ES327 Project

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Contents

1 Abstract

Molecular communication for plant systems is a comparatively new field of research, trying to define cell states using the input, and outputs via a range of mechanisms. Transport of a signal via diffusion is assesssed here, with a model developed using MATLAB to assess the behaviour of an impulse signal propagating between two points in a grid based diffusion network. The effects of this are then evaluated when varying the properties of the system, changing the biological pathways and the internal cell behaviour to rank changes upon system behaviour, concluding that the underlying biological featured controlled by the cell have the largest effect. This is then applied to missing connections, to establish the impact upon decison making, with an increase in missing pathways having a non linear increasing effect on the distance travelled. The antagonistic interactions between opposite signals are compared for adjacent auxin and cytosine signals, with a positive relationship with increasing initial molecules and final system state.

2 Introduction

Plants need to respond to a variety of external stimuli to operate, including detecting light, food, and pathogen detection. The ability to identify and discriminate between these positive and negative environmental behaviour is essential for maximising growth and responses for success, i.e. signalling growth when sunlight is detected. These require the detection of these external events via sensory cells, to allow the conversion of external stimuli events via signal transduction to signalling molecules, which can be detected and induce behaviour across a range of target cells.This triggered response from the cell often involves a change in the state of the cell, ranging from a modification in hormone production, shape, gene expression and individual cell outputs including further propagating signalling molecules. It is from measuring this network of propagation, that the final state of a system, and overall system behaviour can be described, and thus predictions on the behaviour of whole cell tissues are possible. The impulse response of a signal is an essential part of this.[1]

This project objectives are to evaluate the propagation behaviour of signalling molecules through a mesh of cells, comparing the optimal pathway routes whilst changing assumptions on modelling parameters and route limitations to mimic realistic scenarios. As the structure of the network and the distance travelled changes, the behaviour of the plant changes as multiple signals and their interactions are combined, resulting in unique states. The influence of each parameter will be assessed, understanding the impact of modifying them on the whole system. As such this project is intended to be a preliminary investigation into the behaviour of single signal propagation, opening further investigation for modelling multiple molecules. The aim to describe these propagations in a way that can be integrated into large scale models of tissue structures, and full organ behaviour.

The aim of an individual signal is to transfer information between two locations, and for most higher plants, the change occurs when the nucleus of a plant, receives a signal and changes output. The first interrcellular signalling via diffusion is initially triggered when an extracellular cue causes the first messenger molecule to bind to its receptor. This causes an influx of ions across the plasma membrane, and transmit through the cytoplasm to the nucleus. There are multiple mechanisms which are responsible for transport through the cytoplasmic bulk between the cell boundary through to

specific organelles, depending on the specialised receptor. The most common methods of intercellular communication being GTP binding, protein kinade cascades, and membrane ion channels.[2] Each signal has an impact on the nucleus, causing a change in the DNA-binding proteins, which in turn trigger changes in the regulatory regions of genes, leading to changes in overall tissue behaviour, and plant behaviour.

Each cell in a multicellular organism is typically exposed to multiple external signals, which result in a dynamic, constantly changing cell state to address environmental concerns. In order to determine which signals are relevant to respond to for a given cell, the use of specific signalling receptors are used, each attuned to a specific signalling molecule, thus restricting the range of cells that could affect a given cell.[3] However, signalling interplay does occur, and where a combination of stimuli are present, different outputs are produced than would occur in isolation. An example of this is the signalling interaction between LRP-1 and LDL, combining to reach the threshold for ERK phosphorylation, neither of which individually reach this.[4] This in turn leads to changes in feedback mechanisms, (both positive and negative), amplifying or attenuating the signal. As such when the distance and trigger order change, the overall system behaviour changes.

The exchange of these small signalling molecules is carried out differently in plant cells as compared to other organisms, with the lack of a gap-junction separating it from animal cells. The absence of this transport mechanism is due to the existence of the cell wall. The rigid cell walls existence as a partially permeable membrane makes diffusion through extracellular space slower, and in many cases less important, whilst the plasmodesmata forms the other, transportation network. Plasmodesmata comprise of a series of thin channels that connect the cells through the cell wall (connecting the cell protoplasts).[2] This allows for the individual transport of macromolecules to occur which ignores the cell wall, known as symplasmic transport. This in combination with apoplasm (movement through cell walls and intercellular spaces) are the two primary methods of sending signals on a cellular level, whilst other specialised systems are used to transport signalling molecules via the phloem for long distance transport.[5]

The symplastic movement through the plasmodesmata occurs via diffusion, down concentration gradients existing between cells, in a passive system. This is in contrast to other mechanisms for actively moving particles, e.g. Ca2+ uptake in root cells, and applies as the most energy efficient

route of transport.[6] Even within plasmodesmata there are several potential methods of transport with the key one discussed in this project being simple diffusion down concentration gradients through the cytoplasmic sleeve. The other key aspects of plasmodesmata transport can be broadly categorised as: (i) Diffusion with channelling effects (ii) advection (iii) active symplastic transport (iv) diffusive transport within the desmotubule. [7] A brief discussion of these is carried out and possible further extension of this project could integrate the behaviour of all these competing factors into a single cohesive model. Despite the number of alternative propagation mechanisms, simple diffusion still forms an essential part of intercellular transport, and in most cases is the preferred mechanism, because of greater energy efficiency, and lower threshold for failure as compared to other methods. [8]

There are significant variations between cells. The variance is inherently caused by the random diffusion, with changes in the speed of propagation along the different routes. The difference between the time at which the destination is reached which cause changes in the fastest route between iterations. This when coupled with the other biological differences between the cells in the form of cellular pathway differences, and internal cellular processing times changes the final impulse pathway. The cellular pathway changes depending on the plasmodesmata protein structure changing over the course of its lifetime, and the deposits of callose that surround the pathways. This changes the width, and opening size of the pathways, affect the permeability, and randomly affects the behaviour of cell pathways.The preconfigured state of these structures due to external factors can be modelled and the assumptions made for modelling each of these variances compared, with comparisons between uniform or gaussian approximations, a key feature investigated in this report

