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Mathematical Modelling of Auxin Signalling and Transport

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ABSTRACT

Background: Auxin, a plant hormone, has been shown to be integrally linked to plant growth. However, due to the small size of auxin molecules it is experimentally difficult to estimate the concentration of auxin within a cell. Therefore, mathematical modelling is a useful tool in testing predictions and understanding auxins behaviour. Two important systems relating to auxin are: auxin transport, the way auxin moves through plant tissue and the patterns it forms; and auxin signalling, the pathway through which auxin affects the activation and repression of genes through auxin sensitive proteins. However, the exact dynamics of auxin transport and auxin signalling remain unclear.

Aims: The aim of this thesis is to explore experimentally observed interactions relating to auxin signalling and transport in a modelling environment. This is in the pursuit of understanding their potential ramifications and interactions. Therefore, to address this aim we study several pre-existing auxin transport and signalling models, altering them to introduce related phenomena and examining how they react.

Chapters (3-5): In Chapter 3 we examine the effects that cell growth and elongation has on concentration-based auxin transport models. In Chapter 4 we investigate how oscillations in auxin concentration affect auxin signalling models to study how oscillatory behaviour in an auxin transport model might affect auxin signalling. Finally, in Chapter 5 we explore auxin signalling in the ancestral plant *Marchantia polymorpha*, testing whether our current understanding of its auxin signalling system is sufficient to explain some experimentally observed behaviour.

Conclusions: Findings show that the introduction of growth may induce oscillations in auxin concentration within a concentration-based auxin transport model. We also go on to show that oscillations in auxin concentration, like the

aforementioned ones, can alter the period of oscillations in an auxin signalling system and exhibit behaviour similar to resonance. Finally, we show that a mechanistic auxin signalling model for *Marchantia* is currently unable to produce the expected results at this time.

DECLARATION

I, Simon Bellows, declare that this thesis and the work presented in it are the results of my own research. I thank Shubhajit Das, Anthony Bishopp and George Janes for their collaboration in supplying scientific data. This work was wholly done whilst I was registered as a PhD student at the University of Nottingham.

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guided me since further back. I'm reasonably sure that I only came to mathbio and reaped the life I have now due to subconsciously wishing to be like you. I mean why else would I have decided to use maths to study squishy plant things.

Parents, I thank you for the great opportunity cost incurred by deciding to raise me into such an amazing person, that has been very helpful and whilst I know I won't pay you back I will give you the dubiously rewarding honour of my gratitude. With a RRP of £160,692 in 2021 according to the Child Poverty Action Group, I hope I was worth it.

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Chapter 1

Introduction

Auxins are a family of plant hormones that have been shown to be integrally linked to plant growth [Teale et al., 2006, Estelle, 2011, Leyser, 2018, Paque and Weijers, 2016]. However, due to their small molecular size, auxin distribution is hard to track directly [Pařízková et al., 2017]. Therefore, there is a need for mathematical modelling to test hypotheses concerning the manner in which auxin concentrates, within a plant, by producing testable results; for example, analysing models is a key tool to understanding the mechanisms of auxin transport, as discussed below. In this thesis, there is a focus on exploring experimentally observed interactions in a modelling environment in the pursuit of understanding their potential ramifications and interactions.

Furthermore, there are wide-reaching implications associated with having a greater, and more precise, understanding of the nature of auxin. For example, due to how integral auxin is to plant growth, a greater level of mastery over its associated processes could be impactful in finding solutions to issues of food security and sustainable agriculture. For instance, we could genetically alter or breed plants to enable plants to grow better in poorer soil conditions. In addition, we could improve herbicides, leading to an increase in crop yields.

Moreover, as populations and the quantity of arable land required to support them increases we must find new ways to prove Malthus, and his prediction that food production would fail to meet our needs as population grow, wrong

[Malthus, 1798]. To this end, auxin has been shown to be potentially pivotal in the improvement of crops. One recent example is that an accumulation of auxin in the roots of wheat has been associated with an activation of the plants water-saving traits and an increased yield during drought conditions [Sadok and Schoppach, 2019]. It is areas like this which show how important auxin might be in improving the efficiency of arable land with low precipitation or reducing the need for irrigation.

Auxin herbicides were first introduced in the 1940s, and whilst auxin at physiological concentrations promotes growth, it has been found to be detrimental to plants at artificially high concentrations. Since their introduction they have had a relatively low rate of resistance considering how long they have been around. However, as their usage increases so does the risk of greater levels of resistance and the risk of said resistance spreading to more weed species over greater geographical areas [Busi et al., 2018]. Thus, a greater understanding of the mechanisms at play could improve our understanding of how to craft more effective and efficient herbicides and how to overcome the resistance to auxin herbicides.

Auxin transport models have been around for just over 40 years with the first model published in 1980 [Mitchison, 1980]. This was a flux-based model, which was designed to represent the manner in which vein like networks would form, thereby transporting auxin in a self reinforcing pattern. However, in 2006 a new type of model, known as a concentration-based model, was developed to represent the manner in which spots of high auxin concentration form [Jönsson et al., 2006, Smith et al., 2006]. These models have been investigated with a focus on realistic domains (using computationally intensive simulations) or rigorous analysis on more static domains (with idealised geometry) [Mourik et al., 2012, Draelants et al., 2015, Fujita and Kawaguchi, 2018].

However, what is not seen in the literature are investigations on what can be learned by studying the interaction of auxin transport in greater isolation of other factors, such as complex cell geometries. Furthermore, to the best of

our knowledge there has been little mathematical analysis done with growing domains. One exception to this is [van den Berg et al., 2021], which investigated the effect of growth on a domain that, different to ours, was 2d, had different rules for different types of cells, and which did not permit feedback between auxin and the distribution of the proteins that actively transport auxin in or out of the cell. For a more full review of auxin transport and signalling see [Cieslak et al., 2022, Rutten et al., 2022]. One intended outcome of this work was to improve our understanding of the implications of the behaviour represented in concentration-based models. Specifically, we wanted to put forward a potential cause for the temporal oscillatory patterns of auxin in roots.

Downstream elements of the auxin signalling pathway have been observed oscillating [De Smet et al., 2007, Moreno-Risueno et al., 2010a]. Similarly, auxin signalling models have been shown to produce limit cycles that mimic this, and auxin transport models are able to produce periodic solutions and travelling waves. However, auxin signalling models have primarily been investigated in single cells in the absence of auxin transport. One exception being a 2020 paper [Allen and Ptashnyk, 2020], which considered how auxin signalling might affect auxin distribution in a flux-based model.

Nonetheless, what has not been investigated, to the best of my knowledge is how auxin transport may affect auxin signalling. As both the periodic solutions and travelling waves in auxin transport entail oscillatory inputs of auxin at the single cell level. This raises the question of how oscillations of auxin itself interact with its downstream elements. Furthermore, can there be resonance or can signalling filter out the periodic inputs at some specific frequencies?

The vast majority of modelling work on auxin so far has relied on experimental data from the model plant *Arabidopsis thaliana* (cf. next chapter). More recently, the plant *Marchantia polymorpha* has been found to have a much simpler auxin signalling network. Whilst we go into more detail in Chapter 2, *Marchantia* is especially useful as a model organism due to having the simplest auxin signalling

system possible whilst still being analogous to other plants such as *Arabidopsis*. For example, whilst *Arabidopsis* has tens of some of the different types of proteins, *Marchantia* has but one. However, the area of auxin signalling within *Marchantia* is also a relatively new one, with the primary works on the interaction and classification of relevant molecules being published within the last decade [Kato et al., 2015].

As a result, this is still a quickly evolving field and there is, to the best of my knowledge, no literature on the specific modelling of auxin signalling in *Marchantia*. Therefore, to facilitate bridging this gap, we have worked with a research group in Wageningen who are producing data on auxin signalling in *Marchantia*. Thus, the intended outcome was to review the effectiveness of the currently identified auxin signalling elements in *Marchantia* thought to cause the behaviour observed.

This thesis will be structured as follows. In Chapter 2 a review of the current literature and relevant information is presented. This focuses on the elements related to auxin, its transport, and auxin signalling. Chapter 3 explores auxin transport on a growing domain. It looks at the behaviours that arise and juxtaposes them with similar behaviour found in the Swift-Hohenberg equation, a pattern forming PDE (partial differential equation). The result is an interesting use of continuation to understand the mechanisms behind spatio-temporal patterns and a hypothesis as to the cause of the oscillatory behaviour of auxin in the root tip.

In Chapter 4 the interaction between auxin signalling and auxin transport models are explored. The hypothesis being investigated was that instead of perceived oscillations in auxin being caused either by oscillations in the auxin signalling system or by auxin transport alone, it might be caused by both in conjunction with one another, and that there might be a non-trivial interaction between them.

Chapter 5 concerns the modelling of auxin signalling in *Marchantia* and an

exploration of how current understanding of the relevant dynamics compares to experimental results. It starts by trying to adapt and apply existing models to *Marchantia*, before fitting a series of models to time series data provided by our experimental colleagues and seeing if the identified interactions are sufficient to explain the behaviour.

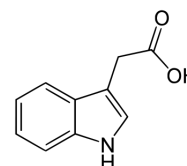
The thesis also includes a final chapter with some conclusions, and two appendices.

Chapter 2

Literature Review

2.1 Auxin

Auxins are a family of plant hormones that include indole-3-acetic-acid (IAA, the most common in higher plants, as shown on the right) and other chemicals which affect plant development in a similar way to IAA [Paque and Weijers, 2016]. Though only four are known to be synthesised by plants: IAA, indole-3-butyric acid (IBA), 4-chloroindole-3-acetic acid (4-CL-IAA) and phenylacetic acid (PAA) [Simon and Petrášek, 2011]. However for simplicity, it is customary to use the singular form *auxin*, or the abbreviation IAA. Nonetheless, despite being a small, rather simple molecule, auxin is involved in a significant number of distinct processes and is, therefore, vital to plant growth. As such, its activity and function have been major topics of research since its postulated existence by Darwin in the late 19th century and its discovery by Fritz Warmolt Went in 1928[Teale et al., 2006, Estelle, 2011, Leyser, 2018, Paque and Weijers, 2016, Abel and Theologis, 2010], as the location of plant organs are dictated by the pattern of auxin concentration during growth (Figure 2.1). For example, this can be observed in the root tip, where cell division and expansion is centred around peaks of auxin [De Smet et al., 2007, Moreno-Risueno et al., 2010b, Grieneisen et al., 2007], or in the shoot meristem, where the well-known



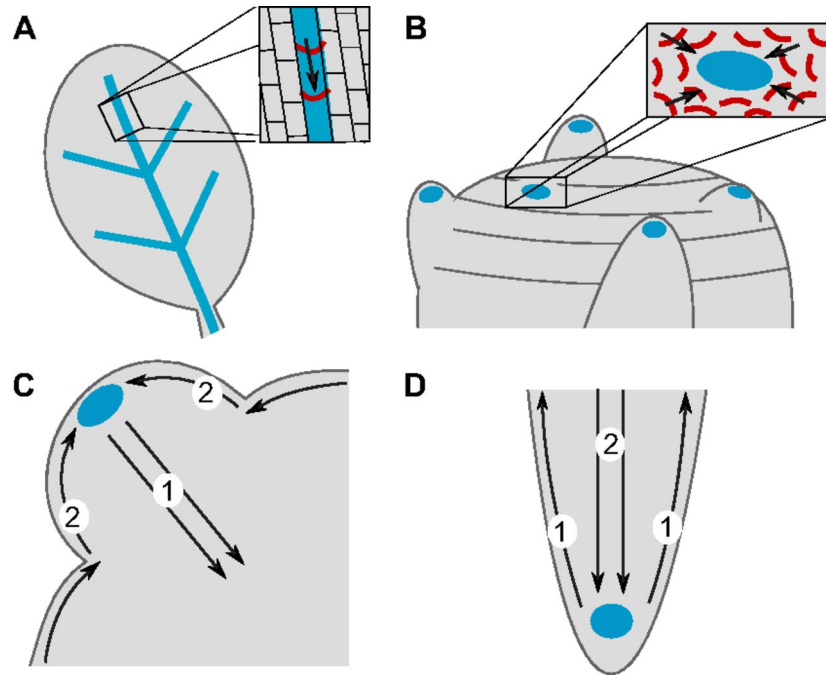


Figure 2.1: Diagrams showing predicted auxin concentration patterning and direction of flow. (A) Shows canalisation of auxin in a growing leaf. (B) Shows auxin patterning in the shoot apical meristem. (C) Shows reverse fountain auxin flow in a developing shoot primordia. (D) Shows fountain like auxin patterning in the root tip. Figure from [van Berkel et al., 2013]

symmetrical arrangements of plants and flower organs are first formed as distributions of auxin maxima [Reinhardt et al., 2003, Galvan-Ampudia et al., 2020].

Thus, the main processes involving auxin can be broadly classified as:

- Auxin metabolism, in particular biosynthesis, which is believed to take place largely in leaves and other aerial parts. In addition, auxins are closely related to tryptophane¹, and therefore their synthesis interferes with a number of core pathways [Ljung, 2013].
- Auxin signalling which involves several steps, from perception by a receptor, to the interference of auxin with a protein-protein network involving transcription factors and feedback loops, as discussed in more detail below.
- Auxin transport, which takes place both over long distances (between root and shoot) and within tissues. The latter involves a partly unknown, com-

¹One of the 20 amino acids used as building blocks for proteins

plex network of molecular events involving active transporters and feedback with auxin itself, as detailed below as well.

Nonetheless, although each of the three topics above is of great importance to biology, and raises interesting modelling questions, it would be too big of a task to tackle all three in a single project. Furthermore, auxin metabolism is the least understood of the topics above and there is less data available to start developing models [Ljung, 2013]. Therefore, in this thesis we shall discuss auxin transport and signalling as the main processes by which auxin achieves its function, as they are the two main processes by which auxin can lead to complex spatio-temporal patterns. In addition, such patterns can lead to many intriguing mathematical questions and are the motivation for the mathematical components of this thesis. It should be noted that recent experimental results have shown that the patterning of auxin is a highly dynamic process, taking place at least as fast as tissue growth itself, making the study of spatio-temporal auxin patterns timely and relevant for biology.

In the remainder of this chapter, one first discusses the main facts currently known about auxin spatio-temporal patterns in growing roots and shoots. Then, one specifically reviews in more details the processes of auxin signalling and transport. As expected for a biological process, which involves complex and partially unknown components, mathematical modelling has played an important role in understanding these aspects of auxin function so far. In fact, perhaps owing to the ancient use of mathematics in the study of plants², computational models are an integral part of contemporary auxin biology; they have indeed been coined its ‘imaginary friend’ [Garnett et al., 2010]. Therefore, these biological facts are discussed with a strong focus on mathematical modelling and perhaps without all the biological details that can be found in the literature, though the text is always aiming for accuracy. Thus, the main points which are reviewed are those relevant to the mathematical modelling of spatio-temporal patterns driven by auxin,

²[Prusinkiewicz and Runions, 2012] traces back to Theophrastus (370-285 BCE) the use of mathematics in plant biology.

and to conclude a summary of the open questions that motivated this thesis are proposed.

2.2 Root and Shoot Meristems

2.2.1 *Arabidopsis thaliana*

Although auxin is found in virtually any plant [Blázquez et al., 2020], its small size has made it difficult to observe, therefore, a number of indirect methods have been developed over the years [Liao et al., 2015, Brunoud et al., 2020]. These methods require sophisticated genetic manipulations, which has led the auxin community to mainly focus on a particular model organism known as *Arabidopsis thaliana*. This model organism is in the crucifer, or mustard, family [Meyerowitz and Somerville, 1994] and it has been intensively used in the past two decades: as of 2015 there have been over 50,000 articles concerning *Arabidopsis* of which over 15,000 have been cited in one or more papers not focused on *Arabidopsis* [Provart et al., 2016].

One reason why *Arabidopsis* has been so well studied is that it has a comparatively simple genome compared to other flowering plants (angiosperms). For example, it has approximately 1.35×10^8 base pairs [Slotte, 2014] compared to tobacco which has over ten times as many [Sierro et al., 2013], and approximately 60% of the genome is protein-coding DNA [Cheng et al., 2017]. As a result, mutations that do occur after mutagenesis screens are likely to be in coding sequences, and as *Arabidopsis* is a diploid, and has five chromosome pairs [Koch and Kiefer, 2005], this makes it easier to discern whether genes are dominant or recessive.

Beyond its genetic simplicity *Arabidopsis* is also well suited to the lab environment, as it is relatively small in size and is self-fertile which benefits homozygous line maintenance. Additionally, it has a short life cycle; as low as six weeks from seed to seed, producing thousands of seeds per plant [Woodward and Bartel, 2018]. Furthermore, due to being non-seasonal multiple generations may be

grown within a year [Wilson, 2000]. Moreover, another important reason for its suitability is that it is a 'weed' [Meyerowitz, 1989] and its lack of market value would limit interference from industry.

Arabidopsis thaliana is named after a 16th century German physician Johannes Thall, who included them in a book of the flora of the Harz mountains. Furthermore, *Arabidopsis* is a winter annual found growing in temperate climates, often along the routes by which European traders transported grain, and although its geographical origin has not been proven, it may originate from the Central Asian highlands or the Western Himalayas. In addition, Columbia wild type originates from samples of *Arabidopsis* collected for Professor Györfy by Professor Laibach from Landsberg, Germany [Csaba Koncz, 1992].

The growth of plants occurs in specialised tissues called *meristems*, which are mainly located at the extremities of plants and comprise of populations of undifferentiated cells undergoing constant cycles of growth and division. Therefore, in the following section, one discusses basic facts about root and shoot meristems in *Arabidopsis*.

2.2.2 Roots

The root meristem is made up of several distinct areas (see Figure 2.2), and at the heart of the meristem is the quiescent centre; a cluster of stem cells which sit at the highest peak of auxin in the root. The quiescent centre is surrounded by the 'initials' for the other tissues in the root tip, such as Epidermis initials. These initials are partially specialised stem cells that divide frequently producing the cells further along the cell column. At the core of the root, above the quiescent centre, are vascular cells called 'stele'. The stele is surrounded by the cortex, vacuolate³ cells whose inner limit is known as the endodermis. In turn, the cortex is surrounded by the epidermis which protects the root [Shipunov, 2018].

At the bottom of the root is the root cap which can be divided into two

³Vacuolate, or vacuolated cells are cells containing vacuoles, i.e. membrane-bound organelles containing water and dissolved substances such as salts, sugars, enzymes, and amino acids.

categories, columella in the centre and the lateral root cap surrounding it [Lack, 2005]. Whilst the primary role of the root cap is to protect the root as it burrows into the soil, the columella additionally produces mucilage, a lubricant which protects the root from bacteria and aluminium, which can be toxic [Maeda et al., 2019, Hawes et al., 2000].

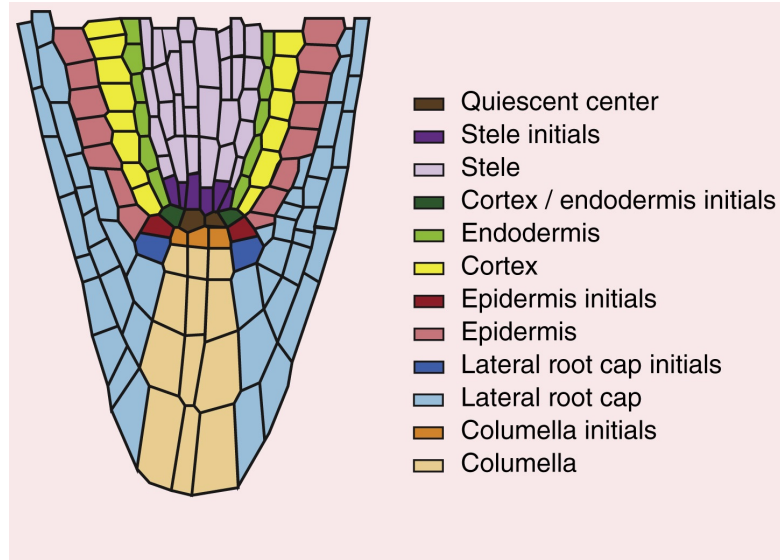


Figure 2.2: The classification of cells within the root apical meristem of *Arabidopsis thaliana*. Figure from [Greb and Lohmann, 2016].

Within the root meristem, the location of the quiescent centre, surrounding stem cells and areas where cell differentiation and division occur are all determined by a gradient of auxin observed along the meristem axis. [Overvoorde et al., 2010, van Berkel et al., 2013]. The quiescent centre is at the point of the highest auxin concentration. Further up the root, as the auxin concentration drops, cells stop dividing and start differentiating and after this point the cells elongate, forming the elongation zone. The region where the rate of cell division begins to slow down and leave place to cell expansion is known as the root apical meristem. The peak of the auxin gradient is in the root tip and it is this gradient that is thought to, over time, affect and shape the plant root as a whole: in addition to this global gradient, local molecular events lead to the formation of auxin peaks, which play a crucial role in triggering local differentiation events allowing the formation of organs such as root hairs or lateral roots

[Overvoorde et al., 2010]. Although an active area of research, and in particular relying on mathematical modelling [Péret et al., 2009, Savage et al., 2008], we do not provide further details on these processes, in order to focus on the spatio-temporal patterns of auxin and its immediate targets, rather than the subsequent developmental events that auxin triggers. After this brief overview of the spatial structure of *Arabidopsis*' root, one can now give a succinct account of temporal, rhythmic events of cell divisions and auxin periodic fluctuations.

It has been observed in a study of *Arabidopsis thaliana* root meristems that the division rates of cells that make up the meristem are spatially uniform [Beemster and Baskin, 1998]. Cell length analysis, which involves comparing the distribution of lengths of cells with respect to their distance from the quiescent centre, was used and it was found that cells in the meristem only stop dividing within one cell cycle of their surrounding cells. For instance, the analysis demonstrated that within the apical meristem, and the mature part of the meristem, only 17% and 15% of cells were more than twice the size of the surrounding cells respectively. This is due to cells being unable to slide past one another; therefore cells which stop dividing early would be growing by a factor of 2^n for each missed mitotic cycle. A time lapse study of cell division of the columella of *Arabidopsis thaliana* showed that cell division was more closely correlated with cell length than it is with diurnal rhythm [Campilho et al., 2006].

Experiments involving ablation, in this case the removal of cells with a laser, show that the quiescent centre cells have a strong effect on neighbouring cells. With results indicating they repress differentiation in cells within a distance as small as one cell, potentially working through cell contact [Van Den Berg et al., 1997].

It has been noted that for cell divisions, the new cell walls are commonly at right angles to the pre-existing walls and divisions rarely occur level with the dividing line between two cells next to them, i.e. four corners do not often meet in the same location. This creates a brick wall like pattern [Shapiro et al., 2015].

A study of cell division in the shoot apical meristem showed that cell division, as a percentage of the dividing cell, had a standard deviation of 9.3% with cell divisions ranging from 24% to 76%, 65% falling within 40% to 60% [Shapiro et al., 2015]. It is also noted by Shapiro et al. that of the bottom 20% for uniform division, 76% occurred within three cells of the border of the meristem and could be in areas that are going to become flower primordia and as such be caused by related phenomena .

Towards the root tip, in the root apical meristem and elongation zone, there is an area where experiments using DR5:luciferase, a luminescent reporter of auxin activity (see Section 2.3 for more details), indicate there is a recurrent pulsing of auxin with a period of approximately six hours [Moreno-Risueno et al., 2010b]. This area is sometimes termed ‘oscillatory zone’. The pulses result in the formation of local peaks of auxin response, which remain active as the root grows and mark the position of potential lateral roots, termed ‘prebranch sites’ [Moreno-Risueno et al., 2010b].

A previous study looking at auxin in roots of seedlings had used DR5:GUS, another reporter of auxin activity, and also observed oscillations although with a period of 15 hours [De Smet et al., 2007]. Although these periods of oscillations differ significantly, this could be due to factors such as light level or sucrose in the medium they are grown on. More recently [Xuan et al., 2015] shows that growth rate controls the period of auxin oscillations, a fact also seen in computational simulations [van den Berg and ten Tusscher, 2018, van den Berg et al., 2021]. However, how precisely the growth and division patterns affect auxin oscillations remain elusive [Laskowski and ten Tusscher, 2017, Xuan et al., 2020] and a better understanding will require further modelling of the processes of auxin signalling and transport.

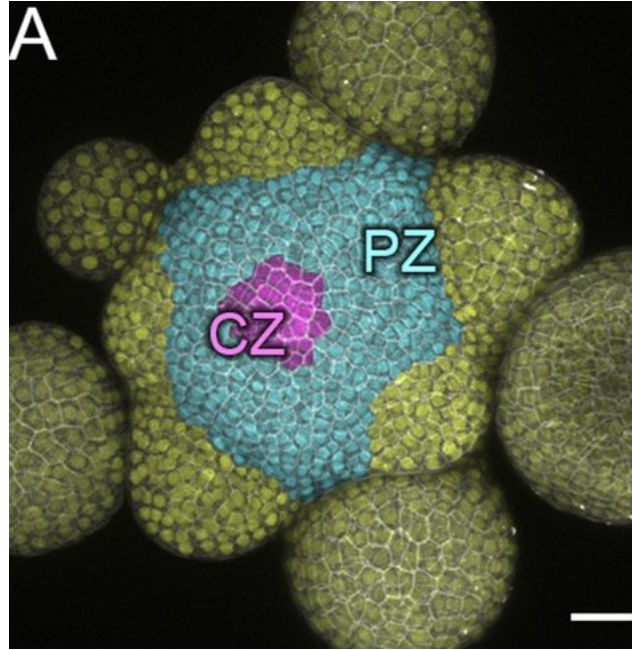


Figure 2.3: SAM radial organisation. The CZ (magenta) is surrounded by the PZ (cyan). Emerging flower primordia and flowers are coloured in yellow, and follow a spiral pattern which remains visible at macroscopic scales. Reproduced from [Galvan-Ampudia et al., 2020].

2.2.3 Shoot Meristems

A simplistic view of shoot apical meristems (SAMs) is that they are dome shaped areas, comprising of a central zone (CZ) of growing and dividing cells, which differentiate as they enter the peripheral zone (PZ) under the effect of growth (Figure 2.3). The reality is more complex, with a three dimensional layered structure and multiple sub-areas regulated by partially known molecular networks [Somssich et al., 2016, Shi and Vernoux, 2019]. To keep within the scope of this thesis, one omits many of these aspects to focus on patterns driven by auxin and its immediate targets in the SAM. More specifically *phyllotaxis*, the geometric arrangement of plant organs, with its well-known regularity, originates in the SAM where sequences of auxin maxima form along a spiral which is the origin of the well-known patterns visible at macroscopic scales [Reinhardt et al., 2003].

It has long been observed that the spirals found in the patterns of leaves, bracts, or florets follow the Fibonacci series [Mitchison, 1977]. This is one of the two most common patterns created by phyllotaxis, where each consecutive leaf

grows at roughly a constant angle apart around the axis of the stem. The other pattern, decussate, is one in which at each level of new growth there are two new leaves at opposite sides of the stem turned 90 degrees from the last level. In the paper 'Phyllotaxis and the Fibonacci Series' G.J.Mitchison [Mitchison, 1977], makes a geometric argument for the necessity for phyllotaxis to follow the Fibonacci series given a few assumptions, namely that each leaf has a short range of influence in determining the location of the next leaf.

This descriptive point of view may account for the reason why phyllotaxis occurs as it does. However, how this is actually realised requires a dynamical point of view, as phyllotaxis is first visible in the ever-changing context of growing meristems. In the 1990s, it became clear that simple local rules allowed for phyllotactic patterns to emerge spontaneously [Douady and Couder, 1992]. Namely, a disk shaped area including a central zone, where no organs are created, and in which existing organs (points moving centripetally) emit a radial repulsion field (limiting the potential positions of organ creation) was enough to reproduce virtually any known phyllotactic pattern.

In modern days, it is commonly accepted that these simple rules are indeed realised as auxin distribution patterns in the SAM. Computational studies have been able to reproduce phyllotaxis as a result of (modelled) auxin patterns in the SAM [Smith et al., 2006, Stoma et al., 2008]. However, thanks in particular to the development of experimental auxin reporters, it now appears that auxin patterns are more intricate than was believed a decade ago, with rapid (comparable to the cell-cycle) waves across the whole SAM [Galvan-Ampudia et al., 2020]. A full understanding of SAM patterning is yet to come, but it requires understanding of both auxin and its immediate effect through a core signalling pathway, as reviewed in the next two sections.

2.3 Auxin Signalling

Auxin affects cells via a signalling pathway, which ultimately activates the transcription of a number of target genes. More precisely, auxin acts by interfering with a protein-protein network involving two families of proteins, called ARF and Aux/IAA. There are 29 different Aux/IAA genes in *Arabidopsis thaliana* [Remington et al., 2004], and 23 different ARFs [Rademacher et al., 2011]. ARF and Aux/IAA proteins are defined by four conserved domains [Finet et al., 2013, Luo et al., 2018], including the shared Domains III and IV (together forming what is known as PB1 domain) which allow these proteins to bind and form dimers [Tiwari et al., 2004], and in principle longer oligomers [Guilfoyle, 2015], though experimental evidence *in planta* is still incomplete [Weijers and Wagner, 2016, Leyser, 2018]. In addition to PB1:

- ARF proteins contain a DNA-binding region and a variable middle region that make them able to activate or repress gene transcription.
- Aux/IAA proteins contain a DI domain able to repress gene transcription, and DII which can lead to Aux/IAA degradation as detailed below.

A consequence of the above is that ARFs are transcription factors on their own, while Aux/IAA can only affect transcription via binding to ARFs, since they lack a DNA binding domain. Although a majority, repressor ARFs play a role which is not fully understood, and in the following ARFs will be mostly interpreted as activators [Vernoux et al., 2011b]. ARF:Aux/IAA dimers are strong transcriptional repressors (stronger than repressor ARFs) [Korasick et al., 2014], so that in the presence of Aux/IAA auxin responsive genes are shut down. These genes are characterised by the presence of specific sections of genetic code termed Auxin-response elements (AuxREs) [Ulmasov et al., 1995]. ARFs directly bind to AuxREs either activating or repressing transcription of the gene depending on the gene in question [Guilfoyle and Hagen, 2012] and the local distribution of ARF and Aux/IAA.

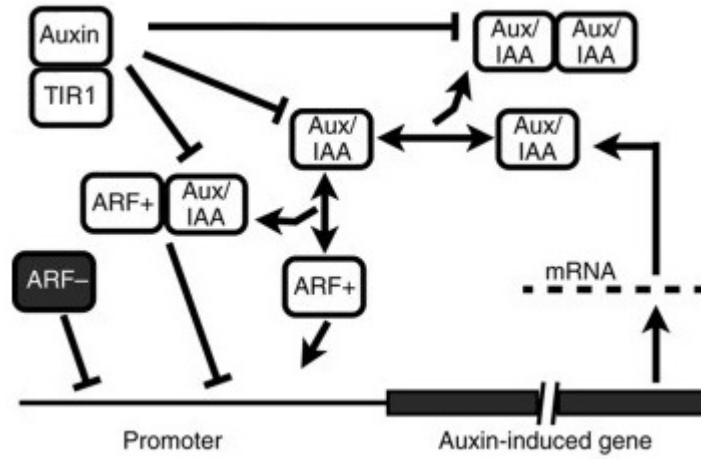


Figure 2.4: Representation of the auxin signalling pathway, adapted from [Vernoux et al., 2011b].

At a molecular scale, the action of auxin is to directly promote the interaction between a receptor complex called SCF^{TIR1} and the DII domain of Aux/IAA in a manner that is dependent on the quantity of auxin added. Auxin has been termed a ‘molecular glue’ between SCF^{TIR1} and Aux/IAA [Tan et al., 2007]. This SCF^{TIR1} -Aux/IAA interaction leads to the proteolysis⁴ of the Aux/IAA proteins [Gray et al., 2001], which are thus rapidly removed from the cell upon auxin input. At first sight, surprisingly, Aux/IAA proteins are generally up regulated within 5 to 30 minutes of auxin being applied to the plant tissue [Tiwari et al., 2004]. The reason for this effect is that Aux/IAA themselves are among the ARF targets (i.e. their sequence include AuxREs), resulting in a negative feedback loop. The structure of this signalling network is schematised in Figure 2.4.

Due to its small size, auxin is very difficult to observe experimentally. For this reason, understanding the signalling pathway above has allowed biologists to develop reporters, to indirectly infer auxin levels in cells. The two main reporters are based on DR5 and DII-Venus, which provide complementary information.

DR5 is a synthetic AuxRE, created by repeating the AuxRE TGTCTC with suitable spacing between [Guilfoyle et al., 1998b, Guilfoyle et al., 1998a]. It has been shown to be a very active AuxRE, several-fold times more active than

⁴Proteolysis is a regulated process of ‘recycling’, whereby proteins are broken down into smaller peptides

natural AuxREs [Ulmasov et al., 1997]. DR5 has been used to make synthetic auxin reporter genes which combine a reporter gene with repeats of the DR5 element, these include DR5::GFP, DR5::RFP, DR5::GUS and DR5::luciferase [Otenshläger et al., 2003, Chen et al., 2013, Moreno-Risueno et al., 2010b], which emit different wavelengths and have different persistence times. Given the structure of the auxin pathway, this reporter does not account for auxin directly, but for the transcriptional activation of a typical auxin target gene. Given the negative feedback in the system and the presence of a permanent level of repression (from both repressor ARFs and ARF:Aux/IAA dimers), this can lead to artefacts such as high auxin and reporters not emitting any signal, or significant delays between auxin input and reporter output.

The DII-Venus reporter, on the other hand, combines the DII domain to a luminescent signal [Brunoud et al., 2012]. As DII is the degradation region on Aux/IAA proteins, the presence of the signal indicates low auxin, while high auxin will remove any signal. It is, therefore, a more direct reporter of auxin itself. It does not indicate whether auxin activates the transcription of genes, but combined with DR5 can provide very valuable information on both auxin and its signal. A semiquantitative version has also been recently developed [Liao et al., 2015].

In summary, the auxin signalling pathway involves a negative feedback loop and a number of its molecular details are still unknown; in particular, which oligomers, of which size and among which partners can form, and what function they have. To cite only one example, would a polymer involving both activator and repressor ARFs activate, or repress? There is currently no clear answer to this question, and many others. This includes the fact that the auxin pathway is far from being isolated and interferes with other plant hormone signals, in particular with cytokinins, whose interactions with auxin have been long known to be crucial to plant development [Dello Ioio et al., 2008, Nordström et al., 2004]. It is believed, however, that auxin is central among plant hormones, as a potential

integrator of various signals from other pathways [Jaillais and Chory, 2010].

A key difficulty is the high number of ARF, Aux/IAA molecules in *Arabidopsis*, which leads to a high number of combinatorial possibilities of molecular complex formation. To overcome this difficulty, in addition to on-going research in *Arabidopsis thaliana*, biologists have used synthetic biology, implementing a minimal pathway in yeast [Pierre-Jerome et al., 2014]. More recently, experimental tools have also started to appear in an ancient plant, *Marchantia polymorpha*, whose auxin pathway is minimal [Kato et al., 2020a, Alam and Pandey, 2016] and, unlike in synthetic approaches, has been designed by evolution.

Modelling is widely recognised in the biological literature as having a key role to play in deciphering auxin signalling [Leyser, 2018, Weijers and Wagner, 2016]. The first proper mathematical investigation of the auxin pathway was published in 2010, in the form of a systems of ordinary differential equations (ODEs) [Middleton et al., 2010], a formalism adopted in all subsequent studies. It showed how the feedback loop could produce different temporal profiles of response to auxin, in different parameter regimes, including stable periodic solutions. Further studies, based on data not available in 2010, have shown that variations on which ARFs and Aux/IAA proteins bind *in planta* might differ from the modelling choices made in [Middleton et al., 2010]. This includes a specific role for repressor ARFs of attenuating the response, for the purpose of fine-tuning, noise cancelling, or localised suppression of the auxin response [Vernoux et al., 2011b]. Additional modelling studies indicate that different sub-networks within the auxin pathway may have a specific function, e.g. controlling the response speed, its sensitivity, or its amplitude [Farcot et al., 2015, Grigolon et al., 2018].

Beyond research focused on auxin signalling on its own, models have been proposed to integrate auxin signalling and the GH3 enzyme family [Mellor et al., 2016], cytokinins [Muraro et al., 2013] or including explicitly multiple Aux/IAA and multiple ARFs [Bridge et al., 2012]. Recently, some attempts have also been made at combining auxin signalling and its active transport (discussed in the

next section, either based on ODEs and hypothetical targets of auxin amongst its transporters [Allen and Ptashnyk, 2020], or in a computational simulation aimed at integrating multiple process in a root-shaped numerical template [van den Berg and ten Tusscher, 2018].

2.4 Auxin Transport

Active transport is the process by which molecules are transported across cell boundaries in a manner that requires energy. This is a difference between active transport and diffusion which does not require energy [world encyclopedia, 2001]. Auxin may enter cells via free diffusion (auxin *influx*) but requires active transport to leave cells (auxin *efflux*). This is due to the pH difference between the cells and the inter-cellular matrix [van Berkel et al., 2013]. The low pH in the cell wall protonates auxin, meaning the auxin molecule is cationic outside of the cell, granting it the ability to easily enter the cell via diffusion. Conversely, this pH disparity prevents intracellular auxin from leaving by diffusion. To actively transport auxin out of the cell, plants use efflux carriers, which actively take auxin out of the cell. Important group transporters are the PIN-FORMED proteins (or simply PIN hereafter). Also relevant are proteins from the AUX/LAX family, which act as influx carriers actively transporting auxin into the cell at a much faster rate than passive diffusion [Swarup and Péret, 2012].

Due to the lack of understanding at a molecular level, several mathematical formulations have been proposed. Auxin transport models are traditionally classified into three main variants: passive flux, flux-based and concentration-based models. In passive flux models Pin polarity is predetermined. In flux-based models, PIN polarisation within a cell is dependent on the net flux of auxin between the cell and its neighbours, pointing primarily in the direction of the greatest flux. Finally, in concentration-based models, PIN polarisation is directed towards the neighbouring cells that have the greatest concentration of auxin..

The earliest auxin transport models were simple passive flux models that

modelled diffusion of auxin along a file of cells containing an auxin source [Leopold and Hall, 1966] and comparing the observed behaviour with experimental results. More recent examples of passive flux models include PINs, often investigating how differences in predetermined PIN polarity between types of tissue affect auxin distribution [Kramer, 2004, Grieneisen et al., 2007].

The creation of flux-based models was driven by the desire to recreate the vein-like patterns where the flow of auxin gets canalised. A process where auxin accumulates in the direction of pre-existing fluxes was described informally by Sachs in the late 1960s and first formalised mathematically by Mitchison in 1980 [Mitchison, 1980]. The concentration-based models, on the other hand, were intended to replicate the occurrence of orderly peaks, like those found in phyllotaxis and arose once PIN transporters had been discovered [van Berkel et al., 2013], and therefore always explicitly represent PIN proteins on the membrane. They are based on the observation that PIN polarisation found on the epidermis of the apical meristem of an *Arabidopsis* shoot has the PINs pointing towards the local auxin maxima which will form the locations of incipient primordia [van Berkel et al., 2013]. Therefore, in concentration-based models PINs accumulate on cell membrane sub-domains which are adjacent to cells with the highest auxin concentration, as inspired by patterns observed in the SAM [Smith et al., 2006, Jönsson et al., 2006].

The PIN proteins are indeed typically found distributed in a polar fashion, focused on one side of the cell with a lower concentration at the opposite side [van Berkel et al., 2013]. This results in a directed flow of auxin from the cell in the direction where the PINs are concentrated [Wisniewska et al., 2006]. In files of cells this can lead to canalisation (Figure 2.1), creating auxin transporting veins where the PINs in the cells involved all point in the same direction promoting a directional flow of auxin. The process for the polarised PIN proteins is not currently fully understood, although it is believed that the PIN proteins constantly cycle on and off the plasma membrane allowing them to quickly adapt

to a change in stimuli, such as the auxin concentration in neighbouring cells rising or lowering, and redirect themselves accordingly [Dhonukshe et al., 2007, Geldner et al., 2001].

A different type of flow is found in the root tip or lateral root primordia. Here auxin flows up the gradient along files of cells within the centre of the root and away from the local maximum on the outer layers [van Berkel et al., 2013]. This flow is referred to as a ‘fountain’. Whilst on the epidermis the PINs point up the auxin gradient towards the primordia, in the cells of the inner layers of the primordia the PINs direct auxin flow away from the local maximum, creating auxin carrying veins and connecting the primordium to the shoots vasculature [van Berkel et al., 2013]. This flow of auxin is referred to as a ‘reverse fountain’. Furthermore, due to PIN1, a specific PIN protein, being found in greater quantities in the external layer than anywhere else it may imply that phyllotactic patterns are generated primarily by the auxin pattern on the surface of the shoot apical meristem [Smith et al., 2006].

In experiments comparing the PIN1 mutant to wild-type *Arabidopsis* and applying the auxin transport inhibitor, N-1-naphthylphthalamic acid (NPA), the PIN1 mutant had low levels of DR5::GFP (a reporter of endogenous auxin response, see previous section), while in instances of NPA applied to wild type there was still higher levels of DR5::GFP but without the distinct phyllotactic patterns of the wild-type [Smith et al., 2006]. From this it can be inferred that active transport is important in creating an uneven distribution of auxin for the creation of phyllotactic patterns and that auxin is produced throughout the surface of the peripheral zone.

In the root, PIN proteins have been documented as being regulated by auxin [Smith et al., 2006]. Similarly, it has been assumed that this is the case with PIN1 in the meristem. This is supported by experiments which show that PIN1 expression shares the same maxima, centred on the primordia, as auxin is thought to, and that all cells in the epidermis are auxin sensitive [Smith et al., 2006]. In

the epidermis, PIN proteins point towards the incipient primordia where highest concentrations of auxin are found [Smith et al., 2006]. This leads to the hypothesis that PIN1 in the epidermis polarises towards the neighbour with the highest concentration of auxin. No specific mechanism for this is presented.

In experiments, locations where auxin was artificially added resulted in new primordia with size and position correlating to the concentration and location of the added auxin [van Berkel et al., 2013]. This implies auxin is capable of affecting the distribution of PIN proteins and hence the existence of a feedback loop between auxin and its active transport. In another experiment auxin transport inhibitors were applied to shoot apices. This prevented the growth of lateral organs while the apices grew with strong radial symmetry at a rapid rate [Smith et al., 2006].

Note that data on the distribution of auxin (which cannot be observed directly, see previous section) and its transporters is often difficult to interpret, being mostly based on images with non-quantitative reporters. For this reason, and the complexity of the interplay between auxin and its transporters, there is currently a lack of consensus on the details of auxin polar transport [Kramer and Bennett, 2006, Bennett et al., 2014] and, for instance, alternatives to the two classes of models discussed above have also been proposed [Merks et al., 2007, Wabnik et al., 2010]. However, most of the literature is referring to the flux or concentration-based models and, therefore, in the following two sections, mathematical formulations for these are presented. The most detailed review on auxin transport models so far is [van Berkel et al., 2013].

2.4.1 Concentration-Based Models

The Smith model [Smith et al., 2006] is one of the two first concentration-based models to have been proposed, published in the same issue of PNAS in 2006 [Jönsson et al., 2006, Smith et al., 2006], along with a third relying on flux-based models [Barbier de Reuille et al., 2006]. Being slightly simpler, the Smith model

was used as a basis for a number of subsequent studies. It was developed to model auxin transport in the shoot apical meristem of Arabidopsis and particularly phyllotaxis. Here each cell has two values: the auxin, in this case indole-3-acetic acid (IAA), concentration in the cell, $\mathbf{a}_i(\mathbf{t})$, and the number of PIN, (or PIN1) molecules in the cell, $\mathbf{p}_i(\mathbf{t})$. The PIN concentration in a cell is up regulated by the presence of auxin and it is distributed within the cell relative to the auxin concentration of its surrounding cells. The ODE's that govern the system are

$$\begin{aligned} \frac{d\mathbf{a}_i}{dt} = & \frac{\rho_{IAA}}{1 + \kappa_{IAA}\mathbf{a}_i} - \mu_{IAA}\mathbf{a}_i + \frac{D}{V_i} \sum_{j \in \mathcal{N}_i} l_{ij}(\mathbf{a}_j - \mathbf{a}_i) \\ & + \frac{T}{V_i} \sum_{j \in \mathcal{N}_i} \left[P_{ji}(\mathbf{a}, \mathbf{p}) \frac{\mathbf{a}_j^2}{1 + \kappa_T \mathbf{a}_i^2} - P_{ij}(\mathbf{a}, \mathbf{p}) \frac{\mathbf{a}_i^2}{1 + \kappa_T \mathbf{a}_j^2} \right] \end{aligned} \quad (2.1)$$

$$\frac{d\mathbf{p}_i}{dt} = \frac{\rho_{PIN_0} + \rho_{PIN}\mathbf{a}_i}{1 + \kappa_{PIN}\mathbf{p}_i} - \mu_{PIN}\mathbf{p}_i, \quad (2.2)$$

with the number of PINs pointing at a cells' neighbours

$$P_{ij}(\mathbf{a}, \mathbf{p}) = \mathbf{p}_i \frac{l_{ij} \exp(c_1 \mathbf{a}_j)}{\sum_{k \in \mathcal{N}_i} l_{ik} \exp(c_1 \mathbf{a}_k)}. \quad (2.3)$$

Here D is the diffusion coefficient, T is the active transport coefficient, V_i is the volume of cell i , $l_{ij} = S_{ij}/(W_i + W_j)$ is the ratio of surface area, S_{ij} , between cells i and j , and the sum of the cell wall thicknesses, W_i and W_j , of cells i and j .

Sensitivity analysis undertaken by the models creators showed the exponential term in the PIN distribution equation was the most sensitive element [Smith et al., 2006]. It should also be noted that whilst there is no support from the experimental data for the quadratic dependence of the flux on auxin, they state it does provide an improvement over linear dependence for the spacing of peaks [Smith et al., 2006].

In subsequent studies, the related Jönsson model was shown to have the potential to generate a variety of patterns, reminiscent of reaction-diffusion models [Sahlin et al., 2009]. This includes, in particular, vein-like patterns (or stripes),

contrary to the previous view in the literature that those were mostly obtained in flux-based models. However, in the latter the main patterning mechanism, often termed Turing instability, is based on a homogeneous steady state being unstable and leading to various stable (inhomogeneous) patterns in response to a perturbation [Hoyle, 2006]. It was shown in [Draelants et al., 2015] to differ in concentration-based models, where the homogeneous steady state is always stable, but coexists with numerous other stable steady states. Interestingly, stable periodic patterns have also been observed [Draelants et al., 2015], which take the form of a standing wave with multiple peaks distributed along a linear domain.

2.4.2 Flux-Based Model

The flux-based model was first formalised by G. J. Mitchison [Mitchison, 1980], and then investigated in a number of studies which have generally been concerned with its ability to produce vein-like patterns, for which they were first introduced [Fujita and Mochizuki, 2006, Feugier et al., 2005, Feller et al., 2015], (see [Rolland-Lagan and Prusinkiewicz, 2005] for a review), as well as other patterns [Farcot and Yuan, 2013] including those underlying spiral phyllotaxis [Stoma et al., 2008]. In this model, each cell has a value associated to it for the concentration of auxin, $a_i(t)$. Whilst some models include a fixed distribution of PIN transporters [Barbier de Reuille et al., 2006, Band and King, 2012], they are often represented with a time dependency; then a set of values for the concentration of PINs pointing towards each of the i th cell's neighbours can be denoted by $P_{ij}(t)$. Here PIN concentrations in the cell wall are increased by auxin flowing through to a neighbouring cell. The ODE's that govern the system are:

$$\frac{da_i}{dt} = \frac{1}{V_i} \sum_{j=1}^N A_{ij} \Phi_{ij} + \kappa - \delta a_i, \quad (2.4)$$

$$\frac{dP_{ij}}{dt} = h(\Phi_{ij}) + \rho_0 - \mu P_{ij}. \quad (2.5)$$

where $\Phi_{ij} = (\text{TP}_{ij} + D)\mathbf{a}_i - (\text{TP}_{ji} + D)\mathbf{a}_j$ is the net flux between cells i and j and $h(\Phi_{ij})$ is the rate at which PINs are inserted with respect to the net flux. Two common choices [Feugier et al., 2005, Stoma et al., 2008, Farcot and Yuan, 2013] for h are a saturating term

$$h_1(\Phi) = \rho \frac{\Phi^n}{\Theta^n + \Phi^n} , \quad (2.6)$$

where Θ is a saturating coefficient, or a polynomial (often quadratic)

$$h_2(\Phi) = \rho \Phi^n . \quad (2.7)$$

In a similar way to concentration-based models being able to generate vein patterns, flux-based models have also been shown to have more general properties than the canalisation process they were first intended to model. In particular, they have successfully reproduced phyllotactic patterns, with their spiral arrangements of auxin peaks [Barbier de Reuille et al., 2006, Stoma et al., 2008]. These results are based on computational simulations, and mathematical analysis is still largely open (as it is for concentration-based models). Among existing results, graph based characterisations of stable tree-like patterns have been found [Feller et al., 2014, Feller et al., 2015]. Similar to concentration-based models, it was shown in [Farcot and Yuan, 2013] that patterning does not occur through a Turing instability, here again the homogeneous steady state is unconditionally stable, coexisting with numerous other patterns. The occurrence of stable periodic solutions was also shown to occur in a 1D linear domain [Farcot and Yuan, 2013], taking the form of a peak of auxin travelling back and forth between the two ends of the domain.

2.5 Time scales

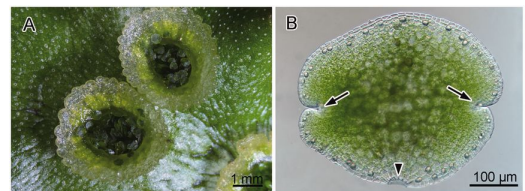
The models discussed above, auxin signalling and transport, investigate the effects of the flux of auxin through plant tissue, the auxin synthesis, cell growth and division, and transcription of proteins that result in feedback. The time scales of some of the processes involved are as follows. Auxin has been shown to be synthesised at a time scale of minutes to hours [Smith et al., 2006] and early experiments showed that if exogenous auxin was added to a plant it moved through the tissue at a rate of 1-2mm per hour [Goldsmith, 1967]. Similarly, the transcription response time of Aux/IAs, an important family of proteins due to its role in the feedback loop at the heart of auxin signalling, vary between 4 to 30 minutes [Abel et al., 1995], a time scale comparable to that of auxin transport or auxin synthesis. By contrast cell divisions within the root apical meristem occur approximately once every 25 hours [Beemster and Baskin, 1998]. This is notably within an order of magnitude of the timescale of oscillations seen in experiments utilising DR5 reporters to estimate auxin concentrations, e.g. 15 hours [De Smet et al., 2007] or 6 hours [Moreno-Risueno et al., 2010b].

2.6 Marchantia Polymorpha

Marchantia Polymorpha (*M. polymorpha*) is a species of dioecious liverwort that is found on all continents barring Antarctica (Figure 2.5a). It was instrumental in the discovery of plant sex chromosomes [Berger et al., 2016]. It is now being used as a model organism due to its relative simplicity, 280Mb, and short maturation period of three-to-four weeks [Poveda, 2020]. In addition to its fast



(a) Picture of *Marchantia Polymorpha* plant [Encyclopædia Britannica, 2022]



(b) Gemma cup (left) and close up image of a gemma (right) [Shimamura, 2016]

growth, it also has the ability to propagate asexually through the use of specialised organs known as gemma cups. In these gemma cups, gemmae form (Figure 2.5b). These masses of cells remain dormant until they are no longer with the gemma cup of a live *M. polymorpha*, and encounter light and moisture. At this time the gemmae become active and grow into genetic clones of the parent plant [Kato et al., 2020b, Eklund et al., 2018].

Whilst *M. polymorpha* has twice as many base pairs as *Arabidopsis thaliana*, which has 138Mb, it is far less complex with respect to auxin signalling. *M. polymorpha* has only three ARFs, one TIR1/AFB and one AUX/IAA compared to *Arabidopsis* which has 23 ARFs and 29 AUX/IAAs [Kato et al., 2015]. This simplicity makes it potentially a good model organism especially as the three ARFs belong to each of the three different clades of ARFs [Kato et al., 2015].

Within the auxin signalling system of *M. polymorpha*, mpARF1 is a promoter ARF as is typical of those in clade A. Similarly, mpARF2 and mpARF3 are thought to be repressor ARFs as indicated by their respective clades [Kato et al., 2015, Finet et al., 2013]. Of the three ARFs, mpARF1 and mpARF2 have the most similar DNA binding domains. As such, unlike mpARF3, mpARF1 and mpARF2 likely compete for similar binding sites. Tests conducted in vitro have shown that mpARF1 and mpARF2 have a comparable disassociation coefficient, of 12nM and 61nM respectively [Kato et al., 2020a]. These are within an order of magnitude of each other allowing mpARF2 to feasibly antagonise mpARF1.

Furthermore, tests involving chimeric proteins with swapped PB1 domains showed that while mpARF1 and mpARF2 may bind to similar promoters, mpARF2 may be less auxin sensitive. This auxin insensitivity seen in mpARF2 and mpARF3 may be explained by the nature in which they bind mpIAA. When mpARF2 and mpARF3 bind to mpIAA they engage both their negative and pos-

itive interaction faces while mpARF1 only engages one interaction allowing for more interactions [Kato et al., 2020a].

Although mpARF3 does not necessarily bind to the same promoters as mpARF1 and mpARF2 all three may affect one another. Y2H Yeast assays have shown that mpIAA can bind to all three ARFs. Furthermore, all three ARFs have been shown to be able to dimerise with one another and themselves, except for mpARF3 that does not appear to be able to homodimerise [Kato et al., 2015].

2.7 Summary

In this chapter, after a brief overview of root and shoot meristems, we have discussed the existing literature on mathematical models of auxin signalling and transport. The vast majority of this literature is concerned with Arabidopsis, and for this plant we have identified some gaps. Mainly, little to no work has been published that integrates signalling and transport in a unified model. Most work on this topic relies on computationally sophisticated simulations, with little rigorous mathematical analysis. Likewise, mathematical analysis of auxin distribution patterns on growing domains is so far almost untouched in the literature. Finally, a relatively newcomer in the world of model organisms, *Marchantia polymorpha*, appears to be an ideal system for the modelling of auxin signalling, with a much simpler structure than Arabidopsis.

These gaps in the literature motivate the three following chapters. First, we will investigate models of auxin transport on growing domains. Then we will discuss the interactions between travelling waves of auxin and oscillations of its response pathway. Thirdly, we will discuss auxin signalling in *Marchantia*, using experimental data provided by collaborators in the Netherlands (Wageningen University).

Chapter 3

Geometric Changes and Growth at the Origin of Spatio-Temporal Patterns in Root-Like Tissues

3.1 Introduction

This chapter is concerned with spatio-temporal patterns in systems which are subject to growth. Our initial focus was on auxin transport models, as this is the most important plant hormone. Although there is a rich literature on reaction-diffusion models on growing domains [Crampin et al., 1999, Crampin et al., 2002, Plaza et al., 2004, Madzvamuse et al., 2010], it does not apply to auxin transport. Instead, auxin transport involves active transport rather than diffusion.

Auxin transporter proteins are distributed anisotropically on cell membranes, where the concentration and location of these proteins are dependent on the concentration of auxin itself [Heisler and Jönsson, 2006]. However, this process is not fully understood at the molecular level, and a number of mathematical models have been proposed. These models are traditionally classified into the two families of “flux-based” and “concentration-based” (or “gradient-based”) models,

see Chapter 2 or [van Berkel et al., 2013] for a review.

For both types of models, patterning has been shown to arise through a different mechanism than a Turing instability. Indeed, in a typical flux-based model the homogeneous steady state is unconditionally stable [Farcot and Yuan, 2013], whereas in a concentration-based model it does not always exist [Draelants et al., 2015]. In both cases, a rich variety of possible patterns arise [Sahlin et al., 2009], and they can be explained by a high level of multistability. This multistability is typically captured by a snaking bifurcation diagram [Draelants et al., 2015], a plot of steady states for a system as a function of a parameter of the system.

Additionally, the effect of growth has not been investigated in a systematic manner, in either class of models, which provided the initial motivation for this chapter. However, in the process of this investigation we observed that the spatial inhomogeneities in the density of cells, which are induced by growth, trigger abrupt changes of auxin distribution. This prompted the question of whether this phenomenon was specific to active transport. Therefore, we implemented similar density inhomogeneities in a classic reaction-diffusion model, the Swift-Hohenberg equations, and report here that similar patterns are found. In both cases, we were able to explain these spatio-temporal patterns by juxtaposing the snaking bifurcation diagrams found on either side of a density change. Further to this, the general mechanism, henceforth described, is shown to provide a plausible explanation for oscillatory patterns, of which auxin distribution in plant roots is a well-documented exemplar [De Smet et al., 2007, Moreno-Risueno et al., 2010b].

