The brain processes information via intricate non-linear interactions between chemical and electrical signals which vary along narrow spatiotemporal scales further constrained by the physiology of the human cellular functioning. The propagation of such signals can be simulated using computational software such as NEURON1,2 and GENESIS3 which are based on theoretical descriptions of neurons by Hodgkin and Huxley4 in their equivalent circuit model, and was later expanded upon by Rall5 who incorporated geometric aspects in Cable Theory. These theories provide valuable predictions of how signals propagate, however are limited in certain respects. Firstly, they are inaccurate in low volume spaces and in areas with rapid ionic fluxes (e.g. dendritic spines)6,7, and secondly fail to adequately account for impermeant anions which can influence disease processes. Herein I propose constructing an electrodiffusion based multicompartmental neuronal model in the Python development environment which will incorporate impermeant anions and adapt to small scale changes to ionic concentrations. Once constructed I shall run simulations to elucidate the impact impermeant anions have on the biophysical and computational properties of dendrites, and further probe how this may be implicated in disease processes such as neurodegenerative disorders and cerebral oedema. This work will provide better theoretical descriptions of normal and pathological neuronal functioning and may provide avenues for therapeutic intervention.

**Current computational models**

State of the art software used to model the biophysical processes involved in neural signalling includes NEURON produced at Yale University and GENESIS produced at Cambridge. Such software enables neuroscientists to cross-validate experimental findings and allows for estimation of values which are not easily attainable through current experimental methods2. Moreover, NEURON and GENESIS allow for theoretical hypothesis testing which can develop theory of the biophysical mechanisms that exist in the nervous system.

Simulation software can only be as accurate and robust as their theoretical backbone. The theoretical underpinnings of software such as NEURON and GENESIS are primarily based on Hodgkin-Huxley principles of equivalent circuits, and Cable Theory by Rall1. Such theories were developed after experimentation on squid giant axons and are generally good at describing the electrical changes that occur across large spatial scales.

Cable theory considers the equilibrium potential of each ion species as a constant battery (i.e. constant driving force)8. The driving force is considered as the difference between the membrane potential and the reversal potential of the ionic species. In larger neurons the membrane potential and reversal potentials change slowly and in a relatively fixed ratio to each other, thus the assumption each ion can be considered a constant battery is relatively sound.

In smaller spaces however, such as in dendritic spines, there are rapid ionic fluxes within a compartment. Therefore, the ionic reversal potentials and membrane potentials will unlikely fluctuate with the same ratio. In such instances modelling ionic driving forces as a constant parameter is inappropriate.

Rapid ion fluxes occur is certain neurological disorders and hence modelling such dynamics is important to studying these conditions. In rapidly progressive depression wh

In order to account for the detailed spatiotemporal changes in ionic concentrations an electrodiffusion based model that is predicated on the Nernst-Plank Equation has been developed9,10. In these instances, the battery is dynamic.

Such theory is excellent at describing the changes that occur with large time scales and large volumes, however, are limited in many respects.

**These theories are limited as…**

**Since these theories are limited we need new software to test hypotheses.**

Consider reorganizing this to discuss the state of the art first and then discuss the problems and how electrodiffusion and impermeant anions can solve it.

Dendrites can be considered cytoplasmic extensions of the neuronal cell body. This is evident as the proximal portion of the dendrite have the same organelles as the soma (barring the nucleus). Distal portions of dendrites too may contain some organelles, specifically endoplasmic reticula and ribosomes11 . As the cellular machinery for protein synthesis occurs throughout the dendrite and soma in a non-uniform manner, it is likely proteins are heterogeneously distributed in the neuron. These proteins are trapped intracellularly, are negatively charged, and have an osmotic pull contributing to the electrical and osmotic properties of the cell. Moreover, these proteins may have different average charges as well as concentrations within different parts of the dendrite which may further contribute spatial difference in the electrical and osmotic properties of the dendrite.

Consider putting parts of the cell membrane modelling here

It is thought that dendritic branching structures also may have functional implications. There are on average 1-9 dendrites per cell body each giving rise to a dendritic tree. The specific architecture of dendritic trees likely relates to the function characteristics of the related neuronal population. Alterations in the dendritic branching structures have been shown to play a role in epilepsy, Down’s Syndrome and certain dementias12. Detailed computational models therefore need to integrate the intricate and diverse branching structures of dendrites in order to comprehensively understand its functioning.