3 Literature Review

3.1 Biological Systems

3.1.1 Plasmodesmata behaviour

Modelling the behaviour of signal propagation through cell networks, requires an understanding of intracellular pathways, and as such plasmodesmata behaviour is an essential area of understanding. Plasmodesmata mediate direct cell-to-cell communication facilitating the movement of a range of differently sized molecules between cells. This includes a mix of small molecules (sugars, ions) and large complex molecules (various RNA species), depending on individual behaviour. The range of exact molecules allowed through varies, with free GFP (molecular mass 27kDa) being unable to pass through under certain circumstances, whilst small molecules such as Ca+ ions pass freely in almost all scenarios.[8] This is because plasmodesmata act as dynamic channels, in higher level plant systems, constantly changing permeability with various physiological, developmental, and environmental cues using a range of different signalling molecules. There are currently over 30 known molecules that play differing roles in the permeability of plasmodesmata, but isolating the individual triggers is difficult, both in attempting to isolate individual triggers, but also in measuring the permeability of the plasmodesmata at any given interval, with a high degree of accuracy. As such it is more effective to measure the effects on plasmodesmata permeability by the mechanisms by which these signalling molecules cause interaction e.g. changes in plasmodesmata physical structure. The current main areas of research for plasmodesmata behaviour are focused heavily on the interaction between viruses and their propagation through plasmodesmata, with a comparatively lower focus on the response to other mobile proteins.[9]

A key example of the effect of signalling, is the changing behaviour of the plasmodesmata aperture by callose (a beta 1,3 glucan polymer), which can cause the pathway to become gated, decreasing the macromolecule trafficking to insignificant levels. Callose is used as a regulatory and structural component for plasmodesmata, deposited during formation in a reversible reaction, that can be changed by the cell e.g. pH. The positioning of callose can be found at the extracellular space between the cell wall and the plasma membrane, at the neck of the plasmodesmata, and has the effect of restricting access to the cytoplasmic sleeve. This is done, because as the callose

deposits increase, since the cell wall is inflexible, the plasma membrane is compressed, physically decreasing the size of the passage.[10] Although the current assumption is the majority of this behaviour occurs at the neck of the passageway, there is significant discussion as to whether the entire plasmodesmata has callose deposits built up, causing overall shrinking of the entire passage rather than specifically the entry points. This is significantly more important when considering the effect this would have on the large signalling molecules passing through the plasmodesmata, with non-uniform molecules notably RNA's propagation affected heavily by this. [11]

For certain signalling molecules such as auxin, this has been noted to act as a feedback system, with the intended effect of the propagation through the plasmodesmata being to induce change in the cell network, changing their state to trigger cell division or other biological effects. This involves the impulse response of the signal being the most important factor to trigger this, but because this simultaneously causes a build-up of callose, itself regulates in a negative feedback loop, as more auxin passes through the permeability decrease. This provides a limit on the signal beyond the physical limitations caused by spatial behaviour, and the internal chemical storages of the cell.[12] This in isolation, has little effect on the impulse response when passing through pathways, but matters when considering the number of individual signalling molecules that trigger changes in width, and thus it can be expected for an impulse signal to have to pass through significantly differently sized apertures, and consequently the optimal routing to change from over unique iterations in accordance with this.[13]

This also has variance with the size of signalling molecule passing through, with varying sources on the properties of small molecules. There are several studies that imply the small ion passage through the plasmodesmata occurs in an unregulated manner, with the regulation of the cytoplasmic sleeve having little influence upon the passage, typically suggesting molecules of ¡1 kDa moving freely (e.g. calcium). These ideas have been discussed extensively, with the use of low-molecular fluorochromes providing some evidence to support this claim. However other results discussing the behaviour of sieve elements and companion cells, and their connection with plasmodesmata act in direct opposition to this claim. These have very different metabolic requirements and require symplastic communication. As such it is necessary for the plasmodesmata between these cells to be selective towards minerals (i.e. calcium) in order to function. This implies some level of ability for the

plasmodesmata to filter small signalling molecules, and hence this assumption was made for the project.

The placement of callose at the neck region of the plasmodesmata has been evaluated to occur in response to exposure to pathogens, as a defense mechanism for the plant, but is also suspected to occur in response to plants stress, with the exact criteria, and manner of release in these case unknown. This means, that when experimental data is collected, often via invasive procedures, it is feasible to assume an increase in the production of callose at the plasmodesmata neck. This means the width of the plasmodesmata is suspected to vary between that observable underneath an electron microscope and in natural environments. This can be identified when comparing the data provided by differing procedures for measuring plasmodesmata permeability, with different methods of microinjection leading to very different data points ranging over a full order of magnitude.[13] A further piece of evidence to suggest this is via photoactivation experiments, using glucan synthase, which have a lower ability to generate, and deposit callose.In these cases, the range of values is far smaller. As a consequence of this, it is difficult to obtain meaningful data on individual plasmodesmata states, with the fluorescent dyes used when identifying the width causing wound response data. Current studies are attempting to compensate for this effect, via tissue level measurement of symplasmic fluxes, but these are limited to very few molecular options.[3]

Extensive research exists surrounding the experimental determination of diffusion through cell to cell transport, across a variety of cell types. The connectivity of a symplast can be studied by observing the movement of florescent probes, that have been microinjected into the cell, and then pass through intercellular transport mechanisms. This is often used in conjunction with electric coupling and photobleaching to generate an estimate of the diffusion constant and the permeativity of the transport mechanism. However, the consensus on the value of these constants varies between experiments, with the type of collection method used having a large impact on the value measured e.g. 1.1micrometers for E. densa permeability, whilst a comparable cell structure of Arabidopsis Meristem gives a value of 8.5micrometers.