We focused on an auxin transport model, hereafter termed the Smith model after its author [Smith et al., 2006]. It is a concentration-based model which has been shown to produce plausible phyllotactic patterns, as seen in the shoot apical meristem (SAM) of *Arabidopsis thaliana*, as well as a variety of well-characterised spotted patterns on linear, one-dimensional domains more similar to root tissues [Draelants et al., 2015]. This model is presented in detail in the next section, followed by a summary of the snaking phenomenon occurring as the transport

rate is used as a bifurcation parameter. After these preliminaries, the same model is considered on a domain subject to growth and cell divisions, and we simulated both a homogeneous growth rate and a position dependent rate as observed in actual plant tissues. In both cases, we observed three main types of spatio-temporal pattern, which are characterised in terms of underlying bifurcation diagrams. We then performed an analogous investigation with the Swift-Hohenberg model, in which a term akin to a density was used in lieu of cell growth and division. Overall, we observed strikingly similar spatio-temporal patterns, which we discuss in the concluding section.

3.2 Auxin Transport Model

3.2.1 Definition

As mentioned earlier, though the notations are slightly adapted, the model presented in this section follows the original proposal of Smith et al. [Smith et al., 2006]. Therefore, we consider the active transport of auxin in a tissue composed of $N = N(t)$ cells at time t . Each cell $i \in \mathcal{N}(t) \doteq \{1 \dots N(t)\}$ has neighbours $\mathcal{N}_i = \mathcal{N}_i(t) \subset \mathcal{N}(t)$, therefore inducing a graph $G = G(t)$ with node set $\mathcal{N}(t)$ and edges (i, j) whenever cells i and j are in physical contact, i.e. $i \in \mathcal{N}_j$ or equivalently $j \in \mathcal{N}_i$, hereafter shortened as $i \sim j$. In the remainder of this chapter all numerical examples will be on linear chains of cells, i.e. $i \sim j \iff |i - j| = 1$. We denote by $V_i(t)$ (m^3) the volume of cell i , and $A_{ij}(t)$ (m^2) the exchange surface area (or length in a 2D model) between cell i and j . Please note that whenever it is not the main point of the discussion, one shall drop the dependence on time to simplify notations.

Furthermore, to describe the dynamics of auxin transport in a tissue, we use a_i ($\text{mol} \cdot \text{m}^{-3}$) to denote the concentration of auxin and p_i ($\text{mol} \cdot \text{m}^{-3}$) to denote the concentration of transporter proteins (PIN), in a cell i . Thus, any variation of auxin a_i in cell i is due to the interplay between five processes:

- auxin synthesis, which for simplicity we suppose occurs at a constant rate κ ($\text{mol} \cdot \text{m}^{-3} \cdot \text{s}^{-1}$) in any cell,
- auxin degradation, with a fixed rate δ (s^{-1}),
- free diffusion towards neighbouring cells $j \in \mathcal{N}_i$, described using Fick's law: $D(\mathbf{a}_i - \mathbf{a}_j)$ ($\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), where D ($\text{m} \cdot \text{s}^{-1}$) is a diffusion coefficient,
- active transport by the transporter (PIN) proteins $T(P_{ij}h(\mathbf{a}_i, \mathbf{a}_j) - P_{ji}h(\mathbf{a}_j, \mathbf{a}_i))$ ($\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), where T ($\text{m}^3 \cdot \text{mol}^{-1} \cdot \text{s}^{-1}$) is a transport efficiency coefficient, P_{ij} is the concentration of PINs in cell i on the cell border with cell j and $h(\mathbf{x}, \mathbf{y})$ a function taking different forms in the literature (cf. e.g. [Mitchison, 1981, van Berkel et al., 2013]), see below for Smith's version.

For each cell $i \in \{1, \dots, N\}$ in the fixed geometry model

$$\frac{d\mathbf{a}_i}{dt} = \frac{1}{V_i} \sum_{j \in \mathcal{N}_i} \Phi_{ji} + \frac{\rho_{IAA}}{1 + \kappa_{IAA} \mathbf{a}_i} - \mu_{IAA} \mathbf{a}_i \quad (3.1)$$

$$\frac{dp_i}{dt} = \frac{\rho_{PIN0} + \rho_{PIN} \mathbf{a}_i}{1 + \kappa_{PIN} p_i} - \mu_{PIN} p_i \quad (3.2)$$

where Φ_{ij} denotes the flux of auxin from cell i to cell j :

$$\Phi_{ij} = \left(TP_{ij} \frac{\mathbf{a}_i}{1 + \kappa_T \mathbf{a}_j^2} + DA_{ji} \right) \mathbf{a}_i - \left(TP_{ji} \frac{\mathbf{a}_j}{1 + \kappa_T \mathbf{a}_i^2} + DA_{ji} \right) \mathbf{a}_j. \quad (3.3)$$

and

$$P_{ij} = p_i \frac{A_{ij} \exp(c_1 \mathbf{a}_j)}{\sum_{k \in \mathcal{N}_i} A_{ik} \exp(c_1 \mathbf{a}_k)}. \quad (3.4)$$

Note that if cells i and j are not neighbours, one shall naturally assume that $A_{ij} = 0$, and in particular $A_{ii} = 0$. With this notation, the summation in (3.1) could be simplified to be over all j values. Also, $\mathbf{A} = (A_{ij})$ is a well-defined matrix and, since $A_{ij} = A_{ji} \geq 0$, it is symmetric and non-negative, with zero diagonal elements.

In the following the main example of tissue will be an idealised file of cells with

homogeneous contact surface (set to 1), so that $\mathcal{N}_i = \{i-1, i+1\} \cap \mathcal{N}$ and $A_{ij} = 1$ iff $j \sim i$.

The boundaries for the following simulations are as follows: On the shootward end of the file of cells, the left side, the shootward most cell is treated as adjacent to it self. Hence, no net flux occurs at the shootward boundary; note how in equation (3.3) if $i=j$ then the two terms on the right hand side cancel out. However, by setting the cell adjacent to itself it has pin polarity representative of a cell within a file of cells rather than that of a cell at the end of a file of cells. On the rootward end of the file of cells, the right side, the rootward most cell is adjacent to only the cell on its shootward side. As a result, no flux occurs out of the file of cells in the rootward direction as there is no adjacent cell for the auxin to be transported to. As a result, unlike the shootward cell, the pin polarity of the rootward most cell is such that all its pins are pointing shootward.

For a file of cells all in order from shootward most to rootward most the adjacency matrix is of the form

$$A = \begin{pmatrix} 1 & 1 & 0 & 0 & \dots & 0 & 0 & 0 & 0 \\ 1 & 0 & 1 & 0 & \dots & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 1 & & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & \ddots & 0 & 0 & 0 & 0 \\ \vdots & \vdots & & \ddots & \ddots & \ddots & & \vdots & \vdots \\ 0 & 0 & 0 & 0 & \ddots & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & & 1 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & \dots & 0 & 1 & 0 & 1 \\ 0 & 0 & 0 & 0 & \dots & 0 & 0 & 1 & 0 \end{pmatrix} /$$

Time-dependent simulations in this chapter are calculated utilising the native MATLAB ode solver ODE23s. This was chosen due to model being stiff caused by the pin polarity changing at a far faster time scale than auxin or pin concentration changes. Another reason why the ode solver was chosen is that it has the capabilities to handle 'events' allowing it to handle discontinuities in simulations. This system of 'events' allows for the handling of cell division or the ending of

cell growth, once the criteria for such an event occurs the ODE solver stops and new cells may be added, adjacency matrices may be updated and the properties of individual cells (such as rate of growth) may be updated.

Variable	Value	Description
ρ_{IAA}	0.85	IAA production rate
κ_{IAA}	1	Saturation of IAA synthesis
μ_{IAA}	0.1	IAA decay rate
D	1	IAA diffusion coefficient
T	Specified with simulations	IAA transport coefficient
c_1	1.099	Exponentiation base for PIN relocation
κ_T	1	IAA transport saturation
$\rho_{\text{PIN}0}$	0	Base production of PIN
ρ_{PIN}	1	IAA dependent rate of PIN production
κ_{PIN}	1	Saturation of PIN production
μ_{PIN}	0.1	Pin decay rate
g	1/3000	Growth rate of the cell
V_{max}	4/3	Volume at which cells divide or stop growing

Table 3.1: Parameter values utilised within this chapter for the Smith model

3.2.2 Typical Patterns on Fixed Domains

Although the Smith model has been shown to present oscillatory solutions [Draelants et al., 2015], we focus here on steady states, with the aim of exploring how they are affected by growth. In brief, the model typically presents multistability, with a high number of co-existing stable, patterned steady states. This is also typical of other auxin transport models and also occurs in reaction-diffusion models [Sahlin et al., 2009, Houghton and Knobloch, 2009, Bramburger and Sandstedt, 2019, Yulin and Champneys, 2010, Champneys et al., 2021, ?]. Thus, on a one-dimensional domain, each stable pattern is comprised of a series of similar, contiguous peaks (Figure 3.1), with a large number of unstable patterns made of permutations of other peak locations. Note that, as shown in [Draelants et al., 2015], due to the boundary conditions there exists no homogeneous steady state. This is in contrast to reaction-diffusion models, where patterns arise through critical bifurcations along a branch containing a fully homogeneous steady state, see

e.g. [Hoyle, 2006].

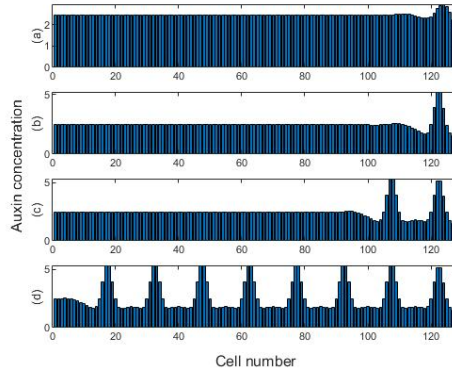


Figure 3.1: Stable solutions including the “proto-peak” (a) and 1, 2 and full peaks in a row of 128 cells (b, c, d resp.). $T = 2.1$, $V = 1$ and other parameters as shown in table 3.1.

A convenient way to summarise the system’s equilibria is to rely on the snaking behaviour it presents, e.g. with the active transport rate T as a bifurcation parameter. To this aim we have constructed bifurcation diagrams using the algorithms in [Rankin et al., 2014], implemented in Matlab in [Avitabile, 2020]. Unlike trying to find steady states via time-dependent simulations which can only find a single steady state per attempt, the code in [Avitabile, 2020] produces a bifurcation diagram and may find many steady states, indicating how they are affected by the change in a bifurcation parameter. The importance of these equilibria will be seen in section 3.2.4 onwards where it is shown how the stable equilibria act as attractors shaping the behaviour of the system.

The algorithm utilises a Runge–Kutta time stepper to generate initial guesses based on previously found solutions to the system. It then uses Matlab’s internal Newton-GMRES method for the root finding step. Should the method fail to converge, the step length used in the initial guess is halved and the root finding step starts anew. Finally the algorithm determines the stability of solutions by spectral analysis, using Matlab’s internal ‘eigs’ function. The input for this being either a numerical approximation of the Jacobian or, as in our case, the Jacobian of the model supplied by the user. Each branch shown in Figure 3.2 represents a state with a specific distribution of peaks. Note that for lower values of T , only

the stable branch continuing the pattern in Figure 3.1a is available.

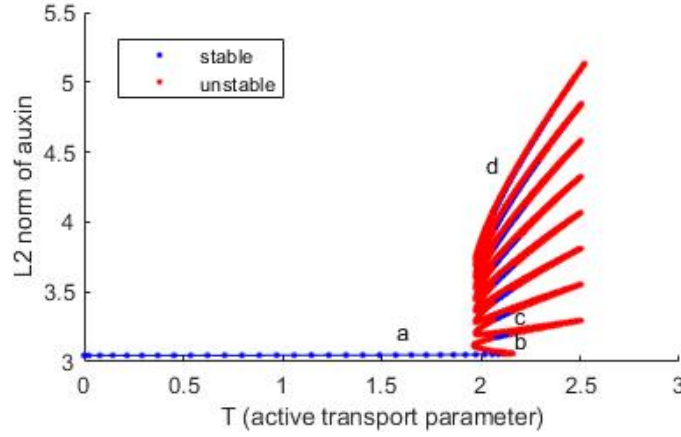


Figure 3.2: Bifurcation diagram showing the snaking behaviour for the system (3.1)-(3.4). a-d stable branches refer to the corresponding patterns in Figure 3.1. Whilst not pictured here there are stable states continuing to the left, though the continuation solver did not find them continuously connected to those shown. $V = 1$ and other parameters as shown in table 3.1

Overall, the number of branches in the snaking region is limited by the length of the domain. In line with this, if the domain was unbounded there would be an infinite number of branches. Though it should be noted that these branches are themselves shadowed by branches containing solutions of permutations of unstable peaks as shown in Figure 3.3. This is likely caused by this taking place on a finite domain, and in [Houghton and Knobloch, 2009] this is shown for the Swift-Hohenberg model, specifically when the peaks get within half a wave length away from the boundary. As such, in the present case this systematically occurs, due to the location of the first peak.

These patterns are unlikely to be important in a stationary domain as they are unstable. Their existence, however, could be of importance in a growing domain, where growth can induce changes of stability over time. We will now explore some effects of growth in the following section.

3.2.3 Implementing Domain Growth for Auxin Transport

Whilst understanding how a system behaves on a fixed domain is important, many realistic dynamical systems occur on growing domains. In biological tissues,

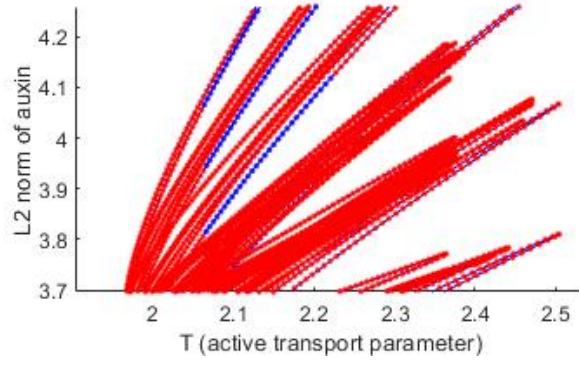


Figure 3.3: Bifurcation diagram showing some of the unstable snaking branches. Legend as in Figure 3.2.

growth is typically comprised of cell elongation and cell divisions. Specifically in plant tissues, these can occur over timescales comparable to auxin variations. Indeed in plant roots, where cell divisions typically occur with a period of a few hours, 25 hours per cell in arabidopsis [Beemster and Baskin, 1998], a periodic oscillation of auxin response is known to occur, also with a period of a few hours, e.g. 15 hours[De Smet et al., 2007] or 6 hours[Moreno-Risueno et al., 2010b], and is critical to the formation of lateral roots. Hence, considering a growing tissue is not only relevant for mathematical purposes, but is also required by the underlying biology. Furthermore, given the nature of auxin transport, with asymmetric membrane-bound transporters, continuum approximations are unpractical and cell divisions have to be represented explicitly.

Regardless of the exact growth model, we must alter the ODEs to take into account dilution due to volume change. Note that concentration is quantity over volume, therefore $\mathbf{a}_i = \frac{\hat{\mathbf{a}}_i}{V_i}$, where $\hat{\mathbf{a}}_i$ is the quantity of auxin in cell i . Differentiating $\mathbf{a}_i$ with respect to time, t gives us:

$$\begin{aligned} \frac{d\mathbf{a}_i}{dt} &= \frac{d}{dt} \frac{\hat{\mathbf{a}}_i}{V_i} \\ &= \frac{1}{V_i} \frac{d\hat{\mathbf{a}}}{dt} - \frac{dV_i}{dt} \frac{\hat{\mathbf{a}}_i}{V_i^2} \\ &= \frac{1}{V_i} \frac{d\hat{\mathbf{a}}}{dt} - \frac{dV_i}{dt} \frac{\mathbf{a}_i}{V_i} \end{aligned}$$

Therefore, the term $-\frac{dV_i}{dt} \frac{\mathbf{a}_i}{V_i}$ must be added to equation (3.1). Similarly, $-\frac{dV_i}{dt} \frac{\mathbf{p}_i}{V_i}$

must be introduced in (3.2).

In the simplest case, growth is homogeneous over the domain. It is more realistic, however, to restrict growth to a sub-domain, as this is what happens in plant roots where cells only divide near the tip of the tissue [Beemster and Baskin, 1998]. Therefore, the two cases we will consider are:

- constant growth rate in all cells, as a simplest case, and
- only the cells within a certain distance to the right end of the domain divide, whilst the others cease growing or dividing. This approximates growth patterns seen in plant roots [Beemster and Baskin, 1998].

In order to avoid duplicating notations, in both cases above we denote the set of dividing cells by $\mathcal{D}(\mathbf{t}) \subset \mathcal{N}(\mathbf{t})$. Then, $\mathcal{D} = \mathcal{N}$ for uniform growth and $\mathcal{D}(\mathbf{t}) = \{\mathbf{N}(\mathbf{t}) - \mathbf{N}_{\text{div}} \dots \mathbf{N}(\mathbf{t})\}$, for a fixed $\mathbf{N}_{\text{div}}$ in the “root-like” case.

To retain simplicity we considered growth to take place linearly over time, i.e. at a constant rate denoted by $\mathbf{g}$. As in previous simulation studies [Stoma et al., 2008, Smith et al., 2006], cells divide when their volume reaches a threshold, here denoted V_{max} . This is in reasonable agreement with experimental data [Beemster and Baskin, 1998, Shapiro et al., 2015, Campilho et al., 2006].

Thus, the resulting extension of equations (3.1)-(3.2) with growth and divisions is:

$$\frac{d\mathbf{a}_i}{dt} = \frac{1}{V_i} \sum_{j=1}^N A_{ij} \Phi_{ji} + \frac{\rho_{\text{IAA}}}{1 + \kappa_{\text{IAA}} \mathbf{a}_i} - \mu_{\text{IAA}} \mathbf{a}_i - \frac{dV_i}{dt} \frac{\mathbf{a}_i}{V_i} \quad (3.5)$$

$$\frac{d\mathbf{p}_i}{dt} = \frac{\rho_{\text{PIN0}} + \rho_{\text{PIN}} \mathbf{a}_i}{1 + \kappa_{\text{PIN}} \mathbf{p}_i} - \mu_{\text{PIN}} \mathbf{p}_i - \frac{dV_i}{dt} \frac{\mathbf{p}_i}{V_i} \quad (3.6)$$

$$\frac{dV_i}{dt} = \begin{cases} \mathbf{g} & \text{if } V_i < V_{\text{max}} \text{ and } i \in \mathcal{D}(\mathbf{t}), \\ 0 & \text{otherwise,} \end{cases} \quad (3.7)$$

with (3.3)-(3.4) still used for Φ_{ij} . Note that the actual cell subscripts, and their

neighbours, have to be updated after each division event. Therefore, we need not specify an algorithm for an arbitrary tissue here. For a linear chain of cells, it is natural that, at any time, cell i is the left neighbour of cell $i + 1$. When a cell divides, its two “daughter cells” are by default initialised with a volume $\frac{V_{\max}}{2}$, where auxin and PIN concentrations are equal to that of the parent cell and all cells to the right of a division have their subscript increased by 1.

3.2.4 Simulation Results

We now report on numerical simulations of the model (3.5)-(3.7) on a growing linear chain, using the procedure described in the previous section. Starting with a pair of cells, we considered both a chain growing homogeneously and a chain whose leftmost cells are neither growing nor dividing. In the latter scenario, only the rightmost eight cells could divide with all cells being able to grow until they hit a maximum volume. Note that the ODE parameters are reported in Table 3.1 where only the transport rate T is left unspecified, since we used it as main bifurcation parameter.

From an exploratory study, where discrete values were assigned to T within a wide range of positive values, we observed three qualitatively distinct behaviours, as represented on Figure 3.4. These three regimes are reached for increasing values of T in the order below:

Case 1 (*Low* T) The system stays locked to a proto-peak similar to Figure 3.1(a), see Figure 3.4(a).

Case 2 (*Intermediate* T) The system switches from the proto-peak state to another attractor comprising peaks or a travelling wave, see Figure 3.4(b)-(c).

Case 3 (*High* T) The system stays locked at a state of maximal peaks with the homogeneous stretching of the domain evenly moving the peaks apart causing the creation of proto peaks, see Figure 3.4(d).

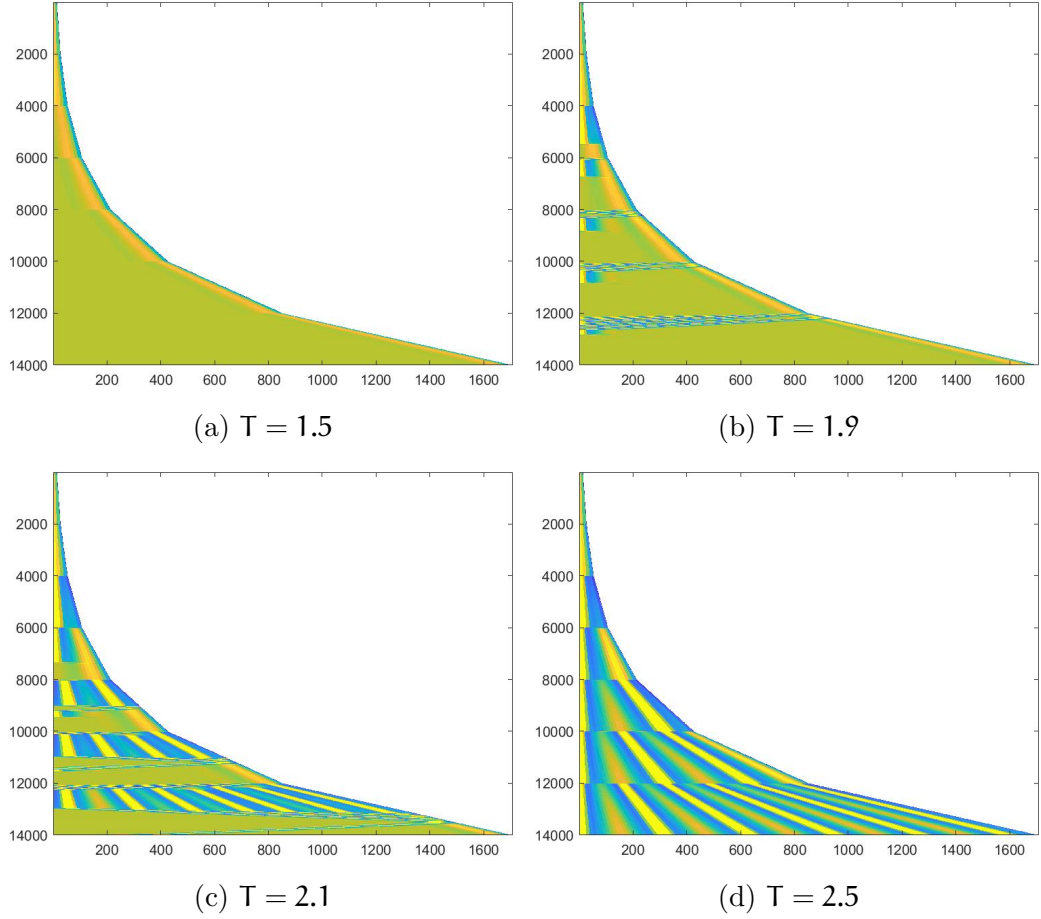


Figure 3.4: Time-dependent simulation of Eq. (3.5)-(3.7) with time in the y-axis, increasing downwards, for different values of T . Growth is homogeneous over the full domain. (a) Case 1: the proto-peak is maintained despite growth. Case 2: the system alternates between the proto-peak and (b) a travelling wave solution, or (c) the fully patterned solution. (d) Case 3: the number of peaks increases as the domain grows. T as shown in subcaptions, initial cell volumes $V = \frac{2}{3}$ and other parameters as shown in table 3.1

Note that the categories above are subjective to an extent, as for instance, one could have chosen to split further the cases covered in Case 2, or merge Cases 1 and 3 as consisting of a single branch of the bifurcation diagram. However, our categorisation can be explained by considering bifurcation diagrams. For example, one can see in Figure 3.5, that the snaking bifurcation diagram is altered as cell sizes are assigned different values. Therefore, the alteration may correspond to a change in the number of equilibria, which is indicative of a bifurcation using cell volume as a parameter, or simply in their location in state space. Though it is difficult to ascertain that all equilibria have been found along the snaking

diagram, one can only assume a combination of both factors from Figure 3.5. We will discuss in a later section how this can be resolved by performing continuation in a parameter representing a characteristic cell size.

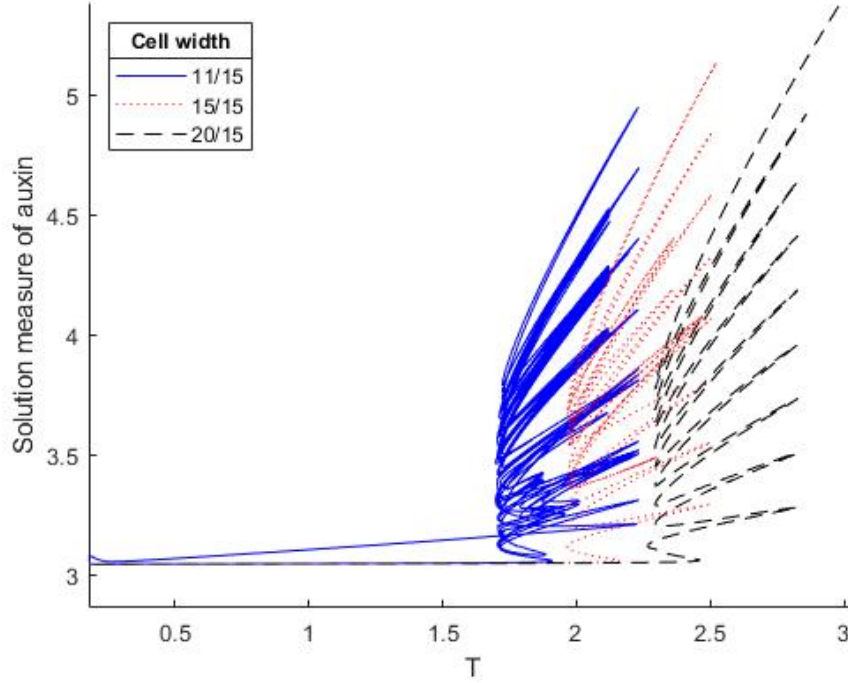


Figure 3.5: Steady state bifurcation diagrams for homogeneous growth in model (3.5)-(3.7). Three different cell widths overlaid, one just after cell division (dashed line), one where the cells are about to divide (dotted line) and one halfway between (full line).

At a cell division event, cell sizes are suddenly halved, so that the steady states available in the system corresponded to different snaking diagrams out of Figure 3.5. Thus, even if the system may not evolve fast enough to actually be close to a new steady state, the fact that steady states are altered entails a re-direction on the transient dynamics, resulting in noticeable qualitative change. As this is well captured by the superposition of snaking diagrams shown in Figure 3.5, we casually refer to this general phenomenon as *snake jumping* in the following, as any prior occurrence of the term is from an unambiguously different context [Socha, 2011].

Over time, the volume V varies in the range $[V_0, V_{\max}]$. Therefore, it follows that the bifurcation diagrams (in T) obtained for fixed volumes of V_0 and $V_{\max}$

respectively, will bound the set of all snaking diagrams obtained for other values of V . As both the V_0 and $V_{\max}$ diagrams have the structure featured e.g. in Figure 3.2, it is expected that a single branch will be available regardless of V for lower and higher values of T , while intermediate values of T will always correspond to a coexistence of attractors allowing snake jumping to occur, hence extending the explanation for three cases in the case of growing cells.

As mentioned earlier, we have also considered a case with inhomogeneous growth, where only the 16 rightmost cells were subject to growth and only the eight rightmost cells were subject to division. As with the homogeneous growth, for our fixed parameters there are three distinct behaviours found by varying T . These are entirely analogous to the three cases above, with the difference that Case 2 corresponds to an alternation between the proto-peak analogue to Figure 3.1(a) and the single peak as in Figure 3.1(b). See some typical values of T in Figure 3.6.

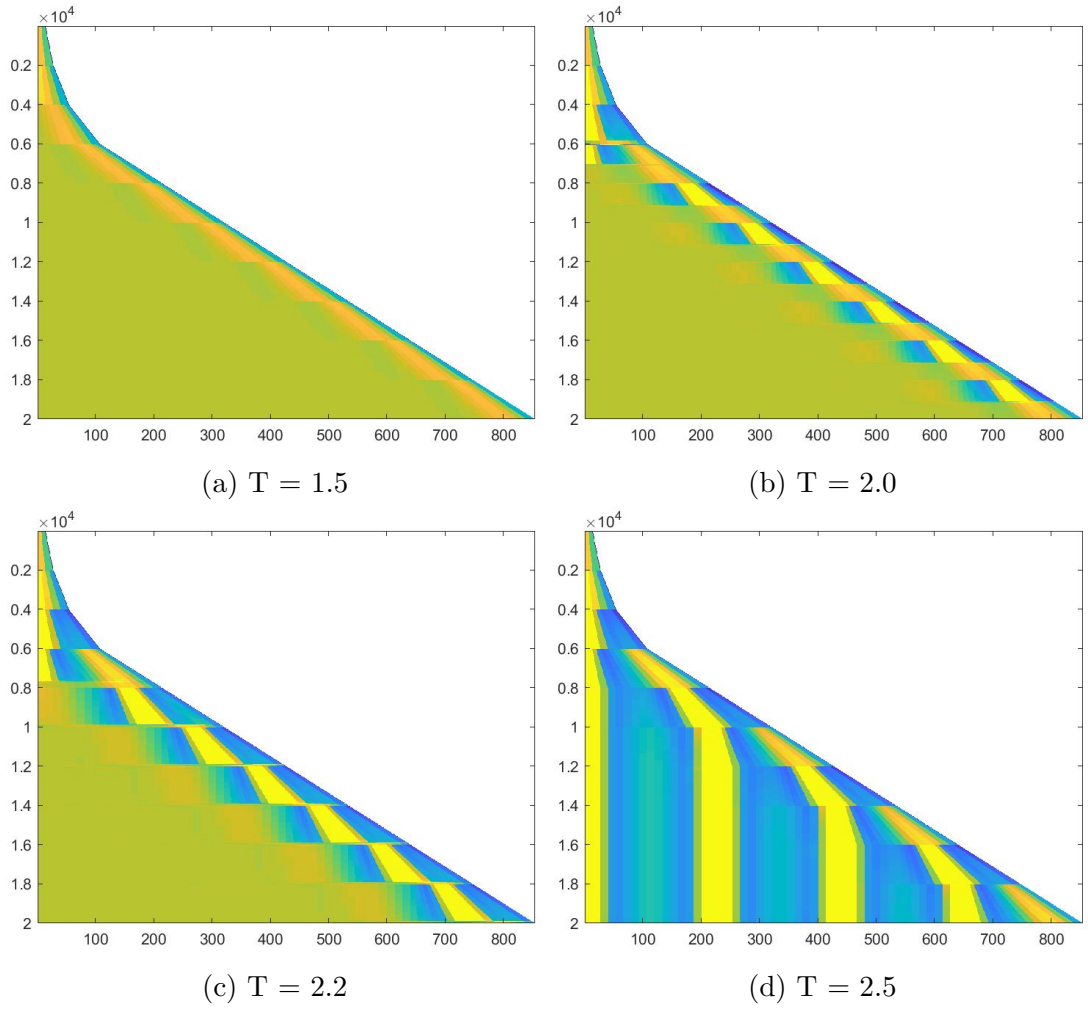


Figure 3.6: Time-dependent simulations of Eq. (3.5)-(3.7) with time in ordinate, increasing downwards, for different values of T as shown above. Growth only occurs for the 16 rightmost cells. (a) Case 1: the proto-peak is maintained despite growth. Case 2: (b)-(c) the system alternates between two distinct one-peak solutions. (d) Case 3: new peaks appear over time near the right end of the domain. T as shown in subcaptions, initial cell volumes $V = \frac{2}{3}$ and other parameters as shown in table 3.1

Here again, the patterns in Figure 3.6 are explained by the bifurcations which the system undergoes as T and the domain geometry are modified. The diagrams in Figure 3.7 illustrate how the first snaking curve occurs for different T as cell volume varies. In the inhomogeneous system, the Case 2 patterns are caused by the state jumping between one and no peaks, instead of many and no peaks, but for the same T value as in the homogeneous model. This is further confirmed in Figure 3.7 (b)-(d): by taking V as a parameter and continuing solutions one can see the occurrence of a bifurcation enabling a snake jump from a single full peak

to a proto-peak. Note that for greater values of T , the system gets locked to a state of full peaks, like in the homogeneous case albeit a different pattern, due to stretching of the domain only occurring at the tip.

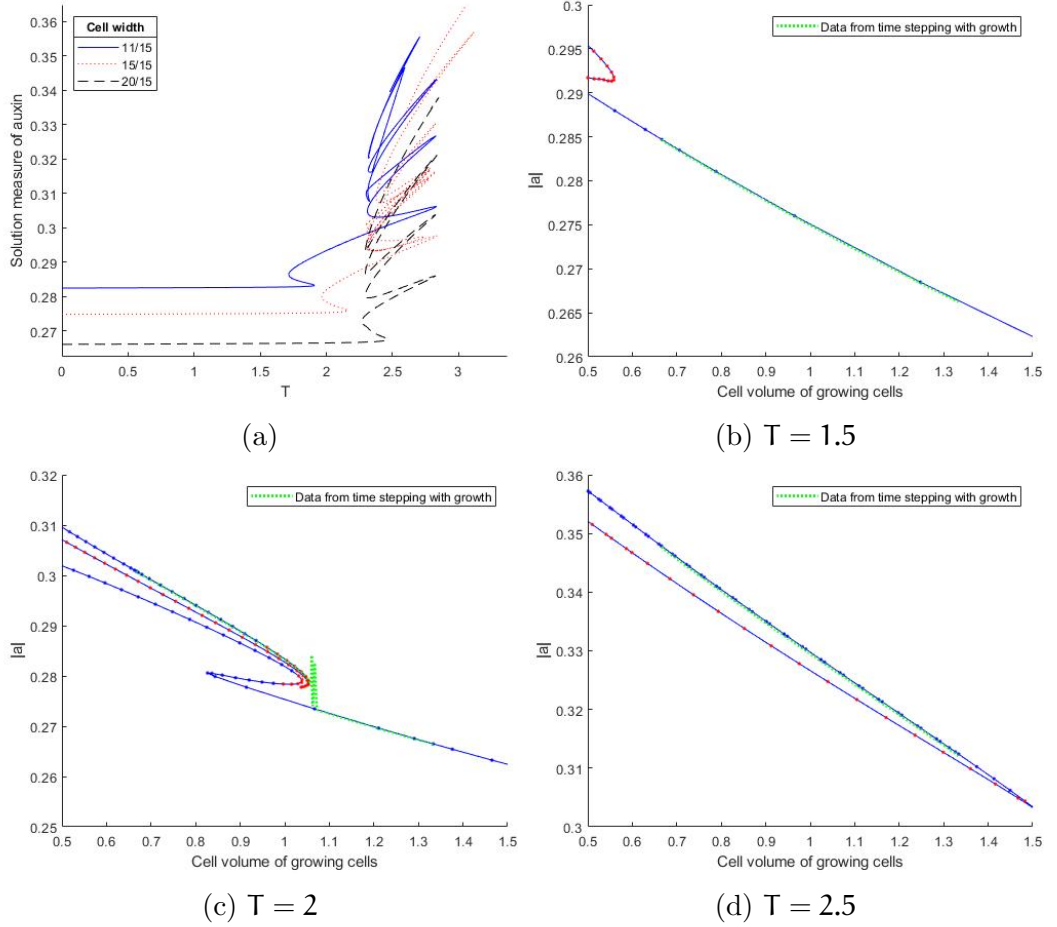


Figure 3.7: Steady-state bifurcation diagrams for inhomogeneous growth, restricted to the right end of the model (3.5)-(3.7). (a) Continuation in T , for three different cell widths overlaid as in Figure 3.5. (b)-(d) Steady-state bifurcation with respect to cell volume, for cells in the dividing domain $\mathcal{D}$, overlaid with a plot from a time dependent simulation with growth. At $T = 2$, the solution “jumps” from a one-peak pattern to the proto-peak, consistently with Figure 3.6(b). T & V as specified, other parameters as shown in table 3.1

3.2.5 Phenomenological Oscillations

One observation that can be made, and is most visible in the inhomogeneous growth model, is that near the tip of the domain auxin concentration may appear to oscillate. This appears in Figure 3.6(b), and is made clearer by fixing the right-end of the domain as in Figure 3.8(a). Oscillations of auxin response in

time have been observed in the model plant *Arabidopsis thaliana* [De Smet et al., 2007, Moreno-Risueno et al., 2010b] and here we showed that with the Smith model similar behaviour could be induced under the effect of growth and cell divisions.

This is not in contradiction with the recent suggestion that the experimental oscillations are due to the occurrence of a limit cycle in the auxin signalling network, i.e. downstream of auxin itself [Perianez-Rodriguez et al., 2021]. Indeed, although [Perianez-Rodriguez et al., 2021] puts most of its focus on oscillations occurring, even for auxin constant with respect to time, it also presents numerical evidence that periodic auxin waves can enhance these oscillations, if they are in phase. The mechanism uncovered here shows how periodic auxin fluctuations can result from the patterns of growth of the tissue. This opens up the possibility for a complex interplay between rhythms of auxin itself and the protein network it controls. Note that in the present context, oscillations are not explained by a limit cycle, but by the periodic change of cell density which occurs locally through growth and division events. Ultimately, it is the cell cycle, and the spatial distribution of its phase, which determine oscillations of auxin.

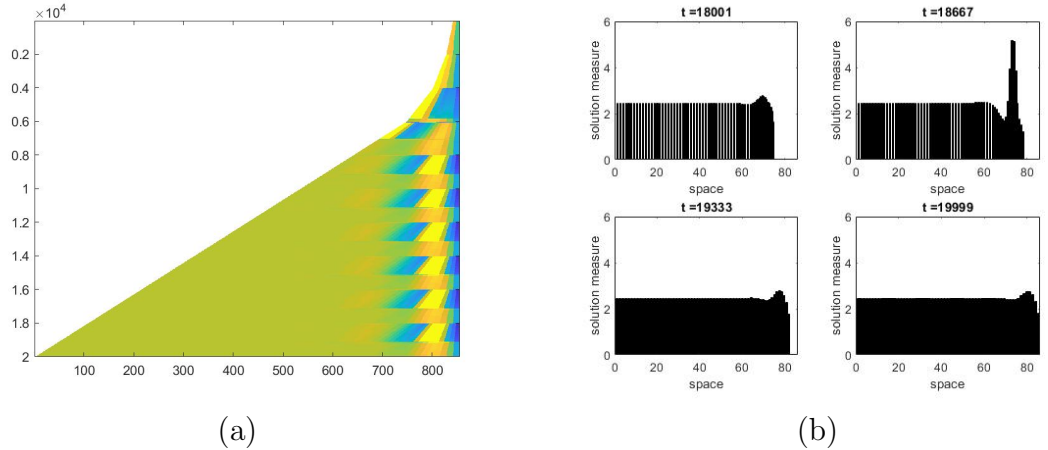


Figure 3.8: (a) Graph of time-dependent simulation with the tip held in place on the right for an elongation zone of 16 cells and $T = 2$. (b) Auxin distribution at specific time points; horizontal position on x-axis and time on y-axis. Initial cell volumes $V = \frac{2}{3}$ and other parameters as shown in table 3.1

3.2.6 Effect of Noise

A limitation of the simulations discussed in the previous section is that within the dividing zone $\mathcal{D}(t)$, all cells divide simultaneously since they are initialised at the same size, grow at the same rate and divide at the same threshold. This is not only unrealistic, but it may invalidate our reasoning about snake jumping. Indeed, the well separated bifurcation diagrams in Figures 3.5 or 3.7 correspond to domains where a significant number of cells have a different size. Therefore, one may expect less distinctly separated diagrams for domain geometries, with less drastic differences, obstructing snake jumps.

Thus, given the key role played by cell divisions, and the fact that cell divisions are certainly subject to noise, we focused on this aspect. Therefore, to observe what effect uneven cell divisions might have on our dynamical system we carried out simulations where the size of the daughter cells were normally distributed between 24% and 76% of their mother's size. Note that the percentage for one of the daughter cells is $50+x\%$ for $x \in \mathcal{N}(0, (9.3)^2)$ given $|x| < 26$. This distribution was chosen due to a study of cell division in the shoot apical meristem [Shapiro et al., 2015] in which a histogram of the proportion daughter cells were of their parents size was a bell curve with a reported standard deviation of 9.3. Hence, this represents the full range of observed values in the paper even though the majority of divisions in the bottom 20th percentile were cells on the periphery of the meristem and may not be representative of cells in the meristem.

What we see below by varying the standard deviation of daughter cell size percentages is that the cells can reach a point where they stop dividing in as distinct a pulse and this correlates with a decrease in the regularity of auxin peaks (Figure 3.9). Whilst this shows that this behaviour is not wholly resistant to noise, with either a standard deviation lower than that seen in the shoot apical meristem or some other factor that forces a greater uniformity of cell size, the behaviour can persist with regularity in a system containing noise.

As reported in Figure 3.9, the predictions of our modelling are, to some extent,

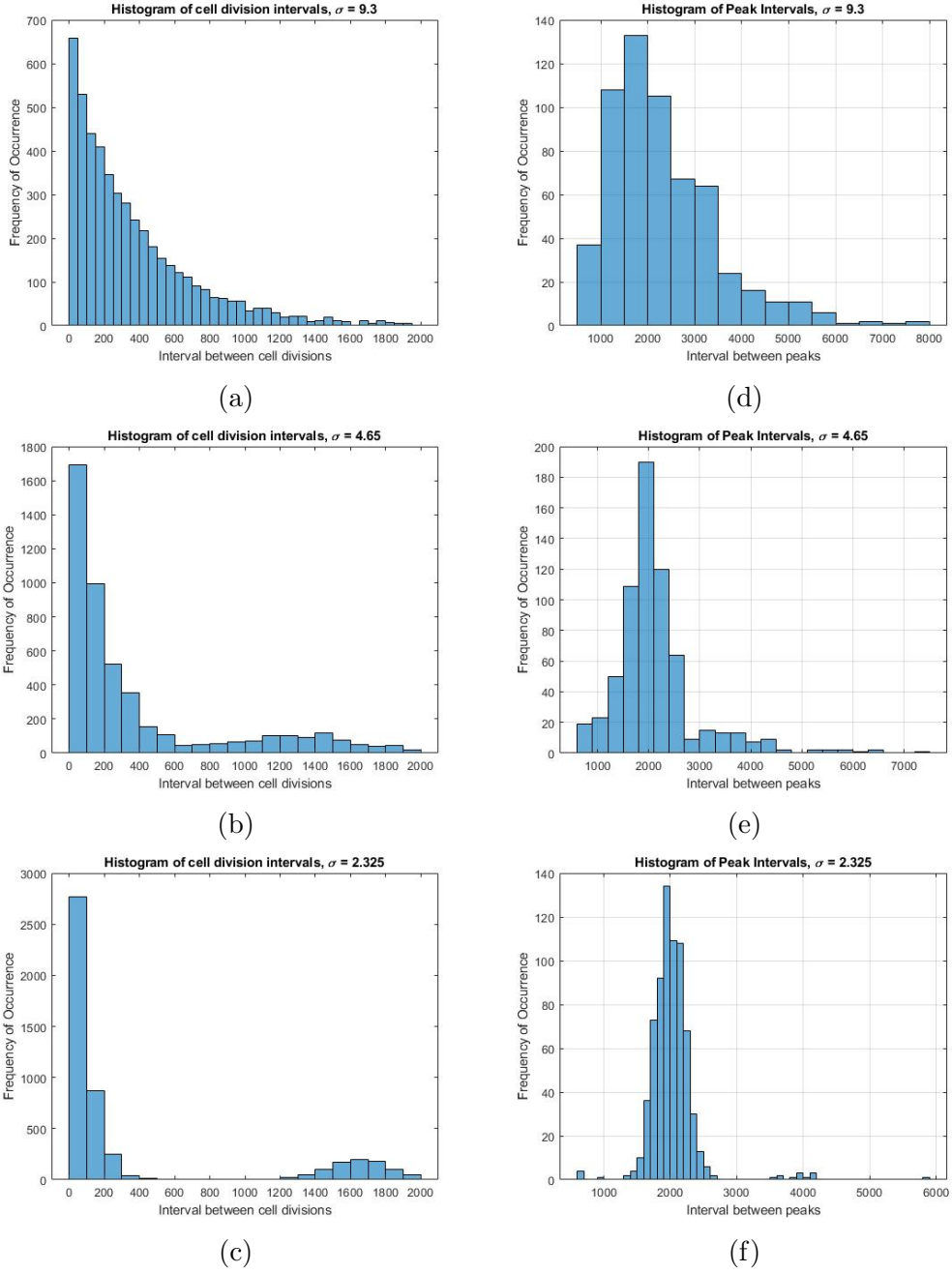


Figure 3.9: Graphs showing how differing levels of standard deviation for daughter cell proportions. (a-b) represent the standard deviation observed in the shoot apical meristem with (c-d) half that value and (e-f) showing results for one quarter of the observed standard deviation, $T = 1.9$, initial cell volumes $V = \frac{2}{3}$ and other parameters as shown in table 3.1

robust to this addition of noise since the main qualitative outcome, which is the occurrence of phenomenological oscillations, is maintained. Whilst differences between the different bifurcation diagrams are less distinct, like in the example shown in Figure 3.10, snake jumps are often possible in many cases for a more

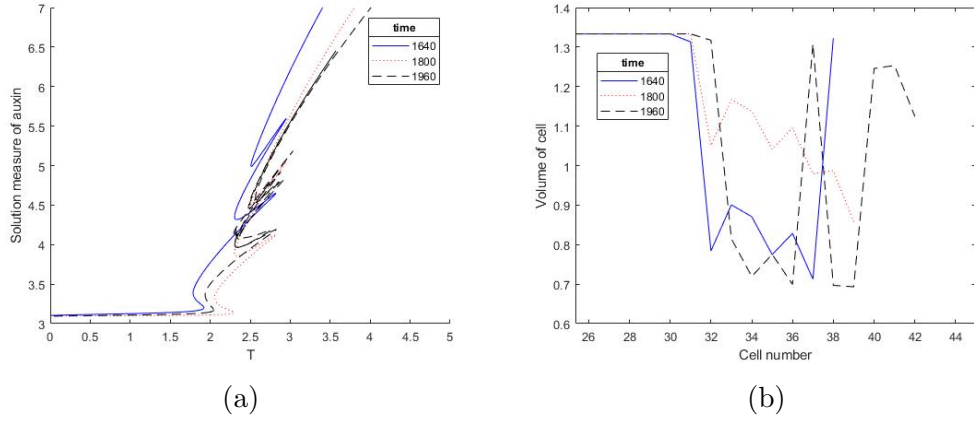


Figure 3.10: (a) Overlaid steady-state bifurcation diagrams for cell volumes (b) taken from a time-dependent simulation with stochastic cell division used in figure 3.9a-b

narrow set of T values.

3.2.7 Investigation on Noisy Domains

The initial hypothesis that was being tested was that the synchronicity of cells in the growing region might be a good indicator for whether or not a full peak would form during a cell cycle. This was supported by peaks becoming more irregular in time and duration as noise in cell divisions increased.

To better understand the effects of non homogeneous cell volumes we decided to run a series of simulations in which a domain of 24 cells included random cell sizes. The first eight cells, those furthest from the tip, were given volumes of $4/3 = V_{\max}$ and were set to not grow whereas the rightmost 16 cells, which represent those that might be growing in one of the previous simulations, were set to have volumes generated stochastically with a uniform distribution between $2/3$ and $4/3$. Other parameters as stated in table 3.1.

From an initial condition of no auxin or PINs in any cell, the system would be run without any cell growth to reach an initial steady state before beginning the main test simulation. In the main test simulation the system was ran for the duration of a cell cycle, which is the time it would take a cell to grow from minimum size $2/3$ to maximum size $4/3$. The difference compared with previous

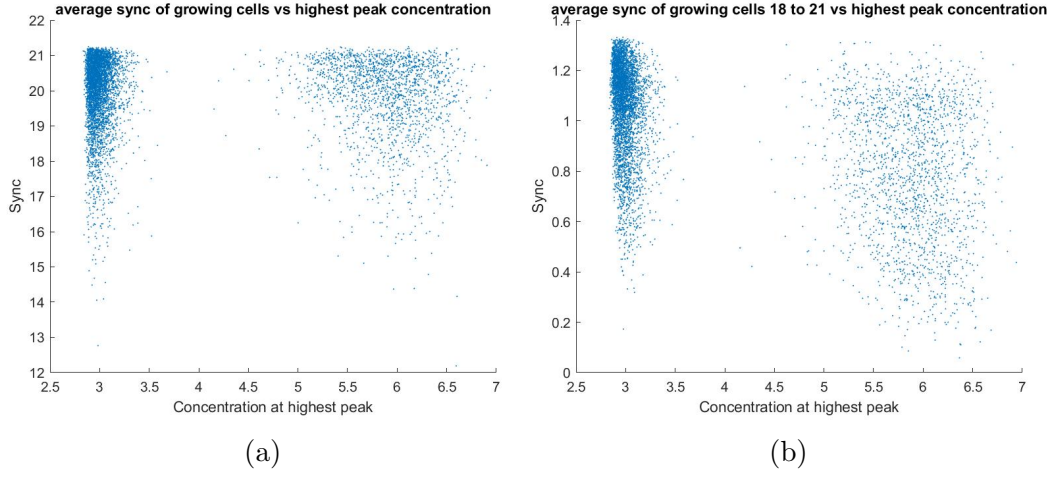


Figure 3.11: Scatter graphs plotting the average synchronicity of different groups of cells in a domain against the highest peak achieved by a simulation upon said domain

growth simulations is that the number of cells is maintained by setting it so that once a cell reaches $v_{\max}$ it would then have its volume set to $2/3$ and the simulation would continue. This allowed for the synchronicity of each cell to any other, barring those that are not growing, to stay constant.

Here the synchronicity of two cells with volumes V_i & V_j is defined as:

$$\text{sync}(V_i, V_j) = \min(V_i - V_j \pmod{\frac{2}{3}}, V_j - V_i \pmod{\frac{2}{3}}),$$

this is proportional to the shortest time between each of two cells having their volume reset to $2/3$ which is itself representative of the time difference between each cells parent cell dividing.

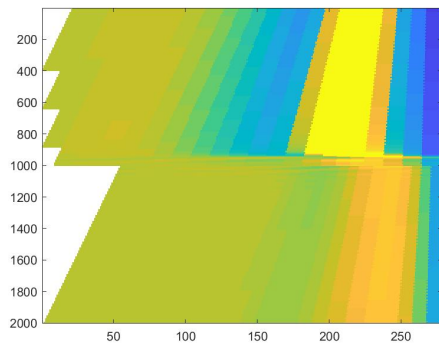
To test the hypothesis, 10,000 simulations were run in the manner above and a Pearson correlation of only -0.1132 was discovered for maximum peak height against average sync for all cells in Figure 3.11. This showed the hypothesis to be largely incorrect.

After ruling out that hypothesis, I next looked at a way to categorise the simulations to better understand the mechanics of what was going on. This commenced with the plotting of a scatter graph of the value for the highest auxin concentration of a simulation against the cell in which it occurred. This

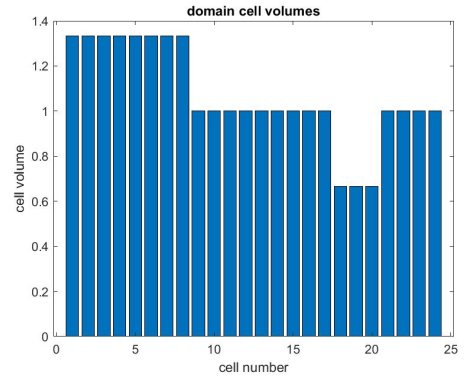
divided up the simulations into a number of groups based on the cell at which the simulation had its highest auxin concentration and whether or not the auxin concentration at its peak was greater or less than four. For each of these categories a box and whisker graph was plotted for the cell size of each cell at the time of the highest auxin concentration for each group. A couple of individual plots of the simulations from each of the groups were looked at and it was discovered that the majority of simulations that had auxin peaks that were greater than four, and occurred in cells 9 to 17, contained travelling waves. These travelling waves were found to be moving up the file of cells away from the tip, and it was said travelling waves that contained the highest point of auxin concentration in the scenario rather than a stable static peak.

From studying these graphs of cell geometries which corresponded to different peak locations it was hypothesised that cells in a stepped formation, where cell volumes descend toward the left in groups of three or more, led to travelling waves. Furthermore, due to the same mechanisms that lead to the peak and proto-peak in the homogeneous domain, there is a preference for peaks forming near the tip at cells 18 and 19 and that a pair of cells of similar size are often found where a peak forms. In line with this, a correlation of -0.5493 was found between peak concentration and average sync of cells 18, 19 and 20.

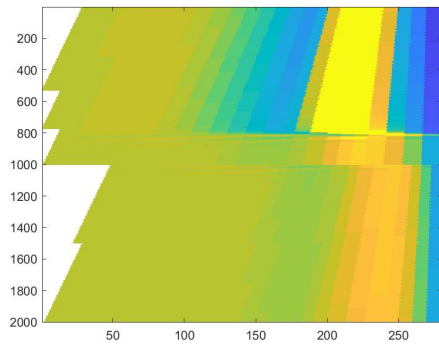
To test the hypothesis on a cause for the formation of travelling waves, I tried to generate domains with predictable results. First a domain that would contain a full auxin peak was generated by setting cells 18 to 20 in sync and out of sync with the other cells, as reported on Figure 3.12b. Next the domain was altered by the addition of sets of cells whose volume descends to the left. By implementing a series of such sets, or a alternating pattern of high and low volumes, the domain was able to induce travelling waves (see Figure 3.12f), hence confirming the plausibility of the hypothesis.



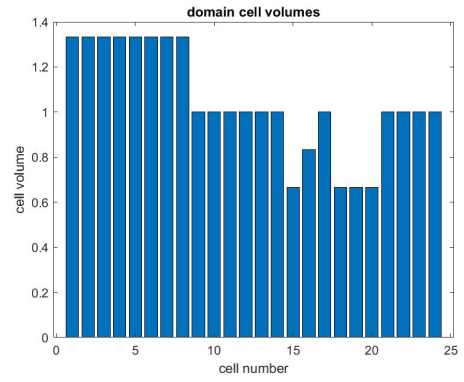
(a)



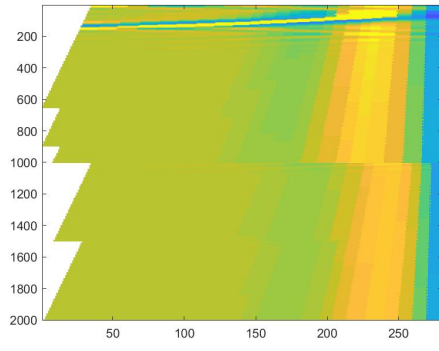
(b)



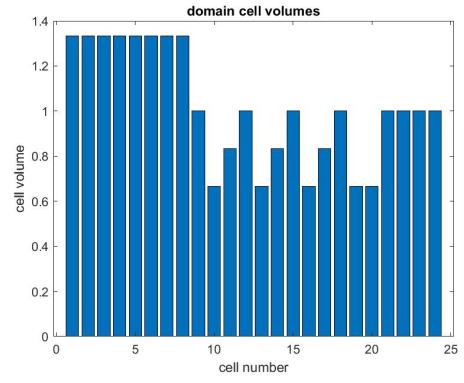
(c)



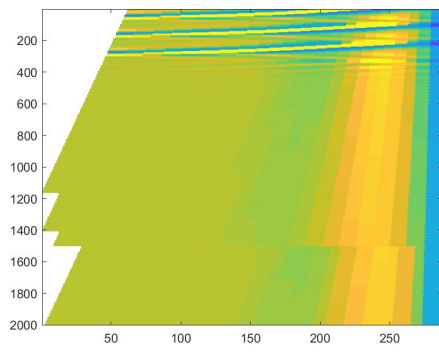
(d)



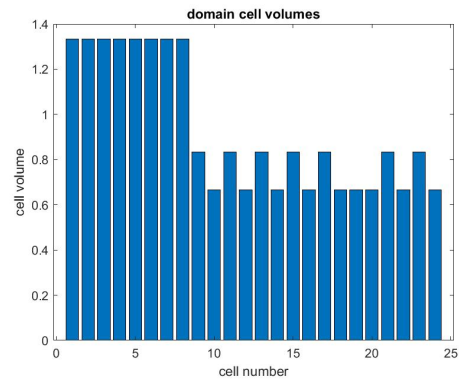
(e)



(f)



(g)



(h)

Figure 3.12: Paired graphs detailing a snapshot of the purpose built domain on the right, where the cells are numbered in ascending order towards the tip on the x-axis and cell volume is measured in the y-axis. The left hand side depicts a time-dependent simulation of a full cell division period, space on the x-axis, time ascending down the y-axis.

