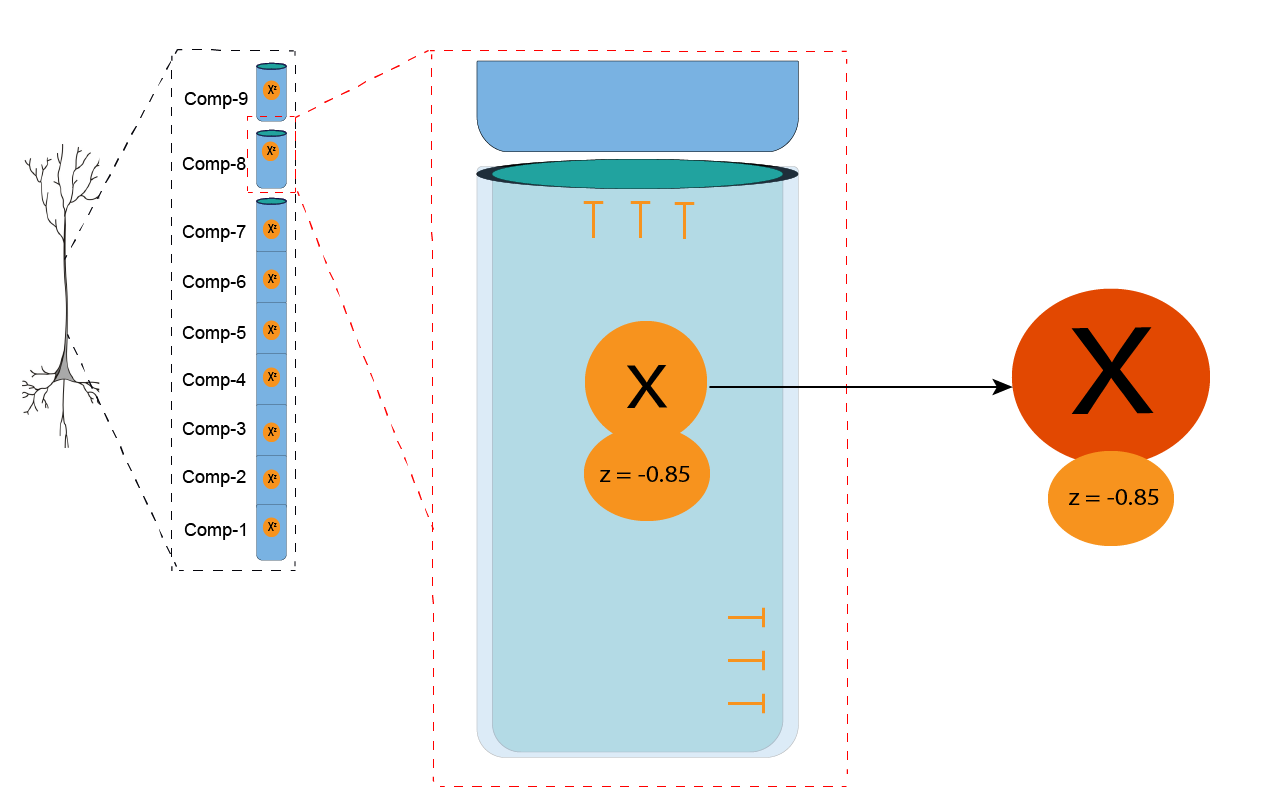
## Changing impermeant anion concentration sets local compartment volume with fixed electrical properties of the dendrite

A multicompartmental model consisting of 9 identical cylindrical compartments linearly arranged in the longitudinal direction was used to evaluate the impact of changing impermeant anion (IA) concentration in a single compartment. The compartments were linked via electro-diffusion and were surrounded by an extracellular bath with fixed ion concentration. The concentration of IAs, “[X] “, in compartment number 8 was increased at a fixed rate of 300mM/min (*In experiment F6)* between 120 -140s whilst keeping the average charge (valence) of IAs in all compartments constant (z = - 0.85) (Schematic 1). The timestep used was 10-6 s, in line with other multi-compartment simulations.



**Schematic 1:** Impermeant anion concentration altered in compartment 8, while impermeant anion charge (z) is held constant.

In the top row of Figure 1A below, shown in orange, IA concentration, charge, and volume for compartment 8 is plotted against simulation time. Following the addition of IAs to compartment 8 there was an increase by XXX (pane 1) while the average valence of IAs remained constant (pane 2). The IA concentration returned to steady state once the addition was removed. To ensure this return to steady state concentration the volume of compartment 8 increased permanently (pane 3)

A comparison between all compartments with respect to IA concentration, valence and volume across simulation time is displayed in the middle row. Although the concentration of IAs was manipulated only in compartment 8, there were uniform changes in the concentration of IAs in all the other compartments likely due to small volume changes occurring due to the movement of ions (pane 1). Only the volume of compartment 8 showed an increase due to the addition of IAs which persisted beyond the manipulation period and subsequently reached a new higher equilibrium volume.

Lastly, the concentrations of the permeant ions (Cl, Na, and K) were compared between compartments across simulation time. Although there were transient changes in permeant anion concentrations in all compartments during the addition of IAs, once this addition ceased the concentrations of all permeant ions returned to their state values as predicted by the analytical solution for single compartment models. As the volume changed in compartment 8 it is expected that the absolute molar quantity of permeant anions would differ from steady state.

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**Figure 1A** – Increased impermeant anion (IA) concentration in compartment 8 between 120-180s results in persistent local change in compartment volume whilst permeant anion concentrations in all compartments return to steady state values following IA manipulation.

Next, we sought to evaluate how the addition of IAs may alter the electrical properties of the dendrite (Figure 1B) using the same simulation described above.

In the top row the membrane potential (Vm) is compared to the ionic reversal potentials for compartment 8 across simulation time. The addition of IAs into compartment 8 resulted in a more negative charges in the compartment and hence a decrease in the membrane potential for the compartment. To maintain electrical and osmolar homeostasis during the addition of IAs, there were transient shifts in the concentrations of permeant ions resulting in changes to their respective reversal. As the volume equilibrated and ionic concentrations returned to steady state values (as seen in Figure 1A), the membrane potential and ionic equilibrium potentials also returned to steady state following the termination of IA addition.

Although IAs were only manipulated in compartment 8 there were near identical changes in all the compartments with respect to membrane potentials (second row). As the compartments are linked via electro-diffusion, changes in one compartment affect the electric field across the longitudinal axis of the dendrite. This result is expected as in Figure 1A it was shown that the permeant anion concentration changes in all the compartments mimic the changes seen in compartment 8 where the manipulation occurred. Similarly, the ionic reversal potentials across the dendritic compartments showed near identical changes (third row).

The changes observed to the membrane potential and ionic reversal potentials meant that there was a change to the respective ionic driving forces during the manipulation of impermeant anions. The changes were again in proportion to across the length of the dendrite and returned to steady state values once the addition of IAs ceased. Therefore, local addition of impermeant anions does not change the steady state excitability of the dendrite.

**Figure 1B** – Increased impermeant anion concentration in compartment 8 leads to unchanged ionic driving forces and thus no change to the excitability of the dendrite.