The structure of each plasmodesmata is comprised of a cytoplasmic sleeve, with a surrounding plasma membrane, with an appressed endoplasmic reticulum (also known as a desmotubule) at the centre. The exact morphology of a plasmodesmata varies, but each can often be approximated as a single

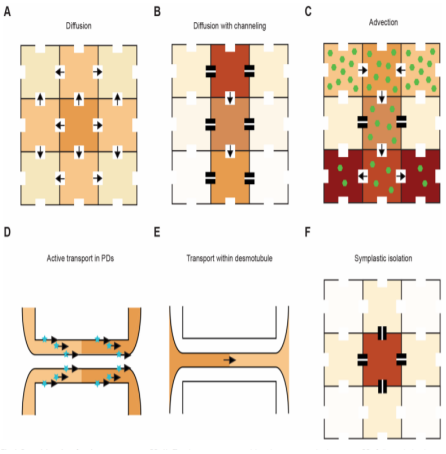


Figure 1: Auxin Propagation Diagram detailing the most common methods of signal movement

cylindrical structure, with a width of 3-4 nm microchannels. The internal structure is often affected by specific spoke like myosin protein structures, that restrict the internal flow of the molecules through the cytoplasmic sleeve. The mechanisms and number of these structures are unknown, with their formation occurring after cytokinesis during the lifespan of the cell. These in combination with actin layered around the desmotubule has an impact on permeability, and likely act as a secondary control mechanism for regulating molecular trafficking. Since their presence, and the magnitude of their effect is unknown and difficult to isolate, it is often easier to model them as a single factor, during propagation, or just to ignore their influence as marginal. [14] [15] [7]

However, since multiple plasmodesmata exist between 2 adjacent cells, mutations and morphology of the structures change interactions heavily. The default case involves the plasmodesmata scattered along sections where the cell wall is thinnest, with a varying density. Where these merge together more complex structures are formed, most commonly involving twinned plasmodesmata or branched plasmodesmata. These involve the combination of primary plasmodesmata with secondary plasmodesmata, which aren't formed during cytosis but during cell wall extension. The mechanisms for plasmodesmata formation after cytosis are unknown, but their effects can be understood experimentally to some extent. The more complex plasmodesmata are the result of these primary and secondary plasmodesmata interacting in different

ways, and result in unique behaviour, often filtering molecules, and changing the diffusion pathways. These complex plasmodesmata exist, but are ignored for the purpose of the investigations, but should be recalled as a major difference between any experimental results and those calculated. [5]

The cell concentrates plasmodesmata into specific regions known as pit fields, where they connect from the cytoplasm into the cytoplastic sleeve. This grouping, has the positive effect of ensuring the distance from the release point of each plasmodesmata to the target organelle is similar, allow the release of the molecules to be treated at occurring at the same point. This means the threshold value for triggering the response by the cell will be triggered more consistently. [16]This assumption is generally made regarding the location of the plasmodesmata, but where multiple pit fields linking two cells exist, distances vary, changing the time-taken for the threshold value to be reached.

Potentially the largest variance in the behaviour between plasmodesmata, is the degradation as part of cell operations. This occurs over time by lowering the density of plasmodesmata via signalling mechanisms or naturally over constant expansion, eventually closing them over the period of time. This has the effect of isolating individual pathways, sometimes cutting off cells and groups of cells from the overall network, symplastically isolating them from surrounding cells. The research into this phenomenon is relatively low despite its importance, with the consequences of it changing the optimal pathing through cells.[17] In cases where cells are isolated, the propagation route between points changes, with longer routes required, and triggering new configurations of cells. The modelling methods used to identify this trend are to justify the removal of the random cells located in frequently used pathways. As with frequent changes of state and constant exposure to intermolecular trafficking, the stresses placed on the cell increase, and thus the cell is more likely to be affected by this behaviour. The exact timing of a cell's isolation and a pathways deterioration is unknown, with weighted random probability acting as the most intuitive method of measuring this.

3.1.2 Signalling Transport Mechanisms

The influence of plasmodesmata transport versus other forms of intracellular transport is an active area of contention, with the biological significance of it contested in many cases. This discussion currently revolves around the

specific behaviour of auxin as a biological signalling molecule but can be applied more broadly to other molecules. The molecule of auxin has a central role in development regulations and growth of the plant, and often travels large distances over several centimetres, and thousands of cells.

The pre-existing literature until very recent studies, place significant emphasis on the behaviour of transporter driven auxin movement as the dominant factor behind cell to cell transmission. This relies on the behaviour of auxin as a weak acid, where the protonated auxin molecules could diffuse outside the cell membrane, with active transporters allowing deprotonated auxin molecules to diffuse outside. The polar localisation of the active transporters allowed for the auxin fluxes to be directed as required through the plant structure. However, studies highlight that investigations using purely transporter driven signalling do not mimic experimental results. These typically implied inaccuracy due to plasmodesmata, formerly suggesting that the auxin transport hindered the ability for active transport mechanisms to carry out movement.[17] More recent studies suggest otherwise, placing significant emphasis on the plasmodesmata based transport, as accounting for the discrepancy between experimental data, and theoretical modelling. [15]

The other mechanisms of transport via plasmodesmata include channelling effects for diffusion. This involves changes in the control mechanisms of plasmodesmata beyond random aperture variation, with specific prior changes between individual cells for directionality, likely because of setting directions of growth; gravitropism. This is best established by the asymmetrical movement of molecules in a horizontal versus vertical direction, determined by increased callose deposits on the transverse direction, compared to the longitudinal direction. Some other effects such as the advection behaviour of molecule transportation are relevant in specific contexts, when considering the interaction between the plasmodesmata and the phloem; a mechanism for transporting signals long distances within the plant. The other main mechanism of transport not being considered, is the diffusion behaviour through the desmotubule. This occurs in parallel with the diffusion through the cytoplasmic sleeve but is very difficult to evaluate the significance of. The size of the desmotubule of 10nm fits around 20 water molecules, which will likely heavily hinder flow through the passage, and will likely be heavily slower than diffusion through the cytoplasmic sleeve, with far fewer signalling molecules able to travel through it. [16]

During specific testing using auxin for Arabidopsis thaliana leaves it was

established that the specific topology had a large impact on the reliability between directions, after finding the permeability was several degrees higher on one side than the other.[18] This was then found to apply to other cell groups include sheath cells and elongated parenchyma cells, with ratios changing from 2 to 5.9 depending on cell.[19] By using electron microscopy this was experimentally evaluated, with calculated diffusion coefficients changing across similar cells. The relevance of this is to highlight the unpredictability of data collection and emphasise the range of advancements still taking place in this field.