Dendritic spines are a further consideration in dendritic modelling. Dendritic spines are pedunculated appendages, roughly 1µm long13, along the length of dendrites in certain areas of the brain. ‘Spiny’ neurons are those that have a high density of dendritic spines which can be in the range of 40 000 – 100 000 per neuron. Inside dendritic spines there are rapid ionic fluxes including calcium transients which aren’t accounted for in traditional neuronal models. The function of dendritic spines is still not precisely known however synapses directed towards the dendritic spines are thought to be predominantly excitatory in nature, while the synaptic input to the rest of the dendrite is generally inhibitory.

Adequate modelling of dendritic spines in the context of the entire dendrite and its branching structure may help shed light on the emergent functions of dendritic spines. It has been speculated that alterations to spine morphology and density could be a substrate for memory based on the supposition that memory can be modelled as changes to synaptic weights14. Other proposed functions for dendritic spines include chemical and/or electrical isolation; attenuation of electrical signal; amplification of electrical signal; increasing the surface area of dendrites; impedance matching; and other forms of signal modulation.

To begin advancing theoretical models, the fundamental and well-established principles of cell membrane and ion channel dynamics should be retained. Dendrites are walled by a 3-4 nano meter thick lipid that is impermeable to most charged molecules. Subsequently, charges build up on either side of the cell membrane in a similar way charges would build up on the plates of a capacitor. Ions can escape through various channel proteins and pumps which extend across the membrane. These channels can be modelled as resistors. The inverse of resistance (conductance) can be thought of as an ion’s ability to move across a membrane. A membrane with a high conductance for a certain ion is one that has an abundance of ion channels and is therefore ‘leaky’.

Ionic conductances across the membrane as well as along the length of the dendrite are established by electrostatic and chemical gradients. Ions move down these gradients in order to reach a steady state where there is no net ionic movement. In most conditions the resting membrane potential is in the range of -90mV to +50mV. The primary ionic species which contributes to the overall charge are sodium (Na+), potassium (K+), and chloride (Cl-); bicarbonate (HCO3-). All these ionic species can move between the intracellular and extracellular compartments. Another electrical species which is often neglected are impermeant anions. These are negatively charged molecules such as proteins and nucleic acids which exist both intracellularly and extracellularly but cannot move between these compartments. Due to the vast extracellular space volume relative to the intracellular volume the concentration of the anions is much larger intracellularly and may play an important role in the electrical properties of the dendrite.

In the traditional computational models two main assumptions are made; firstly, that the extracellular soup is far larger than the subcellular space; and that there are no external sources of electric fields. Both assumptions used to simplify the complexity of the brain are likely invalidated in living tissue. Traditional models also are unable to adequately account for the rapid ionic changes which occur in dendritic spines as these models consider the reversal potential of various ions to remain constant where in reality it various significantly.

According to Savtchenko et al.7 there are 3 major sources of electric fields: (1) fields as a result of electric current flow also referred to local field potentials, (2) fields to the heterogeneity in the distribution of channels causing net submembrane currents, (3) fields across the synaptic cleft

Electrodiffusion based models incorporate the electrical driving forces (drift) of ions, as well as the movement of ions along their concentration gradients (diffusion). Albert Hodgkin, one of the pioneers in the field of neuronal modelling, makes the following analogy: “*diffusion is like a hopping flea… electrodiffusion is like a flea that is hopping in a breeze”*  15*.* To make this analogy more complete we need to consider different fleas moving at different speeds with different breezes. Moreover, each flea is impacting the other as a function of time.

Clearly modelling this highly dynamic and complex process requires significant computational power. This factor has dissuaded computational biologists from pursuing such models, however with the computational resources publicly available in the year 2020/2021, such computations can be performed in a few hours as opposed to days or weeks.

The Gibbs-Donnan effect describes the broad implications of impermeant anions (proteins, nucleic acids, metabolites etc.) have on the cellular compartments enclosed by a semi-permeable membrane16,17. As impermeant anions are trapped intracellular they require cations of equal net ionic charge to move intracellularly to ensure electroneutrality. This will bring water into the cell via osmosis and subsequently dilute the intracellular compartment. The concentration gradient of permeant anions will then also be driven inwards. This repetitive cycle would ultimately lead to uncontrolled cell swelling and bursting if not for active sodium extrusion via Na-K-ATPases. Another possible cellular strategy could be to pump water out of the cell however there is no evidence of aquaporins or similar structures in neurons.

