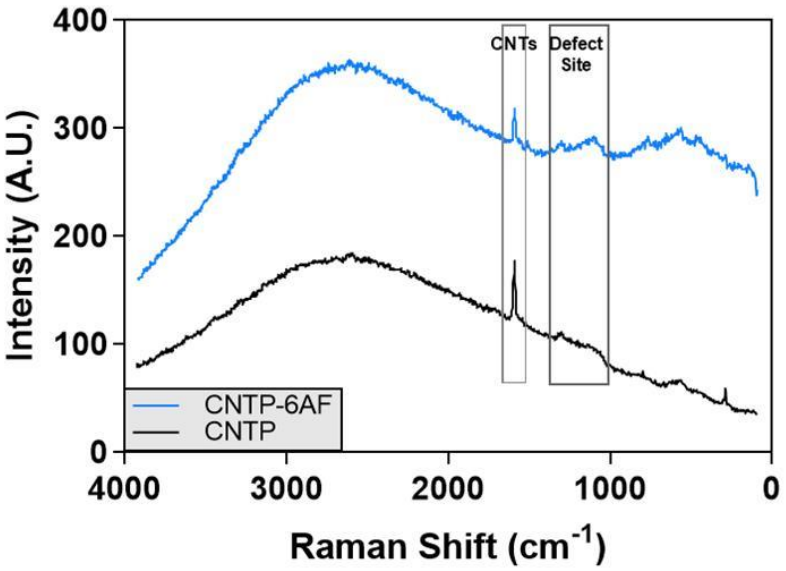
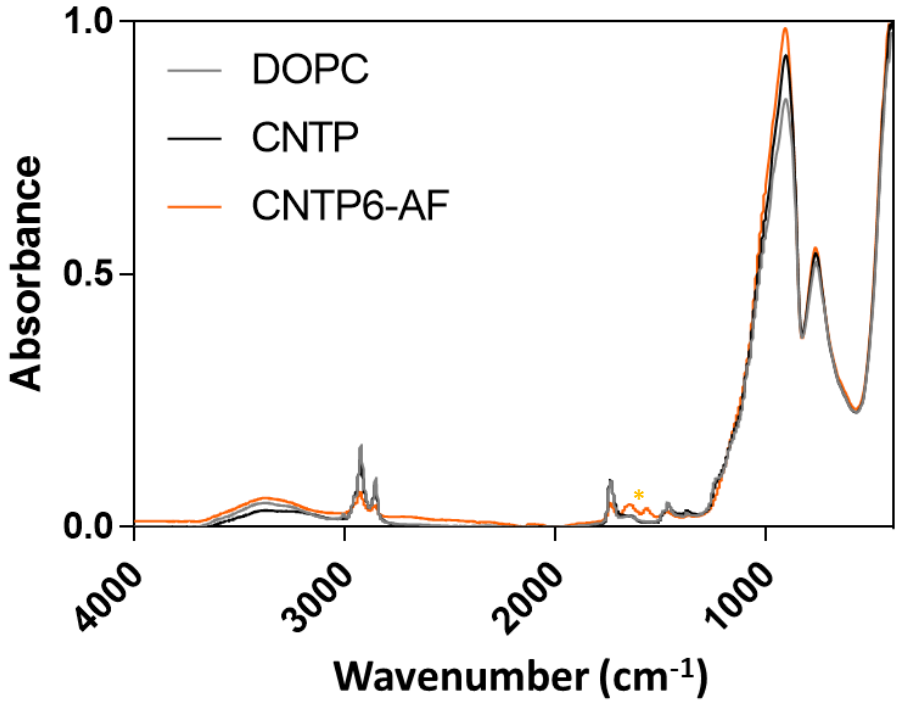
CNTP-6AF Binding

The Raman spectra were measured using a Nicolet Almega XR micro-Raman spectrometer at laser wavelengths of 633 nm. The CNTP samples (ca. 10 µL) were dried on glass slides in a vacuum desiccator for 1 hr to form a film spot for Raman measurements. The laser power used was set less than 10% (100 W/cm^2) to avoid heating the CNTP sample.