3.1.3 Threshold behaviour of signals, and signal responses

The transport from one side of a plasmodesmata to another does not comprise the full length of the signal, with additional distance required for travelling through the cytoplasmic bulk. There is a marked change between the behaviour when in the cytoplastic sleeve vs the cytoplasm, with significantly lower resistance to travel in the cytoplasm. The distance travelled when in the cell also changes from both cell-to-cell depending on topology, but also from signal to signal. This is because of the different internal processing mechanisms that exist for each cell which carry the intermediate role of decoding the message and transmitting it to the relevant organelle. This often requires significant numbers of decoder proteins, and the time-step for molecular release is difficult to evaluate due to the large number of complex interactions.[17] Hence, it is often done to model the behaviour of the cell after leaving the plasmodesmata as a black box, with the effect of the internal cell mechanisms between the membrane and the nucleus outside the scope of this project. There are specialist models that have been developed, and potentially the investigation can be combined with these to accurately model, protein cascades post transmission and thus more accurately assess cell mechanisms.[16]

3.1.4 Antagonistic Hormone Propagation

Beyond the impulse response of a signal travelling from one point to another, the other method of evaluating a signal's behaviour is the final state of each cell after an indeterminate period of time, following different stimuli introduction. This matters for mutually exclusive states, since the time taken for the plant to switch from one of these states to the other is high, due to

the impact on internal chemical stores, and the similarity of the communication methods. The best example of this is the interaction between auxin and cytosine, where they act as inhibitors of each other's effects of growth and growth inhibition respectively. These both are naturally present in plant cells but after threshold values are reached, they cause state changes in the plant's behaviour. Often both signals are sent at the same time in the plant by internal feedback mechanisms, causing a range of different states to occur in similar locations of the plant's tissues. This has interesting applications from a systems perspective, with the mechanisms of cytokinin comparatively unknown, but interactions between them and how these affect the plant's growth. [20]

3.1.5 Interpretations of existing models

There are extensive models for plasmodesmata as part of larger systems, generally focusing on hydrodynamic systems, and interactions in the plasmodesmata for loading into the phloem. These link with the studies on the modification of the permeability of the membrane during these processes but do not generally focus on diffusive transport. The models which consider generalised plasmodesmata structures adopt a number of common conventions, and assumptions when specifically modelling the geometry of the shape which are detailed below:
1. The plasmodesmata as a straight channel assuming the direction of transportation between the two cells to be directly next to each other
2. The pathway between the cells is unimpeded, ignoring the effect of the internal differences between plasmodesmata. This ignores desmotubule interactions, and ignores deviations in protein structures that exist within the passage as a whole
3. The geometry of the passage is a perfect isometric shape i.e. a cone, or funnel
4. Cytoplasmic vs cytoplastic sleeve assumptions are ignored, the typical environment considered is the cytoplasm generally providing a faster diffusion time than reality
5. Gating effects of plasmodesmata are ignored, with the initial state assumed to be identical on every location.
In addition to this, there are common features regarding the lighting, temperature, and cell behaviour which are assumed to be identical between experiments, but in practise tend to vary when comparing experimental datasets,

leading to large ranges between data. This in combination with the difficulties mentioned earlier in the report, lead to high uncertainties when calculating the accuracy of the model. [15] [21] [22]

3.2 General Diffusive Systems

3.2.1 Molecular Diffusion

There exists a wide range of applications for molecular diffusion, with the transfer of information between small devices via molecular communication being applied in manufacturing and component design, particularly on the scales used for nanotechnology. This field of research is separate from the studies carried out using electromagnetic waves, that form the main focus in communications research forming a far smaller, and less researched field. The general method of diffusive communication is the movement of physical molecules, produced by a transmitter, to act as information carriers. These are monitored at a receiver, which triggers a response. This general case is the mechanism employed by diffusion for plant cells, and as such an understanding of information transfer forms an essential part of the investigation.[23]

The general cases considered for information transport, consider the information transmitted both by the time of dispersal but also in the number of molecules dispersed, with the bit rate of the signal used to decode the message at the receiver. The pattern encoded by the receiver is in these cases contain more information than just via number of molecules detected. This is not as applicable to plant cell communication networks because of the biological factors involved, the quantity of information encoded is far below the theoretical maximum and molecule transmission can be treated as a simple binary transmissions leading to on/off states, after a set threshold concentration is reached. The complexity arising from the interactions between different signals. [24]

Molecular diffusion can be explained by using the continuous motion of particles in conjunction with each other, and their constant collisions to cause a change in trajectory. The direction after each collision being random, the mean squared distance travelled becomes proportional to the time taken, where the constant is the molecular diffusivity. This explanation can be used to produce the Stokes-Einstein model, used to model diffusion on a molecular scale, and to produce the diffusion equation.[25]

The diffusion equation is well established in literature, with various solutions applied to all fields, from modelling biological systems, to commercial applications in economics. The most general form of the equation is listed below:

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2}$$

The equation can be solved for a variety of cases, but since the flux is changing across the course of the investigation, applications of Fick's laws are used. The case of diffusion through a finite cylinder are available, and the extensions of these equations have been applied. The calculations used are taken from research studies based on modelling transportation through organ tissues, but with modifications, are broadly applicable to this case as well[26] [27]

The diffusivity coefficient is dependant on the behaviour of the diffusant, and the medium. This factor is dependant on the size, mass and charge of the molecule used, and prexisting lists of experimentally determined diffusion coefficients are used for this. The other parameters needed include the initial concentrations of the diffusant in the system, which in this case is assumed to be trivial.[28] Although not precisely accurate, the difference between the initial concentration in the system and the input amount, is high enough that this assumption is valid. The diffusion coefficient is assumed to remain constant over the course of the experiment. This is because the diffusion coefficient, varies depending on the localised behaviour of molecules and their surroundings, and thus would vary from point to point. The assumed diffusion coefficient is assumed to be the same over the entirety of the cell mesh, ignoring the variations caused by individual effects. [29]

General diffusion behaviour is typically modelled over an infinite period of time and also modelled over an infinite slab for solving problems, calculating the steady state, and transient response of various systems. For each of these cases specific boundary conditions are required. For the case of applying these equations to finite sizes, then analytical solutions are often required. These analytical solutions can be derived from variations of the general forms, and studies exist detailing the methods of doing so. [30] [20]

4 Methodology

4.1 Systems Parameters and Modelling

4.1.1 Single Connection Modelling

The platform used to carry out analysis and modelling was MATLAB, with the uses of Simulink and the inbuilt PDE's solving extensions on the platform. These were used because of ease of use, and ease of integration with other models. This in combination with the software's strong analysis capabilities make it easier to model both individual passageways and the overall large structure.