Dusterwald et. al18 showed that the addition of impermeant anions in a multicompartmental model effects the electrical and osmotic functions of dendrites. In a single compartmental model, altering the concentrations of impermeant anions intracellularly or extracellularly did not change the steady state concentrations of the major ionic species due to balanced osmotic changes. However, when the average charge of impermeant anions changes there are significant changes to reversal potentials of various ions, as well as changes to the membrane potential. Due to the relatively constant ratio of these changes the driving force (Vm – Reversal potential) of the various ions do not significantly change.

Similar effects were demonstrated in a multicompartmental model, however the changes to the driving force were even less due to the impact electrodiffusion had on the sodium ion concentrations thus impacting the Na-K-APTase. In both the single and multicompartment simulations, impermeant anion concentrations were key determinants of cell/compartment volumes. Similarly changing the average charge of impermeant anions had a persistent impact on cell volume.

As impermeant anions had significant effect on cell volumes it was postulated that adding impermeant anions in the apical portions of the dendrites may mimic the increases in cell size of a growth cone. This too was shown via simulations thus illustrating the potential ability of impermeant anions to grow neuronal processes, and it may be possible that neurons could use the transport and tethering of impermeant anions to grow or modify the volume of neuronal compartments

**Impermeant anions in disease**

As physiological osmotic balance in the brain is highly regulated by impermeant anions, in cerebral oedema it is likely that impermeant anions play a role in the pathophysiological processes as well. After a stroke or a traumatic brain injury (TBI) the brain swells leading to an increase in intracranial pressure (ICP). High ICPs result in the paradoxical occlusion of blood vessels leading to worsening ischaemia. Ischaemia leads to further swelling which compresses the brain leading to more cell death in a self-perpetuating and destructive manner.

The transition from ischaemia to swelling can be partially attributed to the impact of impermeant ions. When ATP (adenosine triphosphate) is depleted in ischaemia, the sodium-potassium pumps fail leading to an inability to pump cations out of the cell. Impermeant anions drive the inward movement of cations via the Donnan osmotic pressure. This flow causes water to enter the cell and result in cell swelling. Although there are other hypotheses to explain the swelling in ischaemia, it is likely that impermeant anions are perpetuating the pathological processes that are occurring19.

Impermeant anions also contribute to the pathology seen in several neurodegenerative disorders, most prominently are the Tauopathies where Tau protein is one of the hallmarks of several diseases including Alzheimer’s Disease. The Tau protein begins as a soluble intracellular protein but as it becomes phosphorylated and bundled together with microtubules these proteins become insoluble and thus are trapped within the intracellular compartment. Indeed, the clinical progression from short term memory loss to executive dysfunction in Alzheimer’s Disease closely mirrors the accumulation and spread of Tau proteins through specific brain regions (described by Braak’s staging)20. Interestingly, Tau proteins can also accumulate in glial cells and play a role in Progressive Supranuclear Palsy (PSP) and Corticobasal Degeneration (CBD), while increased Tau proteins in astrocytes have been linked to aging.

Several other neurodegenerative disorders are also characterised by subcellular protein build up. In Parkinson’s Disease intracellular alpha-synuclein deposition and is correlated to disease progression and symptomatology. Similarly, in Pick’s Disease, Pick bodies can be found inside neurons. Extracellular protein deposition also occurs in Huntington’s disease, Multiple Sclerosis, Spinocerebellar Ataxia and Transmissible Spongiform Encephalopathy (the most common being Jakob-Creutzfeldt disease)21.

In summary, dendrites convey electrical signals from the periphery towards the cell body. Traditional computational models of neuronal signal propagation are imprecise in certain conditions, and thus electrodiffusion based model may be more suitable. Moreover, traditional models may not adequately appreciate the influence of impermeant anions on the electrical properties of the dendrite. Impermeant anions play a role in several disease processes such as that occurring in cerebral oedema and neurodegenerative disorders. Modelling the role impermeant anions play in normal and pathological states may help lead to a better pathophysiological understanding of the conditions and ultimately to better therapies.

# Aims and objectives

The overall aim of my thesis is to develope a biophysically accurate computational neuronal model incorporating electrodiffusion and investigate the influence of impermeant anions on the electrical and information processing properties of neurons.