SI 1 Raman spectra of CNTP and CNTPs modified with 6-amino fluorescein (6AF). Regions highlighted indicated peaks assigned to either the CNTs or the defect site of the CNTs where the modification occurs.

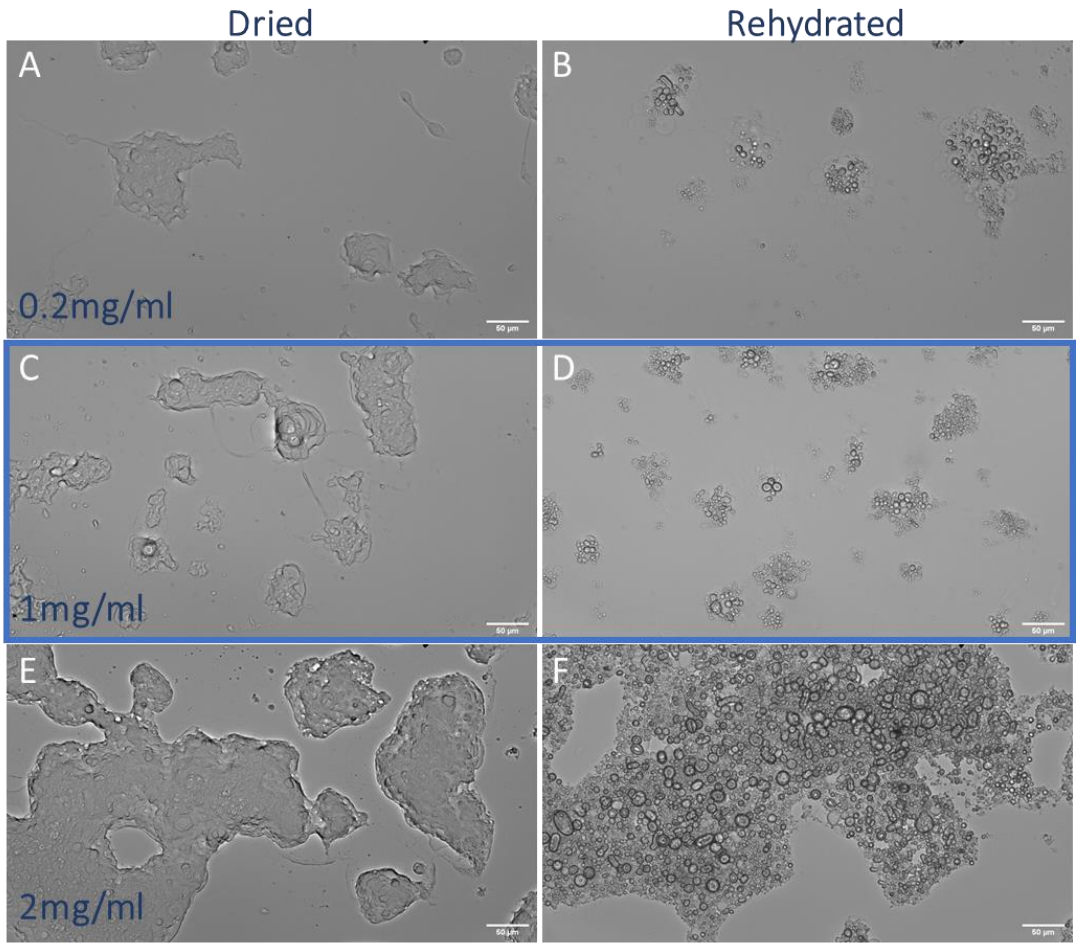
Fourier transform infrared spectroscopy (FTIR) was conducted on using an Agilent Cary 630 with ATR attachment. Samples (ca. 10 µl) were pipetted onto glass slides that had been cleaned with acetone then dried in a vacuum desiccator for 1 hour. Multiple layers (up to 5) of sample were dried in order to increase the concentration on the surface with the contact of the ATR attachment made at the edge of the dried area where concentration was greatest.