In order to obtain an accurate understanding of the structure, the plasmodesmata is treated as a uniform, perfect cylinder, and modelled using a 1D solution. Similar reasoning has been found for modelling funnels in previous studies, with variations upon cylindrical models being used prior to this. The difficulty in obtaining exact dimensions, and rough approximations justify this. The effect of all internal structures within the plasmodesmata are ignored, with the boundary of the structure assumed to be hard, rigid, and impassable. The diffusion is treated as occurring in a single direction along a longitudinal axis, with the input assumed to occurring at one end of the passage, and diffusing through to the end, using Fick's laws to model the results of the changing flux. [31]

The key variable parameter across a single channel, are the molecule choice, which changes the diffusion coefficient of the channel and the diffusion behaviour of a cell. This is initially set to auxin, for the base cases but applies to cytosin, Ca and other signals. The dimensions of the tube are another modifiable area and are varied in accordance with the range found in experimental data. These are grouped as follows: (i) variation in tube length (ii) variation in aperture diameter (iii) variation in the tube diameter.

1. Tube Length: The tube length varies depending on how the cells are packed but range from 90nm to 200nm, with a range of the normal and uniform distribution. [19]

2. Tube Aperture Diameter: The initial aperture state is variant between the cells, with plasmodesmata ranging from 20nm to 60nm depending on the callose deposits. This can also be modelled using uniform or normal distributions

3. Tube Diameter: The callose deposits occurring across the full length of

the plasmodesmata cause a varying length across the entire plasmodesmata, along with fluctuations between adjacent cell dimensions. The change in length is 40nm to 60nm but must be larger than the aperture diameter. This is also modelled using the uniform and normal distributions

Generic diffusion systems have been established to contain four subsystems, (i) Transmitters (ii) Propagation Medium (iii) Receiver (iv) Transmission of information.[32]

1) Transmitter: The Transmitter in this case is assumed to disperse a set number of molecules at one end of the tube, at a constant rate, that continues until the receiver is triggered. The molecules are dispersed randomly with no information encoded in the time of dispersion.

2) Propagation Medium: The propagation medium is the cytosolic sleeve, which has known properties. The cytosolic sleeve is assumed to have zero velocity, with the dispersion of the fluid entirely via molecular diffusion. This is because diffusion through a plasmodesmata is bidirectional in most cases, so there is no net movement of fluid in either direction.

3) Receiver Behaviour: The behaviour of the receiver used is just to detect the concentration of the signalling molecule at the end point of the tube. The receiver is assumed to not have any effect in interacting with the signalling molecule, but when the threshold concentration is released, the cell state change is triggered.

4) Transmission of Information: The information encoded by the signal is a binary Y/N signal which is intended to trigger the state of the end cell from the default N state to Y.[1]

There are multiple noise sources used to model the behaviour of the plasmodesmata, with the first being the addition of random Gaussian noise for the movement of particles from one side to the other. The number of collisions allows for a suitable approximation of Gaussian noise to mimic the uncertainty of the behaviour of molecules. This overall cancels out over the large number of particles, but has to be considered because the only method of movement is dispersion.

The other sources of variance that occur for the individual plasmodesmata are by the dimensions of the cell passageways to affect the cell dimensions for modelling described above, along with a gaussian noise factor added to the threshold detection barrier to mimic the variance in the detection mechanisms.

When grouping plasmodesmata together the number makes a big differ-

ence to the final result, with the number set varying between 100 and 500 plasmodesmata. The increased number of plasmodesmata increases the number of molecules diffusing per unit time, which are modelled simultaneously. An extension would be to model more plasmodesmata, but the time taken to carry out the modelling increases exponentially as the number of pathways increase, so a scaling factor is used to simulate the effects of multiple pathways.

4.1.2 Cell Unit Modelling

Each set of tubes is used to connect to connect between cells, linking a mesh of nodes through which connections travel. When a receiver threshold has been met, the internal communication mechanism of the cell begin to interact with the signal. These are modelled together, with the interactions of the cell's detections molecules, the transportation to the organelle, and the time to generate the response, all combined as a single step process. This use of a simple time-step function describes the varying response of the cell to act on the signal, and then start its own individual propagation of the signal. The time taken for internal mechanisms to occurs vary, and is modelled to vary, depending on the type of structure used.

The cell state was considered to be changed between a Y/N state using state-based modelling tools. The default state was set to be N with Y occurring when the threshold concentration was reached. When the Y trigger occurred the cell continued the propagation chain, until the end signal is given. When a state is triggered to Y then it cannot be retriggered by another set of molecules, with the propagation pathway occurring in all directions except the one just travelled down. The limitations on second waves of propagation is because of the internal chemical stores of the cell, both of the molecule that is then propagated, but also of the molecule that is The overall structure is assumed to have a grid structure of cells with plasmodesmata connecting each cell to the adjacent neighbours in an (x,y,z) coordinate grid. The majority of the modelling data displays the same results in both 2D and 3D cell structures, so the z axis was removed before collecting data, despite being present in the model code. The propagation chain is set to continue until a given cell is triggered e.g. if signal started from (0,0) the signal propagates through to (8,8) which stops all the cells in the respective states. This mimics the behaviour of a signal arriving at the final location where it is able to reach, because of tissue boundaries. [33]

4.1.3 Method

The experimental method can be broken down into individual sections testing different areas of the modelling.