The objectives are as follows:

1. Develop a computational tool to dynamically model ion homeostasis, volume regulation, and electrical changes that occur within a neuron.
   1. Create a single compartment model.
   2. Create a multicompartment model incorporating the properties of electrical drift and diffusion.
   3. Create a tool to visualize the changes to the ionic concentrations, electrical properties and cell volume within each compartment as these properties vary with time.
2. Investigate the effects of adding excitatory or inhibitory synaptic input on each of the dendritic compartments as well as at the soma.
3. Investigate the effect of impermeant anions may have on the osmotic regulation of neurons.
4. Investigate the effect non-isopotential neurons have on information processing and dendritic computation
5. Explore how any observed effects may be relevant to disease processes.
   1. How do impermeant anion concentrations change in diseases. Can this be modelled.

# References

1. Hines ML. The NEURON Simulation Environment. 1997;1209:1179-1209.

2. Hines ML, Carnevale NT. NEURON : A Tool for Neuroscientists. 2001:123-135.

3. Bower J, Beeman D. *The Book of GENESIS: Exploring Realistic Neural Models with GEneral NEural Simulation System*. Springer; 1998.

4. Hodgkin, Huxley. A Quantitative Description of Membrane Current and its Application to Conduction and Excitation in Nerve. *J Ph*. 1952;117:500-544.

5. Rall W. Wilfrid Rall: A short autobiography.

6. Pods J, Schönke J, Bastian P. Electrodiffusion models of neurons and extracellular space using the poisson-nernst-planck equations - Numerical simulation of the intra- and extracellular potential for an axon model. *Biophys J*. 2013;105(1):242-254. doi:10.1016/j.bpj.2013.05.041

7. Savtchenko LP, Poo MM, Rusakov DA. Electrodiffusion phenomena in neuroscience: A neglected companion. *Nat Rev Neurosci*. 2017;18(10):598-612. doi:10.1038/nrn.2017.101

8. Lopreore CL, Bartol TM, Coggan JS, et al. Computational modeling of three-dimensional electrodiffusion in biological systems: Application to the node of Ranvier. *Biophys J*. 2008;95(6):2624-2635. doi:10.1529/biophysj.108.132167

9. Ellingsrud AJ, Solbrå A, Einevoll GT, Halnes G, Rognes ME. Finite Element Simulation of Ionic Electrodiffusion in Cellular Geometries. *Front Neuroinform*. 2020;14(March):1-18. doi:10.3389/fninf.2020.00011

10. Solbrå A, Bergersen AW, van den Brink J, Malthe-Sørenssen A, Einevoll GT, Halnes G. A Kirchhoff-Nernst-Planck framework for modeling large scale extracellular electrodiffusion surrounding morphologically detailed neurons. *PLoS Comput Biol*. 2018;14(10):1-26. doi:10.1371/journal.pcbi.1006510

11. Hammond C. *Cellular Neurophysiology*. Vol 480.; 1994. doi:10.1113/jphysiol.1994.sp020392

12. Wu J and. *Foundations of Cellular Neuroscience*.

13. Dayan P, Abbot L. *Theoretical Neuroscience*. Vol 4.; 2014. doi:10.1080/2153599X.2014.951911

14. Johnston, Wu. *Foundations of Cellular Neurophysiology*.; 1995. doi:10.1097/00004691-199703000-00009

15. Koch C. *Biophysics of Computation Information Processing in Single Neuron*. Vol 11.; 2004. doi:10.3902/jnns.11.157\_2

16. Kay AR. How cells can control their size by pumping ions. *Front Cell Dev Biol*. 2017;5(MAY):1-14. doi:10.3389/fcell.2017.00041

17. Blaustein M, Kao J, Matteson D. *Cellular Physiology and Neurophysiology*. 2nd ed. Philadelphia; 2012.

18. Dusterwald KM, Currin CB, Burman RJ, Akerman CJ, Kay AR, Raimondo J V. Biophysical models reveal the relative importance of transporter proteins and impermeant anions in chloride homeostasis. 2018:1-30.

19. Elkin BS, Shaik MA, Morrison B. *Fixed Negative Charge and the Donnan Effect: A Description of the Driving Forces Associated with Brain Tissue Swelling and Oedema*. Vol 368.; 2010. doi:10.1098/rsta.2009.0223

20. Goedert M, Eisenberg DS, Crowther RA. Propagation of Tau Aggregates and Neurodegeneration. *Annu Rev Neurosci*. 2017;40:189-210. doi:10.1146/annurev-neuro-072116-031153

21. Schaffert LN, Carter WG. Do post-translational modifications influence protein aggregation in neurodegenerative diseases: A systematic review. *Brain Sci*. 2020;10(4):1-37. doi:10.3390/brainsci10040232