*SI 2 FTIR Spectra of DOPC lipid, CNTP and CNTP modified with 6 amino fluorescein (6AF). Additional peaks found in the CNTP6-AF sample are indicated by the orange * and are in the NH range.*

GUV Formation

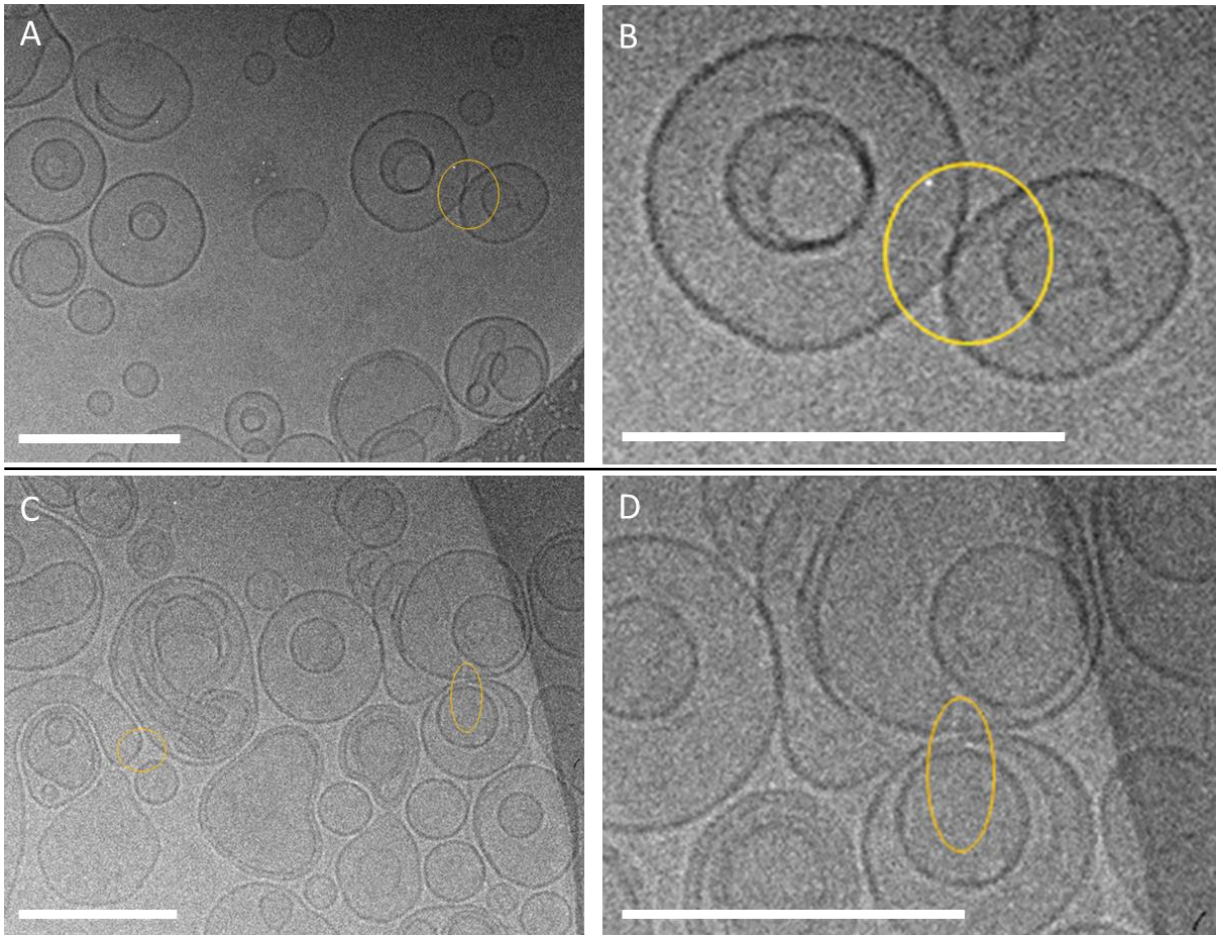
Giant unilamellar vesicles (GUVs) were formed from large unilamellar vesicles (LUVs) as described previously in the literature[57-59]. This involved the formation of LUVs in solution which were then dried onto a surface under vacuum before being rehydrated with water. This rehydration step allows the LUVs to fuse into GUVs, attached to the surface by ionic interactions, within minutes. GUVs made with varying concentrations of lipid were then analysed by brightfield imaging GUVs form within minutes of rehydration and the composition of the lipids can be altered to the requirements. The lipid content here was comprised of 98% DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) and 2% 18:1 Liss Rhod PE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl)) to allow the detection of the lipids by fluorescence.