3.3 Swift-Hohenberg Reaction Diffusion Model

The patterning mechanisms found in the previous section results from the combination of (1) a snaking bifurcation diagram and (2) the growth of and cell divisions within the underlying tissue. They are, therefore, in principle not specific to auxin transport, and in particular do not seem to actually require active transport. To investigate these processes in a more general context, we considered a reaction diffusion model, in which feature (1) was well-documented and included a rich variety of patterns: the Swift-Hohenberg model [Knobloch, 2008, Lloyd et al., 2008]. We considered a one-dimensional compact domain, denoted $[-L, 0]$ with Neumann boundaries and a common form of Swift-Hohenberg equation:

$$\frac{\partial \mathbf{u}}{\partial t} = -(1 + \nabla^2)^2 \mathbf{u} - \mu \mathbf{u} + \nu \mathbf{u}^3 - \mathbf{u}^5. \quad (3.8)$$

To keep some analogy with the previous sections, we considered this model on a discrete tissue, rather than a spatial continuum. Thus, the discretised form of the equation above is:

$$\frac{d\mathbf{u}_i}{dt} = - \left((\mathbf{I}_n + \mathbf{D}_{xx}^2)^2 \mathbf{u} \right)_i - \mu \mathbf{u}_i + \nu \mathbf{u}_i^3 - \mathbf{u}_i^5, \quad (3.9)$$

where: $\mathbf{u} = (\mathbf{u}_1 \dots \mathbf{u}_N)$ and $i \in \{1, \dots, N\}$ ranges over a finite set of discrete locations $\mathbf{x}_i \in [-L, 0]$. $\mathbf{D}_{xx}$ is the Laplacian matrix for the domain, expressed in terms of the matrix of adjacencies $\mathbf{A}$ defined under equations (3.1)-(3.4) as:

$$(\mathbf{D}_{xx})_{ij} = \begin{cases} \mathbf{A}_{ij} & \text{if } i \neq j, \\ -\sum_k \mathbf{A}_{ik} & \text{if } i = j. \end{cases}$$

Although not explicitly a model of biological substrates and processes, the Swift-Hohenberg model accounts for the motion of mass in a spatial domain, hence allowing for a representation of density. Furthermore, we relied on the only spatial operator featured in this model, the Laplacian operator, multiplying it by

a re-scaling factor which could be used to mimic the local changes of density that would result from growth and cell divisions, whilst fixing the domain to remain $[-L, 0]$ at all times. The intuition was that by multiplying D_{xx} by this factor, an increase in ‘density’ at point x has the effect of artificially reducing the gradient of u at x in a way that is analogous of growth.

We denote ‘density’ by $\rho(x, t)$, or in discretised form $\boldsymbol{\rho} = (\rho_1 \dots \rho_N)$, where $\rho_i(t) = \rho(x_i, t)$. Then one adapts (3.9) as follows:

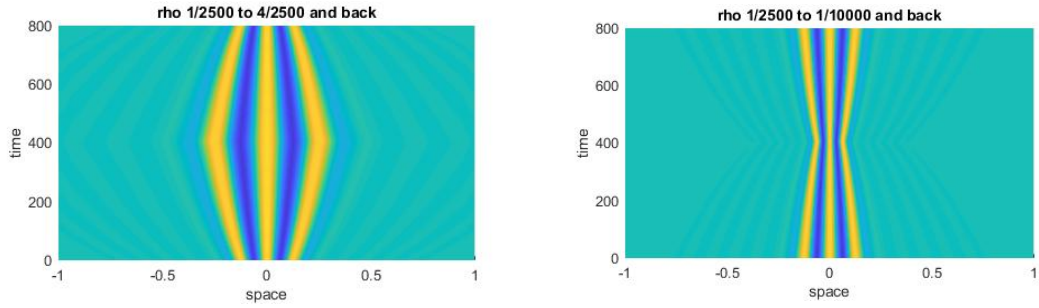
$$\begin{aligned} \frac{du_i}{dt} &= - \left((I_n + \boldsymbol{\rho}^T D_{xx}^2)^2 \mathbf{u} \right)_i - \mu u_i + \nu u_i^3 - u_i^5 \\ \frac{d\rho_i}{dt} &= g h(x_i), \end{aligned} \quad (3.10)$$

with the initial density $\rho_i = \frac{1}{L^2} \forall i$ at time $t = 0$. The term $h(x_i)$ accounts for potential inhomogeneity in density, and if density varies in a spatially homogeneous fashion, one simply sets $h \equiv 1$. However, to represent localised changes of density, we chose a sigmoid function to get a smooth transition:

$$h(x) = \frac{1}{(1 + \exp(-\frac{(x-\gamma)}{\delta}))}, \quad (3.11)$$

where δ governs the gradient of the sigmoid function and γ governs the centre of the transition point where on one side “cells” are changing density and on the other side they are not. Furthermore in this chapter $\mu = 1.5$ and $\nu = 3$.

Firstly, simulations were run where the ρ_i were increased and decreased homogeneously and smoothly over time for different numbers of peaks. In these simulations the solutions were qualitatively unchanged. This is to say, in the experiments none of the large peaks were affected but neither did they get close to the boundary. This was to be expected since in this instance altering $\boldsymbol{\rho}$ is akin to altering the length of the domain. Moreover, as indicated by the relationship between ρ and L when ρ quarters in size the width of the waves halve, as though the domain is twice as wide (Figure 3.13).



(a) ρ linearly increases from $t = 0$ to 400 and linearly decreases from $t = 400$ to 800. $g(t) = 3E-6$ if $t \in [0, 400)$, $g(t) = -3E-6$ if $t \in [400, 800]$.
(b) ρ linearly decreases from $t = 0$ to 400 and linearly increases from $t = 400$ to 800. $g(t) = -7.5E-7$ if $t \in [0, 400)$, $g(t) = 7.5E-7$ if $t \in [400, 800]$.

Figure 3.13: Graphs showing time-dependent simulations for the discrete Swift-Hohenberg featuring continuous and homogeneous alteration of ‘density’ for the whole domain, i.e. $h \equiv 1$.

In addition, continuation was also performed for different values of ρ_i with respect to the parameter μ and this revealed that the snaking behaviour was qualitatively identical for different ρ for solutions that are sufficiently far from the boundaries. This inhomogeneous deformation has the potential to result in snake jumps, notably for $\gamma = -0.125$ and $L = 1$. For example, for $\delta = 8$ (Figure 3.14), we see a snake jump from two peaks to one peak, one of the peaks collapsing as it passes through the transition between high and low density.

In Figure 3.14 the bifurcation appears to occur when the peak crosses the centre of transition between high and low density. Therefore, to investigate whether this was caused by discontinuity due to the discretisation used in the simulation method, simulations were run with different meshes to compare. However, due to the computational costs of the simulations, the meshes used were non-homogeneous with a loose mesh everywhere except close to the density transition point and varying sizes of mesh around the transition point.

Consequently, the result of these mesh refinements was a trend that appeared to indicate that the bifurcation may be caused by the discretisation of the domain. This is because for the limited domains we used we saw a trend where the bifurcation occurred at later times for tighter meshes.

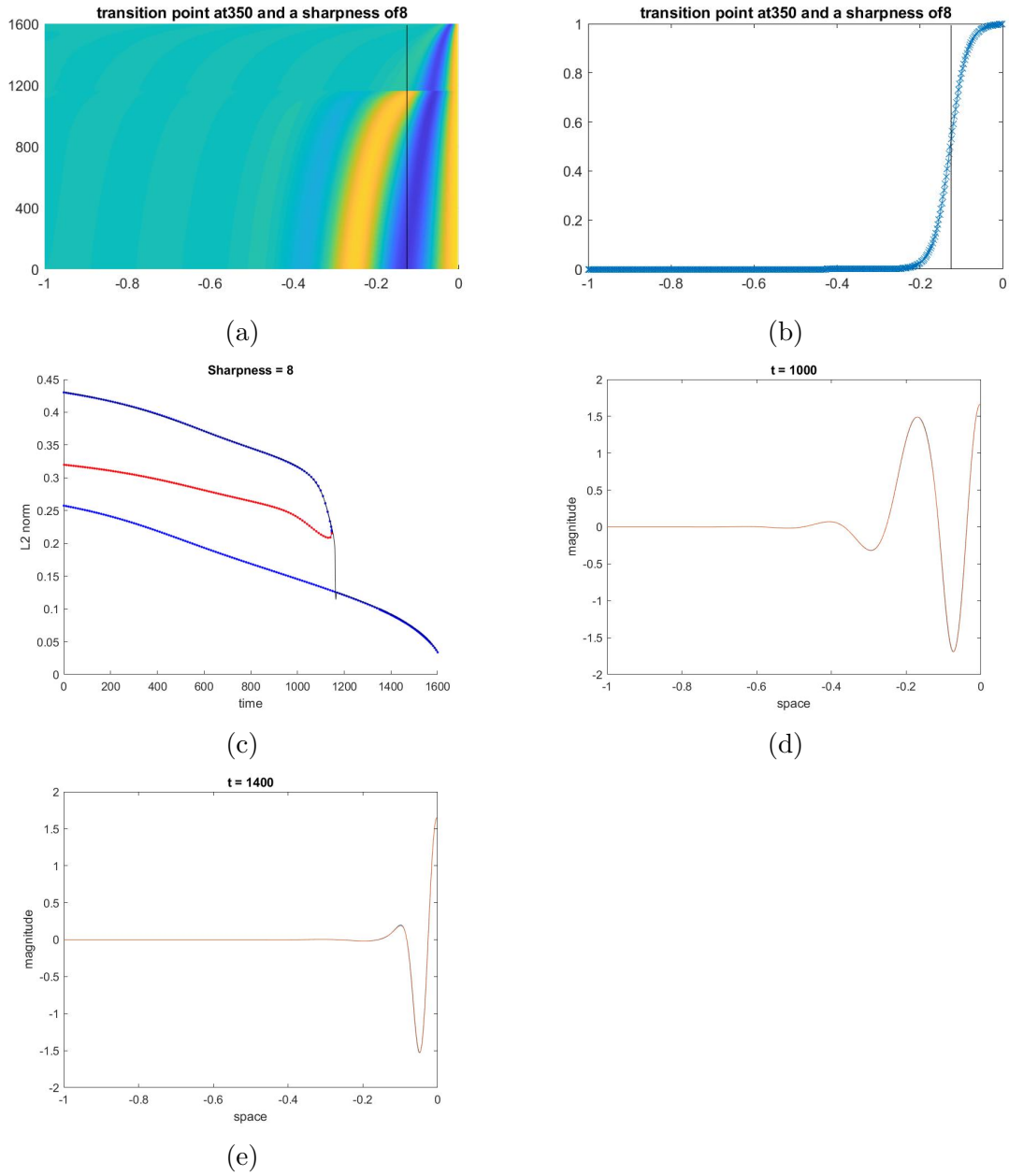


Figure 3.14: Graphs showing a snake jump in a time-dependent simulation of a discrete approximation of the Swift-Hohenberg equation. Here h is in the form given in equation (3.11), with $L = 1$, $\delta = 8$, $\gamma = -.125$. (a) Shows a surf map of the state variable over time in the simulation. (b) Graphically shows $h(x)$. (c) Shows a steady-state bifurcation diagram where the bifurcation parameter is time with a plot of the L2 norm of the time-dependent simulation in (a) overlayed on top. (d-c) Show examples of the two steady states from the time-dependent simulation shown in (a) before and after the snake jump.

3.4 Initial Data on DII Oscillations

Initially there were plans to run a series of experiments using the aforementioned DR5:luciferase reporter to track auxin response elements in growing arabidopsis

roots. This would have given a representation of the quantity of auxin in the root at a given time. In addition, by varying the rate of growth in the roots, by altering the concentration of sugar in the agar medium they grew in, we would have attempted to test if our hypothesis of a connection between the rate of growth and the period of auxin pluses was correct. This was first attempted prior to the advent of Covid, but due to difficulties in replicating the results of [Moreno-Risueno et al., 2010a] no results were gathered before lockdowns and protocols limited lab access resulting in the whole experiment being put to one side till just after the start of 2022.

In the final weeks of this thesis some progress has been made. However, this time rather than using DR5:luciferase, DII:Venus has been used. This was due to difficulties in making a set up similar to that described in [Moreno-Risueno et al., 2010a] work, even after a consultation with the authors. Whilst DR5:luciferase is representative of ARF concentration, DII:venus is degraded in the presence of auxin and thus is more directly representative of auxin concentration. However, due to the requirements of its implementation one cannot capture real time continuous data. Instead 18 observations were taken 60 minutes apart for eight roots, by our colleagues Anthony Bishopp and George Janes (University of Nottingham) as reported on Figure 3.15.

The current data does not show especially pronounced peaks of auxin, at least in comparison to the Smith model, however, this is not discouraging. Firstly, at this time cell selection for observation has not necessarily been optimised; time allowing, more than five cells could be sampled from the raw data set, which consists of stacks of images comprising dozens of cells. Secondly, the DII:Venus curves whilst not qualitatively the same profile as those seen in the inhomogeneous Smith model are visually similar to those generated by the inhomogeneous Smith model with stochastic cell division percentages, as reported on Figure 3.16.

To compare the experimental data with the simulations the experimental data as seen in Figure 3.15 must be inverted as the florescence is inversely proportional

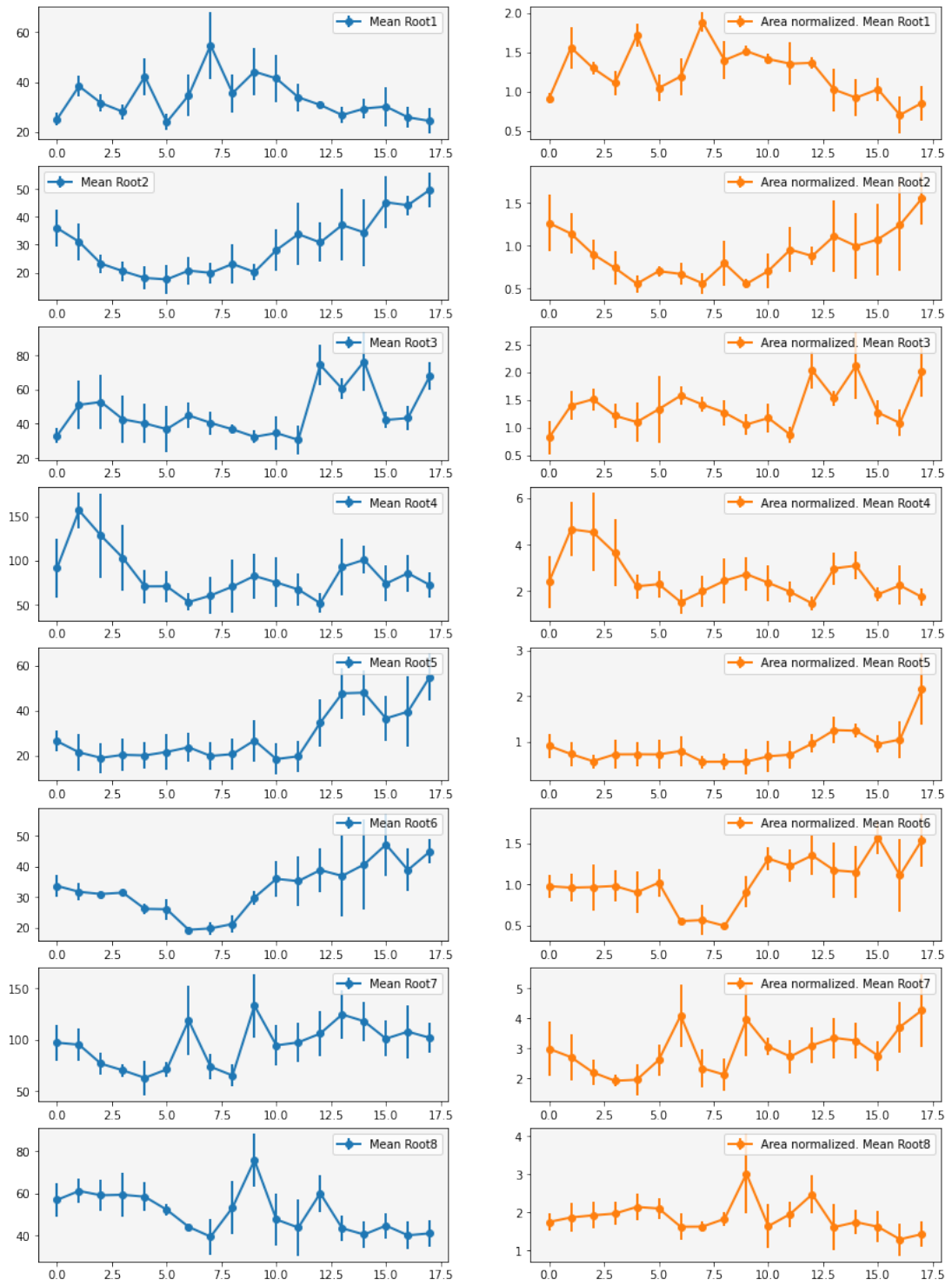


Figure 3.15: Time series plots of DII:Venus fluorescence in five cells of the root tip, for eight roots over a period of 17 hours. Due to the way that auxin degrades DII:Venus, there is an inverse relationship between magnitude of fluorescence and auxin concentration. Curves represent the average over the five cells, and error bars the corresponding standard deviation.

to auxin concentration. The experimental data is then compared against the average auxin concentration of 5 cells near the tip from a time dependent simulation.

The simulation includes stochastic daughter cell generation and uses the same parameters as those found in the beginning of Section 3.2.6, with the exception of cell growth rate being increased by a factor of ten and $T = 2.1$ instead of 1.9, full time series data available in Appendix A.2. These parameters were chosen after a short parameter search trialling $T = 1.9$ and then values slightly higher based on a visual assessment of how well they matched experimental data. The increase in cell growth was motivated by observations of auxin concentration changing slower with respect to time in experimental data than that seen for the original cell growth rate. One time-dependent simulation was run for the aforementioned parameters, and from said simulation including roughly 10 cell cycles. From this single simulation two cell cycles were observed to have similar auxin concentration profiles with respect to time as those found in experimental data.

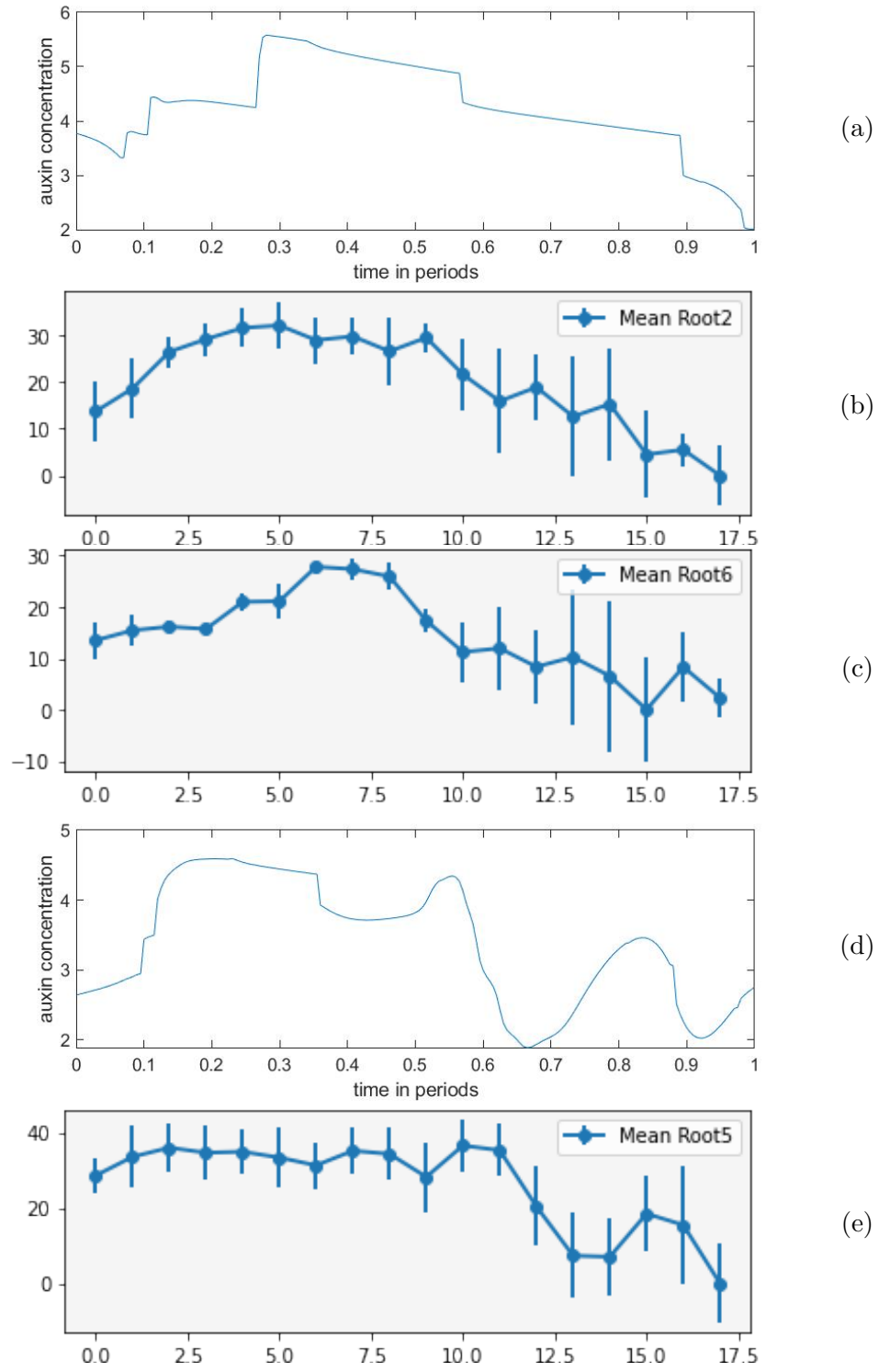


Figure 3.16: Comparison between the mean auxin value of five cells near the tip from a single time-dependent simulation (a,d) and the inverse of the experimental data, for which Y values represent the maximum magnitude of fluorescence for a time series minus the magnitude of fluorescence at that time for (b,c,e) as the florescence is inversely proportional to auxin concentration.

3.5 Conclusion

In this chapter we have looked at variations on the Smith active transport auxin model. In analysing said model we have displayed a useful technique in understanding behaviour on growing domains where we performed continuation, using the active transport parameter T , on “snapshots” of the domain to understand how growth affects the patterning of the system. Any such snapshot presents a specific set of stable steady states (i.e. patterns), forming a snake-like curve. Over time, changes in this curve induce changes in which a particular pattern exists to attract the system, leading to potential alternations of patterns across time. This can supply complementary information about the interaction of growth with the steady states, rather than just continuation in domain size, which only follows one specific pattern for one among those possible.

This viewpoint was used, in particular, to show that the oscillatory behaviour of the auxin peak at the tip of the domain is driven by growth. For a subset of T values, the cell geometry present at the beginning of a new cell cycle had only one steady state which was different to the single steady state found for the cell geometry at the end of the cell cycle, resulting in the system oscillating between these two states. This is important as it is a testable observation due to the rate of oscillation being long enough, approximately the rate of the cell cycle, to be observable via current methods of inferring auxin concentration. This oscillation mimics an observed behaviour [Moreno-Risueno et al., 2010b] where auxin responsive genes (experimentally accounted for using a reporter construct called DR5:luciferase) appear to oscillate at the root tip.

However, this scenario is not the only plausible explanation. For instance, it is known that auxin transport models on a fixed tissue can present oscillatory solutions [Draelants et al., 2015, Farcot and Yuan, 2013] involving travelling or stationary waves of auxin. As another potential explanation to observed oscillations, the auxin signalling pathway, at the level of a single cell, is potentially able to enter a limit cycle through a Hopf bifurcation [Middleton et al., 2010]. As a di-

rection for future work, one could investigate whether oscillations found to occur from auxin transport and signalling could interfere, and perhaps present some resonance. This could be creating the large fluctuations in the DR5:luciferase instead triggered by minute oscillations in auxin.

Unfortunately, Covid has delayed the progression of experiments looking at how the growth rate of a root interacts with the periodicity of fluctuations in auxin concentration within the root. To this date only experiments on a 17 hour time frame have occurred. Whilst the change in auxin over the observed period is comparable to the profile of auxin within simulations ran in Section 3.2.6 as shown in Section 3.4 the time series data does not appear to cover a full cycle. As such, future work should cover a longer window and for different rates of growth to allow for the use of Fourier transforms to estimate periodicity and how growth might alter it. Furthermore, it could also potentially cover cells from different locations using the quiescent centre as a point of reference to understand the spatial pattern.

Chapter 4

Input/Output Behaviour of the Auxin Signalling Pathway

4.1 Introduction

In this chapter, models of the auxin signalling pathway (see also Chapter 2) are investigated with a specific focus on the response of this pathway to temporal signals of auxin. This dynamical input/output viewpoint has never been attempted systematically, and yet it is highly relevant biologically. Indeed, existing modelling work considers either constant values of auxin as main scenario [Middleton et al., 2010, Vernoux et al., 2011a, Farcot et al., 2015, Grigolon et al., 2018], or complex computational simulations mostly aimed at mimicking auxin patterns, and where the specific role of the auxin signalling pathway is hard to decipher [van den Berg and ten Tusscher, 2018, Allen and Ptashnyk, 2020]. In real plants though, despite the difficulty in measuring auxin itself experimentally, there is a lot of evidence that it is a highly volatile signal. This is particularly clear, at least, in meristems which are themselves undergoing constant transformations due to never-ending growth and cell divisions.

For instance, in shoot apical meristems (SAMs), waves of auxin have been observed and shown not to directly correlate with growth patterns [Galvan-Ampudia

et al., 2020]. This study, therefore, suggests that at the level of individual cells, the temporal profile of auxin is likely to be complex, depending on the location in the tissue and most certainly not constant even relative to the typical response time of the auxin pathway (a few minutes to an hour [Vernoux et al., 2011a]). Furthermore in roots, auxin levels have been shown to have temporal oscillations as indicated by oscillating levels of auxin targets (downstream of the auxin pathway) [De Smet et al., 2007, Moreno-Risueno et al., 2010b]. Chapter 3 explored how this could be driven by auxin transport alone.

An auxin signalling model developed by A.M. Middleton, J.R. King, M.J. Bennett and M.R. Owen [Middleton et al., 2010], henceforth referred to as the Middleton *et al.* model, shows the capability for auxin signalling to drive ARF oscillations (i.e. can produce a limit cycle). These two potential causes for the oscillations in auxin response, auxin transport and auxin signalling, are not necessarily mutually exclusive. Therefore, in this chapter we consider, more specifically, how periodic auxin waves generated via auxin transport could affect auxin signalling pathway within an individual cell and, in particular, how such inputs interfere with the oscillating levels of ARF found in the Middleton *et al.* model.

The motivation for this specific line of inquiry is that oscillations have already been observed and are easier to control than the complex, ever-changing patterns observed in shoots. Furthermore, as discussed in Chapter 2, auxin concentrations are not directly accessible experimentally, but may be estimated by observing the transcription rate of reporter genes which have similar promoters activated by ARFs. Therefore, reporter levels are indicative of ARFs concentrations. As such, the interaction between ARFs and auxin are very important for understanding to what degree ARF levels accurately portray auxin levels and relate experimental data to actual auxin patterns.

Hence, to better understand this dynamic and to contextualise the results from auxin transport modelling, this chapter combines results from auxin transport with auxin signalling models. The models are not combined, but instead the

results are transferred from one model to the other, and whilst this omits any feedback between the two models, it reduces complexity and difficulties arising from re-scaling variables. In addition, it will also allow for a more systematic enquiry into the function of auxin transduction in converting temporal auxin inputs into transcriptional responses and the biological processes they trigger.

To investigate the dynamics between auxin behaviour within the cell and its behaviour within a tissue, such as the root tip, two auxin signalling models and the results from the auxin transport model from Chapter 3 will be used. The first of the two auxin signalling models is the aforementioned Middleton *et al.* model [Middleton et al., 2010]. The second auxin signalling model was developed by E. Farcot, C. Lavedrine and T. Vernoux and shall henceforth be referred to as the Farcot auxin signalling model [Farcot et al., 2015], both reviewed in detail below. These two models differ in a number of ways. Firstly, auxin in the Middleton *et al.* model is governed by an ODE, whereas in the Farcot auxin signalling model auxin is prescribed. Secondly, the Middleton *et al.* model has been shown to, for a portion of its state space, contain oscillatory solutions whilst the Farcot model has not been shown to contain such behaviour.

First, the Middleton *et al.* model is explicitly stated and some of its properties are outlined. After this, the effects of periodic inputs are investigated, first for purely sinusoidal inputs whose main parameters are readily controlled, and second for signals extracted from a single cell in the previously discussed spacial model (Chapter 3). For comparison, the Farcot auxin signalling model is stated and contrasted with the Middleton *et al.* model. For the Farcot auxin signalling model we proceed to investigate how the system responds to artificial and controlled auxin pulses. Finally, the same signals extracted from Chapter 3 are applied to the Farcot auxin signalling model, and the overall conclusions are summarised in the final section.

Throughout this chapter time-dependent simulations are conducted in Matlab utilising the stiff ode solver ode23s. This was chosen due to the stiffness imposed

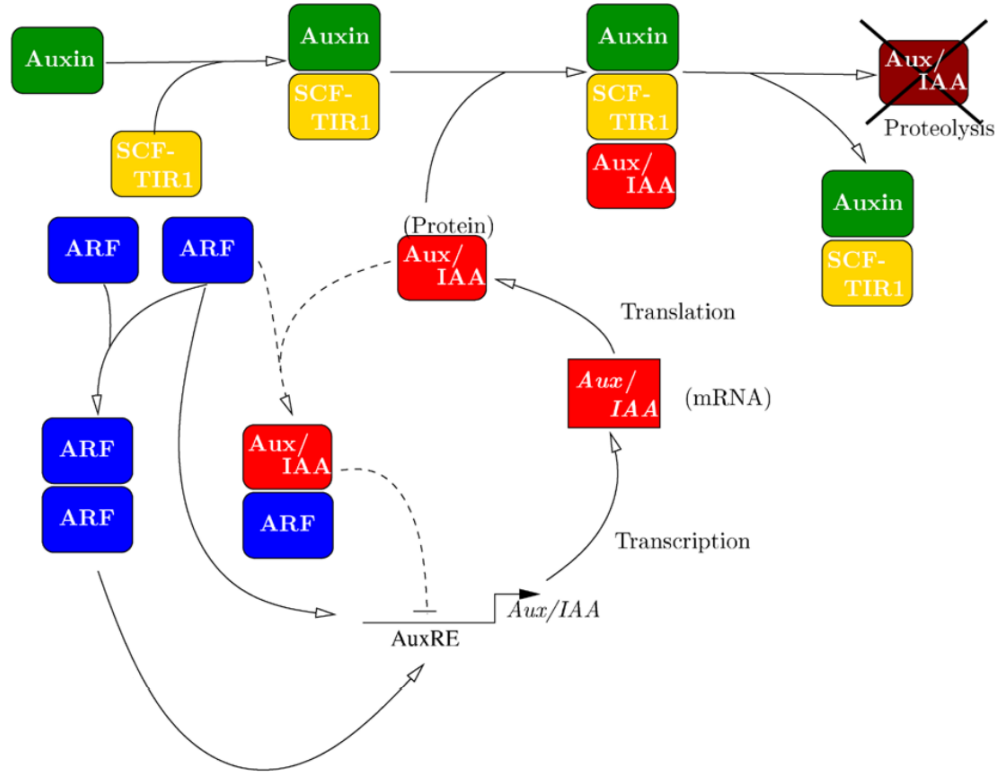


Figure 4.1: Figure modified from [Middleton et al., 2010]

by the different time scales inherent to the models used.

4.2 The Middleton *et al.* Model

The Middleton *et al.* model is an auxin signalling model. The model describes the interactions between an AUX/IAA gene with an AUXRe, a gene that transcribes AUX/IAA and is promoted by ARFs. In addition, the AUX/IAs suppress their own transcription creating a negative feedback loop. Furthermore, the model also contains dynamics for auxin which interacts with and leads to the decay of AUX/IAA proteins, disrupting the negative feedback loop. These dynamics allow for a parameter space containing a stable limit cycle.

The model [Middleton et al., 2010] represents the following interactions (see also Figure 4.1):

- The binding and unbinding of auxin and TIR1
- The binding of the auxin-TIR1 complex and an AUX/IAA protein

- The subsequent disassociation of the AUX/IAA protein, which is now tagged with ubiquitin and the auxin-TIR complex.
- The generation of auxin and its degradation
- The degradation of ubiquitin tagged AUX/IAA
- The forming of and disassociation of heterodimers of AUX/IAA and ARFs
- The forming and disassociation of homodimers of ARFs
- The generation and degradation of AUX/IAA mRNA
- The generation of AUX/IAA from AUX/IAA mRNA

The stable limit cycle utilised in this chapter requires AUX/IAA mRNA production and decay occurring on a $\mathcal{O}(1)$ time scale, AUX/IAA protein synthesis happening on a faster time scale of $\mathcal{O}(1/\epsilon)$ and ARF disassociation and association parameters, $q_a = q_d = \mathcal{O}(1)$. In the model, AUX/IAAs and ARFs compete to bind with ARFs, and when AUX/IAA levels are low more ARFs are free to form homodimers. This results in an increase in AUX/IAAs and a slow reduction in concentration of unbound ARFs and ARF homodimers, $\mathcal{O}(1)$. At this point there is $\mathcal{O}(1)$ AUX/IAA mRNA which results in an increase of AUX/IAA proteins and so it starts again.

The ODEs of the non-dimensionalised model are:

$$\begin{aligned}
\frac{dI_m}{dt} &= \phi (\lambda F_1(A, I_p, D_{AI}, D_{AA}) + F_2(A, I_p, D_{AI}, D_{AA})) - I_m \\
\epsilon \frac{dI_p}{dt} &= \delta I_m - l_a D_{a\pi_p} + l_d D_{a\pi_p} + \epsilon \eta_{D_{AIp}} (p_d D_{AI} - p_a I_p A) \\
\epsilon \frac{dT}{dt} &= -k_a T a + k_d D_{aT} \\
\epsilon \frac{dD_{aT}}{dt} &= k_a a T - k_d D_{aT} + (l_d + 1) D_{a\pi_p} - l_a D_{aT} I_p \\
\epsilon \frac{dD_{a\pi_p}}{dt} &= l_a I_p D_{aT} - (l_d + 1) D_{a\pi_p} \\
\epsilon \frac{dI^*}{dt} &= D_{a\pi_p} - \mu_{I^*} I^* \\
\frac{dA}{dt} &= -2q_a A^2 + 2q_d D_{AA} - p_a A I_p + p_d D_{AI}
\end{aligned}$$

$$\begin{aligned}
\frac{dD_{AI}}{dt} &= p_a A I_p - p_d D_{AI} \\
\frac{dD_{AA}}{dt} &= q_a A^2 - q_d D_{AA} \\
\epsilon \frac{da}{dt} &= \epsilon \mu_{\text{auxin}} (\alpha_{\text{auxin}} - a) - \eta_{D_{aT}} (k_a a T - k_d D_{aT})
\end{aligned}$$

where

$$F_1(A, I_p, D_{AI}, D_{AA}) =$$

$$\frac{\frac{A}{\theta_{ARF}}}{1 + \frac{A}{\theta_A} + \frac{D_{AA}}{\theta_{D_{AA}}} + \frac{D_{AI}}{\theta_{D_{AI}}} + \frac{A I_p}{\psi_{D_{AI}}} + \frac{A^2}{\psi_A}}$$

and

$$F_2(A, I_p, D_{AI}, D_{AA}) =$$

$$\frac{\frac{D_{AA}}{\theta_{D_{AA}}} + \frac{A^2}{\psi_A}}{1 + \frac{A}{\theta_A} + \frac{D_{AA}}{\theta_{D_{AA}}} + \frac{D_{AI}}{\theta_{D_{AI}}} + \frac{A I_p}{\psi_{D_{AI}}} + \frac{A^2}{\psi_A}}.$$

The parameters are given in Table 4.1.

Variable	Value	Description
δ	1	Dimensionless rate of AUX/IAA transcription
k_a	0.1	Rate of association of TIR1 and auxin
k_d	1	Rate of disassociation of TIR1 and auxin
l_a	10	Rate of association of AUX/IAA proteins and auxin-TIR1 dimers
l_d	1	Rate of disassociation of AUX/IAA proteins and auxin-TIR1 dimers
μ_{I^*}	1	Rate of decay of tagged AUX/IAA proteins
q_a	1	Rate of association of ARFs to form dimers
q_d	1	Rate of dissociation of ARF dimers
p_a	100	Rate of association of ARFs and AUX/IAA proteins
p_d	100	Rate of disassociation of ARFs and AUX/IAA proteins
$\eta_{D_{aT}}$	10	Representative total concentration of TIR1 divided by representative concentration of auxin
$\eta_{D_{AIp}}$	1	Representative total concentration of ARF divided by representative total concentration of TIR1
θ_A	0.1	Binding threshold for ARF
$\theta_{D_{AA}}$	0.01	Binding threshold for ARF dimers
$\theta_{D_{AIp}}$	0.1	Binding threshold for ARF-AUX/IAA dimers/
$\psi_{D_{AIp}}$	0.1	Binding threshold for an AUX/IAA to bind to an ARF bound to a promoter
ψ_A	0.1	Binding threshold for an ARF to bind to an ARF bound to a promoter
μ_a	10	Rate of auxin decay
ϕ	100	The effective rate of auxin transcription
λ	0.1	The rate at which ARF monomers cause AUX/IAA mRNA transcription
α_A	1	Total concentration of ARF divided by a representative total concentration of ARF
α_a	1	Total concentration of auxin divided by a representative total concentration of auxin
ϵ	0.01	Rate of decay of AUX/IAA mRNA divided by the rate of ubiquination of AUX/IAA protein

Table 4.1: Parameter values for the Middleton *et al.* model.

4.3 Adding Auxin Waves to Cellular-Level Auxin Signalling Models

4.3.1 Artificial Auxin Waves

The oscillatory attractors found in the Middleton *et al.* model are themselves separate to how auxin oscillations found in the root tip were replicated in Chapter 3. To begin investigating the interactions of the oscillations produced at the cellular level, with those at a tissue level, we added an oscillating source of auxin in the form of a sine wave:

$$\text{Auxin pulse term} = \frac{m}{2} \left(1 + \sin \left(\left(\frac{t}{p} + s \right) 2\pi \right) \right) \quad (4.1)$$

where m represents the peak value of the pulse, which is always positive. In addition, p and s were used respectively to adjust the periodicity of the auxin pulse, and the phase of the pulse, relative to the auxin oscillations present within the cell, where s was varied from zero to one in increments of 0.05.

From these simulations we then looked at two different quantities, ARF peaks and peaks in the rate of transcription. Note that ARF peaks are local maxima in the concentration of ARF proteins that are not bound by AUX/IAA proteins,

$$A + 2D_{AA},$$

and thus could activate a DR5 reporter gene, whereas peaks in the rate of transcription are local maxima in the rate of production of I_m ,

$$\phi(\lambda F_1 A, I_p, D_{AI}, D_{AA}) + \\ F_2 A, I_p, D_{AI}, D_{AA})).$$

This is a good analogue as the promoter for AUX/IAA in this model was assumed to behave the same as a DR5 reporter gene.

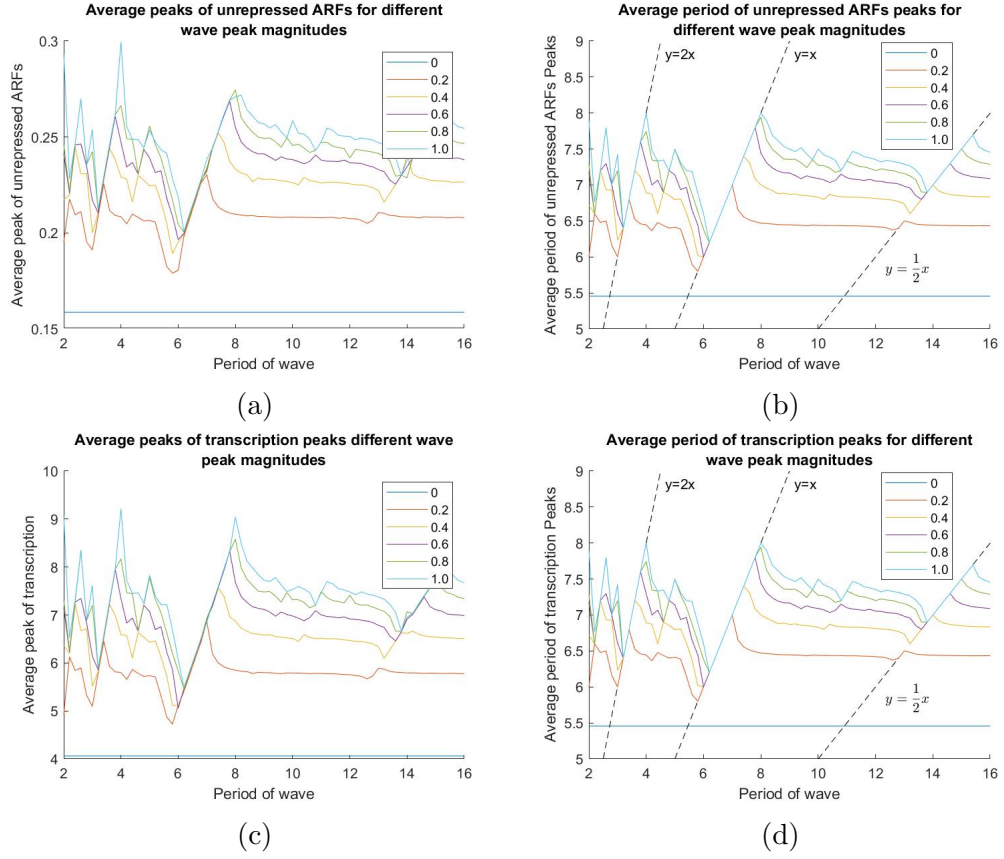


Figure 4.2: The graphs depict how the period of the sine wave (4.1) affects the average magnitude of the ARF peaks (a), the average period of the ARF peaks (b), the peaks of the rate of transcription (c) and the period of the peaks of the rate of transcription (d), respectively. Here peaks were defined as local maxima in the ARF concentration or rate of transcription. The average for each magnitude of auxin influx was found by running a simulation of the Middleton *et al.* model for 1000 time units with the described auxin pulse term (4.1) and taking the average time between local maxima and the average height of local maxima from time 500 to time 1000. Peaks were detected using the native Matlab function `findpeaks`.

Looking at Figure 4.2 it can be seen that the highest average ARF peaks occurred when the periodicity of the external auxin pulse was of a slightly higher periodicity than the stable limit cycle. Additionally, other local maxima of the average ARF peak occurred when the periodicity of the external auxin pulse was a multiple or a divisor of the periodicity of the system. Therefore, this shows that there is the potential for resonance between auxin pulses within a root and within the cell. Furthermore, Figure 4.2 also shows that for wave periodicities close to that of the internal auxin pulses, or very close to half that of the internal auxin pulses, prior to the addition of the auxin pulses the periodicity of the system conforms to that of the external auxin pulse.

Thus, looking at the results from the simulations using sine waves to represent auxin pulses, it appears that changing the magnitude of the auxin pulses affected both the period of the system and the magnitude of the average peaks. Therefore, to better see the relationship, a set of simulations for an auxin pulse were run in the form:

$$\text{Auxin pulse term} = \mathbf{m} \quad (4.2)$$

where, between simulations, $\mathbf{m}$ was varied from zero to two in increments of 0.1 then from two to ten in increments of 0.5. In addition, the size of increment was altered for greater resolution at low magnitude where the rate of change of peak magnitude and peak period was higher. Note that each simulation was ran for 10,000 time units, and the peaks were identified using the Matlab function `find-peaks`. The average peak and average period were then calculated by averaging the height of peaks, and the time between peaks, for peaks after the first 500 time units. This delay was chosen to allow the system to reach equilibrium. Figure 4.3 shows that both peak and period increase in a similar manner. Both the magnitude and peak tend to be limited due to there being a fixed concentration of ARF in the system, i.e.

$$\frac{dA}{dt} + \frac{dD_{AIp}}{dt} + 2\frac{dD_{AA}}{dt} \equiv 0.$$

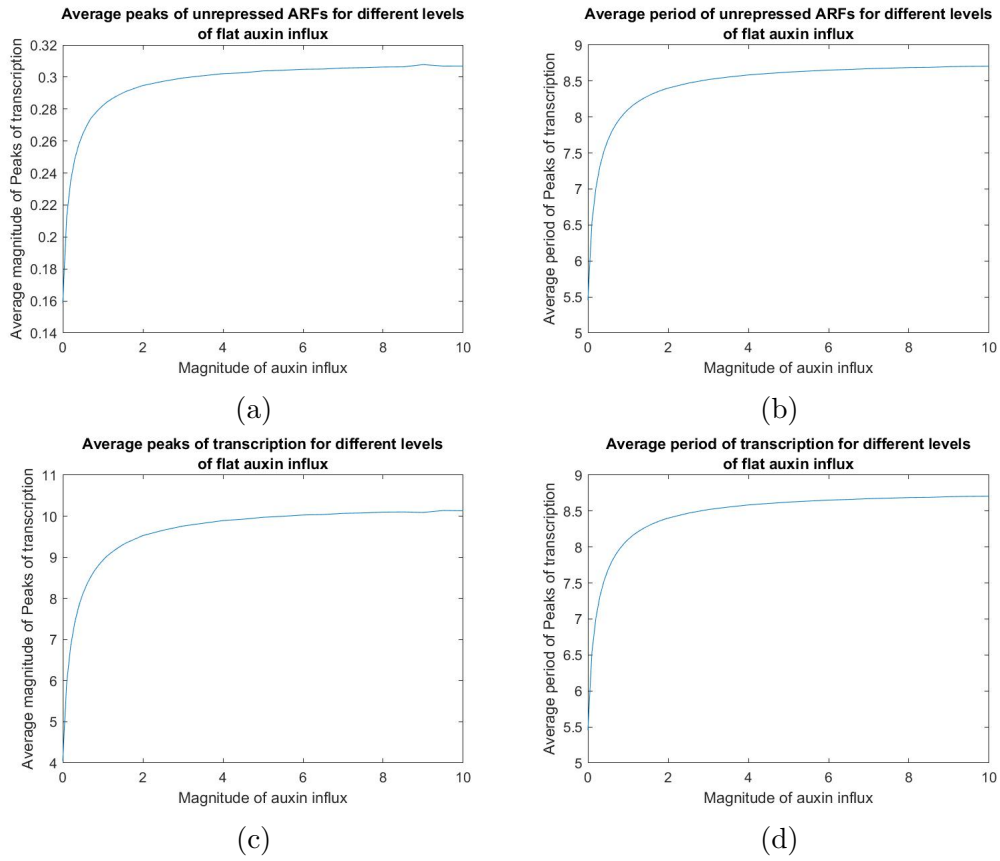


Figure 4.3: The graphs depict how a constant influx of different magnitudes affects the average magnitude of the ARF peaks (a), the transcription rate peaks (c) and the average period of the ARF peaks (b), transcription rate peaks (d) respectively.

4.3.2 Experimentally Derived Auxin Waves

Next, we look at a more realistic auxin pulse, and to this end we modelled the auxin pulse to resemble an auxin peak observed in the root tissue model from Chapter 3. In this model, the observed auxin peaks were localised to specific points within the root relative to the root tip. As such, most cells experienced one auxin peak instead of many as was modelled above using the sin wave. In this chapter we differentiate auxin peak and auxin pulse, auxin peak will refer to the peak of auxin observed in a transport model, whereas auxin pulse will refer to the influx of auxin into the auxin signalling model.

Additionally, for the purpose of reproducing the wave form found in Chapter 3 for $T=2.0$ we looked at auxin levels within the cells at the centre of the auxin peak found in simulations in Chapter 3. This was done by recording the auxin

concentration within a cell at the center of the auxin peak for the duration of one cell cycle, i.e. the time between cell divisions. The profile of auxin peaks with respect to time generated by the model in Chapter 3 differ qualitatively for different rates of cell division. Therefore, in order to account for this variation, the auxin concentration of a cell within the peak was recorded for the duration of one cell cycle for four rates of cell division each differing by a factor of ten. These auxin peaks were then normalised so that the peak magnitude was one and the magnitude of average auxin concentration of cells in the root equates to zero. These normalised peaks (Figure 4.4a) were then used as the auxin pulse added in the following simulations. Simulations were run for auxin pulses representative of the auxin peaks seen for the four rates.

The effects of each of the different auxin pulses was recorded for a specific auxin peak to study how the auxin pulses affected the concentration of ARFs. The auxin pulses were applied at different starting points relative to an auxin peak within the oscillatory system, the magnitude of said peak was then measured. One can see from comparing the profile of the auxin peaks in Figure 4.4.a how the timing affects the magnitude of the ARF peak (Figure 4.4.b) that the former appears to be qualitatively similar to the latter but in reverse. This may suggest that the time within the oscillations where the system is auxin sensitive may be small. This is supported by the fact that while the magnitudes of the ARF peaks are affected their profile remains the same.

$$\text{Auxin pulse term} = \begin{cases} P(t - s) & \text{if } s < t < s + p \\ 0 & \text{otherwise} \end{cases} \quad (4.3)$$

where $P(t)$ is the magnitude of a peak at time t after its start.

Furthermore, the degree to which different concentrations in the model were sensitive to auxin, during one period of the oscillation, were studied. This was done to confirm my postulation that the system is highly sensitive to auxin for a short time close to the local minimum of ARF concentration, but that otherwise

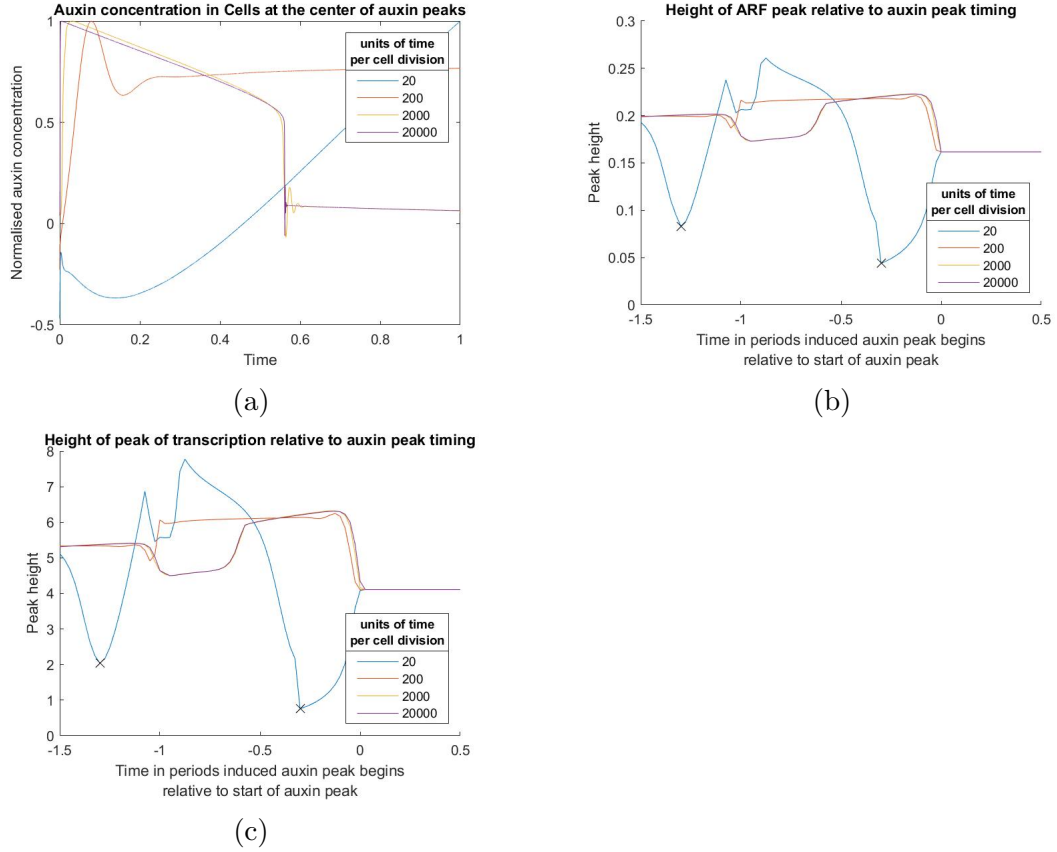


Figure 4.4: (a) Depicts the profile of auxin peaks for differing rates of cell division. Here time on the x axis represents time between cell divisions within the time-dependent simulation the peak is derived from. The time mentioned in the legend references the rate of growth in the time-dependent simulation the auxin peak is derived from. (b-c) Depicts how the timing of the auxin peak affects the ARF peak (b) or the peak rate of transcription (c). Here the duration of the auxin peak is set to that of the periodicity of the oscillations present in the model. In addition the time on the x axis represents the time in periods of oscillation the auxin peak begins relative to the forth local maxima of the concentration or rate measured. In (b) the ARF peak represented is the fourth local maxima of total ARFs not bound by IAA. In (c) the transcription rate peak is the fourth local maxima of the transcription rate of IAA. In (b-c) the x's denote where there are discontinuities due to the auxin pulse generating a new peak.

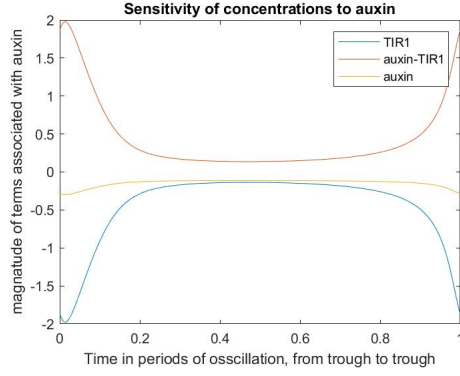


Figure 4.5: Shows the auxin sensitivity of different state variables during a single oscillation from one ARF minimum to the next.

it has a low auxin sensitivity (see Figure 4.5 for the second derivative of the state variables with respect to time and auxin). Whilst this does not take into account any feedback loops, it is clear that the system is far more auxin-sensitive for time close to that of a local minimum of the ARF population. This is understandable as the local minimum of the ARF concentration coincides with an increase in the AUX/IAA population that auxin interacts with.

Finally, we looked at how the system reacted to the Chapter 3 auxin pulse applied repeatedly, to mimic a cell at the centre of the auxin peaks caused by auxin transport. As such, the auxin pulse modelled will be of the form:

$$\text{Auxin pulse term} = P(t - s \pmod{p}). \quad (4.4)$$

Similarly to the sine wave auxin pulse, the highest average peaks in unrepressed ARF concentration and rate of transcription occurred for periods that were comparable to one, half or two times the natural frequency of the system (see Figure 4.6). We also saw once more that for periods of auxin pulse that are near the natural frequency, the frequency of peaks for rate of transcription or unrepressed ARFs conforms to that of the auxin.

4.4 Farcot Auxin Signalling Model

The Farcot model [Farcot et al., 2015] focuses on the effects of dimerisation of AUX/IAA and ARFs on auxin signalling (see Figure 4.7). As in the Mid-

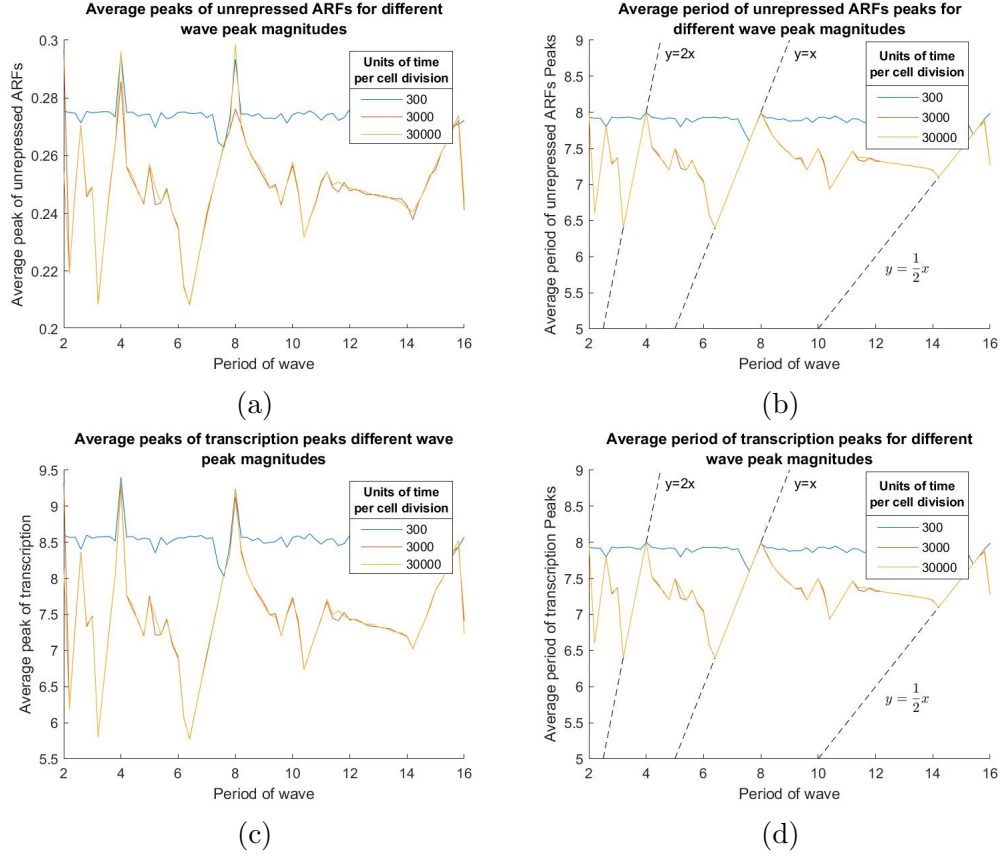


Figure 4.6: The graphs depict how the period of the auxin pulses, designed to mimic those seen in Chapter 3, affect the average magnitude and period of ARF peaks, (a) and (b) respectively, and the average magnitude and period of the rate of transcription, (c) and (d) respectively. The average for each magnitude of auxin influx was found by running a simulation of the Middleton *et al.* model for 1000 time units with the described auxin pulse term (4.4), each period as depicted in Figure 4.4a, and taking the average time between local maxima and the average height of local maxima from time 500 to time 1000. Peaks were detected using the native matlab function findpeaks.