Step (i): Initially testing the fastest pathways to reach between cell A and cell B, after propagating through all the intermediate cells. The signal is started at location (1,1) for a cell group of size (n,n), with the route taken to trigger cell B from cell A detected. This is repeated over a set of 100 iterations marking the fastest pathway between A and B. This is repeated for all iterations for 1¡n¡9 in a 2D configuration. This stage involves the removal of all noise factors, assuming the dimensions of the tube, the internal cell behaviour and the chemical stores are all identical. This to establish a baseline result, against which the modified data can be compared.

Initial Parameters			
Diffusion Coefficient	Plasmodesmata Number	Plasmodesmata Dimensions /nm	Diffusing Molecule
220 µm^2/sec	100	Aperture 30 Length 100 Diameter 40	Auxin

Figure 2: Parameters used for the initial base case

Step (ii): The cells have the individual noise parameters inserted, for each case, starting with the noise from the blackbox, and then adding variations across the length, width, and aperture diameters modelled using uniform distributions. The number of plasmodesmata begins to vary, but this is weighted depending on location within the structure, with the central plasmodesmata containing an increased number on average (mimicking physical behaviour).

Step (iii): The cells have the noise varied from uniform to gaussian distributions, for all the noise factors. The gaussian distributions applied have the mean and variance assessed from the experimental data, with an additional cut-off for where p¡0.01 to prevent complete isolation of cells.

Step (iv): The considerations of isolating individual cells from their neighbours is added, removing passages between adjacent cells, and thus changing the pathways between the routes. The number of missing pathways is varied from 1,5,10,20 connections, located at the most frequently traversed paths in the centre of the structure.

Parameters			
Diffusion Coefficient	Plasmodesmata Number	Plasmodesmata Dimensions /nm	Diffusing Molecule
220-230μm^2/sec (to simulate the precise location differences)	100-500	Aperture 30-50 Length 100-200 Diameter 40-50	Auxin

Figure 3: Parameters used for Uniform Distribution

Step (v): The problem is shifted to considering the behaviour of antagonistic signals, with two alternative signals located at points (1,1) and (n,n) which are then allowed to propagate freely. Rather than detecting the fastest routes of transport between these, the final proportion of cells in each state are measured.

During preliminary testing the signal's emission location played a large part in the final distribution of cell state, with adjacent initial auxin signals having a lower impact than auxin placed on opposite sides. The case that better mimicked realistic signal generation was adjacent cells, as typically adjacent cells would detect external stimuli at the same time, and send a signal, and as such this case is used.

5 Results and Discussion

5.1 Results

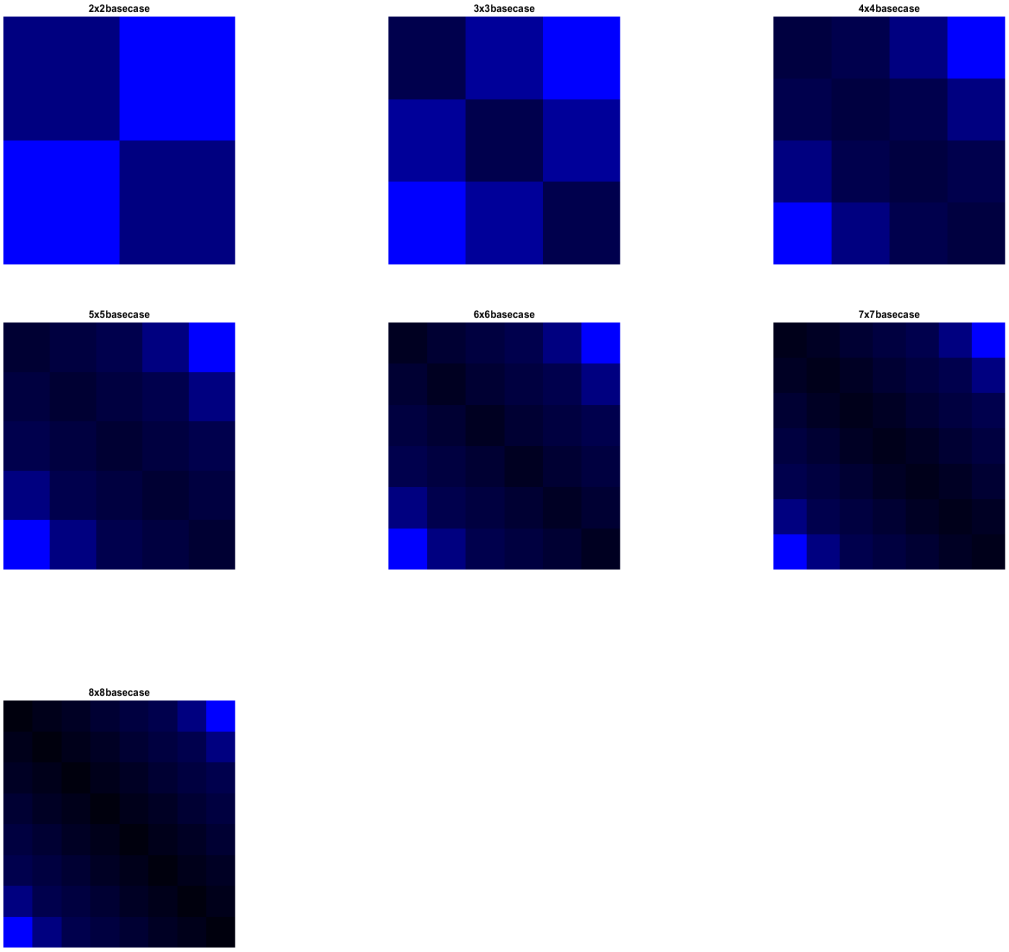


Figure 4: Step (i): For n=1-8: The probability of a square being involved in the fastest route is indicated in the above diagrams. Each square indicates a cell, with the darker colours indicating cells that were along the fastest route in fewer iterations. are repeated over 100 sets, before the fastest routes are displayed. The starting propagation square is in the bottom left, with the final propagation square in the top right.

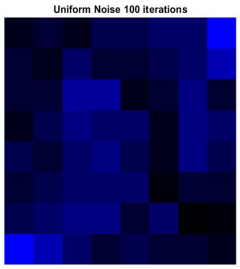


Figure 5: Step (ii) Uniform Noise n=8 : Figure: Diagram showing the cumulative effects of all noise factors modelled with a uniform distribution.