SI 3 Brightfield images of DOPC before and after rehydration at 10X magnification. Left hand column show DOPC dried onto the base of a 24 well plate (5 µL) while right hand column shows DOPC after rehydration with 20 µL water. A and B have a concentration of 0.2 mg/ml of DOPC, C and D 1mg/ml and E and F have 2mg/ml. Scale bars show 50 µm. Highlighted panels indicate the 1mg/ml concentration that was chosen for future experiments.

Imaging of CNTPs by cryo-TEM

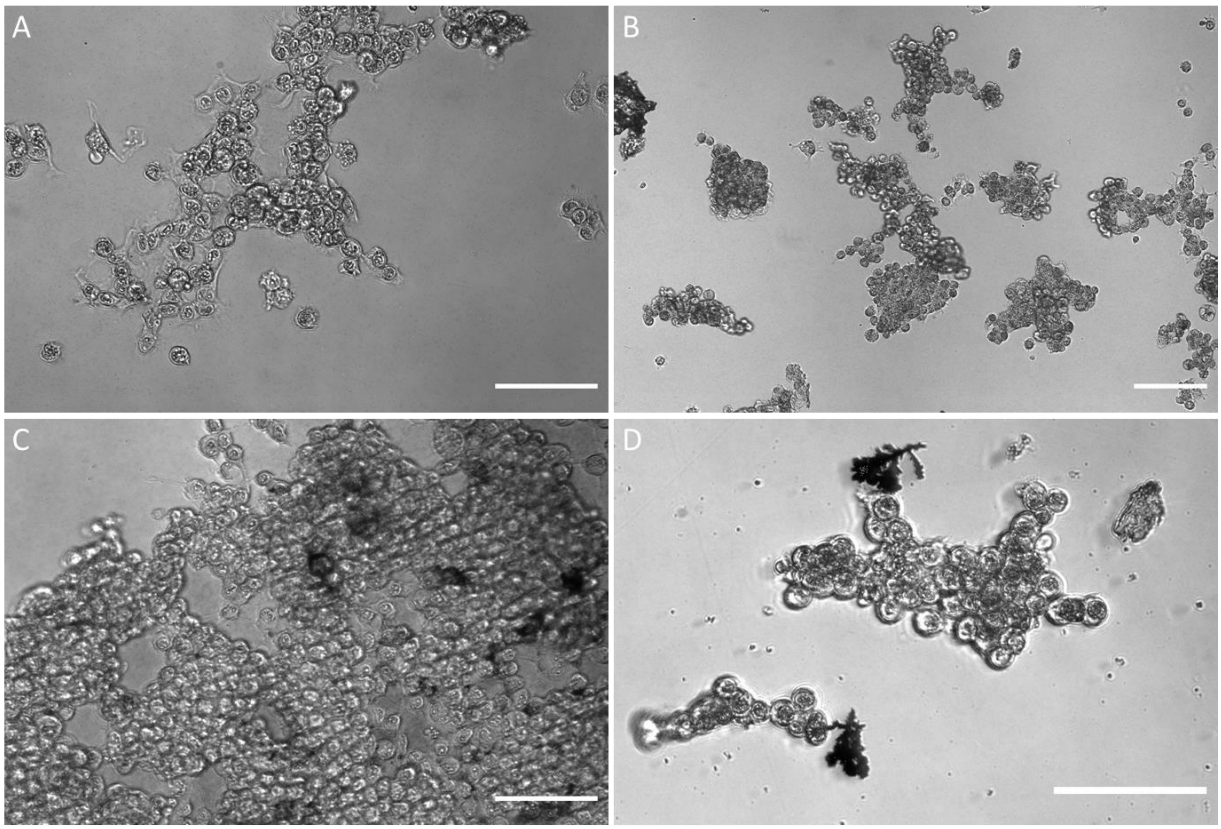
Samples were prepared using a Gatan CP3 Cryoplunge providing a controlled environment (70 – 80 % humidity, 18 ^{o}C), by depositing 3 µL of sample onto a TEM grid (300 mesh Cu, holey carbon or holey carbon / graphene oxide support film, EM Resolutions Ltd). Samples were blotted (1.5 s) before plunging into liquid ethane to vitrify. Samples were maintained under liquid nitrogen (-196 ^{o}C) until transfer to a TEM cryo sample holder (Gatan 926) and held at or below -160 ^{o}C during analysis (Gatan Smartset 900). TEM images were recorded an a JEOL 2100 Plus, operating at 200 kV using a Gatan Ultrascan 100XP camera.