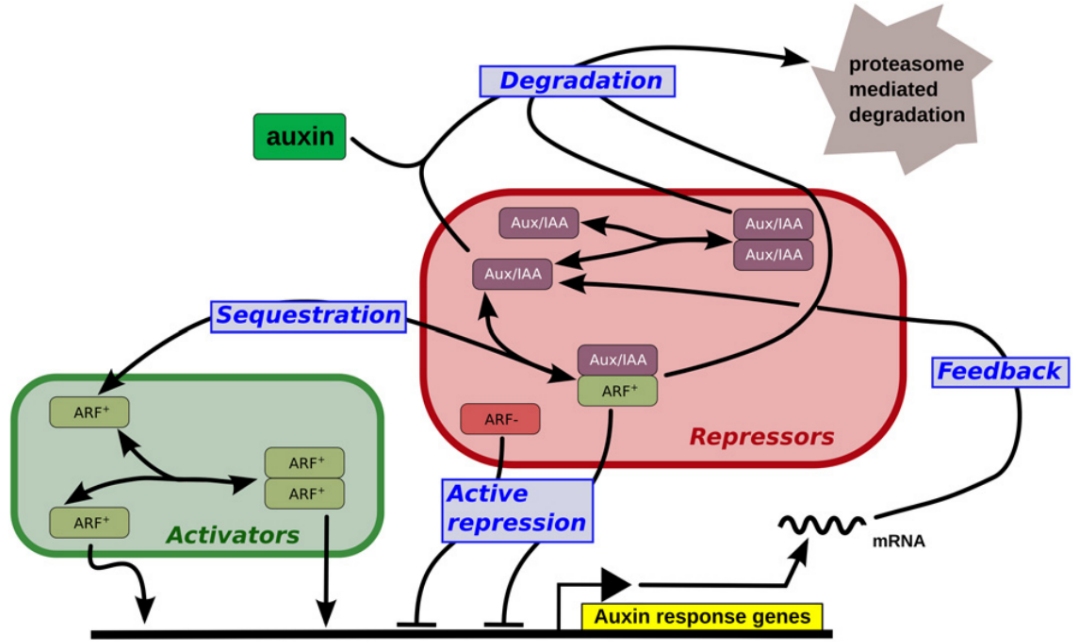


Figure 4.7: Figure showing the interactions of the state variables in the Farcot auxin signalling model, modified from [Farcot et al., 2015]

deletion *et al.* model, ARF dimers and monomers promote the transcription of AUX/IAA mRNA creating a negative feedback loop where the AUX/IAA suppresses its own transcription.

However, the Farcot auxin signalling model differs from the Middleton *et al.* model in that the auxin concentration is prescribed rather than simulated. The Farcot auxin signalling model does, however, contain another dimer, Aux/IAA:Aux/IAA. Furthermore, the Farcot auxin signalling model models transcription by representing the proportion of each possible reporter binding instead of modelling them problematically like in the Middleton *et al.* model. This results in there being a greater delay between the ratios of the protein concentrations changing and a change in transcription rates.

The ODEs of the model are:

$$\begin{aligned} \frac{dI}{dt} &= \pi_I R + 2\theta_{II} D_{II} - 2\alpha_{II} I^2 + \theta_{AI} D_{AI} - \alpha_{AI} IA + \theta_{G_{AI}} G_{AI} - \alpha_{G_{AI}} IG_A \\ &\quad + \delta_{II} D_{II} \kappa_x x - \delta_I (1 + \kappa_x x) I \\ \frac{dA}{dt} &= \pi_A + 2\theta_{AA} D_{AA} - 2\alpha_{AA} A^2 + \theta_{AI} D_{AI} - \alpha_{AI} IA + \theta_{AG} G_A - \alpha_{AG} AG \end{aligned}$$

$$\begin{aligned}
& +\theta_{AG_A} G_{AA} - \alpha_{AG_A} A G_A + \delta_{AI} D_{AI} \kappa_x x - \delta_A A \\
\frac{dD_{II}}{dt} &= \alpha_{II} I^2 - \theta_{II} D_{II} - \delta_{II} (1 + \kappa_x x) D_{II} \\
\frac{dD_{AI}}{dt} &= \alpha_{AI} I A - \theta_{AI} D_{AI} + \theta_{D_{AI}G} G_{IA} - \alpha_{D_{AI}G} G D_{AI} - \delta_{AI} (1 + \kappa_x x) D_{AI} \\
\frac{dD_{AA}}{dt} &= \alpha_{AA} A^2 - \theta_{AA} D_{AA} + \theta_{D_{AA}G} G_{AA} - \alpha_{D_{AA}G} G D_{AA} - \delta_{AA} D_{AA} \\
\frac{dG}{dt} &= \theta_{AG} G_A - \alpha_{AG} A G + \theta_{D_{AI}G} G_{AI} - \alpha_{D_{AI}G} G D_{AI} - \theta_{D_{AA}G} G_{AA} \\
& - \alpha_{D_{AA}G} G D_{AA} \\
\frac{dG_A}{dt} &= \alpha_{AG} A G - \theta_{AG} G_A + \theta_{G_{AI}} G_{AI} - \alpha_{G_{AI}} I G_A + \theta_{AG_A} G_{AA} - \alpha_{AG_A} A G_A \\
\frac{dG_{AA}}{dt} &= \alpha_{AG_A} A G_A - \theta_{AG_A} G_A A + \alpha_{D_{AA}G} G D_{AA} - \theta_{D_{AA}G} G_{AA} \\
\frac{dG_{AI}}{dt} &= \alpha_{G_{AI}} I G_A - \theta_{G_{AI}} G_{AI} + \alpha_{D_{AI}G} G D_{AI} - \theta_{D_{AI}G} G_{AI} \\
\frac{dR}{dt} &= hG + h_A G_A + h_{AA} G_{AA} + h_{AI} G_{AI} - \delta_R R
\end{aligned}$$

where

- I denotes the concentration of AUX/IAA
- A denotes the concentration of ARF
- D_{XY} , $X, Y \in A, I$ denotes the concentrations of an XY dimer
- G , G_A , G_{AA} and G_{AI} denote the probabilities the promoter is free, bound with ARF, an ARF:ARF dimer or an ARF:AUX/IAA dimer. $G + G_A + G_{AA} + G_{AI} = 1$
- x denotes the concentration of auxin. In the original model x is assumed to be constant whereas we will be altering it.

The parameters are given in Table 4.2.

Variable	Value	Description
α_{II}	α	Rate of association of AUX/IAA with itself to form an AUX/IAA:AUX/IAA dimer
α_{AI}	α	Rate of association of AUX/IAA with ARF to form an ARF:AUX/IAA dimer
α_{AA}	α	Rate of association of ARF with itself to form an ARF:ARF dimer
$\alpha_{G_{AI}}$	α	Rate of association of AUX/IAA with an ARF bound to the promoter
α_{AG}	α	Rate of association of an ARF and the promoter
α_{AG_A}	α	Rate of association of an ARF with an ARF bound to the promoter
$\alpha_{D_{AIG}}$	α	Rate of association of an ARF:AUX/IAA dimer and the promoter
$\alpha_{D_{AAG}}$	α	Rate of association of ARF:ARF dimer and the promoter
θ_{II}	θ	Rate of disassociation of an AUX/IAA:AUX/IAA dimer
θ_{AI}	θ	Rate of disassociation of an ARF:AUX/IAA dimer
θ_{AA}	θ	Rate of disassociation of an ARF:ARF dimer
$\theta_{G_{AI}}$	θ	Rate of disassociation of an AUX/IAA with an ARF bound to the promoter
θ_{AG}	θ	Rate of disassociation of an ARF and the promoter
θ_{AG_A}	θ	Rate of disassociation of an ARF with an ARF bound to the promoter
$\theta_{D_{AIG}}$	θ	Rate of disassociation of ARF:AUX/IAA dimer and the promoter
$\theta_{D_{AAG}}$	θ	Rate of disassociation of ARF:ARF dimer and the promoter
δ_I	0.05	Rate of decay of AUX/IAA
δ_A	0.003	Rate of decay of ARF
δ_{AA}	0.003	Rate of decay of ARF:ARF dimers
δ_{II}	0.003	Rate of decay of AUX/IAA:AUX/IAA dimers
δ_R	0.007	Rate of decay for AUX/IAA mRNA
π_I	10	Rate of production of AUX/IAA
π_A	1	Rate of production of ARF
h	1	Rate of transcription for an unbound promoter
h_A	10	Rate of transcription for the promoter bound to an ARF
h_{AA}	10	Rate of transcription for the promoter bound to an ARF:ARF dimer
h_{AI}	0	Rate of transcription for the promoter bound to an ARF:AUX/IAA dimer
κ_x	10	Rate of auxin induced degradation

Table 4.2: Default parameters used for the Farcot *et al.*

4.4.1 Non-Oscillatory Waves

As a comparison to the behaviours seen in the auxin signalling model developed by Middleton *et al.*, we studied the Farcot auxin signalling model. By comparison, the Farcot auxin signalling model was more concerned with the effects of dimerisation, whereas the Middleton *et al.* model had a greater focus on modelling auxin degradation.

To understand how auxin levels translated into the concentration of ARF and ARF:ARF dimers, we tested how the system reacted to an increase in ARF levels represented by a rectangular function. This was done instead of considering sinusoidal curves, due to the lack of stable limit cycle to investigate resonance with, and we set:

$$\chi(t) = \begin{cases} 1 + z & \text{if } s < t < s + d \\ 1 & \text{otherwise} \end{cases} \quad (4.5)$$

where $d = 900$ minutes as this is the duration of the auxin pulses observed in [De Smet et al., 2007]. We set $s = 1500$ to make sure that the system was at a steady state, and the other variables were as described in Table 4.2.

From these simulations we looked at two different quantities, ARF peaks and peaks in the rate of transcription. Note that ARF peaks are local maxima in the concentration of ARF proteins that are not bound by AUX/IAA proteins,

$$A + 2D_{AA} + G_A + 2G_{AA}$$

and thus could activate a DR5 reporter gene, whereas peaks in the rate of transcription are local maxima in the rate of production of R ,

$$hG + h_A G_A + h_{AA} G_{AA} + h_{AI} G_{AI}$$

This is a good analogue as the promoter for AUX/IAA in this model was assumed to behave the same as a DR5 reporter gene.

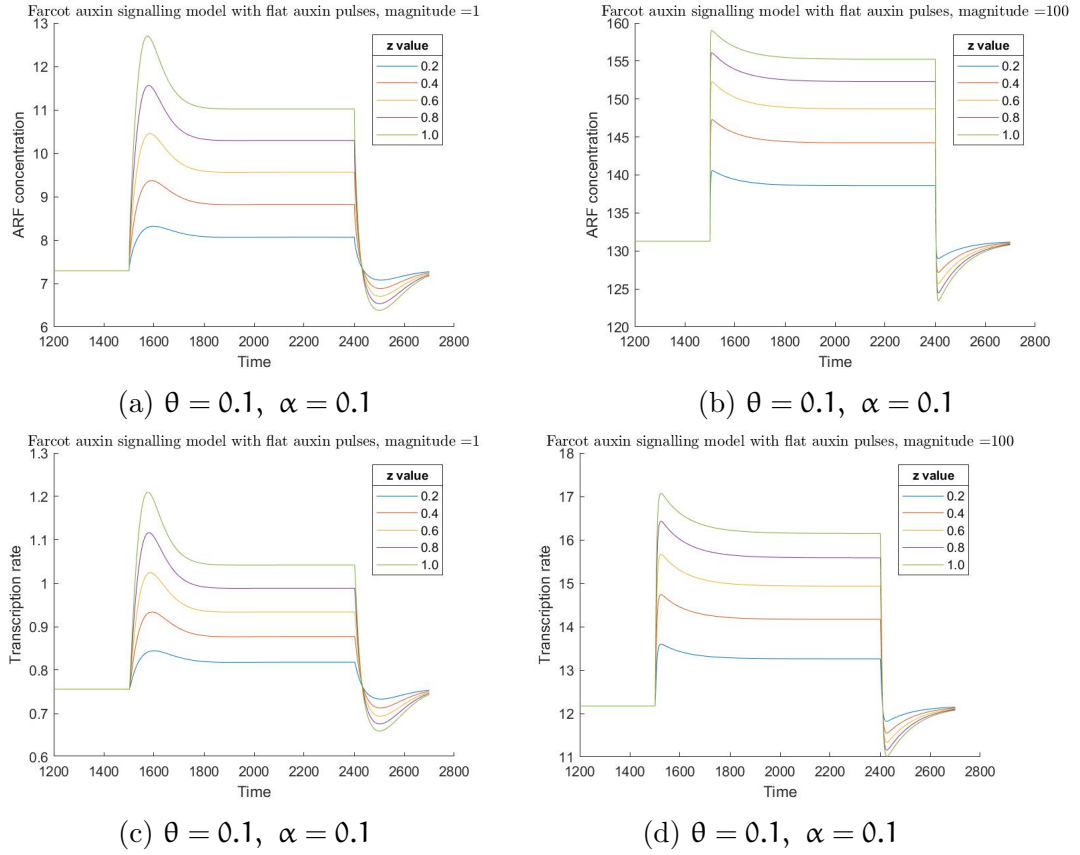


Figure 4.8: This shows how the unbound ARF concentrations and ARF induced transcription for Farcot auxin signalling model reacts to a constant auxin influx for 900 time units. Graph (a) and (c) shows for an initial auxin concentration of one auxin impulses of 0.2, 0.4, 0.6, 0.8 and 1. (b) and (d) shows for an initial auxin concentration of 100 auxin impulses of 20, 40, 60, 80 and 100.

For auxin concentration of $\mathcal{O}(1)$ linear increases in auxin concentration had an approximately linear increase in the stable steady state of the ARF concentration and transcription rate (Figure 4.8.a,c). For auxin concentration of $\mathcal{O}(100)$ or greater increasing auxin concentration showed diminishing returns in terms of peak ARF concentration and transcription rate (Figure 4.8.b,d). This was due to the total number of ARF molecules and promoter sites being finite. Additionally, one may also observe that after a sudden influx of auxin there was an ARF peak produced which was of a greater ARF concentration than the concentration of the new steady state due to the delay time of the negative feedback loop. The same was observed in the transcription rate.

To better understand how the ARF peak generated by a sharp increase in auxin was affected by the rate of increase of the auxin, auxin pulses that reached

the new auxin level at different rates were simulated. To do this auxin concentration, with respect to time, was set thusly:

$$x(t) = \begin{cases} 1 + 1(t - s)/\beta & \text{if } s < t < s + \beta \\ 2 & \text{if } s + \beta < t \\ 1 & \text{otherwise} \end{cases} \quad (4.6)$$

where $\beta = 50n$, $n = 0, 1, 2, \dots, 20$, the time it takes for the auxin concentration to double. Here we set s to zero and the parameters for the Farcot model were as stated in Table 4.2

The results showed that the magnitudes of the peaks were limited and that they were significantly more prominent for faster rates of auxin increase (Figure 4.9). If we compare this to higher auxin concentrations, for example auxin increases from 10 to 20 instead of from one to two, we see that the additional auxin caused the peak to be reached sooner but also decay faster. This would appear to be caused by the feedback loop being faster due to auxin speeding up AUX/IAA decay, and whilst this frees up ARF proteins faster it also results in faster AUX/IAA transcription.

4.4.2 Non-Oscillatory Waves with Chapter 3 Waves

To investigate how the Farcot auxin signalling model interacts with the auxin transport model in Chapter 3 the auxin pulses derived in Chapter 3 were integrated into the model. From this we hoped to derive testable predictions and a point of comparison with the Middleton *et al.* model. In the Farcot auxin signalling model auxin concentration was prescribed, as such x , the concentration of auxin, was replaced with $x(t)$. Here $x(t)$

$$x(t) = \begin{cases} mP(t - s) & \text{if } s < t < s + d \\ m & \text{otherwise} \end{cases} \quad (4.7)$$

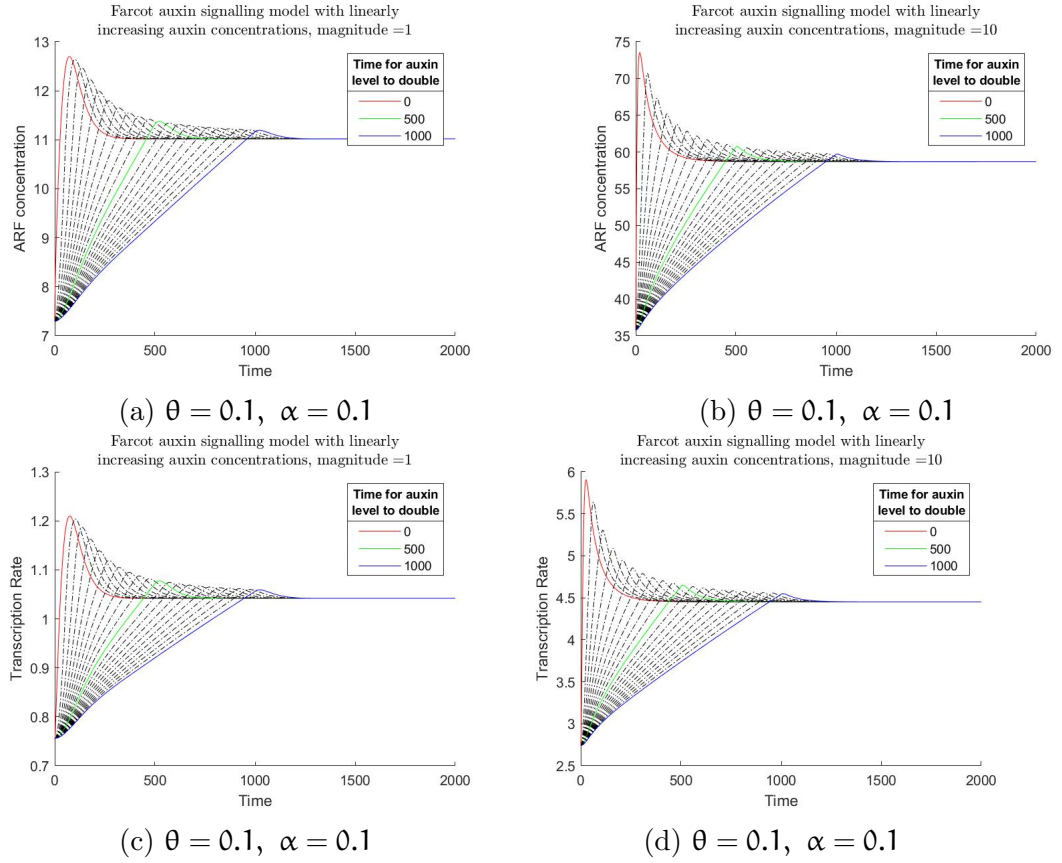


Figure 4.9: This shows how the Farcot auxin signalling model reacts to an auxin influx which linearly increases in magnitude to a set level. (a) and (c) shows for an initial auxin concentration of one the auxin concentration linearly increasing to two. (b) and (d) shows for an initial auxin concentration of two the auxin concentration linearly increasing to 20.

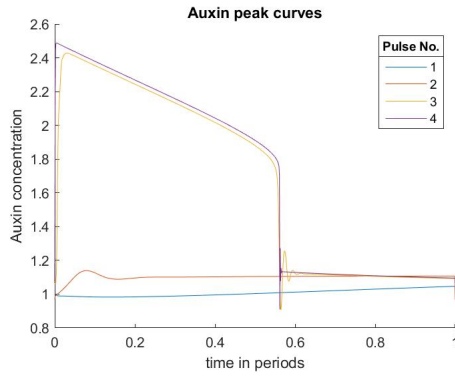


Figure 4.10: Graph showing the auxin concentration of peaks from Chapter 3 normalised such that the average auxin concentration in the rest of the root at the time of the peak is one. Pulse 1 denotes the auxin pulse from chapter 3 where cells divide once ever 20 time units, pulse 2, division very 200 units, pulse 3 division every 2000 units and pulse 4, division every 20,000 units. The units in the x axis represent time in terms of the periods of individual pulses, ie the time between cell divisions.

where $P(t)$ is the magnitude of an auxin pulse at time t after its start, with the pulse lasting d time units. A graphical representation of these peaks is shown in Figure 4.10. However, due to the rates of association and disassociation not being known, the model was ran for numerous combinations of association and disassociation rates, and the d was set to 900 minutes as in the above model.

The auxin pulses are generated from the same auxin pulses in section 4.3.2 however they have been normalised differently as they are being used as auxin concentration rather than rate of change of auxin concentration. In this section the pulses have been normalised by dividing by the average concentration of auxin in the root.

The results shown in Figure A.9 show three types of qualitative behaviour. The first is for θ large and α small (Figure 4.11a and 4.11b), here the profile of ARFs and ARF:ARF dimers over time are very similar to that of the auxin pulse. When θ is large and α small the concentrations of the various dimers were low, and this resulted in a higher quantity of AUX/IAA monomers which have the highest rate of decay due to auxin, over 16 times the other rates of decay for AUX/IAA in oligomers containing AUX/IAA. Furthermore, the low rate of association, and high rate of disassociation, disrupted the negative feedback loop by reducing the rate at which ARFs were binding to the promoter suppressing the transcription of AUX/IAA mRNA.

The second is for θ small (Figure 4.11c and 4.11d), here the low dissociation coefficient led to a high concentration of dimers. The majority of the ARFs were bound in AUX/IAA:ARF dimers, and the affect of auxin on the decay of AUX/IAA bound to ARF was lower, which led to a lower rate of increase in ARF and ARF:ARF dimer concentration. As a result, the ARF concentration rose much slower in response to the auxin pulse.

The third qualitative behaviour was that of the remaining combinations of α and θ (Figure 4.11e and 4.11f). Here the auxin pulse initially effected a sharp reduction in AUX/IAA and AUXIAA:AUXIAA dimers which in turn, via the negative feedback loop, caused a rise in mRNA bringing the ARF population towards an equilibrium lower than that of the initial peak.

Next we applied an oscillating auxin pulse to the model in the form:

$$x(t) = mP(t \pmod{d}) \quad (4.8)$$

to simulate a cell exposed to the oscillating auxin concentration caused by auxin transport as in the previous chapter. Here we saw two things. As shown in Figure 4.12a and 4.12b, if the period of the auxin concentration was too small relative to the speed of the system, the height of the auxin peaks were going to be less than they would be for a faster period. Secondly, as show in Figure 4.12c and 4.12d we see once more that after an increase in auxin concentration the rate of transcription and the concentration of unbound ARFs temporarily exceeded the new steady state. In combination we saw that this suggested that, for a sufficiently large periodicity and suitable parameters, an oscillatory auxin concentration would result in higher spikes in transmission than the system would otherwise produce for constant auxin input.

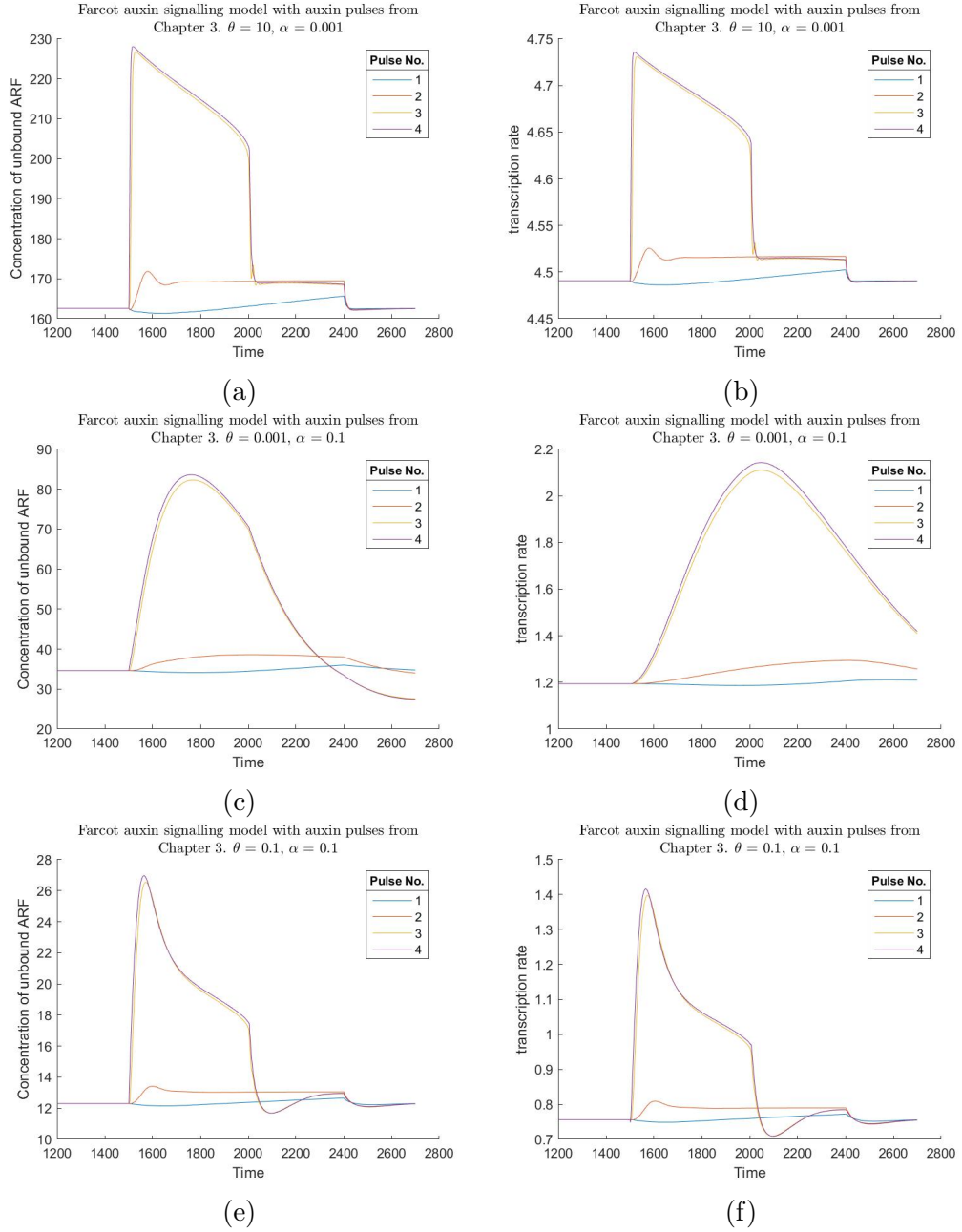


Figure 4.11: Graphs showing the affect of a peak of auxin in the form shown in Chapter 3 on the Farcot auxin signalling model. These are a subset of the graphs shown in Figure A.9. The auxin pulse used is as described in equation (4.7), $s = 1500$, $d = 900$, $m = 1$. Pulse 1 denotes the auxin pulse from chapter 3 where cells divide once ever 20 time units, pulse 2, division very 200 units, pulse 3 division every 2000 units and pulse 4, division every 20,000 units.

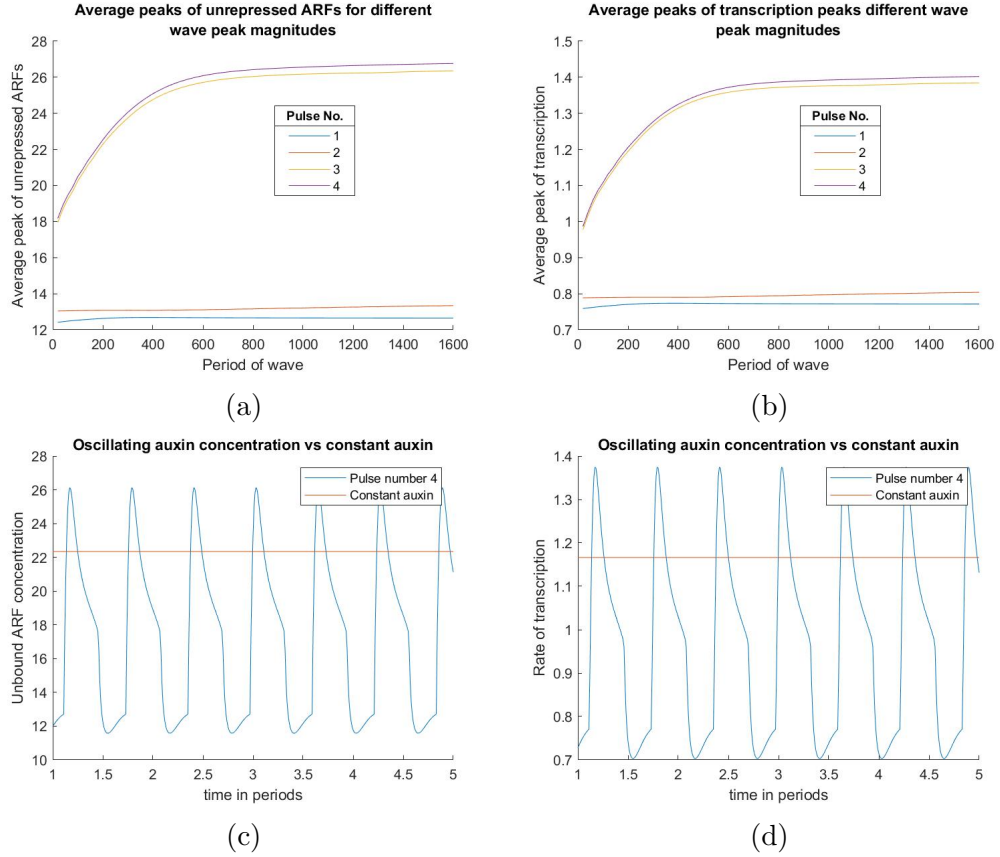


Figure 4.12: Graphs showing the affect of oscillating auxin in the form shown in Chapter 3 on the Farcot auxin signalling model. (a) and (b) are graphs showing how the peaks of auxin transcription and unbound ARF concentrations are affected by different periodicities of the auxin oscillations. (c) and (d) show graphs of how the unbound ARF concentrations or transcription rates is affected by an oscillatory auxin concentration vs a constant auxin concentration. The constant auxin concentration is equal to the maximum auxin concentration of oscillatory auxin pulse. The period of the auxin input is 800.

4.5 Conclusion

In this chapter we looked at how changes in auxin concentration affected auxin signalling by looking at two models, the Middleton *et al.* model and the Farcot auxin signalling model. The addition of auxin was done with a simple constant, or linearly increasing, influx and in a form meant to represent the auxin oscillations seen in the previous chapter.

In the case of the Middleton *et al.* model, within the parameter space required for oscillatory steady states the concentration of unbound ARFs was not directly related to the concentration of auxin for any given time. This is due to the short window of auxin sensitivity during the cycle, the ARF peaks are representative of a short portion of the auxin curve. Furthermore, when auxin pulses derived from Chapter 3 were implemented into the model they affected quantitative behaviour but not the qualitative behaviour of the ARF peaks. In contrast, in the Farcot auxin signalling model, the ARF peak was more representative of the auxin pulse with the degree similarity dependent on the unknown variables determining rates of association and disassociation.

Additionally, the degree to which the perception of auxin levels may be amplified varies between the two models. In the case of the Middleton *et al.* model, much of this was governed by the resonance between the auxin pulses and the auxin signalling pathway. An example of this is shown in Figure 4.4 which shows the maximum auxin peak being 50% greater than the minimum auxin peak when comparing the effects of resonance. It should be noted that the resonance like behaviour observed was interesting and could have strong implications for the existing data on oscillations and how it is interpreted. This is because, should resonance be a factor in auxin signalling, it would mean that a fine coordination of the two rhythms is required for high magnitude auxin (or DR5 as observed [De Smet et al., 2007, Moreno-Risueno et al., 2010b]) peaks to show up at the right time. Since these auxin peaks appeared to coordinate phyllotaxis, that patterning of plant organs, it would indicate that the whole root architecture may

depend on this resonance.

In the case of the Farcot auxin signalling model, the relationship between auxin levels and ARF levels was approximately linear for low auxin concentration. For higher auxin concentrations auxin sensitivity diminished due to total concentration ARFs and promoter sites being finite. More interesting was that if the auxin concentration increased at a sufficiently fast rate it caused a high amplitude oscillation as the ARF concentration tended to the steady state. In addition, the time it took for the system to return to equilibrium was affected by the background auxin in the system, with it doing so faster for higher levels of auxin concentration. Due to this behaviour the system permits a higher peak in both unbound ARF concentration and rate of transcription when auxin oscillates as compared to constant auxin concentration, assuming the rate of the auxin oscillation is sufficiently low.

Thus, both of the models analysed have aspects that the other model is lacking. Therefore, we propose that one future course of action could be creating a hybrid model which combines the best aspects of both models. Due to the nature of what we are investigating, and the active role that auxin plays within it, we would suggest including the AUX/IAA degradation pathway using TIR1 and auxin as state variables. Another aspect of one of the models that we would want to retain would be the inclusion of individual promoter protein pairs being modelled, as it adds more delay to the system of equations in a way we believe to be more physically realistic. It should also be noted that this model has not been explicitly stated here in this chapter as it was my intention to develop this ‘hybrid model’ in conjunction with *Marchantia* data currently being generated by Dolf Weijers in Wageningen.

Chapter 5

Marchantia model

5.1 Introduction

In this chapter the initial goal was to create an auxin signalling model for *Marchantia*. This is in part motivated by a set of data provided by colleagues from Wageningen (Dolf Weijers's lab) covering auxin signalling protein concentrations in *Marchantia*. To do so involved altering the models from Chapter 3 so as to better represent *Marchantia* and to fit the model to data so as to allow for both qualitative and quantitative results. The importance of *Marchantia* is that (as discussed in Section 2.6) it is a very minimal system consisting of only one AUX/IAA and three ARFs, hereafter referred to as mpIAA and mpARFs [Kato et al., 2015]. As such the minimal auxin signalling system of *Marchantia* is similar to simplified auxin signalling models. Due to the data available from Wageningen being specific to gemmae, asexually produced spores, the focus switched to creating a model of mpARFS and transcription in gemmae within the first hours of their removal from the gemma cup. This differs from the first type of model due to the lack of impact of auxin on the system at this time due to negligible concentrations of mpIAA.

In this chapter we started the process of combining two existing auxin signalling models with the intent to create a model for auxin signalling in *Marchantia*. The models are those used in the previous chapter, Middleton *et al.* model

[Middleton et al., 2010] and the Farcot auxin signalling model [Vernoux et al., 2011a]. We then describe the fitting of the experimental data from Wageningen to an altered version of the Farcot auxin signalling model and the shortcomings of the model revealed by doing so. This and the acquiring of new information led to the creation of a revised model, simplified and adapted to the specific data we had.

For this chapter time-dependent simulations are run via Python3.8 utilising primarily SciPy and NumPy packages. Specifically using the ODE solver `scipy.integrate.odeint` and the optimisation function `scipy.optimize.minimize` both of which choose their specific algorithms based on the functions and limits imposed. The switch was made from MATLAB to Python in this chapter due to familiarity with `scipy.optimize.minimize`.

5.2 Comparison of Middleton vs Farcot Models

As in the previous chapter the two existing models that we are considering are the Farcot auxin signalling model and the Middleton *et al.* model. These two models focus on two different aspects of the auxin signalling pathway. Most noticeable of which is the focus the Middleton *et al.* model has on IAA degradation by TIR1. This is clearly a relevant aspect to add to our model due to their being data on the concentrations of mpARF1, mpARF2 and TIR1 in *Marchantia gemmae*. The Middleton *et al.* model also employs a variable ϵ that represents the difference in time scale of IAA degradation and ARF dynamics.

The benefits of these aspects are that the TIR1 pathway allows for better utilisation of data, seeing as we have access to time series data of TIR1 concentrations for the same conditions as data on ARF1 and ARF2 concentrations. The second reason why the TIR1 pathway is important is that whilst the antagonisation of ARF1 by ARF2 is likely an important factor in transcription, studies have shown that as time progresses cells in the centre of gemmae show a very low concentration of ARF2, from which one might infer that IAA antagonisation

would become the dominant form of antagonisation and thus is relevant [Kato et al., 2020a].

In contrast the Farcot auxin signalling model devotes more focus to the binding of promoters. In the Middleton *et al.* model the hypothetical transcription caused by the ARFs is handled by a Hill equation whereas the Farcot auxin signalling model uses state variables to represent each possible combination of an ARF oligomer binding the promoter. The Farcot auxin signalling model also allows for the possibility of IAA-IAA dimers.

The benefit of the Farcot auxin signalling models handling of transcription is that by modelling the binding of the promoter as state variables, rather than as a Hill function, there is a delay imposed upon the change of the rate of transcription with respect to changes in ARF concentrations, instead of it being instantaneous.

Outside of these differences the models are largely similar, both have a feedback loop between transcription and IAA production and model interactions between molecules using the law of mass action.

Initially I intended to set-up a model based on the Middleton *et al.* model due to its simplicity and its inclusion of the TIR1 pathway, and contrast that model against another model that is identical, but for the modelling transcription, in the manner seen in the Farcot auxin signalling model.

5.2.1 Middleton *et al.* Model

The ODEs of the non-dimensionalised model are:

$$\frac{dI_m}{dt} = \phi(\lambda F_1(A, I_p, D_{AI}, D_{AA}) + F_2(A, I_p, D_{AI}, D_{AA})) - I_m \quad (5.1)$$

$$\epsilon \frac{dI_p}{dt} = \delta I_m - l_a I_p D_{aT} + l_d D_{a\pi p} \epsilon \eta_{D_{AI}} (p_d D_{AI} - p_a I_p A) \quad (5.2)$$

$$\epsilon \frac{dT}{dt} = -k_a T a + k_d D_{aT} \quad (5.3)$$

$$\epsilon \frac{dD_{aT}}{dt} = k_a D_{aT} - k_d D_{aT} + (l_d + 1) D_{a\pi p} - l_a D_{a\pi p} \quad (5.4)$$

$$\epsilon \frac{dD_{a\pi p}}{dt} = l_a I_p D_{aT} - (l_d + 1) D_{a\pi p} \quad (5.5)$$

$$\epsilon \frac{dI^*}{dt} = D_{a\Pi_p} - \mu_{I^*} I^* \quad (5.6)$$

$$\frac{dA}{dt} = -2q_a A^2 + 2q_d D_{AA} - p_a A I_p + p_d D_{AI} \quad (5.7)$$

$$\frac{dD_{AI}}{dt} = p_a A I_p - p_d D_{AI} \quad (5.8)$$

$$\frac{dD_{AA}}{dt} = q_a A^2 - q_d D_{AA} \quad (5.9)$$

$$\epsilon \frac{da}{dt} = \epsilon \mu_a (\alpha_a - a) - \eta_{D_{aT}} (k_a D_{aT} - k_d D_{aT}) \quad (5.10)$$

where

$$F_1(A, I_p, D_{AI}, D_{AA}) = \frac{\frac{A}{\theta_A}}{1 + \frac{A}{\theta_A} + \frac{D_{AA}}{\theta_{D_{AA}}} + \frac{D_{AI}}{\theta_{D_{AI}}} + \frac{A I_p}{\psi_{D_{AI}}} + \frac{A^2}{\psi_A}} \quad (5.11)$$

$$F_2(A, I_p, D_{AI}, D_{AA}) = \frac{\frac{D_{AA}}{\theta_{D_{AA}}} + \frac{A^2}{\psi_A}}{1 + \frac{A}{\theta_A} + \frac{D_{AA}}{\theta_{D_{AA}}} + \frac{D_{AI}}{\theta_{D_{AI}}} + \frac{A I_p}{\psi_{D_{AI}}} + \frac{A^2}{\psi_A}} \quad (5.12)$$

and where

- I_m , I_p & I^* denotes the concentration of AUX/IAA mRNA, AUX/IAA protein and AUX/IAA tagged for proteolysis
- T denotes TIR1
- A denotes the concentration of ARF
- D_{XY} , $X, Y \in A, I, a, T$ denotes the concentrations of an XY dimer
- $D_{a\Pi_p}$ denotes the concentration of auxin-TIR1-AUX/IAA oligomers
- a denotes the concentration of auxin.

Variable	Description
δ	Dimensionless rate of AUX/IAA transcription
k_a	Rate of association of T and a
k_d	Rate of disassociation of T and a
l_a	Rate of association of I_p and D_{aT}
l_d	Rate of disassociation of I_p and D_{aT}
μ_{I*}	Rate of decay of tagged IAA proteins
q_a	Rate of association of A to form dimers
q_d	Rate of dissociation D_{AA}
p_a	Rate of association of A and I_p
p_d	Rate of disassociation of A and I_p
$\eta_{D_{aT}}$	Representative total concentration of TIR1 divided by representative concentration of auxin
$\eta_{D_{AI}}$	Representative total concentration of ARF divided by representative total concentration of TIR1
θ_A	Binding threshold for ARF
$\theta_{D_{AA}}$	Binding threshold for ARF dimers
$\theta_{D_{AI}}$	Binding threshold for ARF-IAA
$\psi_{D_{AI}}$	Binding threshold for an IAA to bind to an ARF bound to a promoter
ψ_A	Binding threshold for ARF to bind to an ARF bound to a promoter
μ_a	Rate of auxin decay
ϕ	The effective rate of auxin transcription
λ	The rate at which ARF monomers cause AUX/IAA mRNA transcription
α_A	Total concentration of ARF divided by a representative total concentration of ARF
α_a	Total concentration of auxin divided by a representative total concentration of auxin
ϵ	Rate of decay of AUX/IAA mRNA divided by the rate of ubiquination of AUX/IAA protein

5.2.2 Farcot Auxin Signalling Model

The ODEs of the model as previously stated in [Farcot et al., 2015] are:

$$\frac{dI}{dt} = \pi_I R + 2\theta_{II} D_{II} - 2\alpha_{II} I^2 + \theta_{AI} D_{AI} - \alpha_{AI} IA \quad (5.13)$$

$$\begin{aligned} & + \theta_{G_A I} G_{AI} - \alpha_{G_A I} I G_A + \delta_{II} D_{II} \kappa_x x - \delta_I (1 + \kappa_x x) I \\ \frac{dA}{dt} = & \pi_A + 2\theta_{AA} D_{AA} - 2\alpha_{AA} A^2 + \theta_{AI} D_{AI} - \alpha_{AI} IA + \theta_{AG} G_A \quad (5.14) \\ & - \alpha_{AG} A G + \theta_{AG_A} G_{AA} - \alpha_{AG_A} A G_A + \delta_{AI} D_{AI} \kappa_x - \delta_A A \end{aligned}$$

$$\frac{dD_{II}}{dt} = \alpha_{II} I^2 - \theta_{II} D_{II} - \delta_{II} (1 + \kappa_x x) D_{II} \quad (5.15)$$

$$\frac{dD_{AI}}{dt} = \alpha_{AI}IA - \theta_{AI}D_{AI} + \theta_{D_{AI}G}G_{IA} - \alpha_{D_{AI}G}GD_{AI} \quad (5.16)$$

$$- \delta_{AI} (1 + \kappa_x x) D_{AI}$$

$$\frac{dD_{AA}}{dt} = \alpha_{AA}A^2 - \theta_{AA}D_{AA} + \theta_{D_{AA}G}G_{AA} - \alpha_{D_{AA}G}GD_{AA} - \delta_{AA}D_{AA} \quad (5.17)$$

$$\frac{dG}{dt} = \theta_{AG}G_A - \alpha_{AG}AG + \theta_{D_{AI}G}G_{AI} - \alpha_{D_{AI}G}GD_{AI} - \theta_{D_{AA}G}G_{AA} \quad (5.18)$$

$$- \alpha_{D_{AA}G}GD_{AA}$$

$$\frac{dG_A}{dt} = \alpha_{AG}AG - \theta_{AG}g_A + \theta_{G_{AI}}G_{AI} - \alpha_{G_{AI}}IG_A + \theta_{AG_A}G_{AA} \quad (5.19)$$

$$- \alpha_{AG_A}AG_A$$

$$\frac{dG_{AA}}{dt} = \alpha_{AG_A}AG_A - \theta_{AG_A}G_AA + \alpha_{D_{AA}G}GD_{AA} - \theta_{D_{AA}G}G_{AA} \quad (5.20)$$

$$\frac{dG_{AI}}{dt} = \alpha_{G_{AI}}IG_A - \theta_{G_{AI}}G_{AI} + \alpha_{D_{AI}G}GD_{AI} - \theta_{D_{AI}G}G_{AI} \quad (5.21)$$

$$\frac{dR}{dt} = hG + h_A G_A + H_{AA}G_{AA} + h_{AI}G_{AI} - \delta_R R \quad (5.22)$$

where

- I denotes the concentration of AUX/IAA
- A denotes the concentration of ARF
- D_{XY} , $X, Y \in A, I$ denotes the concentrations of an XY dimer
- G , G_A , G_{AA} and G_{AI} denote the probabilities the promoter is free, bound with ARF, an ARF:ARF dimer or an ARF:AUX/IAA dimer. $G + G_A + G_{AA} + G_{AI} = 1$
- x denotes the concentration of auxin.

Variable	Description
α_{II}	Rate of association of AUX/IAA with itself to form an AUX/IAA:AUX/IAA dimer
α_{AI}	Rate of association of AUX/IAA with ARF to form an ARF:AUX/IAA dimer
α_{AA}	Rate of association of ARF with itself to form an ARF:ARF dimer
α_{GAI}	Rate of association of AUX/IAA with an ARF bound to the promoter
α_{AG}	Rate of association of an ARF and the promoter
α_{AGA}	Rate of association of an ARF with an ARF bound to the promoter
α_{DAIG}	Rate of association of an ARF:AUX/IAA dimer and the promoter
$\alpha_{DAA G}$	Rate of association of ARF:ARF dimer and the promoter
θ_{II}	Rate of disassociation of an AUX/IAA:AUX/IAA dimer
θ_{AI}	Rate of disassociation of an ARF:AUX/IAA dimer
θ_{AA}	Rate of disassociation of an ARF:ARF dimer
θ_{GAI}	Rate of disassociation of an AUX/IAA with an ARF bound to the promoter
θ_{AG}	Rate of disassociation of an ARF and the promoter
θ_{AGA}	Rate of disassociation of an ARF with an ARF bound to the promoter
θ_{DAIG}	Rate of disassociation of ARF:AUX/IAA dimer and the promoter
$\theta_{DAA G}$	Rate of disassociation of ARF:ARF dimer and the promoter
δ_I	Rate of decay of AUX/IAA
δ_A	Rate of decay of ARF
δ_{AA}	Rate of decay of ARF:ARF dimers
δ_{II}	Rate of decay of AUX/IAA:AUX/IAA dimers
δ_R	Rate of decay for AUX/IAA mRNA
π_I	Rate of production of AUX/IAA
π_A	Rate of production of ARF
h	Rate of transcription for an unbound promoter
h_A	Rate of transcription for the promoter bound to an ARF
h_{AA}	Rate of transcription for the promoter bound to an ARF:ARF dimer
h_{AI}	Rate of transcription for the promoter bound to an ARF:AUX/IAA dimer
κ_x	Rate of auxin induced degradation

5.3 Model Adaptions

To adapt the models for modelling *Marchantia* a number of changes were required to better represent the specifics of *Marchantia*. Foremost of these is to add more ARFs to the models. These models each have one promoter ARF but *Marchantia* has three ARFs, one that is generally considered to be a promoter, mpARF1, one

that is commonly thought to be a repressor, mpARF2 and a third one that is currently believed to not interact with the same genes as the first two ARFs and to not play any feedback role in the auxin signalling pathway, mpARF3 [Kato et al., 2015]. The ARFs in these models are renamed mpARF1 as they are both promoter ARFs but another state variable needs to be added to represent mpARF2. Due to the suspected nature of mpARF3 and the fact that there is comparatively little known about it, we will ignore it for now.

Another factor to consider in the models is that experimental results suggest there are different binding affinities for the ARFs to one another and to mpIAA [Kato et al., 2015]. This may simply be a parameter issue though another study suggests that there is a difference in the abilities of the ARFs to oligomerise [Kato et al., 2020a]. At this time oligomerisation is limited to dimers due to a current lack of evidence of larger oligomers in vivo. We add the ability for mpARF2 to bind to any protein mpARF1 can, including itself and mpARF1. However, later in this chapter we describe investigating the affect of more complex oligomerisation.

5.3.1 Middleton *et al.* Model with an Additional ARF

Here using Farcot notation, the ODEs of the non-dimensionalised model are:

$$\begin{aligned} \frac{dI_m}{dt} = & \phi(\lambda F_1(A_1, A_2, I_p, D_{A_1 I}, D_{A_2 I}, D_{A_1 A_1}, D_{A_2 A_2}, D_{AA_2}) \\ & + F_2(A_1, A_2, I_p, D_{A_1 I}, D_{A_1 A_1}, D_{A_2 A_2}, D_{AA_2}) - I_m \end{aligned} \quad (5.23)$$

$$\begin{aligned} \epsilon \frac{dI_p}{dt} = & \delta I_m - l_a I_p D_{aT} + l_d D_{D_{aT} p} + \epsilon \eta_{D_{A_1 I}} (p_d D_{A_1 I} - p_a I_p A_1) \\ & + \epsilon \eta_{D_{A_2 I}} (p_d D_{A_2 I} - p_a I_p A_2) \end{aligned} \quad (5.24)$$

$$\epsilon \frac{dT}{dt} = -k_a T a + k_d D_{aT} \quad (5.25)$$

$$\epsilon \frac{dD_{aT}}{dt} = k_a a T - k_d D_{aT} + (l_d + 1) D_{aT p} - l_a D_{aT} I_p \quad (5.26)$$

$$\epsilon \frac{dD_{aT p}}{dt} = l_a I_p D_{aT} - (l_d + 1) D_{aT p} \quad (5.27)$$

$$\epsilon \frac{dI^*}{dt} = D_{aT p} - \mu_{I^*} I^* \quad (5.28)$$

$$\frac{dA_1}{dt} = -2q_a A_1^2 + 2q_d D_{A_1 A_1} - p_a A_1 I_p + p_d D_{A_1 I} \quad (5.29)$$

$$\begin{aligned} & -q_a A_1 A_2 + q_d D_{A_1 A_2} \\ \frac{dA_2}{dt} = & -2q_a A_2^2 + 2q_d D_{A_2 A_2} - p_a A_2 I_p + p_d D_{A_2 I} \end{aligned} \quad (5.30)$$

$$\begin{aligned} & -q_a A_1 A_2 + q_d D_{A_1 A_2} \\ \frac{dD_{A_1 I}}{dt} = & p_a A_1 I_p - p_d D_{A_1 I} \end{aligned} \quad (5.31)$$

$$\frac{dD_{A_2 I}}{dt} = p_a A_2 I_p - p_d D_{A_2 I} \quad (5.32)$$

$$\frac{dD_{A_1 A_1}}{dt} = q_a A_1^2 - q_d D_{A_1 A_1} \quad (5.33)$$

$$\frac{dD_{A_2 A_2}}{dt} = q_a A_2^2 - q_d D_{A_2 A_2} \quad (5.34)$$

$$\frac{dD_{A_1 A_2}}{dt} = q_a A_1 A_2 - q_d D_{A_1 A_2} \quad (5.35)$$

$$\epsilon \frac{da}{dt} = \epsilon \mu_a (\alpha_a - a) - \eta_{a-T} (k_a a T - k_d D_{aT}) \quad (5.36)$$

where $F_1(A_1, A_2, I_p, D_{A_1 I}, D_{A_2 I}, D_{A_1 A_1}, D_{A_2 A_2}, D_{A_1 A_2}) =$

$$\begin{aligned} & \frac{A_1}{\theta_{A_1}} \\ & 1 + \frac{A_1}{\theta_{A_1}} + \frac{A_2}{\theta_{A_2}} + \frac{D_{A_1 A_1}}{\theta_{D_{A_1 A_1}}} + \frac{D_{A_1 A_2}}{\theta_{D_{A_1 A_2}}} + \frac{D_{A_2 A_2}}{\theta_{D_{A_2 A_2}}} + \frac{D_{A_1 I}}{\theta_{A_1 I}} + \frac{D_{A_2 I}}{\theta_{A_2 I}} + \frac{A_1 I_p}{\psi_{A_1 I}} \\ & + \frac{A_2 I_p}{\psi_{A_2 I}} + \frac{A_1^2}{\psi_{A_1}} + \frac{A_2^2}{\psi_{A_2}} + \frac{A_1 A_2}{\psi_{A_1 A_2}} \end{aligned} \quad (5.37)$$

and where $F_2(A_1, A_2, I_p, D_{A_1 I}, D_{A_2 I}, D_{A_1 A_1}, D_{A_2 A_2}, D_{A_1 A_2}) =$

$$\begin{aligned} & \frac{D_{A_1 A_1}}{\theta_{D_{A_1 A_1}}} + \frac{A_1^2}{\psi_{A_1}} \\ & 1 + \frac{A_1}{\theta_{A_1}} + \frac{A_2}{\theta_{A_2}} + \frac{D_{A_1 A_1}}{\theta_{D_{A_1 A_1}}} + \frac{D_{A_1 A_2}}{\theta_{D_{A_1 A_2}}} + \frac{D_{A_2 A_2}}{\theta_{D_{A_2 A_2}}} + \frac{D_{A_1 I}}{\theta_{A_1 I}} + \frac{D_{A_2 I}}{\theta_{A_2 I}} + \frac{A_1 I_p}{\psi_{A_1 I}} \\ & + \frac{A_2 I_p}{\psi_{A_2 I}} + \frac{A_1^2}{\psi_{A_1}} + \frac{A_2^2}{\psi_{A_2}} + \frac{A_1 A_2}{\psi_{A_1 A_2}} \end{aligned} \quad (5.38)$$

Here the following changes have been made:

- A has been renamed A_1 and now represents mpARF1
- A_2 has been added as a state variable and represents the concentration of mpARF2. As such equation 5.30 has been added to represent it. Its terms mirror those of equation 5.29.
- $D_{A_2 I}$, $D_{A_2 A_2}$ & $D_{A_1 A_2}$ have been added as state variables to represent the concentrations of the respective dimers, mpARF2-mpIAA, mpARF2-

mpARF2 and mpARF1-mpARF2. As such equations 5.32, 5.34 & 5.35 have been added to represent change in their concentration. Their equations mirror equations 5.31, 5.33 & 5.33 respectively

- The terms $\epsilon \eta_{D_{A_2I}} (p_d D_{A_2I} - p_a I_p A_2)$ were added to equations 5.24, 5.30 and 5.32 to represent the binding and unbinding of A_2 to I_p to form the dimer D_{A_2I} .
- the terms $q_a A_1 A_2 + q_d D_{A_1A_2}$ were added to equations 5.29, 5.30 and 5.35 to represent the binding and unbinding of A_1 to A_2 to form the dimer $D_{A_1A_2}$
- terms of the form $\frac{A_2}{\theta_{A_2}}$ & $\frac{A_2 I_p}{\psi_{A_2I}}$ are also added to equations 5.37 & 5.38 represent the new oligomer or monomers that might bind the promoter.