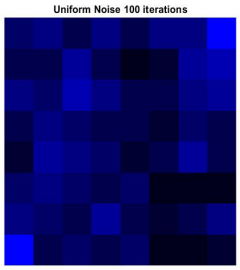
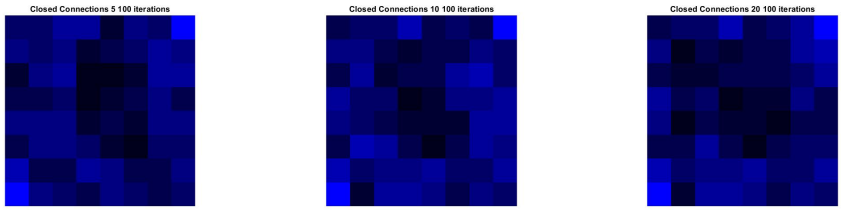


Figure 6: Step (iii) Diagram showing the cumulative effects of all noise factors modelled with a gaussian distribution

5.2 Discussion of Results and Analysis

Where the system had no noise included by any components, then the diffusive pathway indicated showed perfect symmetry, with the time taken to travel directly proportional to the total distance travelled, all other parameters being the same. This is shown for all system sizes tested, from 1-8. When there are multiple routes N that have identical lengths, then the probability of any length being chosen is 1/N, being equally likely to be travelled down, shown by the decreasing certainty of a route being travelled down, as the route number increases. This is represented by the same colour change at each pathway. This serves as a baseline against which the remaining results can be compared, evaluating the impact of the additional mechanisms on the behaviour of the network.

Inspection of the graphs provided in the previous section allow for visual analysis. The variation between the normal, uniform and the base case graphs



(a) 5 missing (b) 10 missing (c) 20 missing

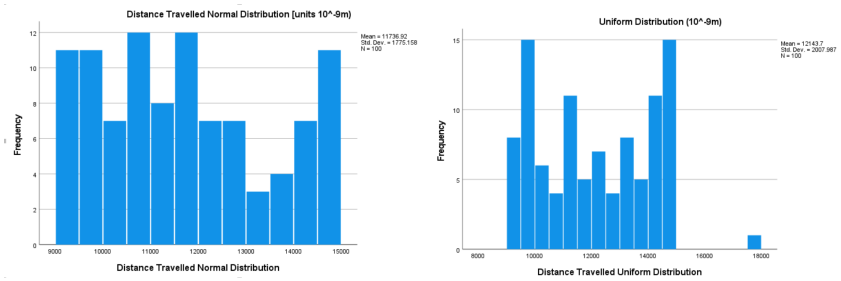
Figure 7: Step (iv): The addition of decayed pathways, and their effect on the trigger order of cells.

provide initial confirmation of the differences in routes that occur. The visible trend between the normal and the base graphs show a increasing probability of travelling through the centre sections of the graph, with a lower percentage of the fastest routes travelling between the edges and the centre involved in the structure. This is likely due to the higher concentration of plasmodesmata in the centre, increasing the probability of the fastest route located there. This also implies that the plasmodesmata number is a large factor when determining the optimal route, as that is the factor that changes the most.

The uniform and gaussian optimal routes share several features, with the increased probability of travelling through the centre reflected in both. The darker edges reflect the decreased chances of travelling at the edges of the structure, which is a common feature. The individual distribution of lighter and darker squares is varied between the two, with no overall consensus.

The modelling of uniform and gaussian distributed noise and the similarities is interesting, when trying to identify how closely the behaviour of uniform noise and gaussian noise mimic each other with 100 samples. A comparison of the means of both data sets suggests the comparison between the two is accurate, suggesting that modelling the noise as either gaussian or normal has a relatively minimal effect at this level.

However, the number of iterations used have to be considered, when analysing the visual distribution of patterns, with only 100 total iterations. This has the effect of evaluating This should in this case trend to a normal distribution for data, according to the Central Limit Theory (CLT), but from viewing the histograms for the data for both a normal, and uniform distribution, this does not appear to have occurred. The most likely cause of this is a small sample size error, with a large number of noise factors, each



(a) Normal (b) Uniform

Figure 8: The histogram distributions of the major routes

having a significant impact on the result. This means that a larger sample is likely needed to perfectly achieve desired results. For the remainder of the analysis, CLT is treated as a valid assumption, with the assumption made that the sample could be modelled as a normal distribution. This is because of the standard use of this assumption, and the underlying assumptions of the model all suggesting it.

A t-test was used to evaluate the difference between the values for the uniform distribution, and the noise free values, to evaluate the significance of biological noise within the system. Several assumptions needed to be made to assess this, (i)normal distribution which is provisionally assessed via the CLT assumption, (ii) the distances are treated as continuous (iii) each iteration is independent (iv) all significant outliers are ignored which was assessed via inspecting a boxplot. When adding the biological noise to the system the increase in noise has a statistically significant effect on the output: $t(99)=9.785$, $p=0.00$. This emphasises the need for considering biological components when modelling overall tissue behaviour, as even over a small array of cells the effect is large, scaling this to other behaviour.

By applying factor analysis to the overall network of cells, it is possible to identify impacts of the underlying properties of the network, and thus weigh their impacts. The underlying pathway structure (assessed by the plasmodesmata structure parameters) can be weighed against the cell structure (assessed by internal cell parameters) and these against diffusion based noise (assessed by the variance in diffusion coefficients, and propagation). This intuitive association was confirmed by exploratory analysis, and then Johnson's relative weight analysis applied to all the parameters. The effect of the weighing on the 6 key parameters are listed below:

Diffusion Coefficient	6.3%
Number of Plasmodesmata	26.2%
Length	21.3%
Width	19.7%
Aperture Diameter	9.0%
Internal Molecular Behaviour	17.3%

Figure 9: Each factor weighed according to impact on final result

The results of this show that the number of plasmodesmata is the most important individual factor, with the important underlying factor being the structure of the pathways between cells. The effect of the internal molecular behaviour and overall cell behaviour is second, with the molecular diffusion parameters ranked third for underlying parameters. This suggests that the largest effect on molecular communication is the one most actively controlled by the plant itself, with the number of plasmodesmata, and dimensions, the easiest factor to modify via callose deposits.

