Supplemental Information

for Jakubek et al., “Neuronal voltage-gated calcium ion channels
are inhibited by trace yttrium released from carbon nanotubes”

Whole Cell Patch Clamp Technique

The whole cell patch recording method is used to measure currents from small cells while maintaining constant voltage (voltage-clamp). In this method inward calcium currents are activated by test voltages applied at values between -60 mV and +60 mV from a holding potential of -100 mV. Characteristic time-dependent changes in current are exemplified by current traces shown in Fig. 1c. The maximum current vs. test voltage relationship is U-shaped (Fig. 1e). Inward current increases with stronger test depolarization because of the steep dependence of channel open probability on voltage. However, the concomitant decrease in driving force on calcium ions with depolarization acts to decrease current flow through each individual channel. The inward current is eventually balanced by an equal and opposite outward current at the channel reversal potential of close to +60 mV (Fig 1e).

Analysis of competitive metal binding to CNT-carboxylate functional groups

The metal adsorption isotherms in Fig. 4e were used to determine equilibrium constants for soluble yttrium binding to CNT-carboxylate, both in DI water and in saline where Na^+ ions compete for the same sites. The relevant reaction and binding equilibrium constant are:

$$Y^{3+} + \text{CNT - COO}^- \leftrightarrow \text{CNT-COOY}^{2+} \qquad \text{(S1)}$$

$$K_Y = \frac{[\text{CNT - COOY}^{2+}]}{[\text{CNT - COO}^-][Y^{3+}]} = \frac{N_Y}{(N_{\max} - N_Y)[Y^{3+}]} \qquad \text{(S2)}$$

where N_Y is the number of bound sites, N_{max} is the total number of surface carboxylic sites, and $[Y^{3+}]$ represents here the total concentration of all soluble yttrium species at equilibrium. Relation S2 fits the experimental isotherm of Fig. 4f well giving N_{max}=0.37 mmol/g and K_Y = 857 l/mmol.   Expressed as a dissociation constant: $K_d = 1/K_Y = 1.2$ μM, which is the soluble yttrium concentration at which 50% of the available carboxylic sites will be occupied. From Figure 2d, yttrium mobilization into the electrophysiology buffer achieves higher concentrations than 1.2 uM, indicating that CNT-surface-bound Y^{3+} is likely another source of bioavailable Y (beyond the discrete nanoparticles), and may explain why whole SWNT suspension has a slightly stronger calcium ion channel inhibitory effect than the supernatant alone (where the CNT-surface-bound yttrium is absent).

In physiological solutions yttrium must compete with other ions for carboxylate binding sites on nanotubes and in the selectivity filter in the ion channel pore. Here simple experiments were conducted in saline solution to probe the competitive binding of yttrium and sodium on CNT-carboxylates. Figure 4e shows that total yttrium binding from 80 uM solutions is reduced but still significant in the presence of 154 mM Na^+, allowing a quantitative analysis of competitive binding. In Y-doped saline two equilibrium expressions must be satisfied simultaneously:

$$K_Y = \frac{[\mathrm{CNT\text{-}COOY^{2+}}]}{[\mathrm{CNT\text{-}COO^-}][\mathrm{Y^{3+}}]} = \frac{N_Y}{(N_{\max} - N_{Na} - N_Y)[Y^{3+}]} \quad \text{(S3)}$$

$$K_{Na} = \frac{[\mathrm{CNT\text{-}COOY^{2+}}]}{[\mathrm{CNT\text{-}COO^-}][\mathrm{Na^+}]} = \frac{N_{Na}}{(N_{\max} - N_{Na} - N_Y)[Na^+]} \quad \text{(S4)}$$

Where K_Y, N_{max} were determined previously (857 l/mmol and 0.37 mmol/g respectively). At 154 mM, $[Na^+]$ is nearly constant since it is much higher than the initial (maximum) yttrium concentration (~0.1 mM). This leaves K_{Na} as the only unknown variable. Fitting the data in Fig. 4e yields $K_{Na} = 0.11$ l/mmol, or a sodium dissociation constant K_d of 9060 μM and provides a satisfactory curve shape (see Figure 4e bottom line). Clearly yttrium binding continues in the presence of the abundant Na^+ ion, but is reduced from the pure water case by ion-ion competition.

Our reported dissociation constants (K_d = 1.2 μM for yttrium/SWNT-COO^-) can be compared to the reciprocal binding constants for Y^{3+} and $Y(OH)^{2+}$ reported by Turkel *et al.* in experiments on salicilic acid, which range from 0.1 to 1000 □ M depending on the soluble yttrium species[29,34]. The metal speciation diagram for yttrium indicates Y^{3+} and $Y(OH)^{2+}$ under the neutral pH conditions of the CES buffer[35]

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