5.3.2 Farcot Auxin Signalling Model with an Additional ARF

The ODEs of the model are:

$$\begin{aligned} \frac{dI}{dt} = & \pi_I R + 2\theta_{II} D_{II} - 2\alpha_{II} I^2 + \theta_{A_1I} D_{A_1I} - \alpha_{A_1I} I A_1 + \theta_{G_{A_1}I} G_{A_1I} \\ & - \alpha_{G_{A_1}I} I G_{A_1} + \theta_{A_2I} D_{A_2I} - \alpha_{A_2I} I A_2 + \theta_{G_{A_2}I} G_{A_2I} - \alpha_{G_{A_2}I} I G_{A_2} \\ & + \delta_{II} D_{II} \kappa_x x - \delta_I (1 + \kappa_x x) I \end{aligned} \quad (5.39)$$

$$\begin{aligned} \frac{dA_1}{dt} = & \pi_{A_1} + 2\theta_{A_1A_1} D_{A_1A_1} - 2\alpha_{A_1A_1} A_1^2 + \theta_{A_1A_2} D_{A_1A_2} - \alpha_{A_1A_2} A_1 A_2 \\ & + \theta_{A_1I} D_{A_1I} - \alpha_{A_1I} I A_1 + \theta_{A_1G} G_{A_1} - \alpha_{A_1G} A_1 G + \theta_{A_1G_{A_1}} G_{A_1A_1} \\ & - \alpha_{A_1G_{A_1}} A_1 G_{A_1} + \theta_{A_1G_{A_2}} G_{A_1A_2} - \alpha_{A_1G_{A_2}} A_1 G_{A_2} \\ & + \delta_{A_1I} D_{A_1I} \kappa_x x - \delta_{A_1} A_1 \end{aligned} \quad (5.40)$$

$$\begin{aligned} \frac{dA_2}{dt} = & \pi_{A_2} + 2\theta_{A_2A_2} D_{A_2A_2} - 2\alpha_{A_2A_2} A_2^2 + \theta_{A_1A_2} D_{A_1A_2} - \alpha_{A_1A_2} A_1 A_2 \\ & + \theta_{A_2I} D_{A_2I} - \alpha_{A_2I} I A_2 + \theta_{A_2G} G_{A_2} - \alpha_{A_2G} A_2 G + \theta_{A_2G_{A_2}} G_{A_2A_2} \\ & - \alpha_{A_2G_{A_2}} A_2 G_{A_2} + \theta_{A_2G_{A_1}} G_{A_1A_2} - \alpha_{A_2G_{A_1}} A_2 G_{A_1} \\ & + \delta_{A_2I} D_{A_2I} \kappa_x x - \delta_{A_2} A_2 \end{aligned} \quad (5.41)$$

$$\frac{dD_{II}}{dt} = \alpha_{II} I^2 - \theta_{II} D_{II} - \delta_{II} (1 + \kappa_x x) D_{II} \quad (5.42)$$

$$\frac{dD_{A_1I}}{dt} = \alpha_{A_1I}IA_1 - \theta_{A_1I}D_{A_1I} + \theta_{D_{A_1I}G}G_{IA_1} - \alpha_{D_{A_1I}G}GD_{A_1I} \quad (5.43)$$

$$-\delta_{A_1I}(1 + \kappa_x x) D_{A_1I}$$

$$\frac{dD_{A_2I}}{dt} = \alpha_{A_2I}IA_2 - \theta_{A_2I}D_{A_2I} + \theta_{D_{A_2I}G}G_{IA_2} - \alpha_{D_{A_2I}G}GD_{A_2I} \quad (5.44)$$

$$-\delta_{A_2I}(1 + \kappa_x x) D_{A_2I}$$

$$\frac{dD_{A_1A_1}}{dt} = \alpha_{A_1A_1}A_1^2 - \theta_{A_1A_1}D_{A_1A_1} + \theta_{D_{A_1A_1}G}G_{A_1A_1} - \alpha_{D_{A_1A_1}G}GD_{A_1A_1} \quad (5.45)$$

$$-\delta_{A_1A_1}D_{A_1A_1}$$

$$\frac{dD_{A_2A_2}}{dt} = \alpha_{A_2A_2}A_2^2 - \theta_{A_2A_2}D_{A_2A_2} + \theta_{D_{A_2A_2}G}G_{A_2A_2} - \alpha_{D_{A_2A_2}G}GD_{A_2A_2} \quad (5.46)$$

$$-\delta_{A_2A_2}D_{A_2A_2}$$

$$\frac{dD_{A_1A_2}}{dt} = \alpha_{A_1A_2}A_1A_2 - \theta_{A_1A_2}D_{A_1A_2} + \theta_{D_{A_1A_2}G}G_{A_1A_2} - \alpha_{D_{A_1A_2}G}GD_{A_1A_2} \quad (5.47)$$

$$-\delta_{A_1A_2}D_{A_1A_2}$$

$$\frac{dG}{dt} = \theta_{A_1G}G_{A_1} - \alpha_{A_1G}A_2G_1 + \theta_{A_2G}G_{A_2} - \alpha_{A_2G}GA_2 + \theta_{D_{A_1I}G}G_{A_1I} \quad (5.48)$$

$$-\alpha_{D_{A_1I}G}GD_{A_1I} + \theta_{D_{A_2I}G}G_{A_2I} - \alpha_{D_{A_2I}G}GD_{A_2I} + \theta_{D_{A_1A_1}G}G_{A_1A_1}$$

$$-\alpha_{D_{A_1A_1}G}GD_{A_1A_1} + \theta_{D_{A_2A_2}G}G_{A_2A_2} - \alpha_{D_{A_2A_2}G}GD_{A_2A_2} + \theta_{D_{A_1A_2}G}G_{A_1A_2}$$

$$-\alpha_{D_{A_1A_2}G}GD_{A_1A_2}$$

$$\frac{dG_{A_1}}{dt} = \alpha_{A_1G}A_1G - \theta_{A_1G}G_{A_1} + \theta_{G_{A_1I}}G_{A_1I} - \alpha_{G_{A_1I}}IG_{A_1} \quad (5.49)$$

$$+ \theta_{A_1G_{A_1}}G_{A_1A_1} - \alpha_{A_1G_{A_1}}A_1G_{A_1} + \theta_{A_2G_{A_1}}G_{A_1A_2} - \alpha_{A_2G_{A_1}}A_2G_{A_1}$$

$$\frac{dG_{A_2}}{dt} = \alpha_{A_2G}A_2G - \theta_{A_2G}G_{A_2} + \theta_{G_{A_2I}}G_{A_2I} - \alpha_{G_{A_2I}}IG_{A_2} \quad (5.50)$$

$$+ \theta_{A_2G_{A_2}}G_{A_2A_2} - \alpha_{A_2G_{A_2}}A_2G_{A_2} + \theta_{A_1G_{A_2}}G_{A_1A_2} - \alpha_{A_1G_{A_2}}A_1G_{A_2}$$

$$\frac{dG_{A_1A_1}}{dt} = \alpha_{A_1G_{A_1}}A_1G_{A_1} - \theta_{A_1G_{A_1}}G_{A_1A_1} + \alpha_{D_{A_1A_1}G}GD_{A_1A_1} \quad (5.51)$$

$$-\theta_{D_{A_1A_1}G}G_{A_1A_1}$$

$$\frac{dG_{A_2A_2}}{dt} = \alpha_{A_2G_{A_2}}A_2G_{A_2} - \theta_{A_2G_{A_2}}G_{A_2A_2} + \alpha_{D_{A_2A_2}G}GD_{A_2A_2} \quad (5.52)$$

$$-\theta_{D_{A_2A_2}G}G_{A_2A_2}$$

$$\frac{dG_{A_1A_2}}{dt} = \alpha_{A_2G_{A_1}}A_2G_{A_1} - \theta_{A_2G_{A_1}}G_{A_1A_2} + \alpha_{A_1G_{A_2}}A_1G_{A_2} - \theta_{A_1G_{A_2}}G_{A_2A_1} \quad (5.53)$$

$$+ \alpha_{D_{A_1A_2}G}GD_{A_1A_2} - \theta_{D_{A_1A_2}G}G_{A_1A_2}$$

$$\frac{dG_{A_1I}}{dt} = \alpha_{G_{A_1I}}IG_{A_1} - \theta_{G_{A_1I}}G_{A_1I} + \alpha_{D_{A_1I}G}GD_{A_1I} - \theta_{D_{A_1I}G}G_{A_1I} \quad (5.54)$$

$$\frac{dG_{A_2I}}{dt} = \alpha_{G_{A_2I}}IG_{A_2} - \theta_{G_{A_2I}}G_{A_2I} + \alpha_{D_{A_2I}G}GD_{A_2I} - \theta_{D_{A_2I}G}G_{A_2I} \quad (5.55)$$

$$\frac{dR}{dt} = hG + h_{A_1}G_{A_1} + h_{A_1A_1}G_{A_1A_1} + h_{A_1A_2}G_{A_1A_2} + h_{A_1I}G_{A_1I} - \delta_R R \quad (5.56)$$

Here the following changes have been made:

- A has been renamed A_1 and now represents mpARF1
- A_2 has been added as a state variable and represents the concentration of mpARF2. As such equation 5.41
- D_{A_2I} , $D_{A_2A_2}$ & $D_{A_1A_2}$ have been added as state variables to represent the concentrations of the respective dimers, mpARF2-mpIAA, mpARF2-mpARF2 and mpARF1-mpARF2. As such equations 5.44, 5.46 & 5.47 have been added to represent change in their concentration. Their equations mirror equations 5.43, 5.45 & 5.45 respectively
- for all new ARF oligomer state variables, corresponding variables representing them binding the promoter. For G_{A_2} , G_{A_2I} , $G_{A_2A_2}$ & $G_{A_1A_2}$ equations 5.50, 5.55, 5.52 & 5.53 have been added respectively, mirroring equations 5.49, 5.55, 5.51 & 5.51.
- The terms $\theta_{A_2I}D_{A_2I} - \alpha_{A_2I}IA_2$ were added to equations 5.39, 5.41 & 5.44 to represent the binding and unbinding of I to A_2 to form the dimer D_{A_2I}
- The terms $\theta_{G_{A_2}I}G_{A_2I} - \alpha_{G_{A_2}I}IG_{A_2}$ were added to equations 5.39, 5.50 & 5.55 to represent the binding and unbinding of I to G_{A_2} to form the bound receptor G_{A_2I}
- The terms $\theta_{A_1A_2}D_{A_1A_2} - \alpha_{A_1A_2}A_1A_2$ were added to equations 5.40, 5.41 & 5.47 to represent the binding and unbinding of A_1 to A_2 to form the dimer $D_{A_1A_2}$
- The terms $\theta_{A_1G_{A_2}}G_{A_1A_2} - \alpha_{A_1G_{A_2}}A_1G_{A_2}$ were added to equations 5.40, 5.50 & 5.53 to represent the binding and unbinding of A_1 to G_{A_2} to form the bound receptor $G_{A_1A_2}$
- The terms $\theta_{A_2G}G_{A_2} - \alpha_{A_2G}A_2G$ were added to equations 5.41, 5.48 & 5.50 to represent the binding and unbinding of A_2 to G to form the bound receptor

G_{A_2}

- The terms $\theta_{D_{A_2I}G}G_{A_2I} - \alpha_{D_{A_2I}G}D_{A_2I}G$ were added to equations 5.44, 5.48 & 5.55 to represent the binding and unbinding of D_{A_2I} to G to form the bound receptor G_{A_2I}
- The terms $\theta_{D_{A_2A_2}G}G_{A_2A_2} - \alpha_{D_{A_2A_2}G}D_{A_2A_2}G$ were added to equations 5.46, 5.48 & 5.52 to represent the binding and unbinding of $D_{A_2A_2}$ to G to form the bound receptor $G_{A_2A_2}$
- The terms $\theta_{D_{A_1A_2}G}G_{A_1A_2} - \alpha_{D_{A_1A_2}G}D_{A_1A_2}G$ were added to equations 5.47, 5.48 & 5.53 to represent the binding and unbinding of $D_{A_1A_2}$ to G to form the bound receptor $G_{A_1A_2}$
- The terms $\theta_{A_2G_{A_1}}G_{A_1A_2} - \alpha_{A_2G_{A_1}}A_2G_{A_1}$ were added to equations 5.41, 5.49 & 5.53 to represent the binding and unbinding of A_2 to G_{A_1} to form the bound receptor $G_{A_1A_2}$
- The term $h_{A_1A_2}G_{A_1A_2}$ was added to equation 5.56 to represent the potential for a mpARF1-mpARF2 dimer to promote the gene.

5.4 Initial Data Fitting

For the initial data fitting for the Farcot auxin signalling model with two ARFs, the data to be fit against was a time series (Figure 5.1) of ARF levels within *Marchantia gemmae*, provided by Shubajit Das of Wageningen University. The data included mpARF1 and mpARF2 levels at six time points within the first 24 hours after the gemmae's removal from the gemma cup. The software utilised to do the fitting is the `minimize` function from the Python package SciPy. As this program utilises root finding algorithms to perform the fitting and it cannot be assumed that there is a global minimum it is important use a variety of initial parameter values to increase the number of basins of attraction tested to increase the number of local minima found. The model, as described above, consists of 58 parameters, and if the fitting algorithm was run for initial value combinations,

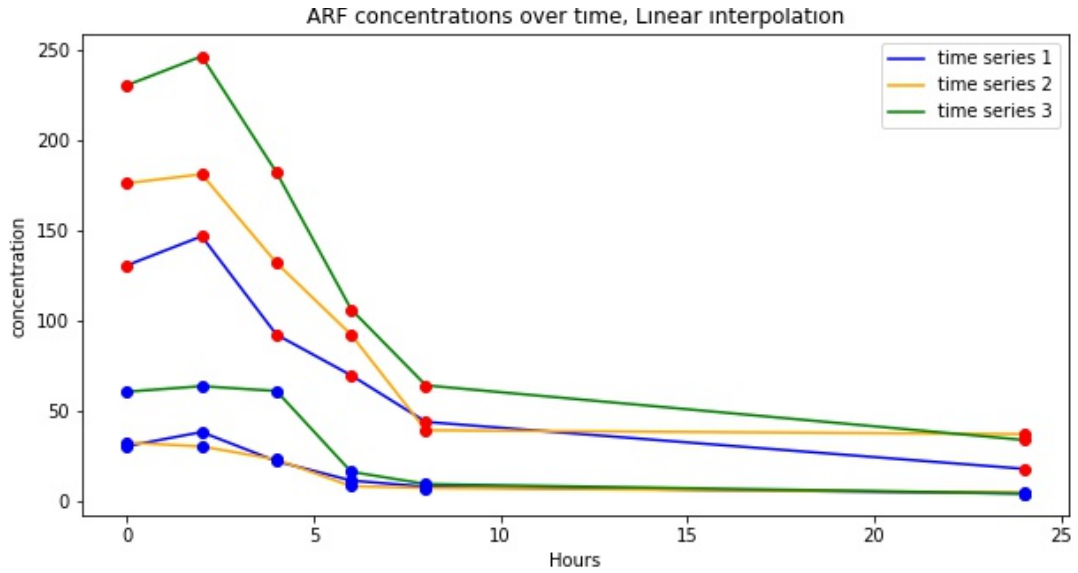


Figure 5.1: Time series data for average ARF1 concentrations, dotted by red markers, and ARF2 concentrations, denoted by blue markers, in cells in a marchantia gemma

allowing for two possibilities for each parameter, the task would be infeasible. If it took one second to run each minimisation the testing would amount to nine billion years of CPU time and my PhD was only four years long. To simplify the task the number of parameters to be fit was reduced to 16 (Table:5.1). This was achieved by setting all the binding and unbinding rates within the model to a single value and by setting many of the rates of decay to a single value. The chosen simplifications were based on the models original paper where these are the parameters for which the author is willing to specify a value. By reducing down to these 16 parameters the fitting only needed to handle 65,536 combinations 2^{42} times less than before. This is approximately only 18 hours of CPU time according to the formula used above. In reality it took over 36 hours using 32 cores.

Variable	Initial values	Description
π_I	1, 10	Production rate of IAA
π_{A_1}	1, 10	Production rate of ARF1
π_{A_2}	1, 10	Production rate of ARF2
θ	0.01, 1	Generic binding rate, replaces all other θ values
α	0.01, 1	Generic unbinding rate, replaces all other α values
δ_I	1, 10	Decay rate of IAA
δ_{II}	1, 10	Decay rate of IAA-IAA homodimers
δ_{A_1}	1, 10	Decay rate of ARF1
δ	1, 10	Generic rate of decay, replaces all δ values not within this table
κ	1, 10	Rate at which auxin removes IAA
χ	300, 3000	Auxin concentration
h	1, 10	Transcription rate of unbound receptor
h_{A_1}	1, 10	Transcription rate of ARF1 bound receptor
$h_{A_1A_1}$	1, 10	Transcription rate of ARF1-ARF1 homodimer bound receptor
h_{A_1I}	1, 10	Transcription rate of ARF1-I heterodimer bound receptor
$h_{A_1A_2}$	1, 10	Transcription rate of ARF1-ARF2 heterodimer bound receptor

Table 5.1

In each individual run of parameter estimation the function `minimize` takes as input a set of initial values and a minimisation criteria. In our case for our minimisation criteria we minimised the 2-norm of the difference between the experimental data and simulated time series data utilising the Python ode solver `scipy.integrate.odeint` and the current iteration of the parameters being minimised. Furthermore, the variables (the parameters for our model) were limited to being non-negative. Based on these inputs the code chose an appropriate root finding algorithm. The algorithm chosen then attempts to, working from the initial values given, find parameters that score as low as possible in the minimisation criteria.

The minimisation code was then run for every combination of the initial values shown in Table:5.1, resulting in the aforementioned 65,536 iterations. This resulted in six fits significantly better than the others, followed by a large number of increasingly worse but similar fits, see Figure 5.2. A visual inspection of the

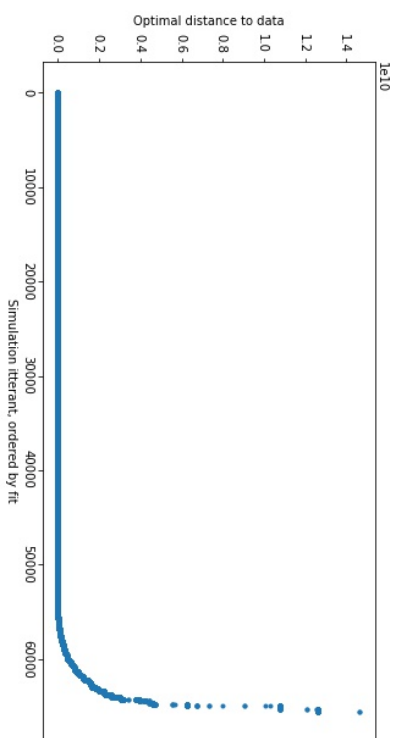
curves created by these fits qualitatively have at least one thing in common. The five superior fits each better captured the apparent increase in ARF concentrations as seen in hour two of the exponential data Figure 5.2. Not all of these six parameter sets led to a positive gradient during the first two hours for all initial conditions. However, they show a much more shallow gradient than the nearest poorer fits which all have their steepest gradients at time zero. Whilst there are parameter sets with yet worse fits that do have an increase in concentration within the first two hours they sharply decay after two hours for a worse overall fit.

5.4.1 Secondary Fitting Based of First Set

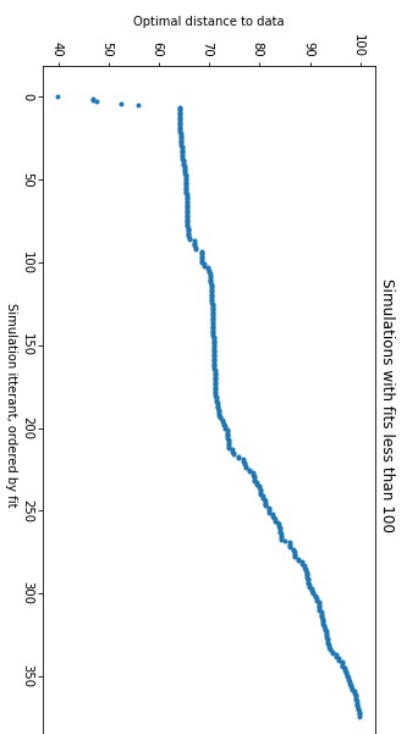
Whilst some parameter sets were better than others at producing curves that fit the observed data, none of them produced time series results that both mimicked the peaks seen at two hours whilst having a slow decay that matched data at latter time points. To test whether this was due to the model or the reduction in distinct parameter values further fitting was done. The results from the initial fitting were used to fit the 58 parameter model, i.e. the initial parameter values for the fitting for each parameter would be set to the fitted value for the parameter from the first fit. These fits were more accurate to the observed data and better matched the ARF1 peak at two hours (Figure 5.3).

Whilst the fit may be improved by the addition of distinct values for the remaining parameters, their addition is not necessarily an improvement to the model. The Akaike Information Criterion (AIC) [Guthery et al., 2003] is a measure that attempts to evaluate the fit of a model whilst weighting it against the number of parameters so as to prevent over-parameterisation. In this regard, the fits using all 58 variables were shown to be significantly worse (Table 5.2) prompting an investigation into what, if any, individual parameters could be considered individually by the model to achieve a superior AIC value.

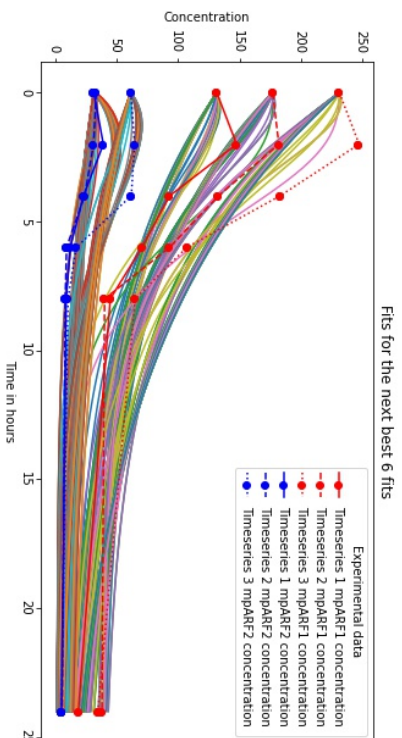
To this end each parameter that was not individually considered was added



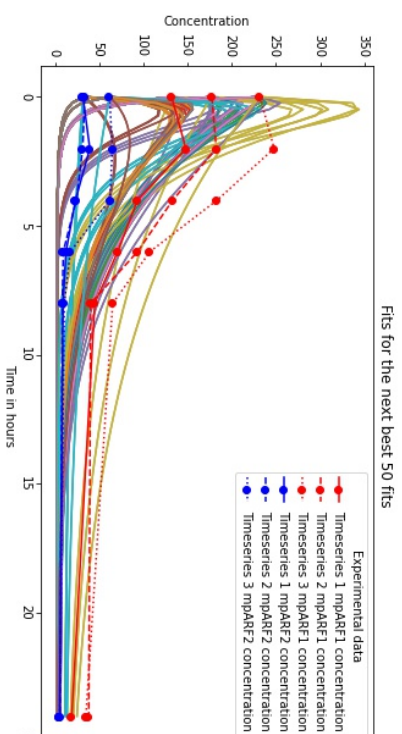
(a)



(b)



(c)



(d)

Figure 5.2: Graphs (a) and (b) show the level of fit achieved by the different 'minimisations' performed on initial value combinations, sorted by fitness value. Graph (c) shows the curves generated from the parameters of the best six fits while (d) shows the curves generated by the parameters of the next best 50

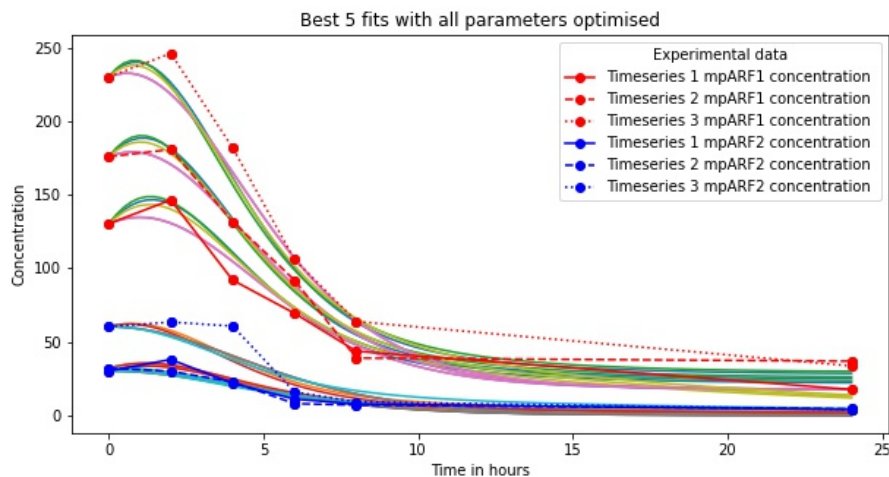


Figure 5.3: Graph showing the best five results from refitting all 58 parameters for the initial fits. Simulations for each of the 5 parameter sets has been plotted in a different colour.

as a distinct parameter and then a new set fittings was done using previous fits as the initial parameters as described above in the 58 parameter case. Out of all the permutations there were seven parameters that when added to the list of distinct parameters lead to a lower AIC value for the best fit (Table 5.2). Of these parameters only one relates to the binding of the promoter and the remaining six are equally split between terms that relate to the binding of ARFs to other ARFs and the protein binding of AUX/IAAs. These terms are quite different from one another and is likely due to the minimisation finding a number of different ways in which the model can achieve comparable fits for the ARFs. There were plans to look further into this, unfortunately discussions with our experimental colleagues prompted a revision of the model as discussed in the next section.

Added Parameter	ΔAIC	Fit
Best initial fit	120.4	39.9
Best 58 parameters fit	193.8	25.6
θ_{II}	117.6	32.6
$\theta_{\text{A}_1\text{I}}$	118.5	33.9
$\theta_{\text{A}_1\text{A}_1}$	117.5	32.4
$\theta_{\text{A}_2\text{A}_2}$	118.3	33.6
$\alpha_{\text{A}_2\text{I}}$	114.5	28.6
$\alpha_{\text{A}_1\text{A}_1}$	112.5	26.4
$\alpha_{\text{G}_{\text{A}_1}\text{I}}$	112.6	26.4

Table 5.2: Table showing the fit and the AIC of parameterisations of the initial altered Farcot auxin signalling model.

5.4.2 Alternative Interpretation of the Data

Whilst presenting the initial fitting and model analysis to our biologist colleagues, it came to light that the data we had to fit to was based on total ARF concentration irrespective of whether it was a monomer or otherwise. The result of this is that the data that we were fitting is not in a form that the models can represent. This is because both models have a relatively constant concentration of ARF; in the Middleton *et al.* model ARFs are conserved and so the concentration does not change, and in the Farcot auxin signalling model there are only simple linear production and decay terms, precluding the non-monotone pattern seen in the data.

To allow for the model to be able to exhibit similar ARF concentrations to those observed in experiments the production terms for the ARFs must be altered. This can be done in at least one of two ways. One would be by adding in other dynamics relating to new state variables previously external to the system to effect change in ARF concentrations. Another would be by assuming some level of feedback between state variables currently in the system and production of the ARFs.

Adding a whole new set of dynamics external to elements currently in the system to control the production of ARFs would essentially be prescribing the very state variables we are fitting at this time. As such, it was decided to have

ARF production affected by preexisting elements. We did this by introducing feedback loops for the ARF concentrations similar in nature to the preexisting AUX/IAA production system. Whilst not yet seen in *Marchantia*, ARF feedback loops are not unprecedented in *Arabidopsis* [Truskina et al., 2021]. This required the addition of an mRNA state variable for each ARF. To implement the feedback loops there are four versions of each model to represent the four combinations of ARF1 directly or otherwise repressing or promoting ARF1 production and repressing or promoting ARF2 production.

5.4.3 New Model Including Feedback

The next iteration of the altered model, now as stated above, includes separate mRNA terms for ARF1 and ARF2. The ODEs of the model are:

$$\begin{aligned} \frac{dI}{dt} = & \pi_I R + 2\theta_{II} D_{II} - 2\alpha_{II} I^2 + \theta_{A_1 I} D_{A_1 I} - \alpha_{A_1 I} I A_1 + \theta_{G_{A_1} I} G_{A_1 I} \\ & - \alpha_{G_{A_1} I} I G_{A_1} + \theta_{A_2 I} D_{A_2 I} - \alpha_{A_2 I} I A_2 + \theta_{G_{A_2} I} G_{A_2 I} - \alpha_{G_{A_2} I} I G_{A_2} \\ & + \delta_{II} D_{II} \kappa_x x - \delta_I (1 + \kappa_x x) I \end{aligned} \quad (5.57)$$

$$\begin{aligned} \frac{dA_1}{dt} = & \pi_{A_1} \mathbf{R}_{A_1} + 2\theta_{A_1 A_1} D_{A_1 A_1} - 2\alpha_{A_1 A_1} A_1^2 + \theta_{A_1 A_2} D_{A_1 A_2} \\ & - \alpha_{A_1 A_2} A_1 A_2 + \theta_{A_1 I} D_{A_1 I} - \alpha_{A_1 I} I A_1 + \theta_{A_1 G} G_{A_1} - \alpha_{A_1 G} A_1 G \\ & + \theta_{A_1 G_{A_1}} G_{A_1 A_1} - \alpha_{A_1 G_{A_1}} A_1 G_{A_1} + \theta_{A_1 G_{A_2}} G_{A_1 A_2} - \alpha_{A_1 G_{A_2}} A_1 G_{A_2} \\ & + \delta_{A_1 I} D_{A_1 I} \kappa_x x - \delta_{A_1} A_1 \end{aligned} \quad (5.58)$$

$$\begin{aligned} \frac{dA_2}{dt} = & \pi_{A_2} \mathbf{R}_{A_2} + 2\theta_{A_2 A_2} D_{A_2 A_2} - 2\alpha_{A_2 A_2} A_2^2 + \theta_{A_1 A_2} D_{A_1 A_2} \\ & - \alpha_{A_1 A_2} A_1 A_2 + \theta_{A_2 I} D_{A_2 I} - \alpha_{A_2 I} I A_2 + \theta_{A_2 G} G_{A_2} - \alpha_{A_2 G} A_2 G \\ & + \theta_{A_2 G_{A_2}} G_{A_2 A_2} - \alpha_{A_2 G_{A_2}} A_2 G_{A_2} + \theta_{A_2 G_{A_1}} G_{A_1 A_2} - \alpha_{A_2 G_{A_1}} A_2 G_{A_1} \\ & + \delta_{A_2 I} D_{A_2 I} \kappa_x x - \delta_{A_2} A_2 \end{aligned} \quad (5.59)$$

$$\frac{dD_{II}}{dt} = \alpha_{II} I^2 - \theta_{II} D_{II} - \delta_{II} (1 + \kappa_x x) D_{II} \quad (5.60)$$

$$\begin{aligned} \frac{dD_{A_1 I}}{dt} = & \alpha_{A_1 I} I A_1 - \theta_{A_1 I} D_{A_1 I} + \theta_{D_{A_1 I} G} G_{A_1 I} - \alpha_{D_{A_1 I} G} G D_{A_1 I} \\ & - \delta_{A_1 I} (1 + \kappa_x x) D_{A_1 I} \end{aligned} \quad (5.61)$$

$$\frac{dD_{A_2I}}{dt} = \alpha_{A_2I}IA_2 - \theta_{A_2I}D_{A_2I} + \theta_{D_{A_2I}G}G_{IA_2} - \alpha_{D_{A_2I}G}GD_{A_2I} \quad (5.62)$$

$$-\delta_{A_2I}(1 + \kappa_x x)D_{A_2I}$$

$$\frac{dD_{A_1A_1}}{dt} = \alpha_{A_1A_1}A_1^2 - \theta_{A_1A_1}D_{A_1A_1} + \theta_{D_{A_1A_1}G}G_{A_1A_1} - \alpha_{D_{A_1A_1}G}GD_{A_1A_1} \quad (5.63)$$

$$-\delta_{A_1A_1}D_{A_1A_1}$$

$$\frac{dD_{A_2A_2}}{dt} = \alpha_{A_2A_2}A_2^2 - \theta_{A_2A_2}D_{A_2A_2} + \theta_{D_{A_2A_2}G}G_{A_2A_2} - \alpha_{D_{A_2A_2}G}GD_{A_2A_2} \quad (5.64)$$

$$-\delta_{A_2A_2}D_{A_2A_2}$$

$$\frac{dD_{A_1A_2}}{dt} = \alpha_{A_1A_2}A_1A_2 - \theta_{A_1A_2}D_{A_1A_2} + \theta_{D_{A_1A_2}G}G_{A_1A_2} \quad (5.65)$$

$$-\alpha_{D_{A_1A_2}G}GD_{A_1A_2} - \delta_{A_1A_2}D_{A_1A_2}$$

$$\frac{dG}{dt} = \theta_{A_1G}G_{A_1} - \alpha_{A_1G}AG_1 + \theta_{AG}G_{A_2} - \alpha_{A_2G}GA_2 + \theta_{D_{A_1I}G}G_{A_1I} \quad (5.66)$$

$$-\alpha_{D_{A_1I}G}GD_{A_1I} + \theta_{D_{A_2I}G}G_{A_2I} - \alpha_{D_{A_2I}G}GD_{A_2I} + \theta_{D_{A_1A_1}G}G_{A_1A_1}$$

$$-\alpha_{D_{A_1A_1}G}GD_{A_1A_1} + \theta_{D_{A_2A_2}G}G_{A_2A_2} - \alpha_{D_{A_2A_2}G}GD_{A_2A_2}$$

$$+\theta_{D_{A_1A_2}G}G_{A_1A_2} - \alpha_{D_{A_1A_2}G}GD_{A_1A_2}$$

$$\frac{dG_{A_1}}{dt} = \alpha_{A_1G}A_1G - \theta_{A_1G}G_{A_1} + \theta_{G_{A_1I}}G_{A_1I} - \alpha_{G_{A_1I}}IG_{A_1} \quad (5.67)$$

$$+\theta_{A_1G_{A_1}}G_{A_1A_1} - \alpha_{A_1G_{A_1}}A_1G_{A_1} + \theta_{A_2G_{A_1}}G_{A_1A_2} - \alpha_{A_2G_{A_1}}A_2G_{A_1}$$

$$\frac{dG_{A_2}}{dt} = \alpha_{A_2G}A_2G - \theta_{A_2G}G_{A_2} + \theta_{G_{A_2I}}G_{A_2I} - \alpha_{G_{A_2I}}IG_{A_2} \quad (5.68)$$

$$+\theta_{A_2G_{A_2}}G_{A_2A_2} - \alpha_{A_2G_{A_2}}A_2G_{A_2} + \theta_{A_1G_{A_2}}G_{A_1A_2} - \alpha_{A_1G_{A_2}}A_1G_{A_2}$$

$$\frac{dG_{A_1A_1}}{dt} = \alpha_{A_1G_{A_1}}A_1G_{A_1} - \theta_{A_1G_{A_1}}G_{A_1A_1} + \alpha_{D_{A_1A_1}G}GD_{A_1A_1} \quad (5.69)$$

$$-\theta_{D_{A_1A_1}G}G_{A_1A_1}$$

$$\frac{dG_{A_2A_2}}{dt} = \alpha_{A_2G_{A_2}}A_2G_{A_2} - \theta_{A_2G_{A_2}}G_{A_2A_2} + \alpha_{D_{A_2A_2}G}GD_{A_2A_2} \quad (5.70)$$

$$-\theta_{D_{A_2A_2}G}G_{A_2A_2}$$

$$\frac{dG_{A_1A_2}}{dt} = \alpha_{A_2G_{A_1}}A_2G_{A_1} - \theta_{A_2G_{A_1}}G_{A_1A_2} + \alpha_{A_1G_{A_2}}A_1G_{A_2} \quad (5.71)$$

$$-\theta_{A_1G_{A_2}}G_{A_2A_1} + \alpha_{D_{A_1A_2}G}GD_{A_1A_2} - \theta_{D_{A_1A_2}G}G_{A_1A_2}$$

$$\frac{dG_{A_1I}}{dt} = \alpha_{G_{A_1I}}IG_{A_1} - \theta_{G_{A_1I}}G_{A_1I} + \alpha_{D_{A_1I}G}GD_{A_1I} - \theta_{D_{A_1I}G}G_{A_1I} \quad (5.72)$$

$$\frac{dG_{A_2I}}{dt} = \alpha_{G_{A_2I}}IG_{A_2} - \theta_{G_{A_2I}}G_{A_2I} + \alpha_{D_{A_2I}G}GD_{A_2I} - \theta_{D_{A_2I}G}G_{A_2I} \quad (5.73)$$

$$\frac{dR}{dt} = hG + h_{A_1}G_{A_1} + h_{A_1A_1}G_{A_1A_1} + h_{A_1A_2}G_{A_1A_2} + h_{A_1I}G_{A_1I} \quad (5.74)$$

$$-\delta_R R$$

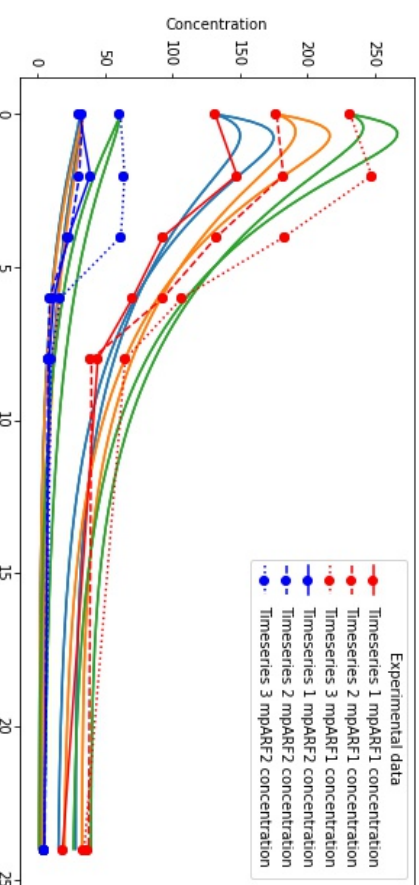
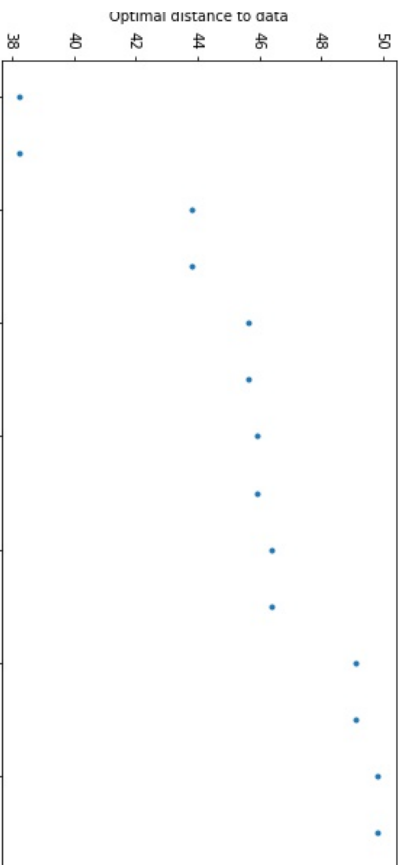
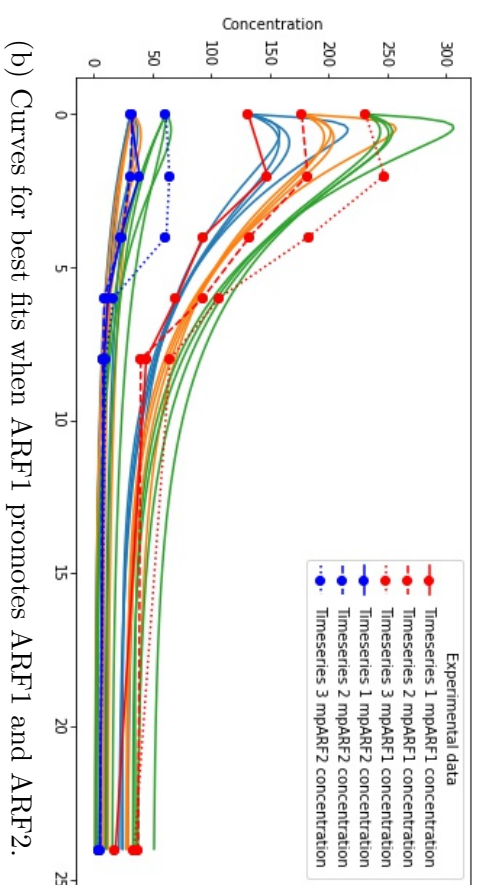
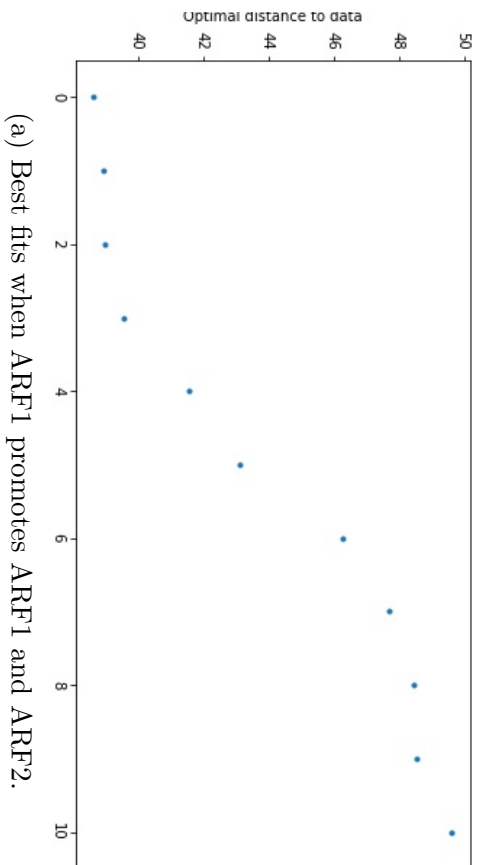
$$\begin{aligned}
\frac{dR_{A_1}}{dt} &= \begin{cases} h_2 G + h_{2A_1} G_{A_1} + h_{2A_1A_1} G_{A_1A_1} + h_{2A_1A_2} G_{A_1A_2} + h_{2A_1I} G_{A_1I} - \delta_{R_{A_1}} R_{A_1} \\ \text{if ARF1 promotes ARF1} \end{cases} \quad (5.75) \\
\frac{dR_{A_2}}{dt} &= \begin{cases} h_3 G + h_{3A_1} G_{A_1} + h_{3A_1A_1} G_{A_1A_1} + h_{3A_1A_2} G_{A_1A_2} + h_{3A_1I} G_{A_1I} - \delta_{R_{A_2}} R_{A_2} \\ \text{if ARF1 promotes ARF2} \\ h_2(G + G_{A_2} + G_{A_1A_2} + G_{A_2A_2} + G_{A_1I} + G_{A_2I}) - \delta_{R_{A_1}} R_{A_1} \\ \text{if ARF1 represses ARF1} \\ h_3(G + G_{A_2} + G_{A_1A_2} + G_{A_2A_2} + G_{A_1I} + G_{A_2I}) - \delta_{R_{A_2}} R_{A_2} \\ \text{if ARF1 represses ARF2} \end{cases} \quad (5.76)
\end{aligned}$$

Where the new state variables R_{A_1} & R_{A_2} represent mRNA concentrations for ARF1 and ARF2 respectively. The terms $\pi_{A_1} R_{A_1}$ & $\pi_{A_2} R_{A_2}$, found in equations 5.58 & 5.59, represent protein synthesis as a result of the respective mRNA. The ODE's governing the rate of change of the concentrations of mRNA for ARF1 & ARF2, $\frac{dR_{A_1}}{dt}$ & $\frac{dR_{A_2}}{dt}$, both come in two forms. The first of the two forms is similar to that seen in $\frac{dR}{dt}$ and represents either ARF1 directly promoting the transcription of the mRNA or the promotion of another protein that promotes the transcription of the respective ARF mRNA. The second of the two forms is for when ARF1 is repressing the transcription of the relevant mRNA. In this instance the transcription of the mRNA is set to be proportional to the percentage of the receptors that are not bound by one of the promoting molecules. This represents ARF1 not promoting a protein that in turn represses the relevant mRNA rather than representing direct repression. These choices are motivated by the nature of ARF1 and ARF2 in that their categorisation within the three clades of ARFs suggests that ARF1 generally promotes and in contrast ARF2 will generally repress requiring equations of theses forms [Kato et al., 2015, Finet et al., 2013].

5.4.4 Results of Data Fitting

As with before the number of free parameters was reduced down to facilitate the fitting of the model to the data. And the model was fitted for each of the four combinations of ARF1 promoting or repressing ARF1 or ARF2.

Whilst the system appears to fit to the ARF1 data with good accuracy, it does not fit to the ARF2 data anywhere near as well. This may be due, in part, to the number of variables that are set to be equal, or the fact that due to the higher concentration of ARF1, percentage discrepancies are weighted higher than those for ARF2. The poor fit may also imply that one cannot replicate the behaviour observed with solely the elements within the system of equations above and that it depends on factors and chemicals that are exogenous to above system of equations. Our experimental collaborators confirmed that such factors were likely to exist, though further experiments would be required to be more specific. These experiments are not going to take place in the foreseeable future.

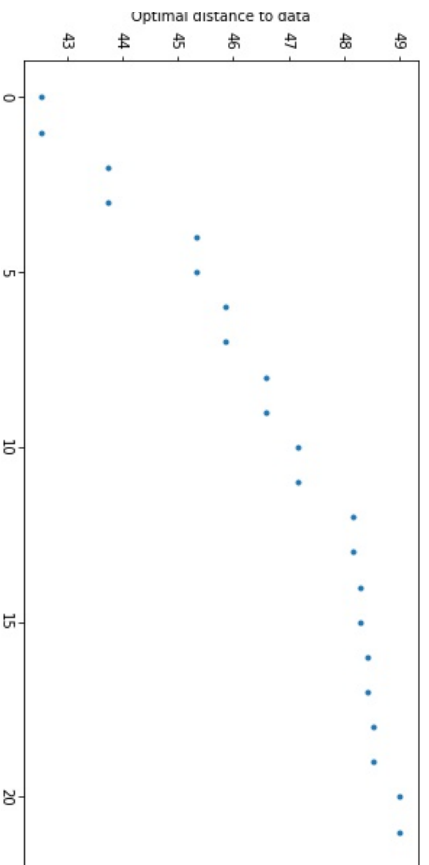


(a) Best fits when ARF1 promotes ARF1 and ARF2.

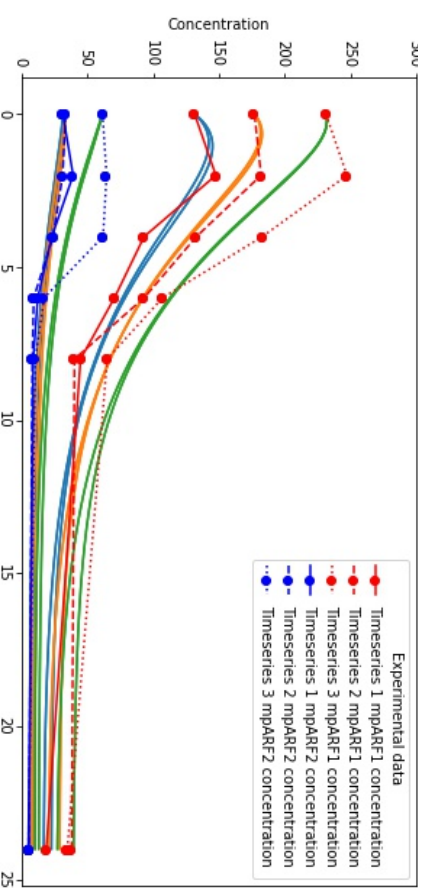
(b) Curves for best fits when ARF1 promotes ARF1 and ARF2.

(c) Best fits when ARF1 promotes ARF1 and represses ARF2.

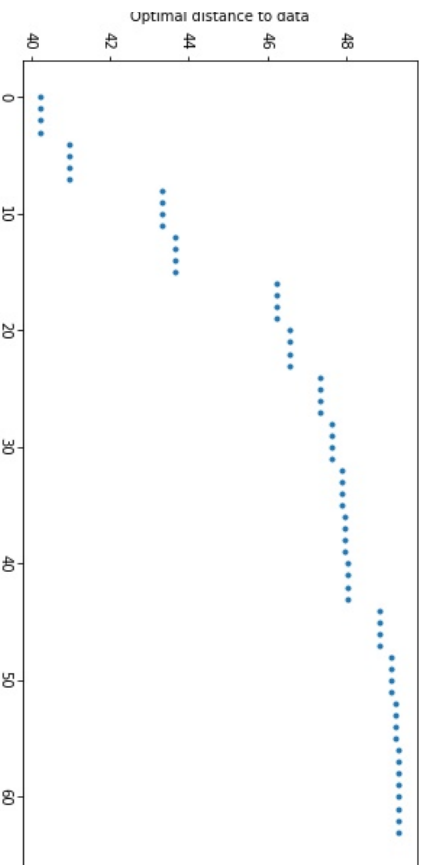
(d) Curves for best fits when ARF1 promotes ARF1 and represses ARF2.



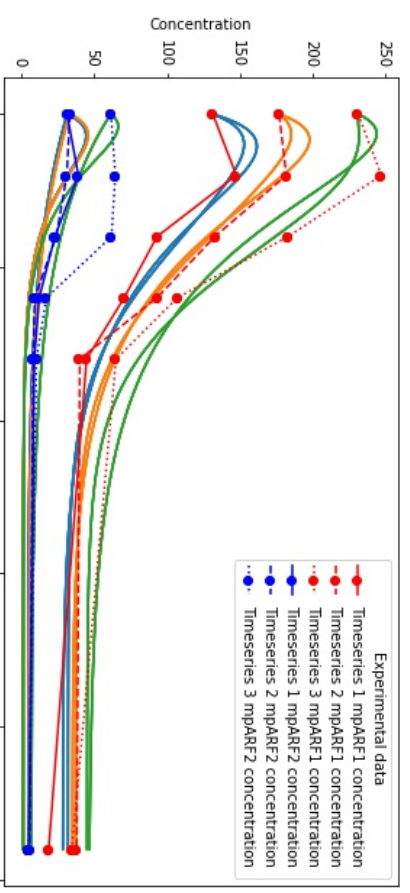
(e) Best fits when ARF1 promotes ARF2 and represses ARF1.



(f) Curves for best fits when ARF1 promotes ARF2 and represses ARF1



(g) Best fits when ARF1 represses ARF1 and ARF2



(h) Curves for best fits when ARF1 represses ARF1 and ARF2.

Figure 5.4: Graphs showing best fits for different positive/negative feedbacks, as indicated in sub-captions. Blue plots denote simulations using time series 1 data. Orange plots denote simulations using time series 2 data. Green plots denote simulations using time series 3 data.

5.5 Using mpARF Data Explicitly

Yet again new revelations (via private communication) altered the course of our research. We were at this time informed that while mpIAA was being produced throughout the first 24 hours, mpIAA is heavily degraded and only starts to appear in measurable quantities at approximately 12 hours. As a result of this, the models we present after this time have had the terms relating to mpIAA, its production and its degradation removed, thus simplifying the model (Full model found in Appendix A.4.1).

Furthermore, due to the relatively poor fit being generated by the previous models, and lack of evidence for a feedback loop affecting production of ARF1 or ARF2 in *Marchantia*, we decided to make alterations to the model, once again. Namely instead of attempting to parameterise the model to generate ARF levels that match those observed we instead utilised the ARF time series data as an input to the system and studied the resulting transcription rates over time. As a result, instead of utilising a state variable that represented a hypothetical promoter that resulted in mRNA, which in turn produced ARFs, ARF concentrations will be prescribed via adding or removing concentration from the ARF1 and ARF2 state variables to keep total concentrations at the correct levels. This has the potential to cause errors should ARF1 or ARF2 concentrations become sufficiently low that this results in negative concentration. Also since we are not algorithmically parameterising the model we will have to set parameter values ourselves (Table 5.3).

According to one of the collaborating experimentalist biologists, the area of ARFs that are targeted for destruction is the PB1 domain, as such monomers are likely more affected than multimers. In fact monomers were not regularly seen in vivo, as such, that is the area I targeted for removal to affect overall concentration change. One solution to the aforementioned problem with potential negative ARF concentration is to use a peicewise function to remove ARF dimers rather than ARF proteins in the case zero concentration of ARF monomers. However, it

Variable	Value	Description
$\alpha_{A_1A_1}$	1	Rate of association of ARF1 with itself
$\alpha_{A_1A_2}$	1	Rate of association of ARF1 with ARF2
$\alpha_{A_2A_2}$	1	Rate of association of ARF2 with itself
α_{A_1G}	K_{on1}	Rate of association of an ARF1 and the promoter
α_{A_2G}	K_{on2}	Rate of association of an ARF2 and the promoter
$\alpha_{A_1A_1G}$	$2K_{on1}$	Rate of association of an ARF1:ARF1 oligomer and the promoter
$\alpha_{A_1A_2G}$	$K_{on1} + K_{on2}$	Rate of association of an ARF1:ARF2 oligomer and the promoter
$\alpha_{A_2A_2G}$	$2K_{on2}$	Rate of association of an ARF2:ARF2 oligomer and the promoter
$\alpha_{A_1A_1A_1G}$	$3K_{on1}$	Rate of association of an ARF1:ARF1:ARF1 oligomer and the promoter
$\alpha_{A_1A_1A_2G}$	$2K_{on1} + K_{on2}$	Rate of association of an ARF1:ARF1:ARF2 oligomer and the promoter
$\alpha_{A_1A_2A_1G}$	$2K_{on1} + K_{on2}$	Rate of association of an ARF1:ARF2:ARF1 oligomer and the promoter
$\alpha_{A_1A_2A_2G}$	$K_{on1} + 2K_{on2}$	Rate of association of an ARF1:ARF2:ARF2 oligomer and the promoter
$\alpha_{A_2A_1A_2G}$	$K_{on1} + 2K_{on2}$	Rate of association of an ARF2:ARF1:ARF2 oligomer and the promoter
$\alpha_{A_2A_2A_2G}$	$3K_{on2}$	Rate of association of an ARF2:ARF2:ARF2 oligomer and the promoter
$\alpha_{A_1A_1A_1A_2G}$	$3K_{on1} + K_{on2}$	Rate of association of an ARF1:ARF1:ARF1:ARF2 oligomer and the promoter
$\alpha_{A_2A_1A_1A_2G}$	$2K_{on1} + 2K_{on2}$	Rate of association of an ARF2:ARF1:ARF1:ARF2 oligomer and the promoter
$\alpha_{A_2A_1A_1A_1A_2G}$	$3K_{on1} + 2K_{on2}$	Rate of association of an ARF1:ARF1:ARF1:ARF1:ARF2 oligomer and the promoter

Variable	Value	Description
$\theta_{A_1A_1}$	1	Rate of disassociation of ARF1 with itself
$\theta_{A_1A_2}$	1	Rate of disassociation of ARF1 with ARF2
$\theta_{A_2A_2}$	1	Rate of disassociation of ARF2 with itself
θ_{A_1G}	$K_{\text{off}1}$	Rate of disassociation of an ARF and the promoter
θ_{A_2G}	$K_{\text{off}2}$	Rate of disassociation of an ARF and the promoter
$\theta_{A_1A_1G}$	$\frac{1}{2}K_{\text{on}1}$	Rate of disassociation of an ARF1:ARF1 and the promoter
$\theta_{A_1A_2G}$	$(K_{\text{on}1}^{-1} + K_{\text{on}2}^{-1})^{-1}$	Rate of disassociation of an ARF1:ARF2 and the promoter
$\theta_{A_2A_2G}$	$\frac{1}{2}K_{\text{on}2}$	Rate of disassociation of an ARF2:ARF2 and the promoter
$\theta_{A_1A_1A_1G}$	$\frac{1}{3}K_{\text{on}1}$	Rate of disassociation of an ARF1:ARF1:ARF1 and the promoter
$\theta_{A_1A_1A_2G}$	$(2K_{\text{on}1}^{-1} + K_{\text{on}2}^{-1})^{-1}$	Rate of disassociation of an ARF1:ARF1:ARF2 and the promoter
$\theta_{A_1A_2A_1G}$	$(2K_{\text{on}1}^{-1} + K_{\text{on}2}^{-1})^{-1}$	Rate of disassociation of an ARF1:ARF2:ARF1 and the promoter
$\theta_{A_1A_2A_2G}$	$(K_{\text{on}1}^{-1} + 2K_{\text{on}2}^{-1})^{-1}$	Rate of disassociation of an ARF1:ARF2:ARF2 and the promoter
$\theta_{A_2A_1A_2G}$	$(K_{\text{on}1}^{-1} + 2K_{\text{on}2}^{-1})^{-1}$	Rate of disassociation of an ARF2:ARF1:ARF2 and the promoter
$\theta_{A_2A_2A_2G}$	$\frac{1}{3}K_{\text{on}2}$	Rate of disassociation of an ARF2:ARF2:ARF2 and the promoter
$\theta_{A_1A_1A_1A_2G}$	$(3K_{\text{on}1}^{-1} + K_{\text{on}2}^{-1})^{-1}$	Rate of disassociation of an ARF1:ARF1:ARF1:ARF2 and the promoter
$\theta_{A_2A_1A_1A_2G}$	$(2K_{\text{on}1}^{-1} + 2K_{\text{on}2}^{-1})^{-1}$	Rate of disassociation of an ARF2:ARF1:ARF1:ARF2 and the promoter
$\theta_{A_2A_1A_1A_1A_2G}$	$(3K_{\text{on}1}^{-1} + 2K_{\text{on}2}^{-1})^{-1}$	Rate of disassociation of an ARF2:ARF1:ARF1:ARF1:ARF2 and the promoter
$\mu_{A_1A_1}, \mu_{A_1A_2}, \dots$	0.05	Rate of decay of ARF oligomers
l_1	1	Rate of transcription caused by a ARF1 monomer
l_2	10	Rate of transcription for the promoter bound by an ARF1 oligomer

Table 5.3: Default parameters used for the altered Farcot models utilised in sections 5.5-5.7.

may also be viewed as a constraint to the parameterisation of the model. For no parameter set chosen does this potential issue arise.

Seeing as the time series data for ARF1 and ARF2 are discrete, to utilise it as an input into this continuous time series of ODEs a continuous approximation will need to be made (Figure 5.5). One way to do this is to approximate it with a piecewise linear function or to use smoother approximation, for instance splines. The former is an acceptable approximation but can potentially be improved upon. The spline approximation does not work very well as the spacing between data points is not even and the gap between the penultimate and last time points is large leading to unlikely fit between time 8 and 24. To try to get a smooth fit I fitted a function that is piecewise polynomial exponential to capture the initial behaviour of early peak followed by decay:

$$\text{ARF}(t) = \begin{cases} at^2 + bt + c & \text{If } t < t_r \\ at_r^2 + bt_r + c - d\exp(e(t_r)) + d\exp(e(t)) & \text{otherwise} \end{cases}$$

where t_r is the time point of the transition between the functions and a, b, c, d, e, f & t_r are all fitted variables. To better compare these continuous approximations one should consider the ratio of mpARF1 to mpARF2. This is because it is the competition between these two proteins that we are predicating the model on. As can be seen in Figure 5.5 baring the peak at around eight hours in the piecewise approximation of time series 3 the sharp changes in ratio of mpARF1 to mpARF2 are due to the data not the fit.