The missing connections data for fastest routes show a increasing difference from the of the normal and uniform results as the number of missing connections increases. For the graphical representation of missing connections, only one case of missing connections is shown per length, but this is sufficient to highlight differences. The initial differences is low with ¡5 connections having no significant variation. For 10 connections, there is a distinct patch in the centre of the array cells (5,3) to (5,5) where the probability is lower than the remainder of the grid. This is where the missing connections are located. The grid for 20 connections missing shows this same behaviour to a larger extent, for (3,4) to (7,6), a larger overall effected region. For routes larger than n=20 the effect is greater, but the overall connectivity between cells begins to be effected, with increasing probabilities that the signal will not be able to propagate to the final receiver at all.

The difference is marked for total average distance travelled as well. By applying multiple tests of missing connections at the (5,10,20) level the effect of the missing connections was calculated to a higher degree of certainty. By carrying out t-tests for the significance of the data, the results for n¿10 show strong statistical significance, showing a difference from n=0 normal distribution. The data trend shows an increase in length as the number of

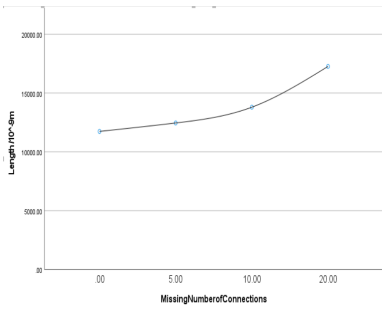


Figure 10: Distances for the cases displayed in figure 7

missing connections increases. This was confirmed using bivariate pearson correlation, showing (0.799, at the p=0.01 level). This result supports the intuitive concept that the number of routes matter when passing signals. When applying the consequences of this to biological systems, this would likely be significant when considering viral infections, where the plant would attempt propagation by closing as many plasmodesmata as possible, as well as when considering the feedback systems for hormone propagation discussed in the literature review.

By extending the results, it is possible to calculate the effect of diffusion through larger systems, and with larger numbers of missing plasmodesmata which are time-intensive to model computationally. This was done via regression analysis, but the statistical significance of this extrapolation is low, and there is significant probability that the behaviour will show increasing non-linearity as the number of missing connections increases. This is because the number of routes available decrease in a non linear manner as n increases.

The addition of antagonistic signals produced interesting effects on the overall system behaviour. The use of multiple propagating signals, and the measurement of the final state rather than propagation distance itself, led to variations as the number of signals increase. The base case displayed a perfect 50/50 distribution between the two signals over 100 iterations. When increasing the number of signals interacting with each other, the numbers shift towards the side with the greater number of initial signals, at a statistically significant level, but the skew is less than initially expected, with a mean increase of 4 percent for auxin, for each added signal source. This is because the initial assumptions used in multiple signal source modelling typically assume either treat signals independantly, or assume signficant starting

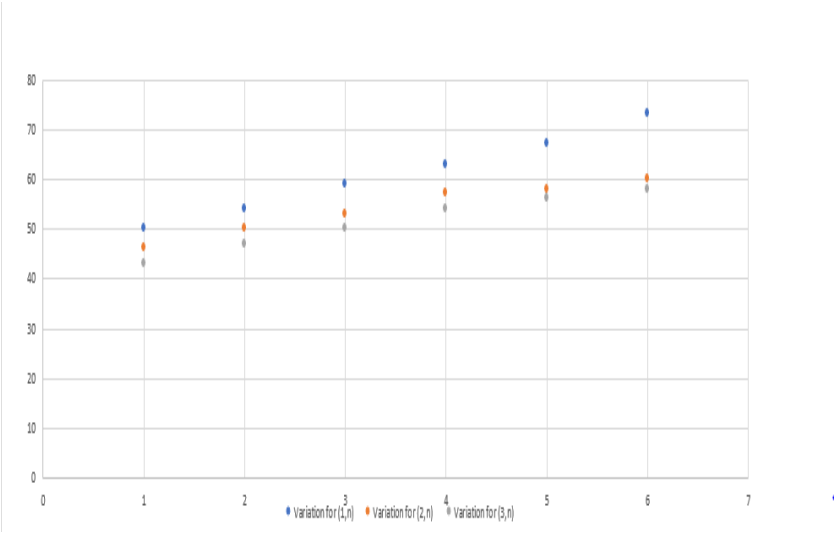


Figure 11: Antagonistic Signal Behaviour for multiple added signals

distance between sources, assumptions which would increase the percentage increase per signal source.

6 Conclusion

To conclude, the project was able to analyse the effects of different noise factors on signal propagation through a cellular mesh, and assess the impact of different noise factors on each case. The effect of variations in cell behaviour was concluded to have a significant impact on the overall rate of signal propagation, both in terms of overall distance travelled, but also the fastest possible route distribution. The normal and uniform approximations are modelled to have similar effect when using T-testing but there is some doubt as to whether the data shows a neat uniform trend, despite CLT, to this end more testing is likely needed on a larger sample of data. The effect of each parameter on the overall response was evaluated, with the number of plasmodesmata having the largest overall effect of the individual factors, whilst the diffusion coefficient and diffusion behaviour had the lowest. This when used to predict the overall underlying factors involved suggests that the parameters actively modified by the plant in plasmodesmata dimensions, and number form the most significant factors for the overall signal transportation.

The project was extended via examining the effect of missing connections on the overall system behaviour, which suggested an increase in the number of missed connections would have an increasing impact on the distance travelled. This could be extended via future investigations by applying the cell mesh to a physical set of cell nodes and connections, with the missing connections based on experimental results, rather than random removal from the network. This would open opportunities into investigating the behaviour of different cell tissues, and contribute to molecular communications in plant based systems.

The behaviour of the antagonistic signals is interesting, and this initial investigation opens furtehr avenues for exploration, using the same model, by changing the behaviour and characteristics of different signalling molecules, and how they change over time with a greater number of cell states. The results shown in this investigation suggest that the number of signals used, has an impact on the overall decision made by the system, and scaling this could lead to understanding behaviour on full tissues.

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