# 2 – Impermeant anion valence change

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Description automatically generated with medium confidenceWe next simulated decreases in impermeant anion average charge (valence) using the same 9 compartment model(Schematic 2). We gradually reduced impermeant valence (z) in Comp8 to z=-1.05 (baseline: z = - 0.85 ), 300s and 350s in a 700s simulation.

## 2.1 Local changes to impermeant anion charge (z) alters local compartment volume and permeant ion concentrations

Dropping impermeant anion average charge in Comp8 resulted in proportional increases in Comp8 volume (and hence decreases in impermeant anion concentration). These changes persisted even once the charge had stabilized at z=-1.05. There were no such changes to any other compartments. Cation concentrations in Comp8 increased despite compartment swelling, while anions concentrations decreased. All ion concentrations reached a new equilibrium in Comp8 once the impermeant anion charge stabilized. Along the rest of the multicompartment model there were no changes to the compartment volumes. Minor transient permeant ion shifts occurred during the impermeant charge flux period but returned to baseline levels thereafter.

Table

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**Figure 2A: Impermeant anion charge alters local compartment volume**

**Top row:** Decreases in IA valence in Comp8 from -0.85 to -1.05 results in proportional compartment swelling and decrease in IA concentration. Red dotted lines indicate flux period

**Middle row:** Heat maps for the first 150s of the simulation indicating no observable changes in IA charges, volumes or IA concentration in other compartments.

**Bottom row:** Heatmaps showing Cl-, Na+, and K+ concentrations during the first 150s of the simulation. Permanent decrease in Cl- concentration and increases in cation concentration in Comp8. Transient shifts in other compartments ion concentrations during flux period but otherwise remain stable over time.

**Middle and right pane:** Compartment 8 volume and impermeant anion concentration changes proportionally to the change in impermeant anion charge in compartment 8. No significant changes are seen in any other compartments.

## 2.2 Local changes to impermeant anion charge (z) create a non-isopotential neuron with no changes to ionic driving force

We examined the electrical changes occurring with a reduction in IA charge in the same simulation as above (Figure 2B). Reducing IA charge from -0.85 to -1.05 led to a permanent decrease in the membrane potential (Vm) of Comp 8, as well as a permanent decrease in ionic reversal potentials for chloride, sodium, and potassium - however, no permanent changes to ionic driving forces. The membrane potential of other compartments transiently shifted during the IA charge flux but returned to baseline once the flux ended. At the end of the simulation, we observe a non-isopotential dendrite at steady state.

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## 2.3 Local changes to impermeant anion charge (z) results in local changes to ionic concentrations

We investigated the transmembrane and axial forces maintaining a resting non-isopotential neuron (Figure 2c). To observe changes independent of volume we plot the total mole of each ion in the various compartments. In Comp8 there is an increase in the amount of Na+  and K+ moles, and a decrease in Cl- moles, while no permanent changes are seen across the rest of the multicompartment model.

We attempted to identify where this difference in moles between Comp8 and the other compartments arises from. There were no major differences between the amount of moles of Na+, K+ or Cl- moving across the membrane per time step. Instead, it was flux secondary to electrodiffusive forces that showed differences between Comp8 and other compartments.

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We then studied the forces that maintain difference in ionic composition across neighbouring compartments. As observed in Figure2B there are no differences in the transmembrane driving forces, so we applied the same principles to calculate an axial (boundary) driving forces. Figure 2D shows an equal but opposite axial/boundary potential at the Comp7/Comp8 boundary and the Comp8/Comp9 boundary. This phenomenon is present for all ionic species measured. No axial driving forces are seem between any of the other compartments.

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