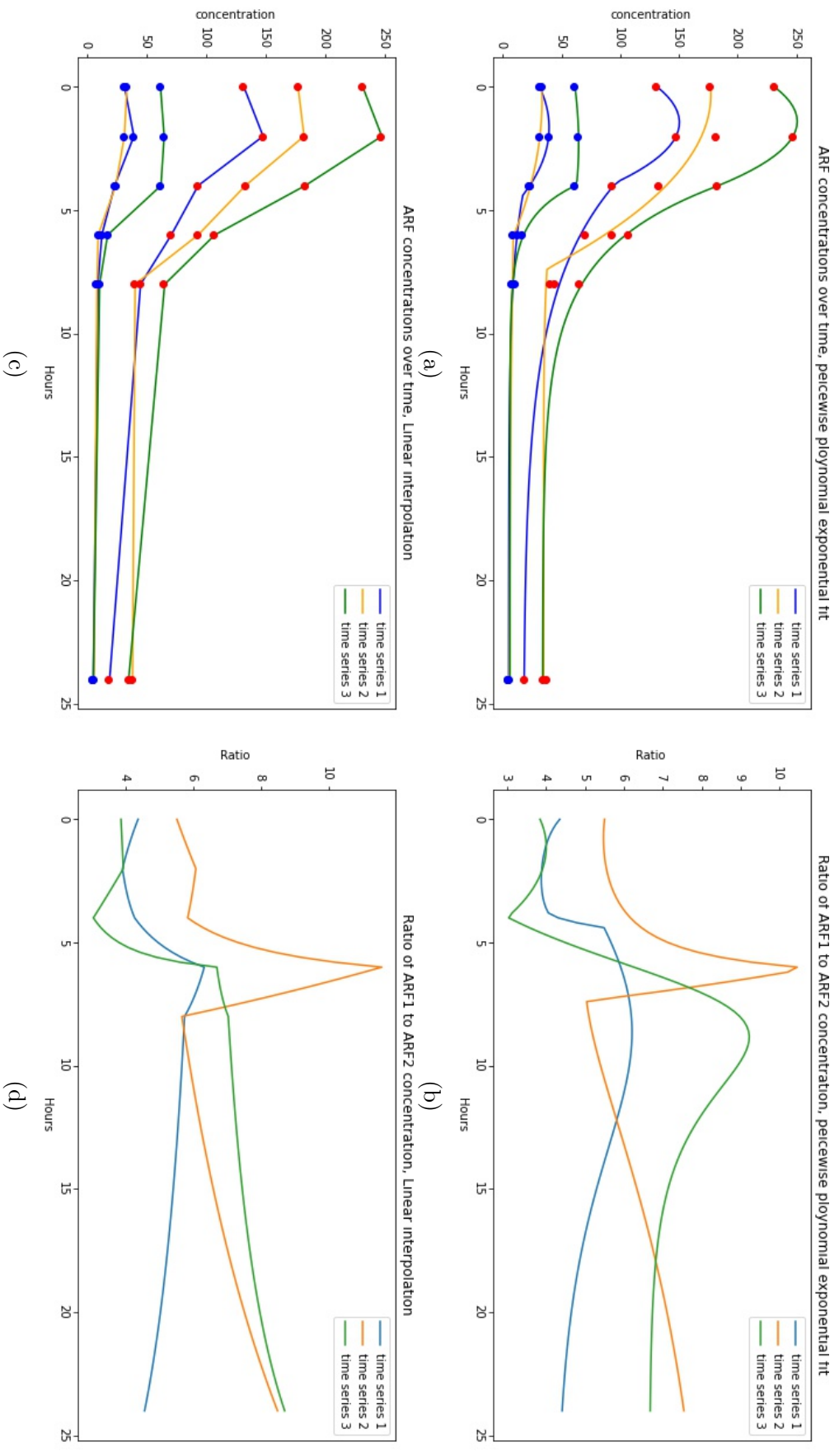


Figure 5.5: First column shows graphs of the continuous time approximations of the time series data of ARF1, denoted by red dots, and ARF2, denoted by blue dots. The second column shows the corresponding ratios between the concentrations of the ARF1 and ARF2 for each time series.

5.5.1 Conflicting Data on Kd

Whilst the literature states that the Kd values for mpARF1 and mpARF2 are 12nM and 61nM respectively, meaning mpARF2 is five times more likely to unbind from a promoter than mpARF1, these were for in vitro tests and only used segments of the proteins rather than whole proteins. As a result, they may not be representative of the actual values in vivo (private communication).

5.5.2 Interaction of ARF1 and ARF2 Ratios

As seen in Figure 5.6 the higher the ratio of ARF1 to ARF2 the lower the gradient of the curve, and as such the percentage change in transcription of the quantity of ARF1 halving from ten times ARF2 to five times ARF2 is less than were ARF1 to halve in concentration from four times being a decrease of 23% and 37% respectively. As such, one might suppose than any measure that would alter the ratio of ARF1 to ARF2 could have an effect on the degree that transcription changes over time in the model.

5.6 Addition of Longer Multimer Chains and Possible Directions

To improve the model I next decided to consider implementing multimers of increased length. Whilst this does have the downside of increasing the complexity of the model it could be quite impactful due to the aforementioned sensitivity to the ratio of ARF1 to ARF2 and the manner in which the forming of dimers would affect the ratio of ARF oligomers that promote and repress.

There are a number of ways to potentially do this. One of the key considerations in this is what oligomers are possible. Whilst we do know that ARF1 and ARF2 can both homodimerise and heterodimerise together [Kato et al., 2015], it is not clear the nature of their abilities to form longer chains. According to a charge homology model, ARF1 can bind an IAA and still oligomerise, whereas

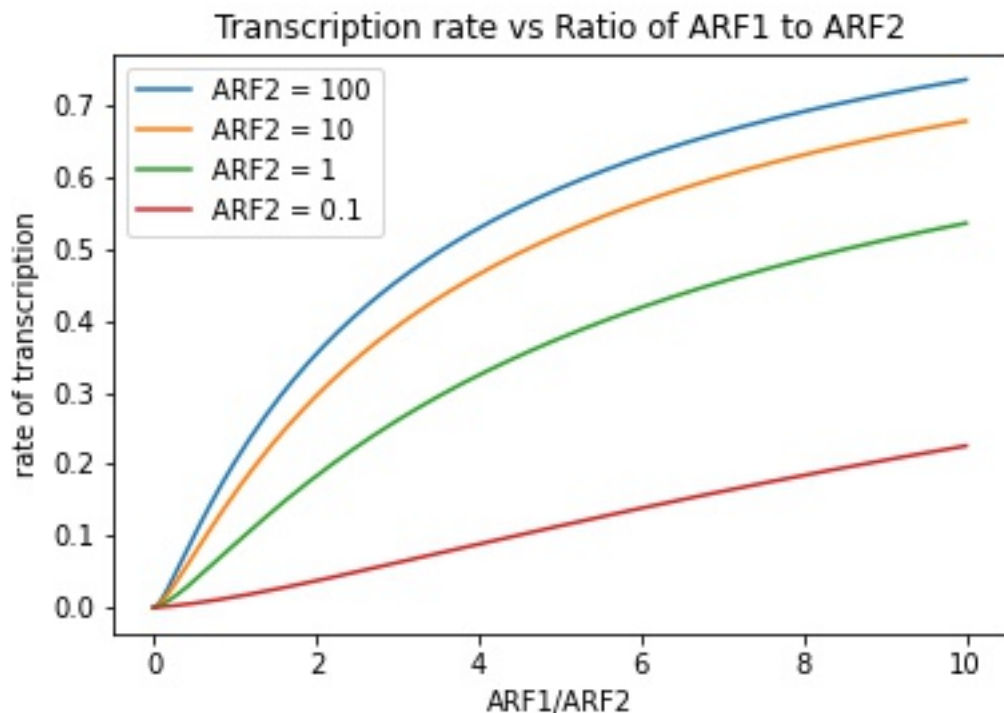


Figure 5.6: ARF2 was kept constant and ARF1 concentration was varied to see how that affected the rate of transcription for different concentrations of ARF2.

ARF2 may not be able to. This may transfer over to the binding of ARFs [Kato et al., 2020a]. To account for this I proposed two variations. In one of these variations the model will permit oligomers up to length three involving ARF1 and ARF2 in all possible permutations. In this model, due to ARF2's ability to recruit TOPLESS (a co-repressor known to pair with auxin response repressors [Szemenyei et al., 2008]), any multimers containing it will be assumed to be repressors. In the second, the only permutations allowed will be ones in which ARF2 is only binding one other element of the oligomer chain. In this instance, instead of just considering oligomers up to length three I considered all oligomer chains that contain two ARF1 molecules .

5.7 Addition of Longer Multimers

Whilst the model does have an increase in transcription as time goes on, the increase in transcription from time 0 to 24 is not very significant. This is in part

due to most of the ARFs being bound up in dimers and giving an approximately linear relationship between ARF ratios and transcription rates. As such, it is potentially significant to consider the case where ARF1 is forming oligomers of lengths greater than two whilst ARF2 is not.

To investigate this I altered the model to allow for ARF1 multimers of length three and four (models not included). The result of this was a greater disparity between initial and final levels of transcription. This is because of the assumption that ARF1 multimers of length three or four promote transcription as effectively as dimers. This should hold true for any increase of effectiveness sufficiently small so as to not offset the change in ratio of oligomers that promote vs repress. As a result of this, I proposed the models found in the following subsections. Note, to describe the meaning of each new term would be impractical due to them being too numerous. However, they are all of a similar format based on mass action.

Terms representing the binding of proteins to one another, including when one of those proteins is already bound to a receptor, or proteins binding to receptors are structured like so.

$$c\alpha XY$$

Where X and Y are the concentrations of the two molecules, or a molecule and a receptor binding together, alpha is the rate of binding between the two elements in question to make the resulting molecule, or molecule receptor complex, and c, the combination factor, is the proportion of possible ways binding sites could be arranged which leads to the formation of the specific complex. For instance, the number of permutations where an mpARF1 binds to the mpARF1 DNA binding domain of a mpARF1-mpARF2 dimer is half the number of permutations which involve an mpARF1 binding to an mpARF1 DNA binding domain of a mpARF1-mpARF1 dimer. This means c would be set to 0.5 in the first case, but 1 in the second case. Hence, to represent A_1 binding to $D_{A_1A_2}$ to create $D_{A_1A_1A_2}$ we would write

$$0.5\alpha_{A_1A_1}A_1D_{A_1A_2}$$

as found in equations A.15, A.18, A.21, A.44, A.45, A.48, A.64, A.65 & A.68. Note that if X or Y is a receptor then $c = 1$ as we assume that every element of a multimer affects how it binds to a receptor rather than modelling the interaction as though only one ARF within a multimer binds to the receptor.

Similarly to represent the reverse, the disassociation of two parts of one of these complexes, we write something in the form

$$c\theta X$$

where θ is the disassociation rate of the bond that is breaking, c , the permutation factor, is the number of ways a bond of that form breaks resulting in the same resulting elements and X is the complex that is breaking down. For example, the term for $D_{A_2A_1A_2}$ breaking down into A_2 and $D_{A_1A_2}$ is expressed as

$$2\theta_{A_1A_2}D_{A_2A_1A_2}$$

as found in equations A.16, A.18, A.27, A.42, A.45, A.46, A.62, A.65 & A.66. This is because there are two A_1A_2 bonds that could break resulting in an A_2 and an $D_{A_1A_2}$.

The final type of term added is one that represents the degradation of a molecule and is of the form

$$\mu X$$

where μ is the rate of degradation of the molecule X . Terms of this form are found in all equations of multimers e.g. the first term in equation A.17.

However, for a molecule binding to a receptor, the alpha term is slightly different because it represents all the possible ways that molecule can bind to the receptor as well as the potential for all of the component parts to in some way affect its disassociation coefficient. For example $D_{A_2A_1A_2}$ binding a receptor would take the form

$$\alpha_{A_2A_1A_2G}D_{A_2A_1A_2}G$$

and the unbinding of the oligomer from the receptor would take the form

$$\theta_{A_2A_1A_2}G_{A_2A_1A_2}.$$

The two example terms above can be found in equations A.27,A.38, A.46, A.55, A.66 & A.78.

5.7.1 ARF Oligomers of Length 3

The first of the models that permit longer oligomers is one that allows for trimers, adding oligomer state variables: $D_{A_1A_1A_1}$, $D_{A_1A_1A_2}$, $D_{A_1A_2A_1}$, $D_{A_1A_2A_2}$, $D_{A_2A_1A_2}$, $D_{A_2A_2A_2}$ which represent the 6 distinct oligomers formed of 3 ARFs and their promoter bound by oligomer state variables $G_{A_1A_1A_1}$, $G_{A_1A_1A_2}$, $G_{A_1A_2A_1}$, $G_{A_1A_2A_2}$, $G_{A_2A_1A_2}$, $G_{A_2A_2A_2}$ (Full model found in Appendix A.4.2).

5.7.2 ARF1² Model

The second of the models that permit longer oligomers was predicated on the possibility that mpARF2 may not be able to form homo-oligomers over length two as discussed in Section 5.6. As a result, this model adds the following oligomer state variables: $D_{A_2A_1A_2}$, $D_{A_1A_1A_2}$ and $D_{A_2A_1A_1A_2}$ along with their promoter bound by oligomer state variables $G_{A_2A_1A_2}$, $G_{A_1A_1A_2}$ and $G_{A_2A_1A_1A_2}$ (Full model found in Appendix A.4.3).

5.7.3 ARF1³ Model

Due to the increased antagonism of ARF1 by ARF2 seen in the ARF1² model I decided to take the model a step further allowing for the formation of ARF1 chains of length three. This allows for a further improved ability for ARF2 to bind a higher ratio of ARF1 into a molecule that will repress transcription. As such, I added the following oligomer state variables: $D_{A_2A_1A_2}$, $D_{A_1A_1A_2}$, $D_{A_2A_1A_1A_2}$, $D_{A_1A_1A_1}$, $D_{A_1A_1A_1A_2}$ and $D_{A_2A_1A_1A_1A_2}$ along with their promoter bound by oligomer state

variables: $G_{A_2A_1A_2}$, $G_{A_1A_1A_2}$, $G_{A_2A_1A_1A_2}$, $G_{A_1A_1A_1}$, $G_{A_1A_1A_1A_2}$ and $G_{A_2A_1A_1A_1A_2}$ (Full model found in Appendix A.4.4).

5.7.4 Comparison of Models

For the purposes of this section we will be referring to the initial model utilising initial data as model 1, the model that allows oligomers up to length three as model 2, the model that allows for homo-oligomers of mpARF1 up to length two as model 3 and the model that allows for homo-oligomers of mpARF1 up to length three as model 4. The discussion is based on Figures 5.7, 5.8 and 5.9 and in Figures 5.7 & 5.8 (a), (b), (c) and (d) correspond with models 1, 2, 3 and 4.

To commence the comparison, we ran simulations with multiple Kon and Koff combinations for each model and each of the three pieces of time series ARF data (Figure 5.7). A comparison of transcription rates in simulations of the models shows that qualitatively the four models perform similarly, one exception to this being the way that the model 1 and model 3 have a sharper trough at approximately seven hours. The reason for this can be seen in Figure 5.8 where there is a greater disparity between rates of transcription for ARF2 concentrations varying between 100 and 10 for models 1 and 3 than for 2 and 4.

Quantitatively, as may be seen in Figure 5.9.a, outside of model 2 for parameter set a (as defined in the figure), model 3 has a significantly larger increase in rate of transcription during the first 12 hours. This is likely due to the increased potential for mpARF2 proteins to bind mpARF1 homo-oligomers rather than oligomers that already contain a mpARF2. Furthermore, this is in addition to the ability for a single mpARF2 molecule to be bound to and repress three mpARF1 rather than just two as seen in models 2 and 3, thus causing the disparity between model 3 and 4.

The differences in how the modelling choices affect the ratio of oligomers that promote versus repress transcription can be clearly seen in Figure 5.8. Here one can see how the reduced chance for a mpARF2 monomer to bind a mpARF1

homo-oligomer affects the rate of transcription for different ratios of mpARF1 to mpARF2, allowing for changes in ratio to have a more pronounced effect on transcription for higher ratios of mpARF1 to mpARF2. Furthermore, as may be seen in Figure 5.9.b the level of increase for model 4 is relatively unaffected by the ratio of mpARF concentration to quantity of promoter. Note that as shown in Figure 5.5.b, the ratio of mpARF1 to mpARF2 varies between approximately ten and four and that as shown in Figure 5.8 for these ratios the plot of transcription vs ratio is approximately linear, particularly for a more acute ratio between mpARF and quantity of promoter.

5.8 Conclusion

In this chapter, conventional models for auxin signalling by Middleton and Farcot were altered to model auxin signalling in *M. polymorpha*. This was done because, much like the auxin signalling models in question, *M. Polymorpha* only has one AUX/IAA like the models. However, unlike the models, *M. Polymorpha* has three ARFs whereas the aforementioned models have one, though this is still significantly less than many other plants like *Arabidopsis* and, as a result, does not constitute a very significant increase in complexity to model.

To serve our purpose they were extended so as to contain an extra repressor ARF. Thus, the model represented the auxin sensitive promoter mpARF1 and the repressor ARF that binds the same DNA sites, mpARF2. Ultimately, little was gained by trying to fit these models to experimental data. The data available were specific to recently activated gemmae, a system that while initially low in TIR1 levels has negligible mpIAA despite being in production. This implies additional systems are likely controlling its degradation, but cannot be modelled explicitly as their exact nature is currently unknown.

The second half of the chapter focused on utilising time series data as an input into variations of the first model in this chapter. Due to the absence of mpIAA, much of the auxin signalling elements were removed, instead focusing on the

antagonisation between mpARF1 by mpARF2. This was explored through considering possible oligomerisation of the ARFs. Allowing for an increase in length of possible oligomers from two to three caused a uniform increase in percentage change in rate of transcription over the first 12 hours. Other models that looked at a scenario where mpARF2 cannot bind to other proteins simultaneously, while mpARF1 can, showed an even more stark increase in rate of transcription over the first 12 hours. However, while mpARF2 can antagonise mpARF1 quite consistently, whilst its disassociation coefficient is within an order of magnitude of that of mpARF1 as observed, it does not seem able to halt transcription. From this we conclude that a simple mass action model that utilises only the current known set of interactions is not able to replicate the predicted lack of ARF promoted transcription.

Whilst little attention has been given in the literature to ARF3, likewise we did not give it any consideration within our models, the fact that it can bind IAA and other ARFs means it may affect the system. If it can bind either mpIAA, mpARF1, or mpARF2 with affinity comparable to the binding affinities the other ARFs have with one another and IAA, it may be able to antagonise mpARF1 or mpARF2 enough that it may be an important element of a stoichiometric model of ARF1 driven transcription. As such I would welcome further investigation into its binding affinities and its concentration over time and, dependent on results, its inclusion into auxin signalling modelling.

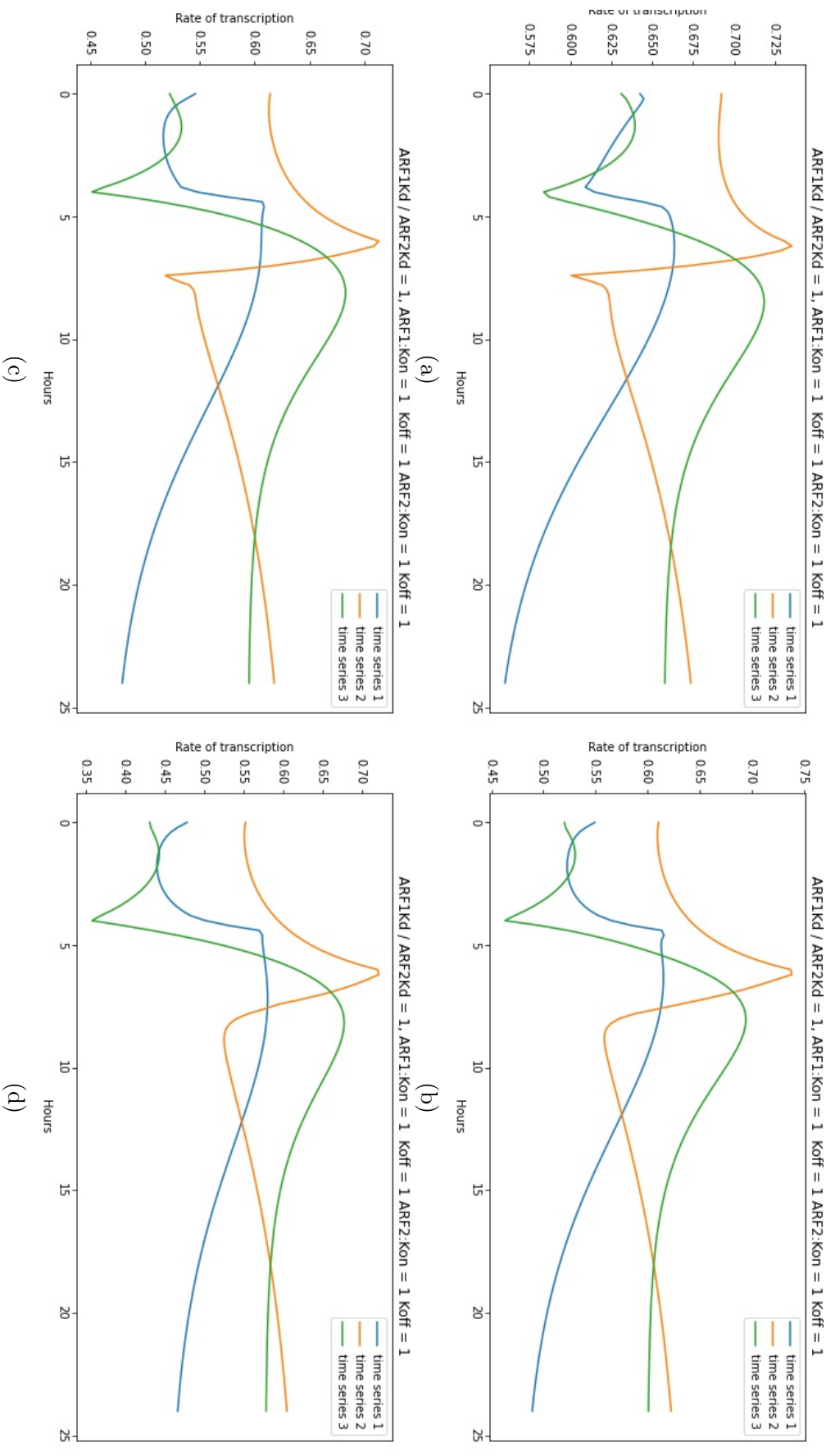


Figure 5.7: Graphs showing time series data for rate of transcription for the four models explicitly using mpARF data presented in this chapter. (a) shows results from the initial model, (b) the model with oligomers of length three, (c) the model with mpARF1 oligomers up to length two, (d) the model with mpARF1 oligomers up to length three.

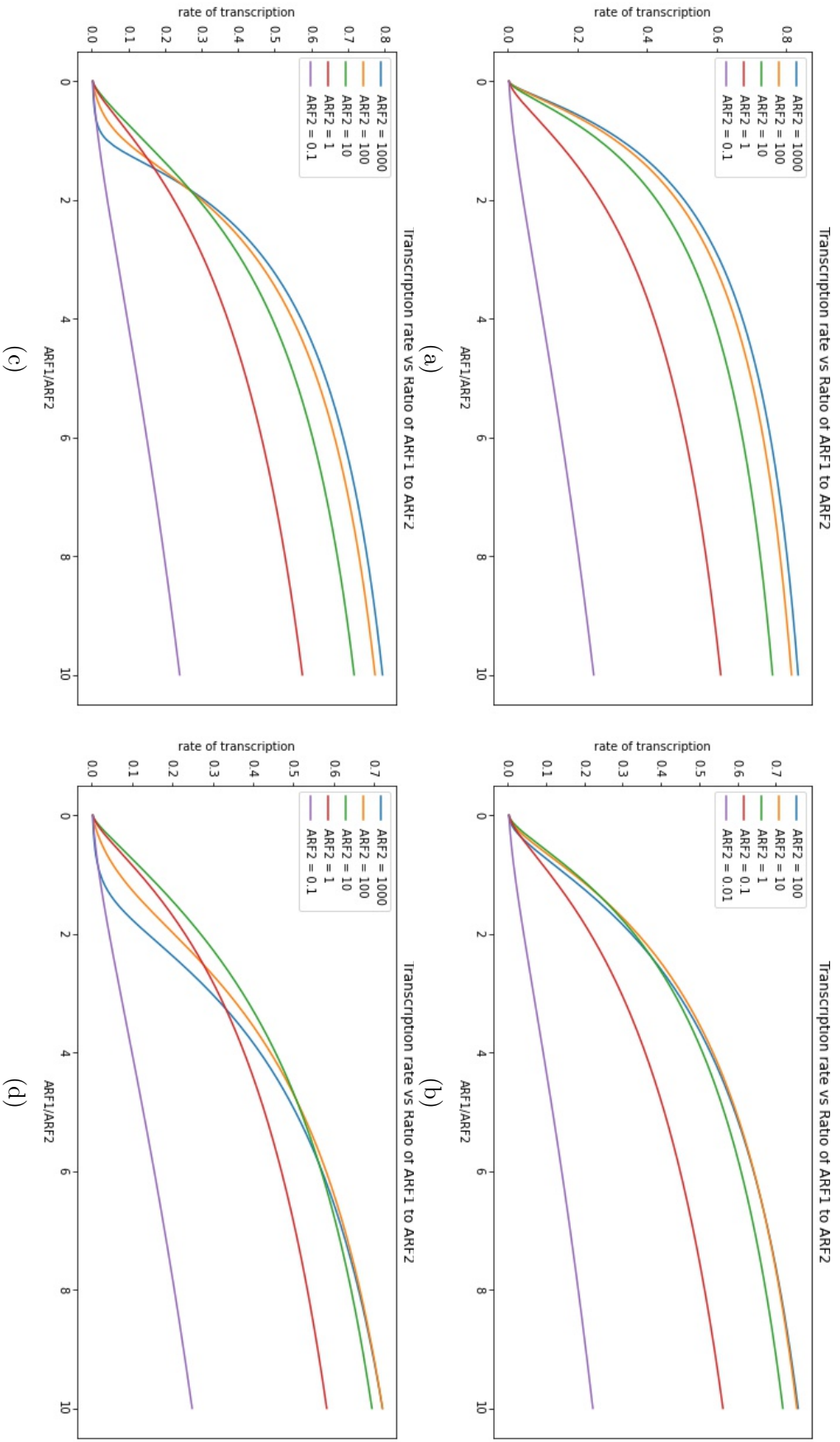
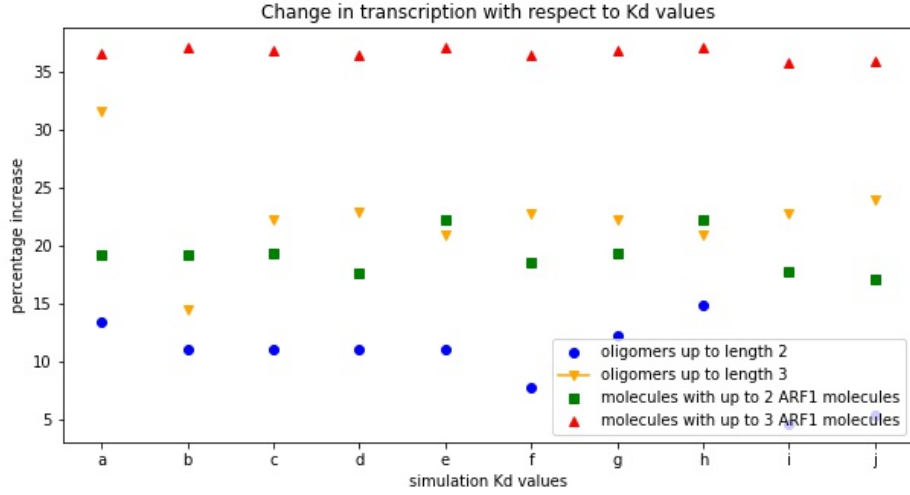
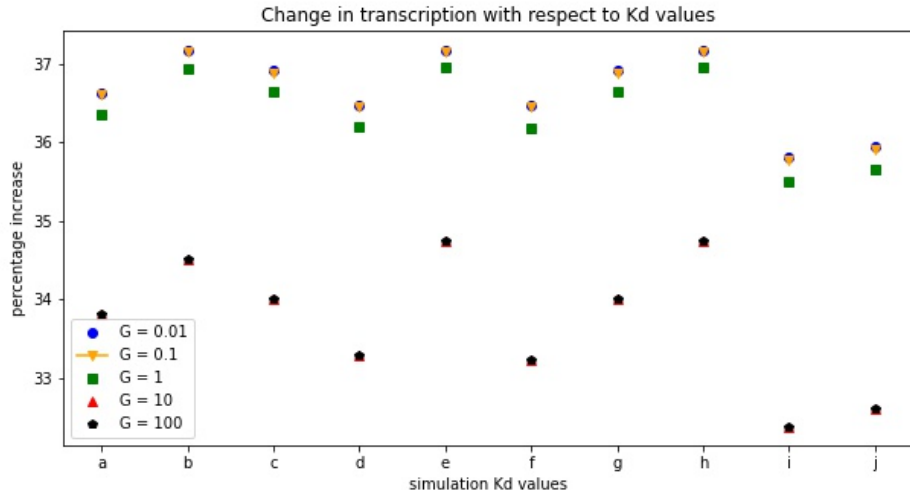


Figure 5.8: Graphs comparing rate of transcription for different ratios of mpARF1 to mpARF2 for different concentrations of mpARFs, for the four models explicitly using mpARF data presented in this chapter. (a) shows results from the initial model, (b) the model with oligomers of length three, (c) the model with mpARF1 oligomers up to length two, (d) the model with mpARF1 oligomers up to length three.

$\text{ARF1:Kd} = 1$, $\text{ARF1:Kon} = 1$, $\text{Koff} = 1$, $\text{ARF2:Kon} = 1$, $\text{Koff} = 1$. Total promoter concentration set to 0.1



(a)



(b)

Figure 5.9: Graphs showing percentage change between rate of transcription when $t = 0$ and the highest rate of transcription across the 24 hours. (a) is done for a total concentration of promoters = 0.1. (b) use the molecules with up to three ARF1 molecules . Kd values:

- a - $\text{ARF1Kd} / \text{ARF2Kd} = 4$, $\text{ARF1:Kon} = 1$ $\text{Koff} = 1$ $\text{ARF2:Kon} = 1$ $\text{Koff} = 4$
b - $\text{ARF1Kd} / \text{ARF2Kd} = 4$, $\text{ARF1:Kon} = 1$ $\text{Koff} = 1$ $\text{ARF2:Kon} = \frac{1}{4}$ $\text{Koff} = 1$
c - $\text{ARF1Kd} / \text{ARF2Kd} = 4$, $\text{ARF1:Kon} = 1$ $\text{Koff} = 1$ $\text{ARF2:Kon} = \frac{1}{2}$ $\text{Koff} = 2$
d - $\text{ARF1Kd} / \text{ARF2Kd} = 4$, $\text{ARF1:Kon} = \frac{1}{2}$ $\text{Koff} = 2$ $\text{ARF2:Kon} = \frac{1}{4}$ $\text{Koff} = 4$
e - $\text{ARF1Kd} / \text{ARF2Kd} = 4$, $\text{ARF1:Kon} = 2$ $\text{Koff} = \frac{1}{2}$ $\text{ARF2:Kon} = \frac{1}{2}$ $\text{Koff} = 2$
f - $\text{ARF1Kd} / \text{ARF2Kd} = 1$, $\text{ARF1:Kon} = 1$ $\text{Koff} = 1$ $\text{ARF2:Kon} = 1$ $\text{Koff} = 1$
g - $\text{ARF1Kd} / \text{ARF2Kd} = 4$, $\text{ARF1:Kon} = 1$ $\text{Koff} = 1$ $\text{ARF2:Kon} = 2$ $\text{Koff} = \frac{1}{2}$
h - $\text{ARF1Kd} / \text{ARF2Kd} = 4$, $\text{ARF1:Kon} = \frac{1}{2}$ $\text{Koff} = 2$ $\text{ARF2:Kon} = 1$ $\text{Koff} = 1$
i - $\text{ARF1Kd} / \text{ARF2Kd} = \frac{1}{4}$, $\text{ARF1:Kon} = 1$ $\text{Koff} = 1$ $\text{ARF2:Kon} = \frac{1}{2}$ $\text{Koff} = 2$
j - $\text{ARF1Kd} / \text{ARF2Kd} = \frac{1}{4}$, $\text{ARF1:Kon} = 2$ $\text{Koff} = \frac{1}{2}$ $\text{ARF2:Kon} = 1$ $\text{Koff} = 1$

Chapter 6

Discussion

6.1 Discussion of Results from Chapter 3

In Chapter 3 we studied the effect of growth on the Smith model, a concentration-based auxin transport model. We then investigated how robust the behaviours that arose were to noise and attempted to juxtapose them to similar behaviours that may be found in the Swift-Hohenberg model. By introducing growth to the Smith model on a file of cells, we observed for a subset of parameter space oscillations in auxin concentration. These oscillations were induced by the change in the domain caused by growth interacting with the balance between diffusion and the active transport of auxin.

However, it should be noted that these oscillations were not wholly robust to stochastically determined cell division ratios. Therefore, the mechanics governing how non-homogeneous cell volumes affect the formation of peaks appears to be complex. However, although it may be difficult to find an equivalent to the cell volume changes, of a discrete model, in a continuous model, we did find a comparable result via changing a 'density' term in the Swift-Hohenberg equations. This may imply a generality to the mechanism we have uncovered. Furthermore, the idea of oscillations in the auxin signalling system are not new, as prior to

this two biology papers [De Smet et al., 2007, Moreno-Risueno et al., 2010b] demonstrated that oscillations in downstream elements were affected by auxin.

Nonetheless, there have been few attempts at investigating the interaction between growth and auxin transport, especially utilising a concentration-based model. Though a more recent paper [Allen and Ptashnyk, 2020] showed that by implementing an auxin signalling model that featured oscillations, those oscillations would be preserved when they were implemented for each cell in a flux-based auxin transport model. In addition, [van den Berg et al., 2021] investigated auxin transport on a growing domain, albeit a more complex one (including different cell types with realistic shapes) and found oscillating concentrations of auxin. However, their model preallocates PIN polarity, whereas our model allows PIN polarity to alter dynamically which we believe to be important as the feedback between auxin distribution and PIN distribution is integral to auxin patterning. It should be mentioned that they tested a variant where the PIN concentration was dependent on the auxin concentration within an individual cell. Nonetheless, in this variant only the total concentration of PINs within a cell changed not its distribution. Furthermore, whilst they did allow for some cells to be out of sync with one another they limited this at 5%, whereas we considered far greater levels of asynchronism with respect to the cell cycle.

However, the research performed within Chapter 3 is not without its limitations. Firstly, the modelling takes place on a one dimensional domain with different properties than a two or three dimensional domain, although this was done to reduce both complexity and computational cost. Secondly, at this stage we do not look at any other type of auxin signalling model, so none of the above results include behaviours such as canalisation, as seen in flux-based models. It is also worth noting that in the section where we consider the effect of noise on the system, we only consider it with respect to cell division ratios and not the threshold for cell divisions themselves. Finally, though done to keep the model simple, and to explore the implications of other modelling decisions before in-

creasing complexity, the model does not consider the potential for auxin to affect the rate of growth of a cell. Time allowing, all these aspects would have been interesting to explore and will hopefully provide topics of future research.

Moreover, unlike all but one of the aforementioned oscillations, the one we study is driven by growth itself, and unlike previous works it is done in a model where auxin drives the PIN distribution, as is known to happen in real plants. Thus, the findings we generated by examining the nature of auxin in auxin transport models has lead us on to two important areas for biologists to consider. Firstly, though delayed by covid, we have initiated experiments examining the interaction between oscillations in auxin outputs and the rate of root growth. These experiments are important as we may learn of a new factor in auxin signalling or we may be able to rule out growth as a factor. They are, to date, on-going experiments and have not led yet to a definite conclusion. Secondly, we have investigated the interaction between transport induced oscillations and auxin signalling. Since modelling indicates both these oscillations may exist, it is important to understand their interplay. Thus, it would be interesting to see how well these findings generalise to other auxin transport models, specifically a flux-based model or a hybrid model.

Finally, in Chapter 3 after introducing growth to the Smith model we utilised continuation to plot bifurcation diagrams for different stages within the growth cycle. By overlaying these bifurcation diagrams, we were able to clearly explain that the cause for the oscillations in auxin concentration was the manner in which two steady states gained and lost stability as the domain deformed. This technique has been something that we have not seen in our review of model analysis, particularly in the area of auxin transport. It is our belief that this technique, along with more use of continuation as part of steady state analysis, can lead to greater understanding in model analysis of systems undergoing parameter change. Potentially it could be implemented in the analysis of growth on a flux-based auxin transport model. However, one limitation with respect

to generalising this technique to other systems is the potential difficulty in finding a bifurcation parameter, that through continuation, can sufficiently map the relevant steady states.

6.2 Discussion of Results from Chapter 4

In Chapter 4 we looked at the interactions between auxin signalling and oscillatory auxin concentration. Specifically, we looked at the case of the Middleton *et al.* model parameterised to contain a limit cycle and introduced oscillating levels of auxin influx, due to auxin transport or otherwise. We showed that strictly speaking there is no resonance between our oscillating input of auxin and the concentration of unbound ARFs, or rate of transmission. However, the system is only sensitive to auxin for a small proportion of each period. Thus, the amplitude of oscillations for transcription and unbound ARF concentration are highest when the period of the auxin oscillations are close to the period, a fraction of the period, or a multiple of the period of the signalling pathway. In fact, much like with resonance for auxin pulses as described above, the periodicity of the auxin signalling system will conform to that of the auxin pulses. This shows the potential for the auxin pulses to strongly affect oscillations in the auxin signalling system. Furthermore, it also allows for a greater tolerance for the two systems to interact, something that is potentially relevant considering the potential for different rates of root growth, e.g. in different environmental conditions.

The results of the first half of the chapter raise a series of important questions. For example, should it be the case that there are oscillations in auxin concentration and that cell growth and auxin transport induce oscillating auxin concentrations, how might this interact with auxin signalling in an experimentally observable sense? In addition, how might affecting the rate of growth affect DR5 reporter levels, which track ARF driven transcription, in comparison to DII reporters, which more directly represent auxin through tracking auxin driven degradation? For instance, would there be experimentally observable disruptions

to the regularity or amplitude of the auxin signalling system? We hope that the on-going experimental work of our colleagues will help answer some of these questions.

In the same chapter we looked at how changes in auxin concentration affected the Farcot auxin signalling model, which is another auxin signalling model that does not exhibit a limit cycle. Again, we measured the effect in two ways, the concentration of ARFs not bound by AUX/IAA and the rate of transcription. Whilst, unlike with the Middleton *et al.* model, there was no issue with the auxin oscillations syncing up with those of the system, we did find that for the parameter sets set out by the authors, time was still an important factor. Specifically, we found that if an auxin peak is sufficiently steep the concentration of unbound ARFs, or the rate of transcription, will temporarily exceed the level they would reach were the auxin concentration constant at a level equal to the auxin peak. This implies that oscillations in auxin may be amplified in the auxin signalling system.

One limitation that is notable in both halves of the chapter is that the auxin signalling system is affected by the auxin transport system, but not vice versa. This is an interesting area for future investigation as it poses the question of whether the auxin signalling system would have a sufficiently considerable effect within a cell to affect distribution. Furthermore, is it possible that in the case of the oscillations in the Middleton *et al.* model, there is a threshold for some parameters such that the natural periodicity becomes dominant in the system? This idea and others could be tested by setting the rate of growth to different values on a model where auxin concentration affects cell growth. It also poses the question of how well oscillations in the auxin signalling system would sync up across multiple cells and what affect that might have.

Prior to this there has been little research done on the interaction between auxin signalling and auxin transport. Of the two papers we could find studying this interaction, one focused more on how auxin signalling might affect auxin

distribution [Allen and Ptashnyk, 2020], and the other appeared limited in the depth to which it varies the auxin input [Perianez-Rodriguez et al., 2021]. Therefore, it could not showcase the ability of the auxin signalling system to conform to periodic auxin input. In addition, the auxin signalling model utilised was phenomenological and did not follow the reaction scheme for the pathway, as it appeared to include a term where the rate of dimerisation of the ARF-AUX/IAA dimer was auxin dependent.

Thus, potential courses for future action include looking at how the system handles a less regular auxin input, such as one generated in Chapter 3 with irregular cell divisions. This is important because up until this point only single auxin pulses, or those of constant period, have been utilised and this is not necessarily biologically realistic. Furthermore, as mentioned in the chapter’s conclusion, it would be interesting to analyse a periodic input into an auxin signalling model that was a hybrid including the respective benefits of the Middleton and Farcot models. Therefore, one might consider analysing a subset of the auxin signalling models proposed in Chapter 5 with an oscillating auxin concentration.

6.3 Discussion of Results from Chapter 5

In Chapter 5 we focused on modelling auxin signalling in a manner specific to *Marchantia*, and this took two forms. The first form was an intent to develop a model in which we would combine two generic auxin signalling models, as they both had features the other did not. This new model was then to be fitted to the ARF data we received from Wageningen. However, during the process we were repeatedly halted due to miscommunication and new information. Thus, the furthest this got was a modified version of the Farcot auxin signalling model which contained feedback for the ARF concentrations. Nonetheless, although there is currently an absence of *Marchantia* specific data, it might also be worth continuing work on such a model. For example, one potential avenue is to take a more systematic attempt by looking at the importance of adding different terms

to the multi-ARF model.

The second form the research took was to create a reduced version of this model which was simplified to only contain terms relevant to the first hours of a gemma leaving the gemma cup. The model was also altered so that ARF concentration was used as an input, replicating the time series we received from Wageningen. Here we focused on how the antagonising of mpARF1 by mpARF2 affected transcription, and during this process we investigated the affect different oligomerisation schemes had on the interaction between the two ARFs. The results showed that allowing longer oligomers increased the effect mpARF2 had in antagonising mpARF1. This was especially true in the cases where mpARF2 was only able to bind to one other protein molecule at a time. Furthermore, whilst this demonstrated that understanding the oligomerisation of proteins in the *Marchantia* auxin signalling pathway is important, none of this was sufficient in halting ARF driven transcription as thought to be the case in gemmae. As a result, we hypothesise that, for at least a short time after a gemma leaves the gemma cup, there are other external, unidentified, elements producing the behaviour observed in *Marchantia*.

The primary limitations of this work are in the lack of certainty in the interactions being modelled. As a result, future work in this area should first involve more investigations into proteins present at this time. This should take the form of what other proteins are present and experiments to glean more about the oligomerisation of currently know proteins. We believe this could lead to a more complete understanding of the auxin signalling pathway.

Moreover, the decision to remove all aspects of the system related to mpIAA and its repression of mpARF1 also has the effect of making the system limited in the scope to which it models. Therefore, another important future area of study is the point of transition where it is believed that IAA takes over as the primary repressor rather than mpARF2.

Finally, another area clearly noticeable during our investigation was the degree

of variation between the concentrations of the ARFs. Thus, it would be quite beneficial to have an increased data set looking at more than three separate roots, potentially with time points between 8 hours and 24 hours as this poses a difficulty in terms of interpolation and data fitting. In addition, we think that, after studying the antagonising of mpARF1 by mpARF2, mpARF3 warrants further attention as to whether it has comparable affinity to the other auxin signalling proteins, and if so, if it is in sufficient concentrations to have a significant affect on the system.

6.4 Conclusion

Overall, this thesis investigates the dynamics inherent in already existing auxin signalling and auxin transport models. This is done by considering a concentration-based auxin transport model on a growing domain, by studying how oscillations in auxin concentration can affect auxin signalling models, and by looking at how current auxin signalling models can be used to model auxin signalling in *Marchantia*.

We found out that cell growth and division can cause oscillations in auxin concentration within a concentration-based auxin transport model. In the process of this, we developed a method that we believe to be novel in this space, to understand these oscillations. We found that oscillations in auxin concentration can alter the period of oscillations in an auxin signalling system and exhibit behaviour similar to resonance. Finally, we found out that the current understanding of auxin signalling in *Marchantia* is either lacking with respect to having a full list of relevant elements, or to how the known auxin signalling elements interact with one another, or possibly both.

These findings have important implications for understanding the cause for perceived oscillations in auxin, a hormone whose behaviour is at the heart of plant growth. They are also important in directing research into how auxin signalling works in general, due to *Marchantia* being an ancestral plant, and as such, a model

organism for the understanding of auxin signalling in all plants. Future research is also needed to better understand how auxin signalling and auxin transport interact.

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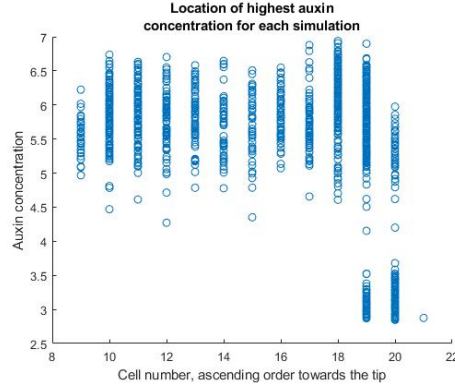
Appendices

Appendix A

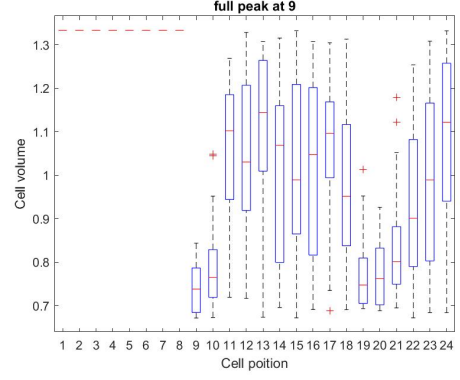
Additional Figures and Plots

A.1 Box and Whisker Plots for Stochastic Domains

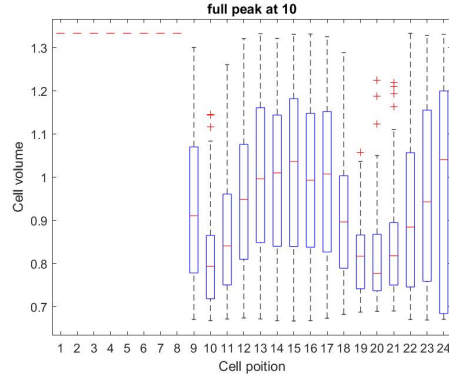
Box and whisker plots for Section 3.2.7



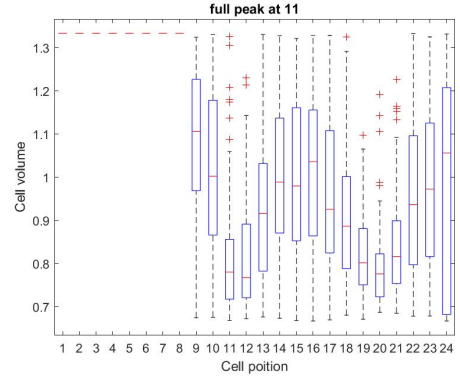
(a) Positions of Highest peaks



(b) Full peak at cell 9

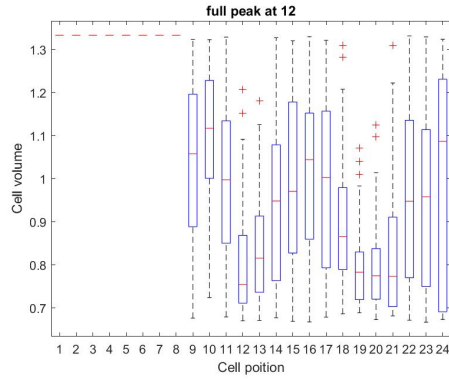


(c) Full peak at cell 10

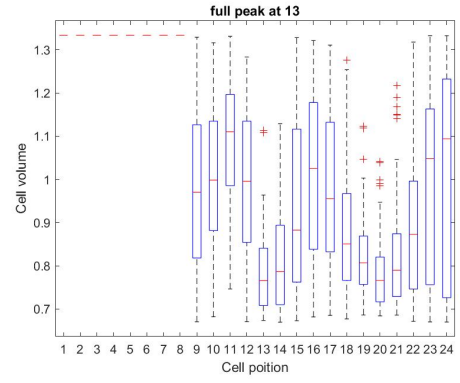


(d) Full peak at cell 11

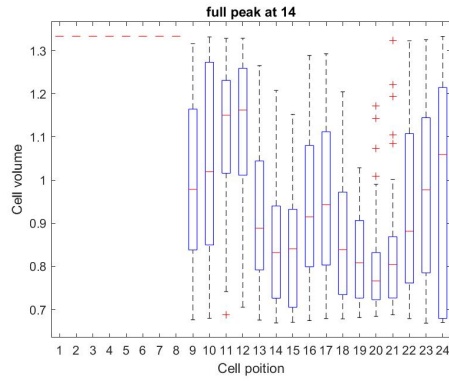
Figure A.1: (a) contains a plot of the locations of the highest peaks for 10,000 simulations on stochastic generated domains as described in Section 3.2.7. Concentration is plotted on the y-axis and cells numbered in ascending order towards the root tip on the x-axis. (b)-(p) Box and whisker plots with cell volume in the y-axis and cells numbered in ascending order towards the root tip on the x-axis. Each plot displays average cell volumes at the time the highest peak was recorded for a simulation, if the peak occurred in the cell corresponding to the graph. Secondly if the highest peak does not past the threshold concentration of four it is labelled a proto peak and displayed in a separate graph.



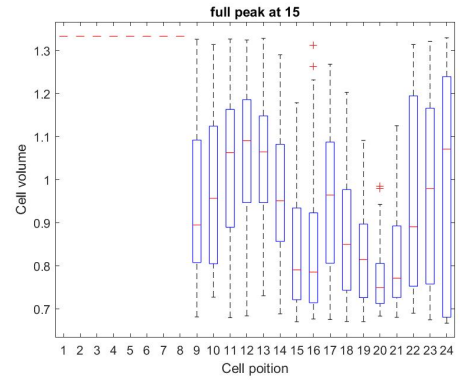
(e) Full peak at cell 12



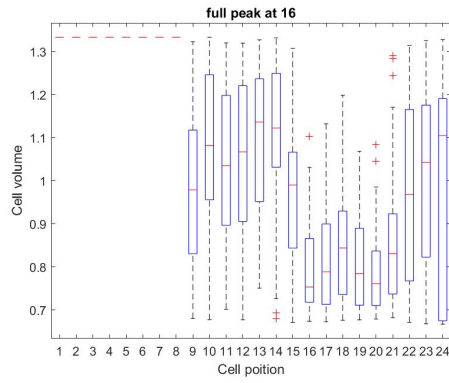
(f) Full peak at cell 13



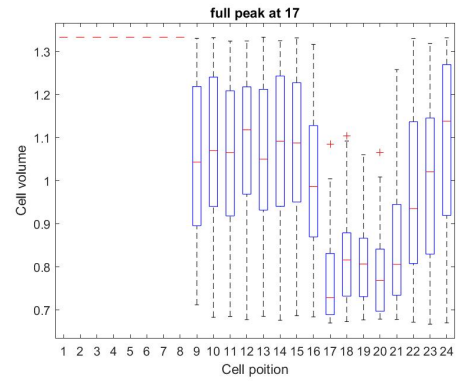
(g) Full peak at cell 14



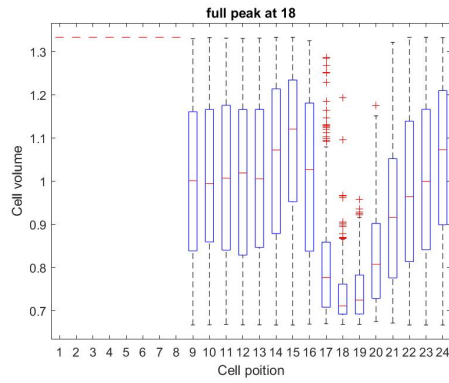
(h) Full peak at cell 15



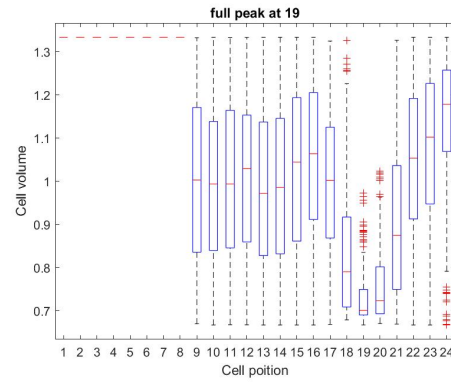
(i) Full peak at cell 16



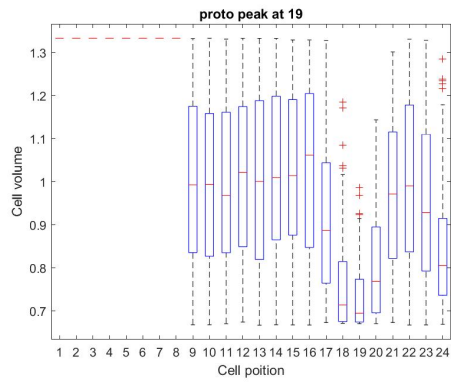
(j) Full peak at cell 17



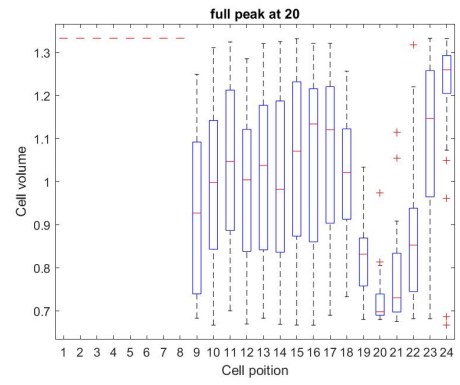
(k) Full peak at cell 18



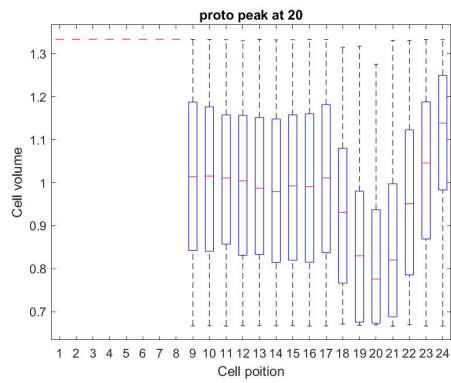
(l) Full peak at cell 19



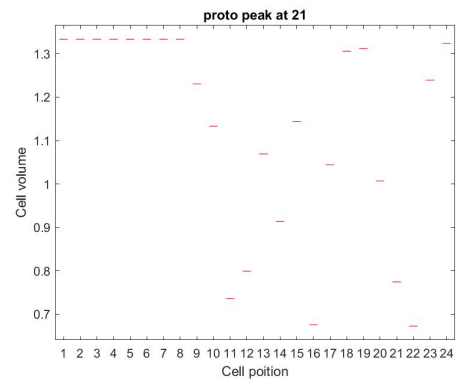
(m) Proto peak at cell 19



(n) Full peak at cell 20



(o) Proto Peak at cell 20



(p) Proto Peak at cell 21

A.2 Time Series Data used in Section 3.4

This subsection contains the full time series data for a simulation used to show DII:Venus data looks similar to the Smith model with growth and noise. The parameters for the simulation are the same as used in the beginning of Section 3.2.6, with the exception of cell growth rate being increased by a factor of ten and $T = 2.1$ instead of 1.9.

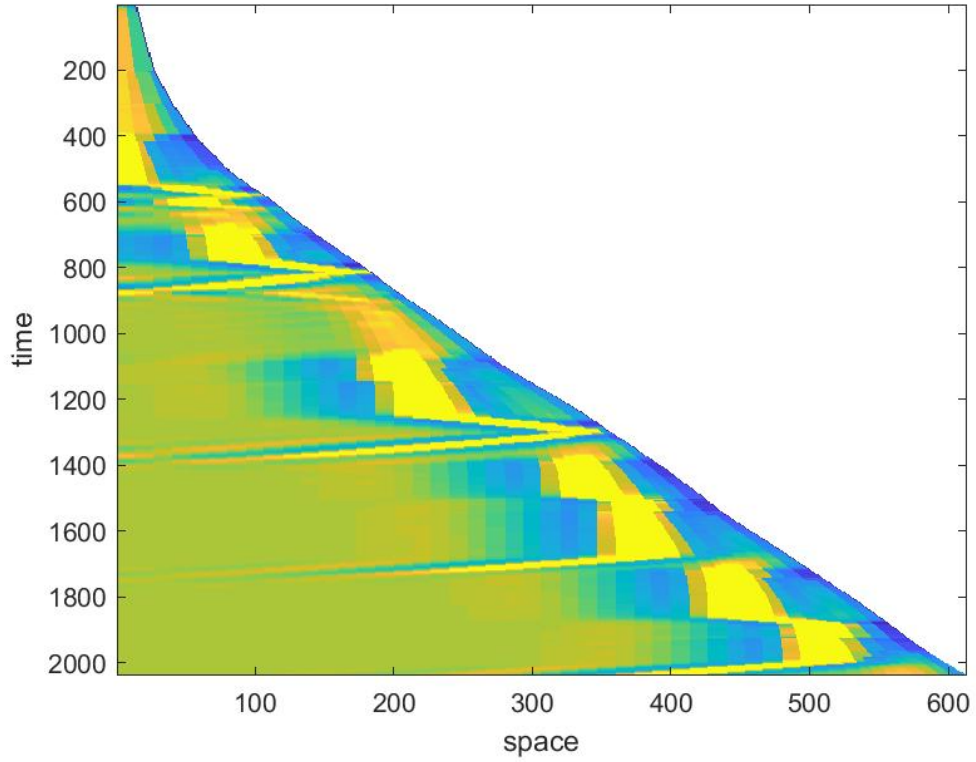


Figure A.2: Graph showing the full time series data utilised in the Section 3.4 and Figure 3.16

A.3 Graphs of Farcot Auxin Signalling Model from Chapter 4

This section contains graphs from Sections 4.4.1 and 4.4.2. The following figures show data for all 25 combinations of α & θ of which graphs in the main text are a subset.