SI 4 Cryo-TEM images of GUVs with CNTPs within the membrane. Panel B is a zoomed in section of panel A while D is a zoomed in section of panel C. All scale bars are 200 nm. CNTs visible within the membrane are circled.

The CNT structures highlighted in figure SI 4 are measured to be approximately 23 nm x 1.6 nm (length x width) and 28 nm x 2.5 nm; the single CNT structure in SI 4D is measured to be between 28 and 40 nm long and 3.4 nm wide. Given the low resolution of the images at that magnification and the distribution of CNT lengths that will be present within a sample these values align with the dimensions of CNTPs which are reported as minimum 0.7 nm wide and approximately 10 nm in length[35].

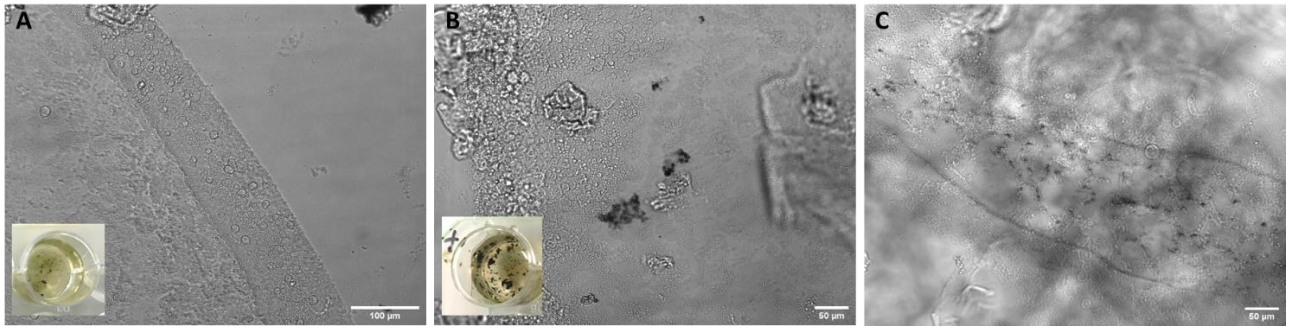
Threshold Potential Application to Neuronal Cells