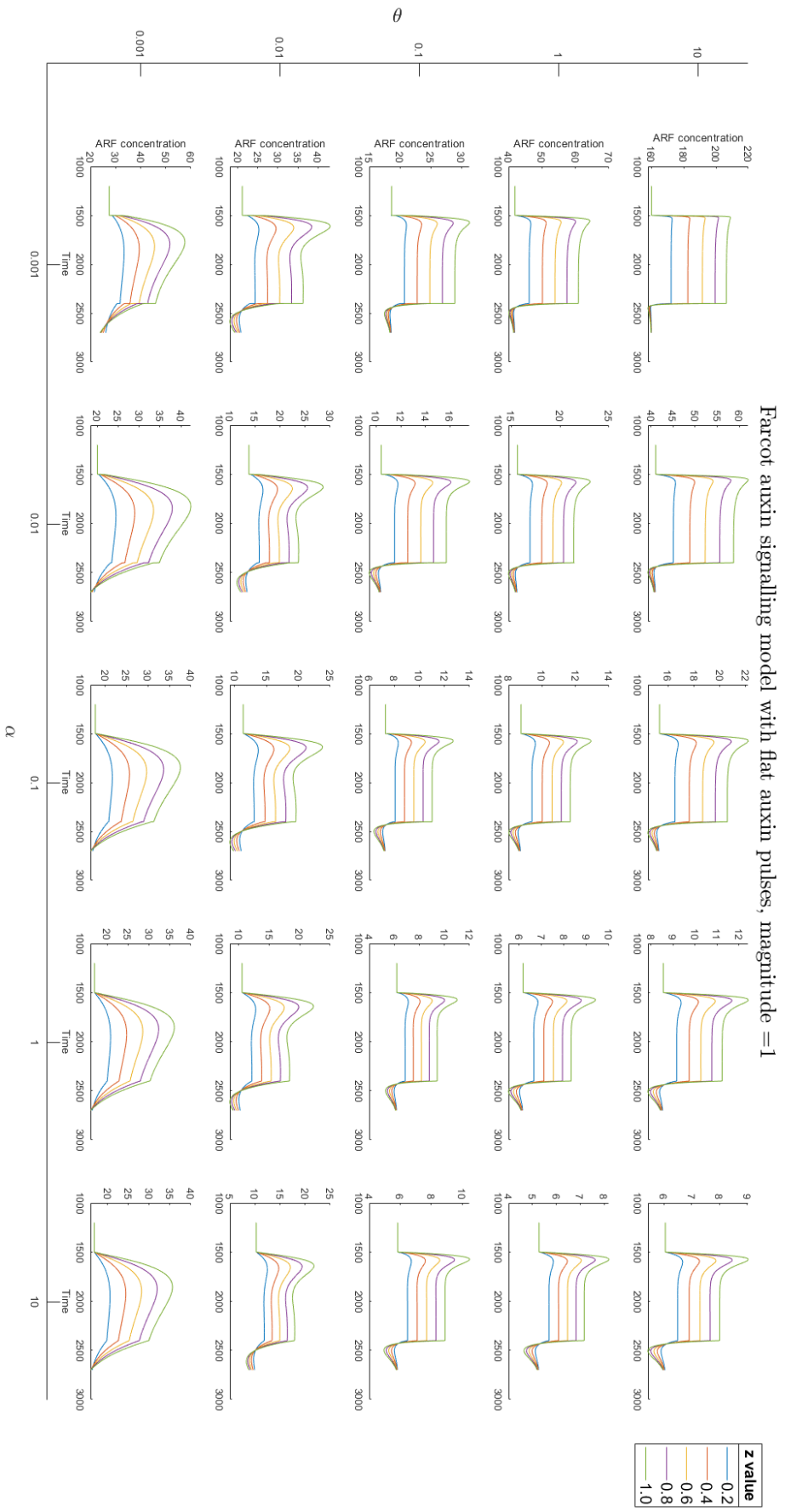


Figure A.3: Farcot auxin signalling model for a range of α and θ values, showing concentrations of ARFs not bound by IAA.

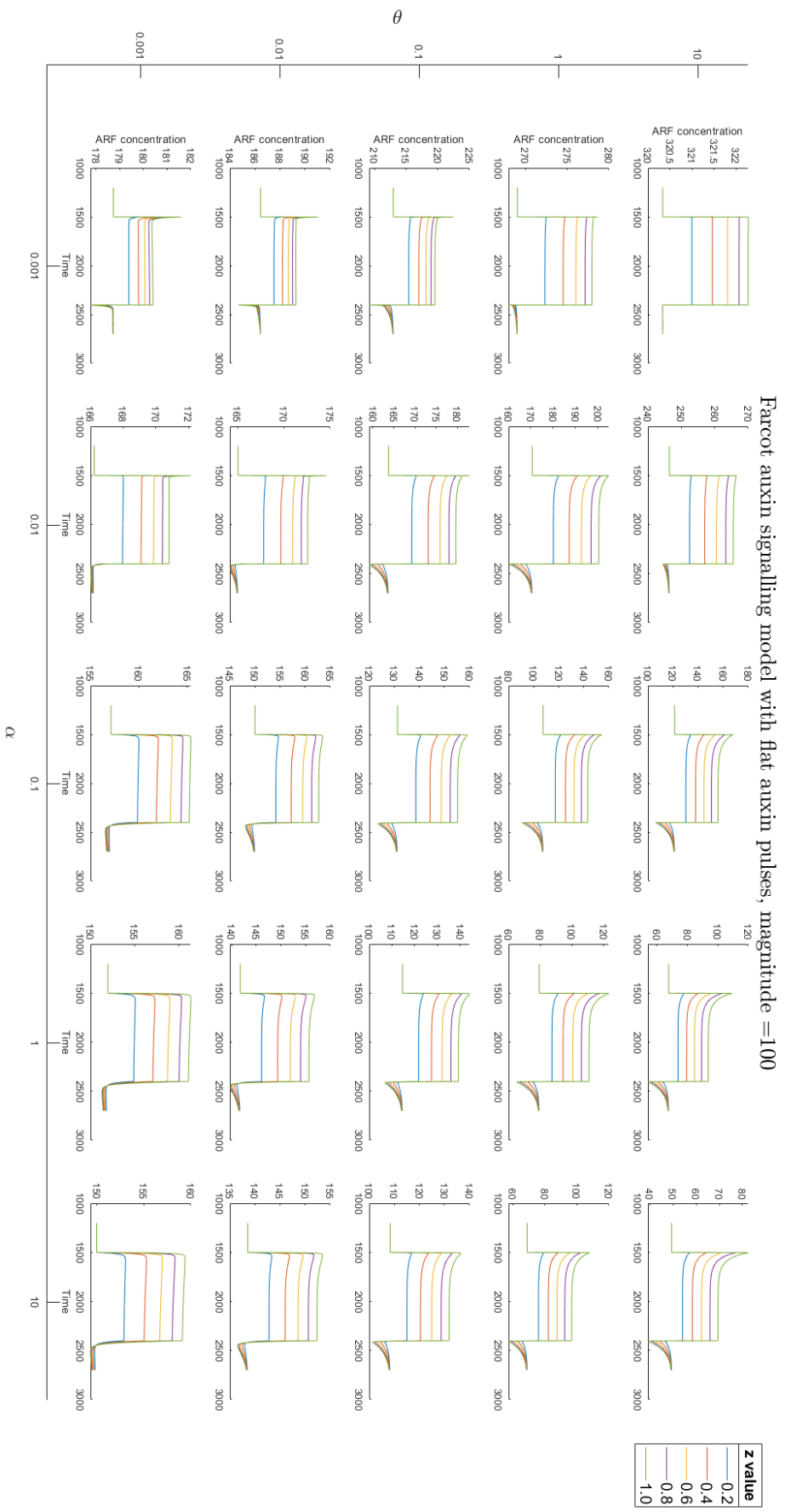


Figure A.4: Farcot auxin signalling model for a range of α and θ values, showing concentrations of ARFs not bound by IAA.

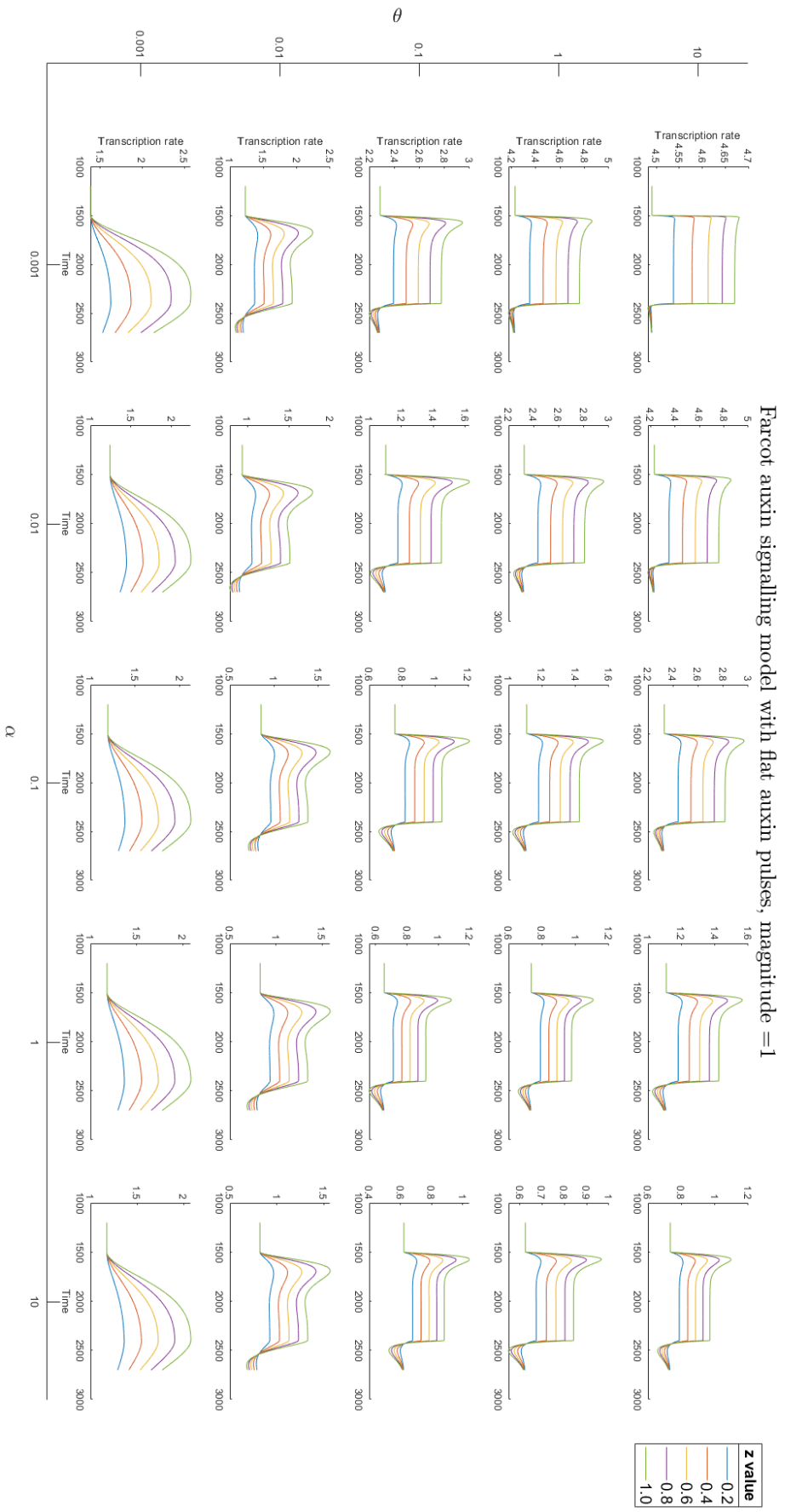


Figure A.5: Farcot auxin signalling model for a range of α and θ values, showing rate of transcription.

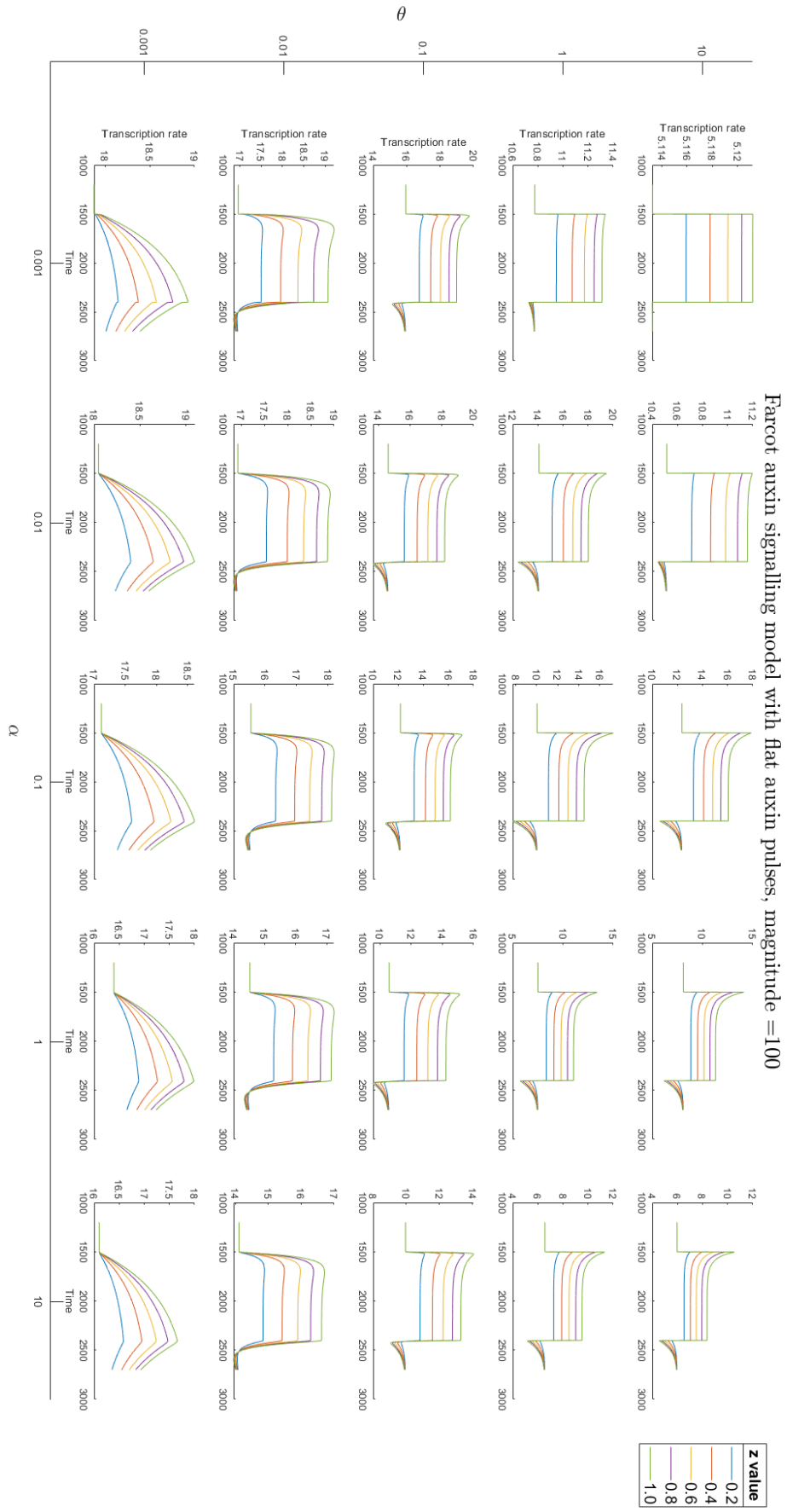


Figure A.6: Farcot auxin signalling model for a range of α and θ values, showing rate of transcription.

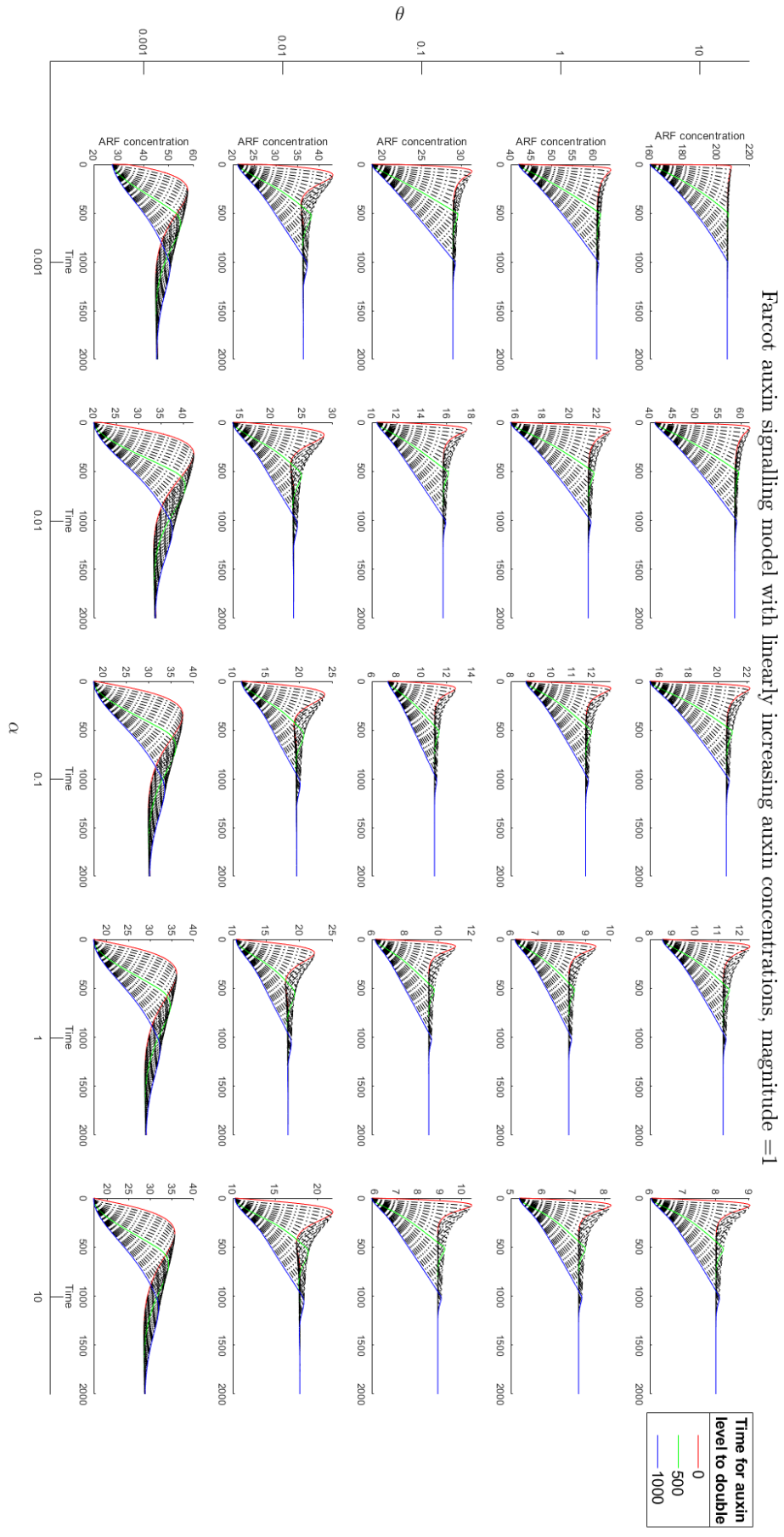


Figure A.7: Farcot auxin signalling model for a range of α and θ values, showing concentrations of ARFs not bound by IAA.

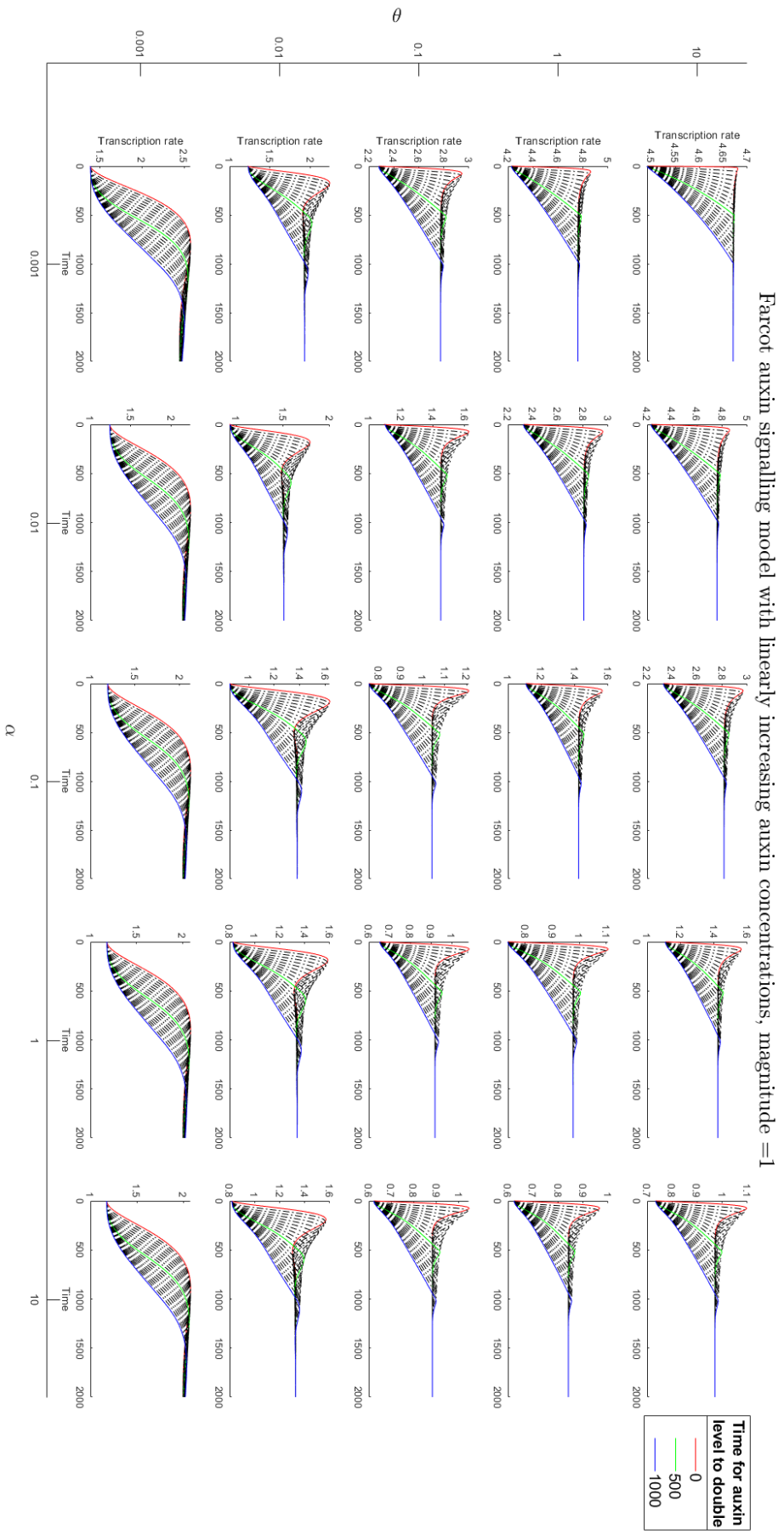


Figure A.8: Farcot auxin signalling model for a range of α and θ values, showing rate of transcription.

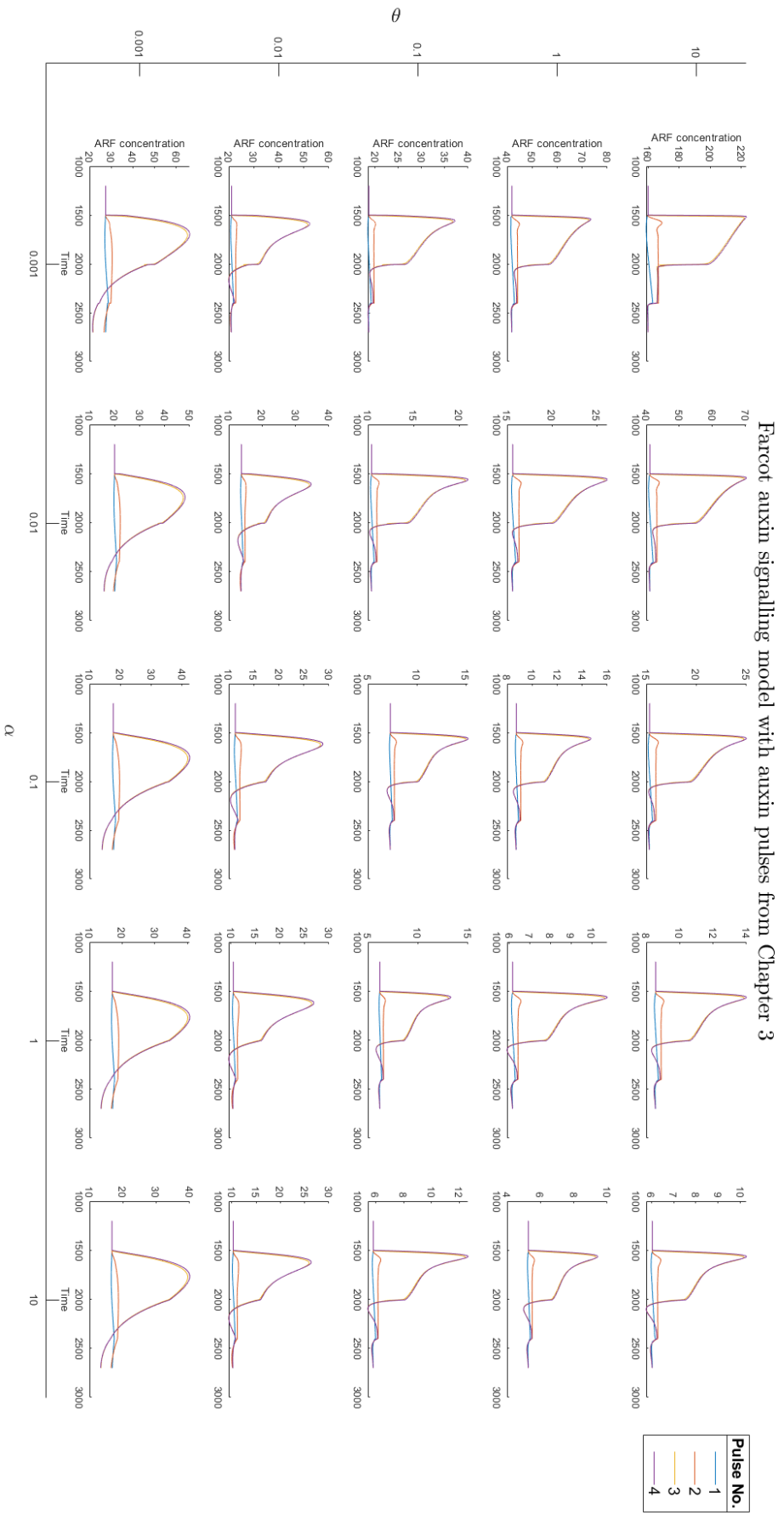


Figure A.9: Farcot auxin signalling model for a range of α and θ values, showing concentrations of ARFs not bound by IAA. Pulse 1 denotes the auxin pulse from chapter 1 where cells divide once ever 20 time units, pulse 2, division every 200 units, pulse 3 division every 2000 units and pulse 4, division every 20,000 units

Farcot auxin signalling model with auxin pulses from Chapter 3

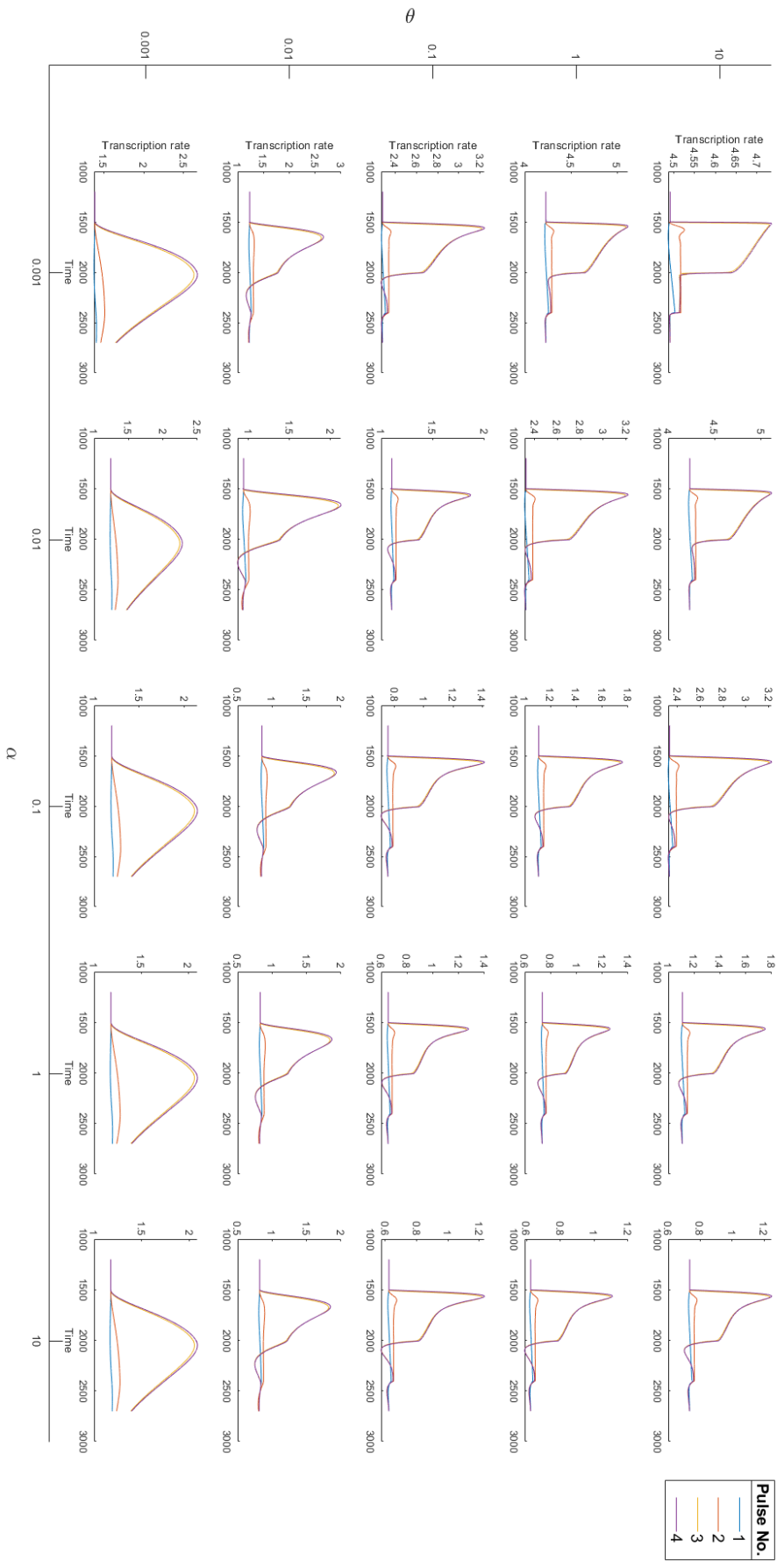


Figure A.10: Farcot auxin signalling model for a range of α and θ values, showing rate of transcription. Pulse 1 denotes the auxin pulse from chapter 1 where cells divide once ever 20 time units, pulse 2, division very 200 units, pulse 3 division every 2000 units and pulse 4, division every 20,000 units

A.4 Equations of Models From Chapter 5

Below are the models for that utilise the mpARF data explicitly from Sections 5.5 and 5.8.

A.4.1 Inital Auxin Signalling Model Using Data Explicitly

$$\frac{dA_1}{dt} = \pi_{A_1} - 2\alpha_{A_1A_1}A_1A_1 + 2\theta_{D_{A_1A_1}}D_{A_1A_1} - \alpha_{A_1A_2}A_1A_2 \quad (A.1)$$

$$+ \theta_{A_1A_2}D_{A_1A_2} - \alpha_{GA_1}GA_1 + \theta_{GA_1}G_{A_1} - \alpha_{A_1GA_1}A_1G_{A_1} \\ + \theta_{A_1GA_1}G_{A_1A_1} - \alpha_{A_1GA_2}A_1G_{A_2} + \theta_{A_1GA_2}G_{A_1A_2}$$

$$\frac{dA_2}{dt} = \pi_{A_2} - \alpha_{A_1A_2}A_1A_2 + \theta_{A_1A_2}D_{A_1A_2} - 2\alpha_{A_2A_2}A_2A_2 \quad (A.2)$$

$$+ 2\theta_{A_2A_2}D_{A_2A_2} - \alpha_{A_2GA_1}A_2G_{A_1} + \theta_{A_2GA_1}G_{A_1A_2} - \alpha_{GA_2}GA_2 \\ + \theta_{GA_2}G_{A_2} - \alpha_{A_2GA_2}G_{A_2}A_2 + \theta_{A_2GA_2}G_{A_2A_2}$$

$$\frac{dD_{A_1A_1}}{dt} = -\mu_{A_1A_1}D_{A_1A_1} + \alpha_{A_1A_1}A_1A_1 - \theta_{D_{A_1A_1}}D_{A_1A_1} \quad (A.3)$$

$$- \alpha_{A_1A_1G}D_{A_1A_1}G + \theta_{A_1A_1G}G_{A_1A_1}$$

$$\frac{dD_{A_1A_2}}{dt} = -\mu_{A_1A_2}D_{A_1A_2} + \alpha_{A_1A_2}A_1A_2 - \theta_{A_1A_2}D_{A_1A_2} - \alpha_{A_1A_2G}D_{A_1A_2}G \quad (A.4)$$

$$+ \theta_{A_1A_2G}G_{A_1A_2}$$

$$\frac{dD_{A_2A_2}}{dt} = -\mu_{A_2A_2}D_{A_2A_2} + \alpha_{A_2A_2}A_2A_2 - \theta_{A_2A_2}D_{A_2A_2} + \theta_{A_2A_2G}G_{A_2A_2} \quad (A.5)$$

$$- \alpha_{A_2A_2G}D_{A_2A_2}G$$

$$\frac{dG}{dt} = -\alpha_{GA_1}GA_1 + \theta_{GA_1}G_{A_1} - \alpha_{GA_2}GA_2 + \theta_{GA_2}G_{A_2} \quad (A.6)$$

$$- \alpha_{A_1A_1G}D_{A_1A_1}G + \theta_{A_1A_1G}G_{A_1A_1} - \alpha_{A_1A_2G}D_{A_1A_2}G + \theta_{A_1A_2G}G_{A_1A_2}$$

$$- \alpha_{A_2A_2G}D_{A_2A_2}G + \theta_{A_2A_2G}G_{A_2A_2}$$

$$\frac{dG_{A_1}}{dt} = \alpha_{GA_1}GA_1 - \theta_{GA_1}G_{A_1} - \alpha_{A_1GA_1}A_1G_{A_1} + \theta_{A_1GA_1}G_{A_1A_1} \quad (A.7)$$

$$- \alpha_{A_2GA_1}A_2G_{A_1} + \theta_{A_2GA_1}G_{A_1A_2}$$

$$\frac{dG_{A_2}}{dt} = \alpha_{GA_2}GA_2 - \theta_{GA_2}G_{A_2} - \alpha_{A_1GA_2}A_1G_{A_2} + \theta_{A_1GA_2}G_{A_1A_2} \quad (A.8)$$

$$- \alpha_{A_2GA_2}G_{A_2}A_2 + \theta_{A_2GA_2}G_{A_2A_2}$$

$$\frac{dG_{A_1A_1}}{dt} = \alpha_{A_1GA_1}A_1G_{A_1} - \theta_{A_1GA_1}G_{A_1A_1} + \alpha_{A_1A_1G}D_{A_1A_1}G \quad (A.9)$$

$$- \theta_{A_1A_1G}G_{A_1A_1}$$

$$\frac{dG_{A_1A_2}}{dt} = \alpha_{A_2GA_1}A_2G_{A_1} - \theta_{A_2GA_1}G_{A_1A_2} + \alpha_{A_1GA_2}A_1G_{A_2} \quad (A.10)$$

$$- \theta_{A_1GA_2}G_{A_1A_2} + \alpha_{A_1A_2G}D_{A_1A_2}G - \theta_{A_1A_2G}G_{A_1A_2}$$

$$\frac{dG_{A_2A_2}}{dt} = \alpha_{A_2A_2G}D_{A_2A_2}G - \theta_{A_2A_2G}G_{A_2A_2} + \alpha_{A_2GA_2}G_{A_2}A_2 \quad (A.11)$$

$$- \theta_{A_2GA_2}G_{A_2A_2}$$

$$\frac{dh}{dt} = l_1G_{A_1} + l_2G_{A_1A_1} \quad (A.12)$$

where

$$\pi_{A_1}(t) = \frac{d\text{TotalARF1}}{dt}(t) + \mu_{A_1A_2} + 2\mu_{A_1A_1} \quad (A.13)$$

$$\pi_{A_2}(t) = \frac{d\text{TotalARF2}}{dt}(t) + \mu_{A_1A_2} + 2\mu_{A_2A_2} \quad (A.14)$$

A.4.2 Model with mpARF Oligomers of up to Length 3

The terms in red represent the alterations made to the equations found above in Appendix A.4.1.

$$\frac{dA_1}{dt} = \pi_{A_1} - 2\alpha_{A_1A_1}A_1A_1 + 2\theta_{A_1A_1}D_{A_1A_1} - \alpha_{A_1A_2}A_1A_2 \quad (A.15)$$

$$+ \theta_{A_1A_2}D_{A_1A_2} - \alpha_{A_1G}GA_1 + \theta_{A_1G}G_{A_1} - \alpha_{A_1A_1}A_1G_{A_1} + \theta_{A_1A_1}G_{A_1A_1}$$

$$- \alpha_{A_1A_2}A_1G_{A_2} + \theta_{A_1A_2}G_{A_1A_2} - \alpha_{A_1A_1}A_1D_{A_1A_1} + 2\theta_{A_1A_1}D_{A_1A_1A_1}$$

$$- 0.5\alpha_{A_1A_1}A_1D_{A_1A_2} + \theta_{A_1A_1}D_{A_1A_1A_2} - \alpha_{A_1A_2}A_1D_{A_2A_2}$$

$$+ \theta_{A_1A_2}D_{A_1A_2A_2} - 0.5\alpha_{A_1A_2}A_1D_{A_1A_2} + \theta_{A_1A_2}D_{A_1A_2A_1}$$

$$- \alpha_{A_1A_1}A_1G_{A_1A_1} + 2\theta_{A_1A_1}G_{A_1A_1A_1} - \alpha_{A_1A_2}A_1G_{A_2A_2}$$

$$+ \theta_{A_1A_2}G_{A_1A_2A_2} - 0.5\alpha_{A_1A_2}A_1G_{A_1A_2} + 2\theta_{A_1A_2}G_{A_1A_2A_1}$$

$$- 0.5\alpha_{A_1A_1}A_1G_{A_1A_2} + \theta_{A_1A_1}G_{A_1A_1A_2}$$

$$\frac{dA_2}{dt} = \pi_{A_2} - \alpha_{A_1A_2}A_1A_2 + \theta_{A_1A_2}D_{A_1A_2} - 2\alpha_{A_2A_2}A_2A_2 \quad (A.16)$$

$$+ 2\theta_{A_2A_2}D_{A_2A_2} - \alpha_{A_1A_2}A_2G_{A_1} + \theta_{A_1A_2}G_{A_1A_2} - \alpha_{A_2G}GA_2$$

$$\begin{aligned}
& +\theta_{A_2G}G_{A_2} - \alpha_{A_2A_2}G_{A_2}A_2 + \theta_{A_2A_2}G_{A_2A_2} - \alpha_{A_1A_2}A_2D_{A_1A_1} \\
& +\theta_{A_1A_2}D_{A_1A_1A_2} - 0.5\alpha_{A_2A_2}A_2D_{A_1A_2} + \theta_{A_2A_2}D_{A_1A_2A_2} \\
& -\alpha_{A_2A_2}A_2D_{A_2A_2} + 2\theta_{A_2A_2}D_{A_2A_2A_2} - 0.5\alpha_{A_1A_2}A_2D_{A_1A_2} \\
& +\theta_{A_1A_2}D_{A_2A_1A_2} - \alpha_{A_1A_2}A_2G_{A_1A_1} + \theta_{A_1A_2}G_{A_1A_1A_2} \\
& -\alpha_{A_2A_2}A_2G_{A_2A_2} + 2\theta_{A_2A_2}G_{A_2A_2A_2} - 0.5\alpha_{A_1A_2}A_2G_{A_1A_2} \\
& +2\theta_{A_1A_2}G_{A_2A_1A_2} - 0.5\alpha_{A_2A_2}A_2G_{A_1A_2} + \theta_{A_2A_2}G_{A_1A_2A_2} \\
\frac{dD_{A_1A_1}}{dt} = & -\mu_{A_1A_1}D_{A_1A_1} + \alpha_{A_1A_1}A_1A_1 - \theta_{A_1A_1}D_{A_1A_1} - \alpha_{A_1A_1G}D_{A_1A_1}G \quad (A.17)
\end{aligned}$$

$$\begin{aligned}
& +\theta_{A_1A_1G}G_{A_1A_1} - \alpha_{A_1A_1}A_1D_{A_1A_1} + 2\theta_{A_1A_1}D_{A_1A_1A_1} \\
& -\alpha_{A_1A_2}A_2D_{A_1A_1} + \theta_{A_1A_2}D_{A_1A_1A_2} - \alpha_{A_1A_1}D_{A_1A_1}G_{A_1} \\
& +2\theta_{A_1A_1}G_{A_1A_1A_1} - \alpha_{A_1A_2}G_{A_2}D_{A_1A_1} + \theta_{A_1A_2}G_{A_1A_1A_2} \\
\frac{dD_{A_1A_2}}{dt} = & -\mu_{A_1A_2}D_{A_1A_2} + \alpha_{A_1A_2}A_1A_2 - \theta_{A_1A_2}D_{A_1A_2} - \alpha_{A_1A_2G}D_{A_1A_2}G \quad (A.18)
\end{aligned}$$

$$\begin{aligned}
& +\theta_{A_1A_2G}G_{A_1A_2} - 0.5\alpha_{A_1A_1}A_1D_{A_1A_2} + \theta_{A_1A_1}D_{A_1A_1A_2} \\
& -0.5\alpha_{A_2A_2}A_2D_{A_1A_2} + \theta_{A_2A_2}D_{A_1A_2A_2} - 0.5\alpha_{A_1A_2}A_1D_{A_1A_2} \\
& +\theta_{A_1A_2}D_{A_1A_2A_1} - 0.5\alpha_{A_1A_2}A_2D_{A_1A_2} + \theta_{A_1A_2}D_{A_2A_1A_2} \\
& -0.5\alpha_{A_1A_2}D_{A_1A_2}G_{A_1} + 2\theta_{A_1A_2}G_{A_1A_2A_1} - 0.5\alpha_{A_1A_2}D_{A_1A_2}G_{A_2} \\
& +2\theta_{A_1A_2}G_{A_2A_1A_2} - 0.5\alpha_{A_1A_1}D_{A_1A_2}G_{A_1} + \theta_{A_1A_1}G_{A_1A_1A_2} \\
& -0.5\alpha_{A_2A_2}D_{A_1A_2}G_{A_2} + \theta_{A_2A_2}G_{A_1A_2A_2} \\
\frac{dD_{A_2A_2}}{dt} = & -\mu_{A_2A_2}D_{A_2A_2} + \alpha_{A_2A_2}A_2A_2 - \theta_{A_2A_2}D_{A_2A_2} + \theta_{A_2A_2G}G_{A_2A_2} \quad (A.19)
\end{aligned}$$

$$\begin{aligned}
& -\alpha_{A_2A_2G}D_{A_2A_2}G - \alpha_{A_1A_2}A_1D_{A_2A_2} + \theta_{A_1A_2}D_{A_1A_2A_2} \\
& -\alpha_{A_2A_2}A_2D_{A_2A_2} + 2\theta_{A_2A_2}D_{A_2A_2A_2} - \alpha_{A_1A_2}G_{A_1}D_{A_2A_2} \\
& +\theta_{A_1A_2}G_{A_1A_2A_2} - \alpha_{A_2A_2}D_{A_2A_2}G_{A_2} + 2\theta_{A_2A_2}G_{A_2A_2A_2} \\
\frac{dD_{A_1A_1A_1}}{dt} = & -\mu_{A_1A_1A_1}D_{A_1A_1A_1} + \alpha_{A_1A_1}A_1D_{A_1A_1} - 2\theta_{A_1A_1}D_{A_1A_1A_1} \quad (A.20)
\end{aligned}$$

$$\begin{aligned}
& -\alpha_{A_1A_1A_1G}D_{A_1A_1A_1}G + \theta_{A_1A_1A_1G}G_{A_1A_1A_1} \\
\frac{dD_{A_1A_1A_2}}{dt} = & -\mu_{A_1A_1A_2}D_{A_1A_1A_2} + 0.5\alpha_{A_1A_1}A_1D_{A_1A_2} - \theta_{A_1A_1}D_{A_1A_1A_2} \quad (A.21) \\
& +\alpha_{A_1A_2}A_2D_{A_1A_1} - \theta_{A_1A_2}D_{A_1A_1A_2} - \alpha_{A_1A_1A_2G}D_{A_1A_1A_2}G \\
& +\theta_{A_1A_1A_2G}G_{A_1A_1A_2}
\end{aligned}$$

(A.23)

$$\begin{aligned} \frac{dD_{A_1A_2A_2}}{dt} = & -\mu_{A_1A_2A_2} D_{A_1A_2A_2} + \alpha_{A_1A_2} A_1 D_{A_2A_2} - \theta_{A_1A_2} D_{A_1A_2A_2} \\ & + 0.5\alpha_{A_2A_2} A_2 D_{A_1A_2} - \theta_{A_2A_2} D_{A_1A_2A_2} - \alpha_{A_1A_2A_2} G D_{A_1A_2A_2} \\ & + \theta_{A_1A_2A_2} G_{A_1A_2A_2} \end{aligned} \quad (A.24)$$

$$\begin{aligned} \frac{dD_{A_2A_2A_2}}{dt} = & -\mu_{A_2A_2A_2} D_{A_2A_2A_2} + \alpha_{A_2A_2} A_2 D_{A_2A_2} - 2\theta_{A_2A_2} D_{A_2A_2A_2} \\ & - \alpha_{A_2A_2A_2} G D_{A_2A_2A_2} + \theta_{A_2A_2A_2} G_{A_2A_2A_2} \end{aligned} \quad (A.25)$$

$$\begin{aligned} \frac{dD_{A_1A_2A_1}}{dt} = & -\mu_{A_1A_2A_1} D_{A_1A_2A_1} + 0.5\alpha_{A_1A_2} A_1 D_{A_1A_2} - \theta_{A_1A_2} D_{A_1A_2A_1} \\ & - \alpha_{A_1A_2A_1} G D_{A_1A_2A_1} + \theta_{A_1A_2A_1} G_{A_1A_2A_1} \end{aligned} \quad (A.26)$$

$$\begin{aligned} \frac{dD_{A_2A_1A_2}}{dt} = & -\mu_{A_2A_1A_2} D_{A_2A_1A_2} + 0.5\alpha_{A_1A_2} A_2 D_{A_1A_2} - \theta_{A_1A_2} D_{A_2A_1A_2} \\ & - \alpha_{A_2A_1A_2} G D_{A_2A_1A_2} + \theta_{A_2A_1A_2} G_{A_2A_1A_2} \end{aligned} \quad (A.27)$$

$$\frac{dG}{dt} = -\alpha_{A_1} G A_1 + \theta_{A_1} G_{A_1} - \alpha_{A_2} G A_2 + \theta_{A_2} G_{A_2} \quad (A.28)$$

$$\begin{aligned} & -\alpha_{A_1A_1} G D_{A_1A_1} + \theta_{A_1A_1} G_{A_1A_1} - \alpha_{A_1A_2} G D_{A_1A_2} \\ & + \theta_{A_1A_2} G_{A_1A_2} - \alpha_{A_2A_2} G D_{A_2A_2} + \theta_{A_2A_2} G_{A_2A_2} \\ & - \alpha_{A_1A_1A_1} G D_{A_1A_1A_1} + \theta_{A_1A_1A_1} G_{A_1A_1A_1} - \alpha_{A_1A_1A_2} G D_{A_1A_1A_2} \\ & + \theta_{A_1A_1A_2} G_{A_1A_1A_2} - \alpha_{A_1A_2A_2} G D_{A_1A_2A_2} + \theta_{A_1A_2A_2} G_{A_1A_2A_2} \\ & - \alpha_{A_2A_2A_2} G D_{A_2A_2A_2} + \theta_{A_2A_2A_2} G_{A_2A_2A_2} - \alpha_{A_1A_2A_1} G D_{A_1A_2A_1} \\ & + \theta_{A_1A_2A_1} G_{A_1A_2A_1} - \alpha_{A_2A_1A_2} G D_{A_2A_1A_2} + \theta_{A_2A_1A_2} G_{A_2A_1A_2} \end{aligned}$$

$$\frac{dG_{A_1}}{dt} = \alpha_{A_1} G A_1 - \theta_{A_1} G_{A_1} - \alpha_{A_1A_1} A_1 G_{A_1} + \theta_{A_1A_1} G_{A_1A_1} \quad (A.29)$$

$$\begin{aligned} & -\alpha_{A_1A_2} A_2 G_{A_1} + \theta_{A_1A_2} G_{A_1A_2} - \alpha_{A_1A_1} D_{A_1A_1} G_{A_1} + 2\theta_{A_1A_1} G_{A_1A_1A_1} \\ & - \alpha_{A_1A_2} G_{A_1} D_{A_2A_2} + \theta_{A_1A_2} G_{A_1A_2A_2} - 0.5\alpha_{A_1A_2} D_{A_1A_2} G_{A_1} \\ & + 2\theta_{A_1A_2} G_{A_1A_2A_1} - 0.5\alpha_{A_1A_1} D_{A_1A_2} G_{A_1} + \theta_{A_1A_1} G_{A_1A_1A_2} \end{aligned}$$

$$\frac{dG_{A_2}}{dt} = \alpha_{A_2} G A_2 - \theta_{A_2} G_{A_2} - \alpha_{A_1A_2} A_1 G_{A_2} + \theta_{A_1A_2} G_{A_1A_2} \quad (A.30)$$

$$\begin{aligned} & -\alpha_{A_2A_2} G_{A_2} A_2 + \theta_{A_2A_2} G_{A_2A_2} - \alpha_{A_1A_2} G_{A_2} D_{A_1A_1} + \theta_{A_1A_2} G_{A_1A_1A_2} \\ & - \alpha_{A_2A_2} D_{A_2A_2} G_{A_2} + 2\theta_{A_2A_2} G_{A_2A_2A_2} - 0.5\alpha_{A_1A_2} D_{A_1A_2} G_{A_2} \end{aligned}$$

$$\begin{aligned}
& +2\theta_{A_1A_2}G_{A_2A_1A_2} - 0.5\alpha_{A_2A_2}D_{A_1A_2}G_{A_2} + \theta_{A_2A_2}G_{A_1A_2A_2} \\
\frac{dG_{A_1A_1}}{dt} = & \alpha_{A_1A_1}A_1G_{A_1} - \theta_{A_1A_1}G_{A_1A_1} + \alpha_{A_1A_1}D_{A_1A_1}G \\
& - \theta_{A_1A_1}G_{A_1A_1} - \alpha_{A_1A_1}A_1G_{A_1A_1} + 2\theta_{A_1A_1}G_{A_1A_1A_1} \\
& - \alpha_{A_1A_2}A_2G_{A_1A_1} + \theta_{A_1A_2}G_{A_1A_1A_2} \\
\frac{dG_{A_1A_2}}{dt} = & \alpha_{A_1A_2}A_2G_{A_1} - \theta_{A_1A_2}G_{A_1A_2} + \alpha_{A_1A_2}A_1G_{A_2} - \theta_{A_1A_2}G_{A_1A_2} \quad (A.31) \\
& + \alpha_{A_1A_2}D_{A_1A_2}G - \theta_{A_1A_2}G_{A_1A_2} - 0.5\alpha_{A_1A_2}A_1G_{A_1A_2} \\
& + 2\theta_{A_1A_2}G_{A_1A_2A_1} - 0.5\alpha_{A_1A_2}A_2G_{A_1A_2} + 2\theta_{A_1A_2}G_{A_2A_1A_2} \\
& - 0.5\alpha_{A_1A_1}A_1G_{A_1A_2} + \theta_{A_1A_1}G_{A_1A_1A_2} - 0.5\alpha_{A_2A_2}A_2G_{A_1A_2} \\
& + \theta_{A_2A_2}G_{A_1A_2A_2}
\end{aligned}$$

$$\begin{aligned}
\frac{dG_{A_2A_2}}{dt} = & \alpha_{A_2A_2}D_{A_2A_2}G - \theta_{A_2A_2}G_{A_2A_2} + \alpha_{A_2A_2}G_{A_2}A_2 \quad (A.32) \\
& - \theta_{A_2A_2}G_{A_2A_2} - \alpha_{A_1A_2}A_1G_{A_2A_2} + \theta_{A_1A_2}G_{A_1A_2A_2} \\
& - \alpha_{A_2A_2}A_2G_{A_2A_2} + 2\theta_{A_2A_2}G_{A_2A_2A_2}
\end{aligned}$$

$$\begin{aligned}
\frac{dG_{A_1A_1A_1}}{dt} = & \alpha_{A_1A_1A_1}D_{A_1A_1A_1}G - \theta_{A_1A_1A_1}G_{A_1A_1A_1} + \alpha_{A_1A_1}A_1G_{A_1A_1} \quad (A.33) \\
& - 2\theta_{A_1A_1}G_{A_1A_1A_1} + \alpha_{A_1A_1}D_{A_1A_1}G_{A_1} - 2\theta_{A_1A_1}G_{A_1A_1A_1}
\end{aligned}$$

$$\begin{aligned}
\frac{dG_{A_1A_1A_2}}{dt} = & \alpha_{A_1A_1A_2}D_{A_1A_1A_2}G - \theta_{A_1A_1A_2}G_{A_1A_1A_2} + \alpha_{A_1A_2}A_2G_{A_1A_1} \quad (A.34) \\
& - \theta_{A_1A_2}G_{A_1A_1A_2} + \alpha_{A_1A_2}G_{A_2}D_{A_1A_1} - \theta_{A_1A_2}G_{A_1A_1A_2} \\
& + 0.5\alpha_{A_1A_1}A_1G_{A_1A_2} - \theta_{A_1A_1}G_{A_1A_1A_2} + 0.5\alpha_{A_1A_1}D_{A_1A_2}G_{A_1} \\
& - \theta_{A_1A_1}G_{A_1A_1A_2}
\end{aligned}$$

$$\begin{aligned}
\frac{dG_{A_1A_2A_2}}{dt} = & \alpha_{A_1A_2A_2}D_{A_1A_2A_2}G - \theta_{A_1A_2A_2}G_{A_1A_2A_2} + \alpha_{A_1A_2}A_1G_{A_2A_2} \quad (A.35) \\
& - \theta_{A_1A_2}G_{A_1A_2A_2} + \alpha_{A_1A_2}G_{A_1}D_{A_2A_2} - \theta_{A_1A_2}G_{A_1A_2A_2} \\
& + 0.5\alpha_{A_2A_2}A_2G_{A_1A_2} - \theta_{A_2A_2}G_{A_1A_2A_2} + 0.5\alpha_{A_2A_2}D_{A_1A_2}G_{A_2} \\
& - \theta_{A_2A_2}G_{A_1A_2A_2}
\end{aligned}$$

$$\begin{aligned}
\frac{dG_{A_2A_2A_2}}{dt} = & \alpha_{A_2A_2A_2}D_{A_2A_2A_2}G - \theta_{A_2A_2A_2}G_{A_2A_2A_2} + \alpha_{A_2A_2}A_2G_{A_2A_2} \quad (A.36) \\
& - 2\theta_{A_2A_2}G_{A_2A_2A_2} + \alpha_{A_2A_2}D_{A_2A_2}G_{A_2} - 2\theta_{A_2A_2}G_{A_2A_2A_2}
\end{aligned}$$

$$\begin{aligned}
\frac{dG_{A_1A_2A_1}}{dt} = & \alpha_{A_1A_2A_1}D_{A_1A_2A_1}G - \theta_{A_1A_2A_1}G_{A_1A_2A_1} + 0.5\alpha_{A_1A_2}A_1G_{A_1A_2} \quad (A.37) \\
& - 2\theta_{A_1A_2}G_{A_1A_2A_1} + 0.5\alpha_{A_1A_2}D_{A_1A_2}G_{A_1} - 2\theta_{A_1A_2}G_{A_1A_2A_1}
\end{aligned}$$

$$\frac{dG_{A_2A_1A_2}}{dt} = \alpha_{A_2A_1A_2G}D_{A_2A_1A_2}G - \theta_{A_2A_1A_2G}G_{A_2A_1A_2} + 0.5\alpha_{A_1A_2}A_2G_{A_1A_2} \quad (A.38)$$

$$\begin{aligned} & -2\theta_{A_1A_2}G_{A_2A_1A_2} + 0.5\alpha_{A_1A_2}D_{A_1A_2}G_{A_2} - 2\theta_{A_1A_2}G_{A_2A_1A_2} \\ \frac{dh}{dt} &= l_1G_{A_1} + l_2(G_{A_1A_1} + G_{A_1A_1A_1}) \end{aligned} \quad (A.39)$$

where

$$\pi_{A_1}(t) = \frac{d\text{TotalARF1}}{dt}(t) + \mu_{A_1A_2} + 2\mu_{A_1A_1} + 3\mu_{A_1A_1A_1} \quad (A.40)$$

$$\begin{aligned} & + 2\mu_{A_1A_1A_2} + 2\mu_{A_1A_2A_1} + \mu_{A_1A_2A_2} + \mu_{A_2A_1A_2} \\ \pi_{A_2}(t) &= \frac{d\text{TotalARF2}}{dt}(t) + \mu_{A_1A_2} + 2\mu_{A_2A_2} + 3\mu_{A_2A_2A_2} \\ & + \mu_{A_1A_1A_2} + \mu_{A_1A_2A_1} + 2\mu_{A_1A_2A_2} + 2\mu_{A_2A_1A_2} \end{aligned} \quad (A.41)$$

A.4.3 Model with Oligomers of mpARF1 of up to Length 2 bound by mpARF2

The terms in red represent the alterations made to the equations found above in Appendix A.4.1. Note also that due to the structure of the ARF oligomers formed the order of some of the equations has changed. Instead of being ordered by length of oligomer they are now ordered first by number of ARF1 proteins then by length.

$$\begin{aligned} \frac{dA_2}{dt} &= \pi_{A_2} - \alpha_{A_1A_2}A_1A_2 + \theta_{A_1A_2}D_{A_1A_2} - 2\alpha_{A_2A_2}A_2A_2 \\ & + 2\theta_{A_2A_2}D_{A_2A_2} - \alpha_{A_1A_2}A_2G_{A_1} + \theta_{A_1A_2}G_{A_1A_2} \\ & - \alpha_{A_2G}GA_2 + \theta_{A_2G}G_{A_2} - \alpha_{A_2A_2}G_{A_2}A_2 \\ & + \theta_{A_2A_2}G_{A_2A_2} - 0.5\alpha_{A_1A_2}D_{A_1A_2}A_2 + 2\theta_{A_1A_2}D_{A_2A_1A_2} \\ & - \alpha_{A_1A_2}D_{A_1A_1}A_2 + \theta_{A_1A_2}D_{A_1A_1A_2} - 0.5\alpha_{A_1A_2}D_{A_1A_1A_2}A_2 \\ & + \theta_{A_1A_2}D_{A_2A_1A_1A_2} - 0.5\alpha_{A_1A_2}A_2G_{A_1A_2} + 2\theta_{A_1A_2}G_{A_2A_1A_2} \\ & - \alpha_{A_1A_2}A_2G_{A_1A_1} + \theta_{A_1A_2}G_{A_1A_1A_2} - 0.5\alpha_{A_1A_2}A_2G_{A_1A_1A_2} \\ & + 2\theta_{A_1A_2}G_{A_2A_1A_1A_2} \end{aligned} \quad (A.42)$$

$$\frac{dD_{A_2A_2}}{dt} = -\mu_{A_2A_2}D_{A_2A_2} + \alpha_{A_2A_2}A_2A_2 - \theta_{A_2A_2}D_{A_2A_2} \quad (A.43)$$

$$+ \theta_{A_2A_2G}G_{A_2A_2} - \alpha_{A_2A_2G}D_{A_2A_2}G$$

$$\frac{dA_1}{dt} = \pi_{A_1} - 2\alpha_{A_1A_1}A_1A_1 + 2\theta_{A_1A_1}D_{A_1A_1} - \alpha_{A_1A_2}A_1A_2 \quad (A.44)$$

$$+ \theta_{A_1A_2}D_{A_1A_2} - \alpha_{A_1G}GA_1 + \theta_{A_1G}G_{A_1}$$

$$- \alpha_{A_1A_1}A_1G_{A_1} + \theta_{A_1A_1}G_{A_1A_1} - \alpha_{A_1A_2}A_1G_{A_2}$$

$$+ \theta_{A_1A_2}G_{A_1A_2} - 0.5\alpha_{A_1A_1}D_{A_1A_2}A_1 + \theta_{A_1A_1}D_{A_1A_1A_2}$$

$$- 0.5\alpha_{A_1A_1}A_1G_{A_1A_2} + \theta_{A_1A_1}G_{A_1A_1A_2}$$

$$\frac{dD_{A_1A_2}}{dt} = -\mu_{A_1A_2}D_{A_1A_2} + \alpha_{A_1A_2}A_1A_2 - \theta_{A_1A_2}D_{A_1A_2} \quad (A.45)$$

$$- \alpha_{A_1A_2G}D_{A_1A_2}G + \theta_{A_1A_2G}G_{A_1A_2} - 0.5\alpha_{A_1A_2}D_{A_1A_2}A_2$$

$$+ 2\theta_{A_1A_2}D_{A_2A_1A_2} - 0.5\alpha_{A_1A_1}D_{A_1A_2}A_1 + \theta_{A_1A_1}D_{A_1A_1A_2}$$

$$- 0.5\alpha_{A_1A_1}D_{A_1A_1}^2 + 2\theta_{A_1A_1}D_{A_2A_1A_1A_2} - 0.5\alpha_{A_1A_2}G_{A_2}D_{A_1A_2}$$

$$+ 2\theta_{A_1A_2}G_{A_2A_1A_2} - 0.5\alpha_{A_1A_1}G_{A_1}D_{A_1A_2} + \theta_{A_1A_1}G_{A_1A_1A_2}$$

$$- 0.25\alpha_{A_1A_1}G_{A_1A_2}D_{A_1A_2} + \theta_{A_1A_1}G_{A_2A_1A_1A_2}$$

$$\frac{dD_{A_2A_1A_2}}{dt} = -\mu_{A_2A_1A_2}D_{A_2A_1A_2} + 0.5\alpha_{A_1A_2}D_{A_1A_2}A_2 \quad (A.46)$$

$$- 2\theta_{A_1A_2}D_{A_2A_1A_2} - \alpha_{A_2A_1A_2G}D_{A_2A_1A_2}G + \theta_{A_2A_1A_2G}G_{A_2A_1A_2}$$

$$\frac{dD_{A_1A_1}}{dt} = -\mu_{A_1A_1}D_{A_1A_1} + \alpha_{A_1A_1}A_1A_1 - \theta_{A_1A_1}D_{A_1A_1} \quad (A.47)$$

$$- \alpha_{A_1A_1G}D_{A_1A_1}G + \theta_{A_1A_1G}G_{A_1A_1} - \alpha_{A_1A_2}D_{A_1A_1}A_2$$

$$+ \theta_{A_1A_2}D_{A_1A_1A_2} - \alpha_{A_1A_2}G_{A_2}D_{A_1A_1} + \theta_{A_1A_2}G_{A_1A_1A_2}$$

$$\frac{dD_{A_1A_1A_2}}{dt} = -\mu_{A_1A_1A_2}D_{A_1A_1A_2} + 0.5\alpha_{A_1A_1}D_{A_1A_2}A_1 - \theta_{A_1A_1}D_{A_1A_1A_2} \quad (A.48)$$

$$+ \alpha_{A_1A_2}D_{A_1A_1}A_2 - \theta_{A_1A_2}D_{A_1A_1A_2} - 0.5\alpha_{A_1A_2}D_{A_1A_1A_2}A_2$$

$$+ \theta_{A_1A_2}D_{A_2A_1A_1A_2} - \alpha_{A_1A_1A_2G}D_{A_1A_1A_2}G + \theta_{A_1A_1A_2G}G_{A_1A_1A_2}$$

$$- 0.5\alpha_{A_1A_2}G_{A_2}D_{A_1A_1A_2} + 2\theta_{A_1A_2}G_{A_2A_1A_1A_2}$$

$$\frac{dD_{A_2A_1A_1A_2}}{dt} = -\mu_{A_2A_1A_1A_2}D_{A_2A_1A_1A_2} + 0.5\alpha_{A_1A_2}D_{A_1A_1A_2}A_2 \quad (A.49)$$

$$- \theta_{A_1A_2}D_{A_2A_1A_1A_2} + 0.25\alpha_{A_1A_1}D_{A_1A_1}^2 - \theta_{A_1A_1}D_{A_2A_1A_1A_2}$$

$$- \alpha_{A_2A_1A_1A_2G}D_{A_2A_1A_1A_2}G + \theta_{A_2A_1A_1A_2G}G_{A_2A_1A_1A_2}$$

$$\frac{dG}{dt} = -\alpha_{A_1G}GA_1 + \theta_{A_1G}G_{A_1} - \alpha_{A_2G}GA_2 + \theta_{A_2G}G_{A_2} \quad (A.50)$$

$$\begin{aligned}
& -\alpha_{A_1 A_1 G} D_{A_1 A_1} G + \theta_{A_1 A_1 G} G_{A_1 A_1} - \alpha_{A_1 A_2 G} D_{A_1 A_2} G \\
& + \theta_{A_1 A_2 G} G_{A_1 A_2} - \alpha_{A_2 A_2 G} D_{A_2 A_2} G + \theta_{A_2 A_2 G} G_{A_2 A_2} \\
& - \alpha_{A_2 A_1 A_2 G} D_{A_2 A_1 A_2} G + \theta_{A_2 A_1 A_2 G} G_{A_2 A_1 A_2} - \alpha_{A_1 A_1 A_2 G} D_{A_1 A_1 A_2} G \\
& + \theta_{A_1 A_1 A_2 G} G_{A_1 A_1 A_2} - \alpha_{A_2 A_1 A_1 A_2 G} D_{A_2 A_1 A_1 A_2} G \\
& + \theta_{A_2 A_1 A_1 A_2 G} G_{A_2 A_1 A_1 A_2} \\
\frac{dG_{A_2}}{dt} = & \alpha_{A_2 G} G A_2 - \theta_{A_2 G} G_{A_2} - \alpha_{A_1 A_2} A_1 G_{A_2} + \theta_{A_1 A_2} G_{A_1 A_2} \quad (A.51) \\
& - \alpha_{A_2 A_2} G_{A_2} A_2 + \theta_{A_2 A_2} G_{A_2 A_2} - 0.5 \alpha_{A_1 A_2} G_{A_2} D_{A_1 A_2} \\
& + 2 \theta_{A_1 A_2} G_{A_2 A_1 A_2} - \alpha_{A_1 A_2} G_{A_2} D_{A_1 A_1} + \theta_{A_1 A_2} G_{A_1 A_1 A_2} \\
& - 0.5 \alpha_{A_1 A_2} G_{A_2} D_{A_1 A_1 A_2} + 2 \theta_{A_1 A_2} G_{A_2 A_1 A_1 A_2}
\end{aligned}$$

$$\begin{aligned}
\frac{dG_{A_2 A_2}}{dt} = & \alpha_{A_2 A_2 G} D_{A_2 A_2} G - \theta_{A_2 A_2 G} G_{A_2 A_2} + \alpha_{A_2 A_2} G_{A_2} A_2 \quad (A.52) \\
& - \theta_{A_2 A_2} G_{A_2 A_2}
\end{aligned}$$

$$\begin{aligned}
\frac{dG_{A_1}}{dt} = & \alpha_{A_1 G} G A_1 - \theta_{A_1 G} G_{A_1} - \alpha_{A_1 A_1} A_1 G_{A_1} + \theta_{A_1 A_1} G_{A_1 A_1} \quad (A.53) \\
& - \alpha_{A_1 A_2} A_2 G_{A_1} + \theta_{A_1 A_2} G_{A_1 A_2} - 0.5 \alpha_{A_1 A_1} G_{A_1} D_{A_1 A_2} \\
& + \theta_{A_1 A_1} G_{A_1 A_1 A_2}
\end{aligned}$$

$$\begin{aligned}
\frac{dG_{A_1 A_2}}{dt} = & \alpha_{A_1 A_2} A_2 G_{A_1} - \theta_{A_1 A_2} G_{A_1 A_2} + \alpha_{A_1 A_2} A_1 G_{A_2} - \theta_{A_1 A_2} G_{A_1 A_2} \quad (A.54) \\
& + \alpha_{A_1 A_2 G} D_{A_1 A_2} G - \theta_{A_1 A_2 G} G_{A_1 A_2} - 0.5 \alpha_{A_1 A_2} A_2 G_{A_1 A_2} \\
& + 2 \theta_{A_1 A_2} G_{A_2 A_1 A_2} - 0.5 \alpha_{A_1 A_1} A_1 G_{A_1 A_2} + \theta_{A_1 A_1} G_{A_1 A_1 A_2} \\
& - 0.25 \alpha_{A_1 A_1} G_{A_1 A_2} D_{A_1 A_2} + \theta_{A_1 A_1} G_{A_2 A_1 A_1 A_2}
\end{aligned}$$

$$\begin{aligned}
\frac{dG_{A_2 A_1 A_2}}{dt} = & \alpha_{A_2 A_1 A_2 G} D_{A_2 A_1 A_2} G - \theta_{A_2 A_1 A_2 G} G_{A_2 A_1 A_2} \quad (A.55) \\
& + 0.5 \alpha_{A_1 A_2} A_2 G_{A_1 A_2} - 2 \theta_{A_1 A_2} G_{A_2 A_1 A_2} + 0.5 \alpha_{A_1 A_2} G_{A_2} D_{A_1 A_2} \\
& - 2 \theta_{A_1 A_2} G_{A_2 A_1 A_2}
\end{aligned}$$

$$\begin{aligned}
\frac{dG_{A_1 A_1}}{dt} = & \alpha_{A_1 A_1} A_1 G_{A_1} - \theta_{A_1 A_1} G_{A_1 A_1} + \alpha_{A_1 A_1 G} D_{A_1 A_1} G \quad (A.56) \\
& - \theta_{A_1 A_1 G} G_{A_1 A_1} - \alpha_{A_1 A_2} A_2 G_{A_1 A_1} + \theta_{A_1 A_2} G_{A_1 A_1 A_2}
\end{aligned}$$

$$\begin{aligned}
\frac{dG_{A_1 A_1 A_2}}{dt} = & \alpha_{A_1 A_1 A_2 G} D_{A_1 A_1 A_2} G - \theta_{A_1 A_1 A_2 G} G_{A_1 A_1 A_2} \quad (A.57) \\
& + 0.5 \alpha_{A_1 A_1} A_1 G_{A_1 A_2} - \theta_{A_1 A_1} G_{A_1 A_1 A_2} + 0.5 \alpha_{A_1 A_1} G_{A_1} D_{A_1 A_2} \\
& - \theta_{A_1 A_1} G_{A_1 A_1 A_2} \alpha_{A_1 A_2} G_{A_2} D_{A_1 A_1} - \theta_{A_1 A_2} G_{A_1 A_1 A_2}
\end{aligned}$$

$$\begin{aligned}
& +\alpha_{A_1A_2}A_2G_{A_1A_1} - \theta_{A_1A_2}G_{A_1A_1A_2} - 0.5\alpha_{A_1A_2}A_2G_{A_1A_1A_2} \\
& +2\theta_{A_1A_2}G_{A_2A_1A_1A_2} \\
\frac{dG_{A_2A_1A_1A_2}}{dt} = & \alpha_{A_2A_1A_1A_2}G_{A_2A_1A_1A_2} - \theta_{A_2A_1A_1A_2}G_{A_2A_1A_1A_2} \quad (A.58)
\end{aligned}$$

$$\begin{aligned}
& +0.5\alpha_{A_1A_2}G_{A_2}D_{A_1A_1A_2} - 2\theta_{A_1A_2}G_{A_2A_1A_1A_2} + 0.5\alpha_{A_1A_2}A_2G_{A_1A_1A_2} \\
& -2\theta_{A_1A_2}G_{A_2A_1A_1A_2} + 0.25\alpha_{A_1A_1}G_{A_1A_2}D_{A_1A_2} - \theta_{A_1A_1}G_{A_2A_1A_1A_2} \\
\frac{dh}{dt} = & l_1G_{A_1} + l_2G_{A_1A_1} \quad (A.59)
\end{aligned}$$

$$\pi_{A_1}(t) = \frac{d\text{TotalARF1}}{dt}(t) + \mu_{A_1A_2} + 2\mu_{A_1A_1} + 2\mu_{A_1A_1A_2} \quad (A.60)$$

$$\begin{aligned}
& +2\mu_{A_2A_1A_1A_2} \\
\pi_{A_2}(t) = & \frac{d\text{TotalARF2}}{dt}(t) + 2\mu_{A_2A_2} + \mu_{A_1A_2} + \mu_{A_1A_1A_2} \quad (A.61) \\
& +2\mu_{A_2A_1A_1A_2}
\end{aligned}$$

A.4.4 Model with Oligomers of mpARF1 of up to Length 3 bound by mppARF2

The terms in red represent the alterations made to the equations found above in Appendix A.4.3. Note also that due to the structure of the ARF oligomers formed the order of some of the equations has changed. Instead of being ordered by length of oligomer they are now ordered first by number of ARF1 proteins then by length.

$$\begin{aligned}
\frac{dA_2}{dt} = & \pi_{A_2} - \alpha_{A_1A_2}A_1A_2 + \theta_{A_1A_2}D_{A_1A_2} - 2\alpha_{A_2A_2}A_2A_2 \quad (A.62) \\
& +2\theta_{A_2A_2}D_{A_2A_2} - \alpha_{A_1A_2}A_2G_{A_1} + \theta_{A_1A_2}G_{A_1A_2} \\
& -\alpha_{A_2G}GA_2 + \theta_{A_2G}GA_2 - \alpha_{A_2A_2}G_{A_2}A_2 + \theta_{A_2A_2}G_{A_2A_2} \\
& -0.5\alpha_{A_1A_2}D_{A_1A_2}A_2 + 2\theta_{A_1A_2}D_{A_2A_1A_2} - \alpha_{A_1A_2}D_{A_1A_1}A_2 \\
& +\theta_{A_1A_2}D_{A_1A_1A_2} - 0.5\alpha_{A_1A_2}D_{A_1A_1A_2}A_2 + \theta_{A_1A_2}D_{A_2A_1A_1A_2} \\
& -0.5\alpha_{A_1A_2}A_2G_{A_1A_2} + 2\theta_{A_1A_2}G_{A_2A_1A_2} - \alpha_{A_1A_2}A_2G_{A_1A_1}
\end{aligned}$$

$$\begin{aligned}
& +\theta_{A_1 A_2} G_{A_1 A_1 A_2} - 0.5\alpha_{A_1 A_2} A_2 G_{A_1 A_1 A_2} + 2\theta_{A_1 A_2} G_{A_2 A_1 A_1 A_2} \\
& -\alpha_{A_1 A_2} D_{A_1 A_1 A_1} A_2 + \theta_{A_1 A_2} D_{A_1 A_1 A_1 A_2} - 0.5\alpha_{A_1 A_2} D_{A_1 A_1 A_1 A_2} A_2 \\
& +2\theta_{A_1 A_2} D_{A_2 A_1 A_1 A_1 A_2} - \alpha_{A_1 A_2} G_{A_1 A_1 A_1} A_2 + \theta_{A_1 A_2} G_{A_1 A_1 A_1 A_2} \\
& -0.5\alpha_{A_1 A_2} G_{A_1 A_1 A_1 A_2} A_2 + 2\theta_{A_1 A_2} G_{A_2 A_1 A_1 A_1 A_2} \\
\frac{dD_{A_2 A_2}}{dt} = & -\mu_{A_2 A_2} D_{A_2 A_2} + \alpha_{A_2 A_2} A_2 A_2 - \theta_{A_2 A_2} D_{A_2 A_2} \quad (A.63) \\
& +\theta_{A_2 A_2} G_{A_2 A_2} - \alpha_{A_2 A_2} G_{A_2 A_2}
\end{aligned}$$

$$\begin{aligned}
\frac{dA_1}{dt} = & \pi_{A_1} - 2\alpha_{A_1 A_1} A_1 A_1 + 2\theta_{A_1 A_1} D_{A_1 A_1} - \alpha_{A_1 A_2} A_1 A_2 \quad (A.64) \\
& +\theta_{A_1 A_2} D_{A_1 A_2} - \alpha_{A_1 G} G A_1 + \theta_{A_1 G} G_{A_1} - \alpha_{A_1 A_1} A_1 G_{A_1} \\
& +\theta_{A_1 A_1} G_{A_1 A_1} - \alpha_{A_1 A_2} A_1 G_{A_2} + \alpha_{A_1 A_2} G_{A_1 A_2} \\
& -0.5\alpha_{A_1 A_1} D_{A_1 A_2} A_1 + \theta_{A_1 A_1} D_{A_1 A_1 A_2} - 0.5\alpha_{A_1 A_1} A_1 G_{A_1 A_2} \\
& +\theta_{A_1 A_1} G_{A_1 A_1 A_2} - \alpha_{A_1 A_1} D_{A_1 A_1} A_1 + 2\theta_{A_1 A_1} D_{A_1 A_1 A_1} \\
& -0.5\alpha_{A_1 A_1} A_1 D_{A_1 A_1 A_2} + \theta_{A_1 A_1} D_{A_1 A_1 A_1 A_2} - \alpha_{A_1 A_1} G_{A_1 A_1} A_1 \\
& +2\theta_{A_1 A_1} G_{A_1 A_1 A_1} - 0.5\alpha_{A_1 A_1} A_1 G_{A_1 A_1 A_2} + \theta_{A_1 A_1} G_{A_1 A_1 A_1 A_2}
\end{aligned}$$

$$\begin{aligned}
\frac{dD_{A_1 A_2}}{dt} = & -\mu_{A_1 A_2} D_{A_1 A_2} + \alpha_{A_1 A_2} A_1 A_2 - \theta_{A_1 A_2} D_{A_1 A_2} \quad (A.65) \\
& -\alpha_{A_1 A_2} G_{A_1 A_2} + \theta_{A_1 A_2} G_{A_1 A_2} - 0.5\alpha_{A_1 A_2} D_{A_1 A_2} A_2 \\
& +2\theta_{A_1 A_2} D_{A_2 A_1 A_2} - 0.5\alpha_{A_1 A_1} D_{A_1 A_2} A_1 + \theta_{A_1 A_1} D_{A_1 A_1 A_2} \\
& -0.5\alpha_{A_1 A_2} G_{A_2} D_{A_1 A_2} + 2\theta_{A_1 A_2} G_{A_2 A_1 A_2} - 0.5\alpha_{A_1 A_1} G_{A_1} D_{A_1 A_2} \\
& +\theta_{A_1 A_1} G_{A_1 A_1 A_2} - 0.5\alpha_{A_1 A_1} D_{A_1 A_1} D_{A_1 A_2} + \theta_{A_1 A_1} D_{A_1 A_1 A_1 A_2} \\
& -0.25\alpha_{A_1 A_1} D_{A_1 A_1 A_2} D_{A_1 A_2} + 2\theta_{A_1 A_2} D_{A_2 A_1 A_1 A_1 A_2} \\
& -0.5\alpha_{A_1 A_1} G_{A_1 A_1} D_{A_1 A_2} + \theta_{A_1 A_1} G_{A_1 A_1 A_1 A_2} \\
& -0.25\alpha_{A_1 A_1} G_{A_1 A_1 A_2} D_{A_1 A_2} + 2\theta_{A_1 A_2} G_{A_2 A_1 A_1 A_1 A_2}
\end{aligned}$$

$$\frac{dD_{A_2 A_1 A_2}}{dt} = -\mu_{A_2 A_1 A_2} D_{A_2 A_1 A_2} + 0.5\alpha_{A_1 A_2} D_{A_1 A_2} A_2 \quad (A.66)$$

$$\begin{aligned}
& -2\theta_{A_1 A_2} D_{A_2 A_1 A_2} - \alpha_{A_2 A_1 A_2} G_{A_2 A_1 A_2} + \theta_{A_2 A_1 A_2} G_{A_2 A_1 A_2} \\
\frac{dD_{A_1 A_1}}{dt} = & -\mu_{A_1 A_1} D_{A_1 A_1} + \alpha_{A_1 A_1} A_1 A_1 - \theta_{A_1 A_1} D_{A_1 A_1} \quad (A.67) \\
& -\alpha_{A_1 A_1} G_{A_1 A_1} + \theta_{A_1 A_1} G_{A_1 A_1} - \alpha_{A_1 A_2} D_{A_1 A_1} A_2 \\
& +\theta_{A_1 A_2} D_{A_1 A_1 A_2} - \alpha_{A_1 A_2} G_{A_2} D_{A_1 A_1} + \theta_{A_1 A_2} G_{A_1 A_1 A_2}
\end{aligned}$$

$$\begin{aligned}
& -\alpha_{A_1 A_1} D_{A_1 A_1} A_1 + 2\theta_{A_1 A_1} D_{A_1 A_1 A_1} - 0.5\alpha_{A_1 A_1} D_{A_1 A_1} D_{A_1 A_2} \\
& + \theta_{A_1 A_1} D_{A_1 A_1 A_1 A_2} - \alpha_{A_1 A_1} D_{A_1 A_1} G_{A_1} + 2\theta_{A_1 A_1} G_{A_1 A_1 A_1} \\
& - 0.5\alpha_{A_1 A_1} D_{A_1 A_1} G_{A_1 A_2} + \theta_{A_1 A_1} G_{A_1 A_1 A_1 A_2} \\
\frac{dD_{A_1 A_1 A_2}}{dt} = & -\mu_{A_1 A_1 A_2} D_{A_1 A_1 A_2} + 0.5\alpha_{A_1 A_1} D_{A_1 A_2} A_1 \quad (A.68)
\end{aligned}$$

$$\begin{aligned}
& -\theta_{A_1 A_1} D_{A_1 A_1 A_2} + \alpha_{A_1 A_2} D_{A_1 A_1} A_2 - \theta_{A_1 A_2} D_{A_1 A_1 A_2} \\
& - 0.5\alpha_{A_1 A_2} D_{A_1 A_1 A_2} A_2 + \theta_{A_1 A_2} D_{A_2 A_1 A_1 A_2} - \alpha_{A_1 A_1 A_2} G D_{A_1 A_1 A_2} G \\
& + \theta_{A_1 A_1 A_2} G G_{A_1 A_1 A_2} - 0.5\alpha_{A_1 A_2} G_{A_2} D_{A_1 A_1 A_2} + 2\theta_{A_1 A_2} G_{A_2 A_1 A_1 A_2} \\
& - 0.5\alpha_{A_1 A_1} A_1 D_{A_1 A_1 A_2} + \theta_{A_1 A_1} D_{A_1 A_1 A_1 A_2} \\
& - 0.25\alpha_{A_1 A_1} D_{A_1 A_1 A_2} D_{A_1 A_2} + 2\theta_{A_1 A_2} D_{A_2 A_1 A_1 A_1 A_2} \\
& - 0.5\alpha_{A_1 A_1} G_{A_1} D_{A_1 A_1 A_2} + \theta_{A_1 A_1} G_{A_1 A_1 A_1 A_2} \\
& - 0.25\alpha_{A_1 A_1} D_{A_1 A_1 A_2} G_{A_1 A_2} + 2\theta_{A_1 A_2} G_{A_2 A_1 A_1 A_1 A_2} \\
\frac{dD_{A_2 A_1 A_1 A_2}}{dt} = & -\mu_{A_2 A_1 A_1 A_2} D_{A_2 A_1 A_1 A_2} + 0.5\alpha_{A_1 A_2} D_{A_1 A_1 A_2} A_2 \quad (A.69)
\end{aligned}$$

$$\begin{aligned}
& -\theta_{A_1 A_2} D_{A_2 A_1 A_1 A_2} - \alpha_{A_2 A_1 A_1 A_2} G D_{A_2 A_1 A_1 A_2} G \\
& + \theta_{A_2 A_1 A_1 A_2} G G_{A_2 A_1 A_1 A_2} \\
\frac{dD_{A_1 A_1 A_1}}{dt} = & -\mu_{A_1 A_1 A_1} D_{A_1 A_1 A_1} + \alpha_{A_1 A_1} D_{A_1 A_1} A_1 - 2\theta_{A_1 A_1} D_{A_1 A_1 A_1} \quad (A.70)
\end{aligned}$$

$$\begin{aligned}
& -\alpha_{A_1 A_2} D_{A_1 A_1 A_1} A_2 + \theta_{A_1 A_2} D_{A_1 A_1 A_1 A_2} - \alpha_{A_1 A_1 A_1} G D_{A_1 A_1 A_1} G \\
& + \theta_{A_1 A_1 A_1} G G_{A_1 A_1 A_1} - \alpha_{A_1 A_2} D_{A_1 A_1 A_1} G_{A_2} + \theta_{A_1 A_2} G_{A_1 A_1 A_1 A_2} \\
\frac{dD_{A_1 A_1 A_1 A_2}}{dt} = & -\mu_{A_1 A_1 A_1 A_2} D_{A_1 A_1 A_1 A_2} + \alpha_{A_1 A_2} D_{A_1 A_1 A_1} A_2 \quad (A.71)
\end{aligned}$$

$$\begin{aligned}
& -\theta_{A_1 A_2} D_{A_1 A_1 A_1 A_2} + 0.5\alpha_{A_1 A_1} D_{A_1 A_1} D_{A_1 A_2} - \theta_{A_1 A_1} D_{A_1 A_1 A_1 A_2} \\
& + 0.5\alpha_{A_1 A_1} A_1 D_{A_1 A_1 A_2} - \theta_{A_1 A_1} D_{A_1 A_1 A_1 A_2} \\
& - 0.5\alpha_{A_1 A_2} D_{A_1 A_1 A_1 A_2} A_2 + 2\theta_{A_1 A_2} D_{A_2 A_1 A_1 A_1 A_2} \\
& - \alpha_{A_1 A_1 A_1 A_2} G D_{A_1 A_1 A_1 A_2} G + \theta_{A_1 A_1 A_1 A_2} G G_{A_1 A_1 A_1 A_2} \\
& - 0.5\alpha_{A_1 A_2} D_{A_1 A_1 A_1 A_2} G_{A_2} + 2\theta_{A_1 A_2} G_{A_2 A_1 A_1 A_1 A_2} \\
\frac{dD_{A_2 A_1 A_1 A_1 A_2}}{dt} = & -\mu_{A_2 A_1 A_1 A_1 A_2} D_{A_2 A_1 A_1 A_1 A_2} + 0.5\alpha_{A_1 A_2} D_{A_1 A_1 A_1 A_2} A_2 \quad (A.72)
\end{aligned}$$

$$\begin{aligned}
& -2\theta_{A_1 A_2} D_{A_2 A_1 A_1 A_1 A_2} + 0.25\alpha_{A_1 A_1} D_{A_1 A_1 A_2} D_{A_1 A_2} \\
& - 2\theta_{A_1 A_2} D_{A_2 A_1 A_1 A_1 A_2} - \alpha_{A_2 A_1 A_1 A_1 A_2} G D_{A_2 A_1 A_1 A_1 A_2} G
\end{aligned}$$

$$\begin{aligned} \frac{dG}{dt} = & -\alpha_{A_1G}GA_1 + \theta_{A_1G}G_{A_1} - \alpha_{A_2G}GA_2 + \theta_{A_2G}G_{A_2} \\ & -\alpha_{A_1A_1G}D_{A_1A_1}G + \theta_{A_1A_1G}G_{A_1A_1} - \alpha_{A_1A_2G}D_{A_1A_2}G \\ & +\theta_{A_1A_2G}G_{A_1A_2} - \alpha_{A_2A_2G}D_{A_2A_2}G + \theta_{A_2A_2G}G_{A_2A_2} \\ & -\alpha_{A_2A_1A_2G}D_{A_2A_1A_2}G + \theta_{A_2A_1A_2G}G_{A_2A_1A_2} - \alpha_{A_1A_1A_2G}D_{A_1A_1A_2}G \\ & +\theta_{A_1A_1A_2G}G_{A_1A_1A_2} - \alpha_{A_2A_1A_1A_2G}D_{A_2A_1A_1A_2}G \\ & +\theta_{A_2A_1A_1A_2G}G_{A_2A_1A_1A_2} - \alpha_{A_1A_1A_1G}D_{A_1A_1A_1}G \\ & +\theta_{A_1A_1A_1G}G_{A_1A_1A_1} - \alpha_{A_1A_1A_1A_2G}D_{A_1A_1A_1A_2}G \\ & +\theta_{A_1A_1A_1A_2G}G_{A_1A_1A_1A_2} - \alpha_{A_2A_1A_1A_1A_2G}D_{A_2A_1A_1A_1A_2}G \\ & +\theta_{A_2A_1A_1A_1A_2G}G_{A_2A_1A_1A_1A_2} \end{aligned} \quad (A.73)$$

$$\frac{dG_{A_2}}{dt} = \alpha_{A_2G}GA_2 - \theta_{A_2G}G_{A_2} - \alpha_{A_1A_2}A_1G_{A_2} + \alpha_{A_1A_2}G_{A_1A_2} \quad (A.74)$$

$$\begin{aligned} & -\alpha_{A_2A_2}G_{A_2}A_2 + \theta_{A_2A_2}G_{A_2A_2} - 0.5\alpha_{A_1A_2}G_{A_2}D_{A_1A_2} \\ & +2\theta_{A_1A_2}G_{A_2A_1A_2} - \alpha_{A_1A_2}G_{A_2}D_{A_1A_1} + \theta_{A_1A_2}G_{A_1A_1A_2} \\ & -0.5\alpha_{A_1A_2}G_{A_2}D_{A_1A_1A_2} + 2\theta_{A_1A_2}G_{A_2A_1A_1A_2} \\ & -\alpha_{A_1A_2}D_{A_1A_1A_1}G_{A_2} + \theta_{A_1A_2}G_{A_1A_1A_1A_2} \\ & -0.5\alpha_{A_1A_2}D_{A_1A_1A_1A_2}G_{A_2} + 2\theta_{A_1A_2}G_{A_2A_1A_1A_1A_2} \end{aligned}$$

$$\frac{dG_{A_2A_2}}{dt} = \alpha_{A_2A_2G}D_{A_2A_2}G - \theta_{A_2A_2G}G_{A_2A_2} + \alpha_{A_2A_2}G_{A_2}A_2 - \theta_{A_2A_2}G_{A_2A_2} \quad (A.75)$$

$$\frac{dG_{A_1}}{dt} = \alpha_{A_1G}GA_1 - \theta_{A_1G}G_{A_1} - \alpha_{A_1A_1}A_1G_{A_1} + \theta_{A_1A_1}G_{A_1A_1} \quad (A.76)$$

$$\begin{aligned} & -\alpha_{A_1A_2}A_2G_{A_1} + \theta_{A_1A_2}G_{A_1A_2} - 0.5\alpha_{A_1A_1}G_{A_1}D_{A_1A_2} \\ & +\theta_{A_1A_1}G_{A_1A_1A_2} - \alpha_{A_1A_1}D_{A_1A_1}G_{A_1} + 2\theta_{A_1A_1}G_{A_1A_1A_1} \\ & -0.5\alpha_{A_1A_1}G_{A_1}D_{A_1A_1A_2} + \theta_{A_1A_1}G_{A_1A_1A_1A_2} \end{aligned}$$

$$\frac{dG_{A_1A_2}}{dt} = \alpha_{A_1A_2}A_2G_{A_1} - \theta_{A_1A_2}G_{A_1A_2} + \alpha_{A_1A_2}A_1G_{A_2} \quad (A.77)$$

$$\begin{aligned} & -\alpha_{A_1A_2}G_{A_1A_2} + \alpha_{A_1A_2G}D_{A_1A_2}G - \theta_{A_1A_2G}G_{A_1A_2} \\ & -0.5\alpha_{A_1A_2}A_2G_{A_1A_2} + 2\theta_{A_1A_2}G_{A_2A_1A_2} - 0.5\alpha_{A_1A_1}A_1G_{A_1A_2} \\ & +\theta_{A_1A_1}G_{A_1A_1A_2} - 0.5\alpha_{A_1A_1}D_{A_1A_1}G_{A_1A_2} + \theta_{A_1A_1}G_{A_1A_1A_1A_2} \end{aligned}$$

$$\begin{aligned} \frac{dG_{A_2A_1A_2}}{dt} = & -0.25\alpha_{A_1A_1}D_{A_1A_1A_2}G_{A_1A_2} + 2\theta_{A_1A_2}G_{A_2A_1A_1A_2} \\ & \alpha_{A_2A_1A_2G}D_{A_2A_1A_2}G - \theta_{A_2A_1A_2G}G_{A_2A_1A_2} \end{aligned} \quad (A.78)$$

$$\begin{aligned} & +0.5\alpha_{A_1A_2}A_2G_{A_1A_2} - 2\theta_{A_1A_2}G_{A_2A_1A_2} + 0.5\alpha_{A_1A_2}G_{A_2}D_{A_1A_2} \\ & -2\theta_{A_1A_2}G_{A_2A_1A_2} \\ \frac{dG_{A_1A_1}}{dt} = & \alpha_{A_1A_1}A_1G_{A_1} - \theta_{A_1A_1}G_{A_1A_1} + \alpha_{A_1A_1G}D_{A_1A_1}G \end{aligned} \quad (A.79)$$

$$\begin{aligned} & -\theta_{A_1A_1G}G_{A_1A_1} - \alpha_{A_1A_2}A_2G_{A_1A_1} + \theta_{A_1A_2}G_{A_1A_1A_2} \\ & -\alpha_{A_1A_1}G_{A_1A_1}A_1 + 2\theta_{A_1A_1}G_{A_1A_1A_1} - 0.5\alpha_{A_1A_1}G_{A_1A_1}D_{A_1A_2} \\ & +\theta_{A_1A_1}G_{A_1A_1A_1A_2} \\ \frac{dG_{A_1A_1A_2}}{dt} = & \alpha_{A_1A_1A_2G}D_{A_1A_1A_2}G - \theta_{A_1A_1A_2G}G_{A_1A_1A_2} \end{aligned} \quad (A.80)$$

$$\begin{aligned} & +0.5\alpha_{A_1A_1}A_1G_{A_1A_2} - \theta_{A_1A_1}G_{A_1A_1A_2} + 0.5\alpha_{A_1A_1}G_{A_1}D_{A_1A_2} \\ & -\theta_{A_1A_1}G_{A_1A_1A_2} + \alpha_{A_1A_2}G_{A_2}D_{A_1A_1} - \theta_{A_1A_2}G_{A_1A_1A_2} \\ & +\alpha_{A_1A_2}A_2G_{A_1A_1} - \theta_{A_1A_2}G_{A_1A_1A_2} - 0.5\alpha_{A_1A_2}A_2G_{A_1A_1A_2} \\ & +2\theta_{A_1A_2}G_{A_2A_1A_1A_2} - 0.5\alpha_{A_1A_1}A_1G_{A_1A_1A_2} + \theta_{A_1A_1}G_{A_1A_1A_1A_2} \\ & -0.25\alpha_{A_1A_1}G_{A_1A_1A_2}D_{A_1A_2} + 2\theta_{A_1A_2}G_{A_2A_1A_1A_2} \\ \frac{dG_{A_2A_1A_1A_2}}{dt} = & \alpha_{A_2A_1A_1A_2G}D_{A_2A_1A_1A_2}G - \theta_{A_2A_1A_1A_2G}G_{A_2A_1A_1A_2} \end{aligned} \quad (A.81)$$

$$\begin{aligned} & +0.5\alpha_{A_1A_2}G_{A_2}D_{A_1A_1A_2} - 2\theta_{A_1A_2}G_{A_2A_1A_1A_2} \\ & +0.5\alpha_{A_1A_2}A_2G_{A_1A_1A_2} - 2\theta_{A_1A_2}G_{A_2A_1A_1A_2} \\ \frac{dG_{A_1A_1A_1}}{dt} = & \alpha_{A_1A_1A_1G}D_{A_1A_1A_1}G - \theta_{A_1A_1A_1G}G_{A_1A_1A_1} + \alpha_{A_1A_1}G_{A_1A_1}A_1 \\ & -2\theta_{A_1A_1}G_{A_1A_1A_1} + \alpha_{A_1A_1}D_{A_1A_1}G_{A_1} - 2\theta_{A_1A_1}G_{A_1A_1A_1} \\ & -\alpha_{A_1A_2}G_{A_1A_1A_1}A_2 + \theta_{A_1A_2}G_{A_1A_1A_1A_2} \end{aligned} \quad (A.82)$$

$$\begin{aligned} \frac{dG_{A_1A_1A_1A_2}}{dt} = & \alpha_{A_1A_1A_1A_2G}D_{A_1A_1A_1A_2}G - \theta_{A_1A_1A_1A_2G}G_{A_1A_1A_1A_2} \\ & +\alpha_{A_1A_2}G_{A_1A_1A_1}A_2 - \theta_{A_1A_2}G_{A_1A_1A_1A_2} + \alpha_{A_1A_2}D_{A_1A_1A_1}G_{A_2} \\ & -\theta_{A_1A_2}G_{A_1A_1A_1A_2} + 0.5\alpha_{A_1A_1}G_{A_1A_1}D_{A_1A_2} - \theta_{A_1A_1}G_{A_1A_1A_1A_2} \\ & +0.5\alpha_{A_1A_1}D_{A_1A_1}G_{A_1A_2} - \theta_{A_1A_1}G_{A_1A_1A_1A_2} \\ & +0.5\alpha_{A_1A_1}G_{A_1}D_{A_1A_1A_2} - \theta_{A_1A_1}G_{A_1A_1A_1A_2} \\ & +0.5\alpha_{A_1A_1}A_1G_{A_1A_1A_2} - \theta_{A_1A_1}G_{A_1A_1A_1A_2} \end{aligned} \quad (A.83)$$

$$\frac{dG_{A_2A_1A_1A_1A_2}}{dt} = -0.5\alpha_{A_1A_2}G_{A_1A_1A_1A_2}A_2 + 2\theta_{A_1A_2}G_{A_2A_1A_1A_1A_2} \\ + \alpha_{A_2A_1A_1A_1A_2}G_{A_2A_1A_1A_1A_2}G - \theta_{A_2A_1A_1A_1A_2}G_{A_2A_1A_1A_1A_2} \quad (A.84)$$

$$+0.5\alpha_{A_1A_2}G_{A_1A_1A_1A_2}A_2 - 2\theta_{A_1A_2}G_{A_2A_1A_1A_1A_2} \\ +0.5\alpha_{A_1A_2}D_{A_1A_1A_1A_2}G_{A_2} - 2\theta_{A_1A_2}G_{A_2A_1A_1A_1A_2} \\ +0.25\alpha_{A_1A_1}G_{A_1A_1A_2}D_{A_1A_2} - 2\theta_{A_1A_2}G_{A_2A_1A_1A_1A_2} \\ +0.25\alpha_{A_1A_1}D_{A_1A_1A_2}G_{A_1A_2} - 2\theta_{A_1A_2}G_{A_2A_1A_1A_1A_2} \\ \frac{dh}{dt} = l_1G_{A_1} + l_2(G_{A_1A_1} + G_{A_1A_1A_1}) \quad (A.85)$$

$$\pi_{A_1}(t) = \frac{dTotalARF1}{dt}(t) + \mu_{A_1A_2} + 2\mu_{A_1A_1} + 2\mu_{A_1A_1A_2} \quad (A.86)$$

$$+2\mu_{A_2A_1A_1A_2} + 3\mu_{A_1A_1A_1} + 3\mu_{A_1A_1A_1A_2} + 3\mu_{A_2A_1A_1A_1A_2} \\ \pi_{A_2}(t) = \frac{dTotalARF2}{dt}(t) + 2\mu_{A_2A_2} + \mu_{A_1A_2} + \mu_{A_1A_1A_2} \quad (A.87) \\ +2\mu_{A_2A_1A_1A_2} + \mu_{A_1A_1A_1A_2} + 2\mu_{A_2A_1A_1A_1A_2}$$

Appendix B

Published Supplementary Information

Supplementary Note 2 to
[Truskina et al., 2021]

Modelling the non-linear feedback of ARF caused
by the presence of multiple transcriptional
regulators.

September 30, 2022

As ARF8 has multiple regulators, many of which are positively or negatively regulated by auxin, we used a mathematical model to predict the levels of ARF8 mRNA if these regulators were knocked out.

We consider a model including a generic ARF (of concentration denoted ARF), a generic Aux/IAA (concentration denoted I). Given that ARFs are under the control of multiple transcription factors (TFs), we also include two such transcription factors, denoted abstractly as X and Y . In the extreme case of ARF8, there are many more TFs, as well as feedback loops between them and ARF8. The main goal of this model is to investigate in a simplified context what could be the potential effects of the interplay between multiple TFs on ARF activity, feedback and interactions between these actors. By including only two regulators, we will be able to investigate the potential outcomes more comprehensively than would be possible with a complete model including all actual TFs. The main goal of this model is to predict the experimentally observed output of ARF mRNA levels when some TFs, particularly within feedback loops, are knocked out.

To describe transcriptional regulation, one also has to include variables for the mRNA of each of the four proteins listed above. We denote by m_W the concentration of mRNA for W where W can be any of ARF , I , X and Y . By using a toggle term (see below), we were able to investigate scenarios when individual transcription factors were either repressed or activated by the ARF. The model

takes the form

$$\begin{aligned}
\frac{d\text{ARF}}{dt} &= \delta_{\text{ARF}} (\beta_{\text{ARF}} m_{\text{ARF}} - \text{ARF} I - \text{ARF}) \\
\frac{dI}{dt} &= \delta_I \left(\beta_I m_I - \frac{\kappa_1 X}{1 + \kappa_1 X} I \right) \\
\frac{dX}{dt} &= \delta_X (\beta_X m_X - X) \\
\frac{dY}{dt} &= \delta_Y (\beta_Y m_Y - Y) \\
\frac{dm_{\text{ARF}}}{dt} &= \delta_{m_{\text{ARF}}} (\beta_{m_{\text{ARF}}} [\alpha_1 h^-(X, t_X) h^-(Y, t_Y) + \alpha_2 h^+(X, t_X) h^-(Y, t_Y) + \\
&\quad \alpha_3 h^-(X, t_X) h^+(Y, t_Y) + \alpha_4 h^+(X, t_X) h^+(Y, t_Y)] - m_{\text{ARF}}) \\
\frac{dm_X}{dt} &= \delta_{m_X} \left(\rho_0 + \beta_{m_X} \frac{\kappa_2 (\lambda + (1-\lambda) \text{ARF}^2)}{1 + \kappa_2 \text{ARF} (I + \text{ARF})} - m_X \right) \\
\frac{dm_Y}{dt} &= \delta_{m_Y} \left(\rho_0 + \beta_{m_Y} \frac{\kappa_3 \text{ARF}^2}{1 + \kappa_3 \text{ARF} (I + \text{ARF})} - m_Y \right) \\
\frac{dm_I}{dt} &= \delta_{m_I} \left(\rho_0 + \beta_{m_I} \frac{\kappa_4 \text{ARF}^2}{1 + \kappa_4 \text{ARF} (I + \text{ARF})} - m_I \right)
\end{aligned} \tag{B.1}$$

where

$$h^+(x, \theta) = \frac{x^p}{x^p + \theta^p} \quad \text{and} \quad h^-(x, \theta) = \frac{\theta^p}{x^p + \theta^p} = 1 - h^+(x, \theta)$$

are activating and repressing Hill functions, respectively, a classical choice for rates of transcriptional regulation. The parameters used are reported in Table B.1, along with a brief interpretation. Note here the particular role of $\lambda \in \{0, 1\}$ in m_X 's equation, which is a toggle parameter, with the effect that X is repressed by ARF when $\lambda = 1$ and activated when $\lambda = 0$, since both cases are found in the data.

Four particular scenarios were investigated, which are summarized in the diagrams in Extended Data Fig.B.1. See also Table B.1 for parameter values. In each case transcription factor X inhibits the transcription of ARF mRNA. The scenarios differ as to whether auxin feeds back on the levels of TF X (left) or not (right), and if so whether it does so in a negative (bottom left) or positive (top

left) manner.

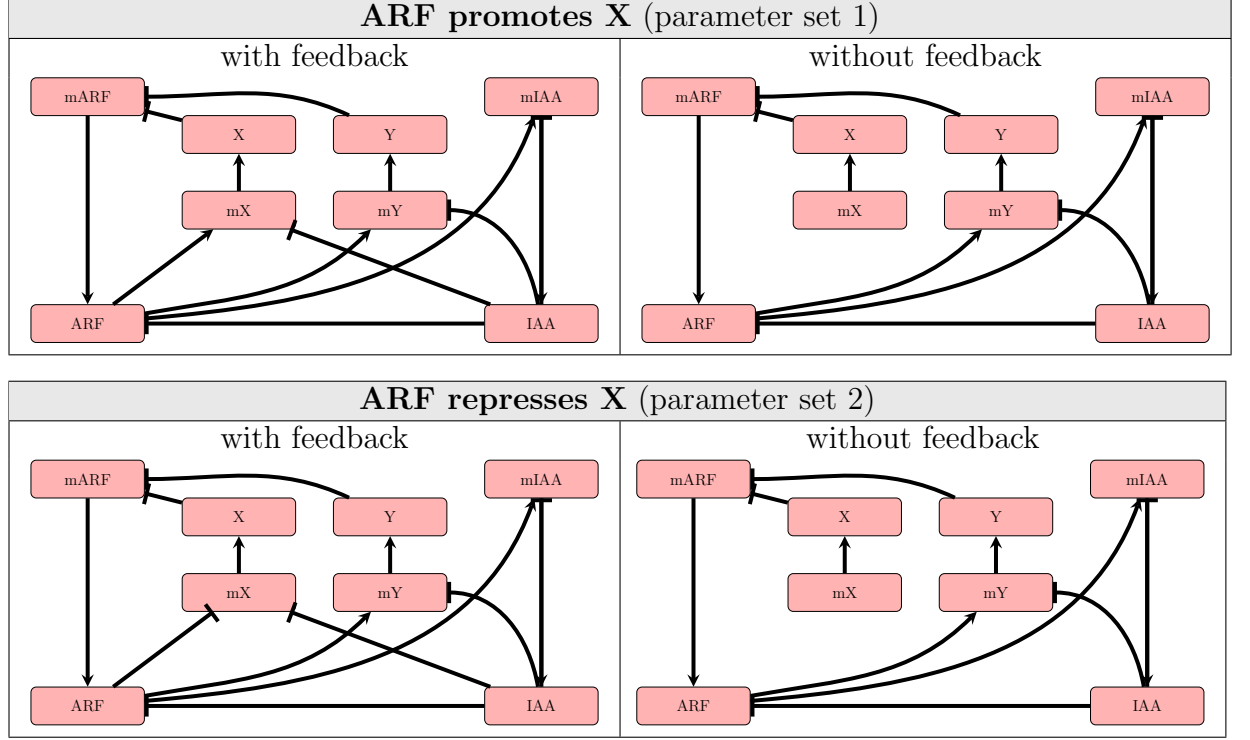


Figure B.1: Diagrammatic representation of the interactions taking place for different instances of model (B.1). The two diagrams on the right (without feedback) are identical. However, for comparison with the models with feedback the parameters used for these differ (see Table B.1).

We report two simulations, for $\lambda = 0$ and $\lambda = 1$ respectively, corresponding to the two columns in Table B.1. All other parameters are identical, except for β_X , which was increased in the repressor case to prevent the X population from being very low.

The main conclusions are that, in the model, knocking out an ARF repressor (here Y), opposite outcomes can be obtained depending on the remaining population of regulators (here X): ARF mRNA can either increase or decrease when certain repressors are removed depending on the feedback and the configuration of the network.

More specifically, Extended Data Fig.B.2, shows how knocking out Y results in a decreased level of transcribed ARF. Though not reported here, it was also observed, for other parameter choices, that knocking out Y leads to increased ARF, as would be expected intuitively (for both values of λ). In the first case

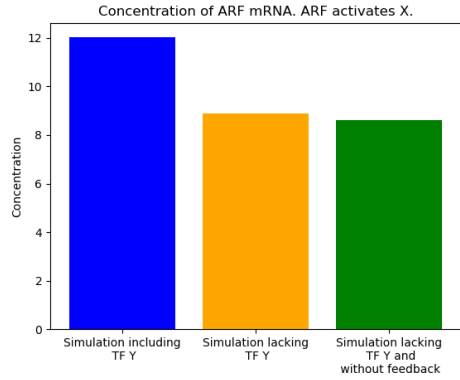
(decreased ARF transcription) is consistent with some observations made with ARF8, whilst the second (increased ARF transcription) was observed with all ARFs.

Finally, we also report the effect of the presence of feedback in Extended Data Fig.B.2a and c (green bars): the absence of feedback does not prevent the qualitative results above. However, it has a quantitative impact on the change in ARF transcription levels. The relatively small effect that feedback has on the system might seem quite surprising. One possible explanation is that, although we have explored a number of parameter values, it is not possible to claim that we have exhausted all possibilities. Though feedback loops might have a much stronger or different effect in parameter regimes not considered in our numerical survey, we did observe a tendency for the feedback to be more effective for TFs which are negatively regulated by auxin than for those that are positively regulated, as seen in Extended Data Fig.B.2.

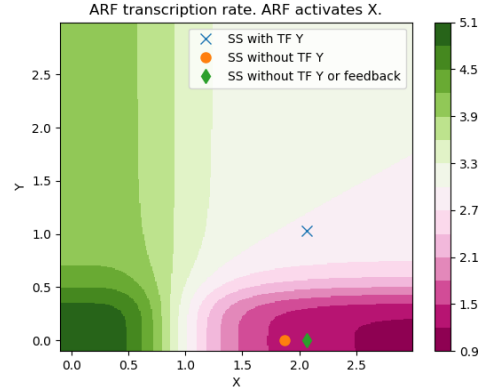
In situations in which multiple TFs regulate the same ARF this may result in (steady state) mRNA values of that ARF going up or down (in response to knocking out negative regulators) in virtually every conceivable way, depending on parameters values taken with a plausible range. Although we have modelled this for two transcriptional regulators, the outcome is likely to be more complex the greater the number of transcriptional regulators.

Variable	Interpretation	Parameter set 1	Parameter set 2
δ_{ARF}	timescale for ARF rate of change	1	1
δ_{I}	timescale for IAA rate of change	1	1
δ_{X}	timescale for X rate of change	1	1
δ_{Y}	timescale for Y rate of change	1	1
δ_{mARF}	timescale for ARF mRNA rate of change	1	1
δ_{mX}	timescale for X mRNA rate of change	1	1
δ_{mY}	timescale for Y mRNA rate of change	1	1
δ_{mI}	timescale for IAA mRNA rate of change	1	1
β_{ARF}	ratio between production and decay of ARF	1	1
β_{I}	ratio between production and decay of IAA	8	8
β_{X}	ratio between production and decay of X	2	5
β_{Y}	ratio between production and decay of Y	1	1
β_{mARF}	ratio between production and decay of ARF mRNA	4	4
β_{mX}	ratio between production and decay of X mRNA	4	4
β_{mY}	ratio between production and decay of Y mRNA	4	4
β_{mI}	ratio between production and decay of IAA mRNA	5	5
κ_1	threshold for auxin induced IAA degradation rate	4	4
κ_2	threshold for m_X transcription rate	8	8
κ_3	threshold for m_Y transcription rate	8	8
κ_4	threshold for m_I transcription rate	8	8
α_1	mARF transcription rate for low X and low Y	4	4
α_2	mARF transcription rate for high X and low Y	1	1
α_3	mARF transcription rate for low X and high Y	3	3
α_4	mARF transcription rate for high X and high Y	2	2
t_{X}	threshold for high vs low X population	1	1
t_{Y}	threshold for high vs low Y population	0.5	0.5
λ	Toggle ARF suppression or promotion of X	0	1
ρ_0	Background production rate	0	0

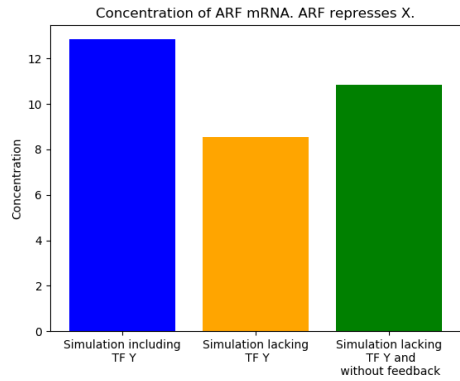
Table B.1: Parameter values.



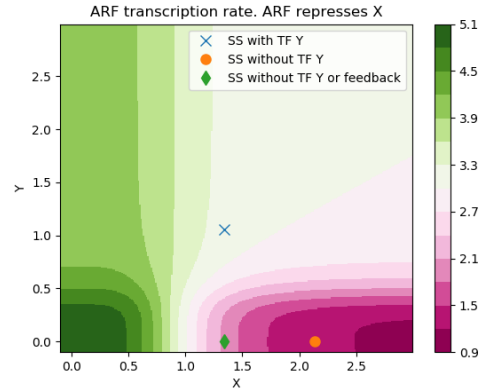
(a)



(b)



(c)



(d)

Figure B.2: On the left: bar chart displaying concentrations before and after knock out of transcription factor X, where Y is activated by ARF (a) or repressed by ARF (c). On the right: Contour plot displaying ARF transcription rate before and after knock out of transcription factor X relative to Y and X populations, where Y is activated by ARF (b), or repressed by ARF (d). Steady state (SS) values corresponding to the bar plot are also reported.