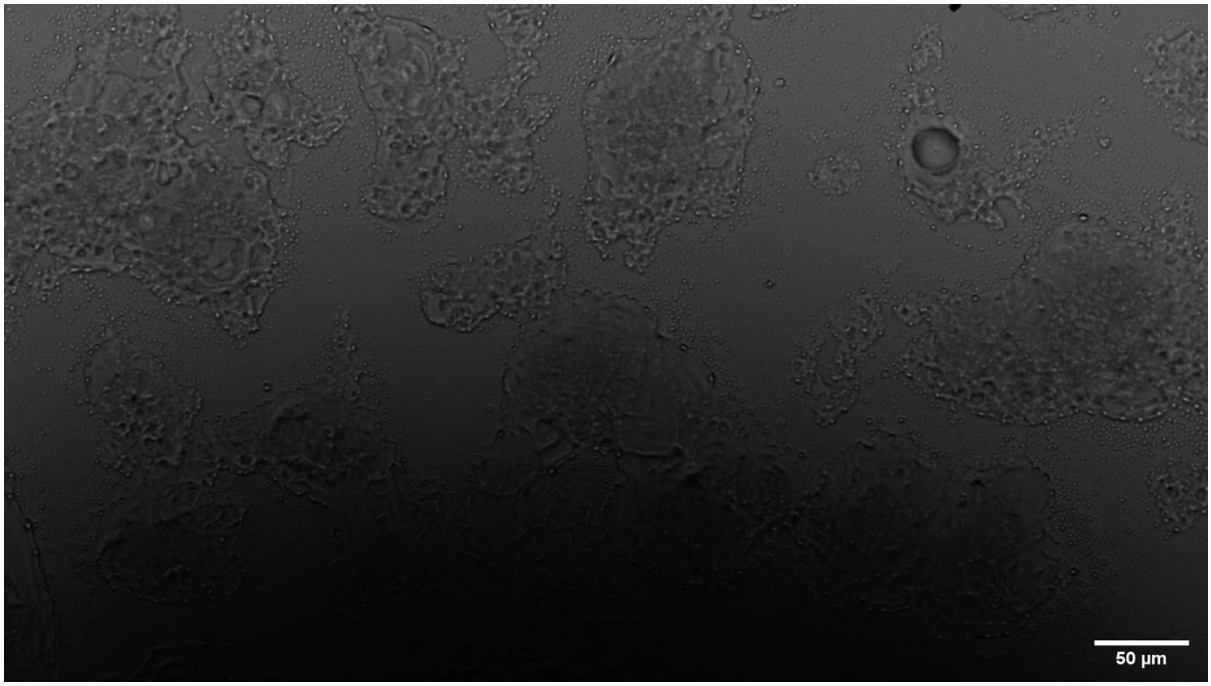
SI 5 NG108-15 cells stimulated at 1.5 V DC for one hour while submerged in 1 mM $AuCl_4$ (in water) Panels A & B are of cells that were incubated with PBS for 4 hours. Panels C and D are of cells incubated with CNTP-6AF for 4 hours. Scale bars are 100 μM.

High Voltage Stimulation with Zinc Chloride

Giant unilamellar vesicles (GUVs) were prepared as described in the main text. As an alternative to gold chloride, zinc chloride was used for metal deposition triggered by the current density at the CNT terminus. Concentration was normalised to take into account the difference in ionic concentration and a higher voltage of 7 V was required to trigger to zinc deposition; this is most likely due zinc being approximately three times more resistive than gold. The inset images show the stark differences in zinc deposition within the solution that is visible by eye while the brightfield images show deposits associated with the lipid membranes when CNTPs are present within the membrane (SI 6 B and C) whereas no membrane-associated deposits are seen in the control (SI 6 A).



SI 6 Brightfield images of GUVs either without CNTPs (A) or containing CNTPs (B) and (C) that were submerged in 2.5 mM $ZnCl_2$ and stimulated with 7 V for 1 hour. (A) and (B) inset images show photographs of the wells immediately after stimulation. Scale bars are 50 µM in length.



Si 7: `Time lapse of GUVs modified with carbon nanotube porins stimulated at 1.5 V DC submerged in 1 mM $AuCl_4$ (in water)

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3. Jesorka, A.; Stepanyants, N.; Zhang, H.; Ortmen, B.; Hakonen, B.; Orwar, O., Generation of phospholipid vesicle-nanotube networks and transport of molecules therein. *Nat Protoc* **2011,** *6* (6), 791-805.
4. Tunuguntla, R. H.; Escalada, A.; Frolov, V. A.; Noy, A., Synthesis, lipid membrane incorporation, and ion permeability testing of carbon nanotube porins. *Nat. Prot.* **2016,** *11* (10), 2029.