Indian Institute of Technology Madras

Raman Lab

Topological Sensitivity Analyses of Target-of-Rapamycin pathway in *S. cerevisiae*

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May 10, 2019

CERTIFICATE

This is to certify that the thesis titled **TOPOLOGICAL SENSITIVITY ANALYSES OF TARGET OF RAPAMYCIN PATHWAY IN S. CEREVISIAE**, submitted by **Abhijeet Mavi (BE14B001)**, to the Indian Institute of Madras, for the award of Dual Degree (B.Tech. & M.Tech.) in Biological Engineering, is a bonafide record of the research work done by him under my supervision. The contents of this thesis, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma.

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ACKNOWLEDGEMENT

I would like to thank Dr. Karthik Raman for his support and guidance during the project. He always kept me on my toes and would push me to my limits for maximum output. I would also like to thank each and every lab member of Raman Lab, IIT Madras. All of them were helpful and always open to discussions regarding the project which gave me a lot of direction at various junctions of the project. Special thanks to Vishnu and Shayantan. Without their coding expertise, I would not have come out of various pitfalls during the project, and they also motivated me to garner the coding skillset.

I am also glad that I had an amazing set of competitive peers like Debayan, Sharika, Saurabh who were always pushing me to my extreme limit. Finally, I would like to thank my parents and brother for their unwavering support during the project.

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ABSTRACT

Identifying the functional capability and quantifying the effects of biological systems on the corresponding phenotypes has been a challenging question for the past few decades. Ordinary differential equations (ODEs) are used to explain the temporal effects of biological systems. The mathematical modelling approach has been successful in defining the Target-of-Rapamycin pathway. The study has been fundamental in forming hypotheses based on reaction kinetics and effect of various components in the model. We investigated the importance of various hypotheses along with perturbations in the core TOR model to find out if there are new effects that have not been accounted for by the core model. The approach followed the perturbations in the topology to discern the temporal changes in the TOR pathway along various protein complexes and metabolites. Various complex formations crucial for the TOR signalling pathway can be perturbed by disturbing the kinetic architecture of the system. Sensitivity analyses provide an answer to look for the parameters to perturb for quantifying such effects.

A mathematical model always entail some level of uncertainty with itself due to various factors. Major factors include human error, paucity of data and knowledge and external factors not accounted for. One of the major problems paucity of data in uncertainty and have tried to quantify the effects associated with the amount of data given for such a study. The hypotheses test requires experimental data which in turn, are used for optimization methods to define the model architecture. We have used PyGMO, a parallel optimization solver, to find the parameters and hence, the uncertainty associated with them. These studies majorly revealed the validity of hypotheses through *in silico* approaches and give a wholesome picture of the TOR model by introducing the concepts of uncertainty and sensitivity analyses.

Keywords: TOR, uncertainty, PyGMO, sensitivity, phosphorylation, ordinary differential equations (ODEs), optimization, bootstrap, rapamycin

1 Mathematical Modelling in Biology

Biology provides a lot of interesting and novel models that invoke questions which have not been answered due to various reasons ranging from lack of technology to lack of data that explain a particular biological phenomena. These biological systems are generally governed by the general rules of nature, and driven in particular chemistry and mathematics.

1.1 Ordinary Differential Equations

Ordinary Differential Equations (ODEs) provide a platform to study the rate equations associated with a model. This is where the intersection of mathematics and biology happens. Each programming platform have their own specific architecture to solve ODEs. ODEs can be largely divided into 2 categories: **stiff** and **non-stiff** ODEs based on the level of complexity and its tendency to fall into unstable pitfalls. Biology-based ODEs are generally seen to be stiff in most cases, even though solutions for the same have not been implemented very well.

1.2 Other Methods to solve ODEs

Various other methods like Euler method, Runge-Kutta n-th order, are also used for the same. These methods largely are based on the variance of timestep required to solve the ODE. These systems largely fail in the presence of stiff systems as they can't give a proper estimate of timestep at each step which varies for a stiff problem [4].

1.3 Uncertainty Quantification in Biology

Biological networks are widely described as mathematical models. These mathematical models are generally coupled ODEs, as mentioned before. Developing informative and realistic information from these models involves suitable statistical infer- ence methods and a bit of luck. The model should also be highly robust and should be compatible with any amount of data generated from experimental analysis [5]. The structure of the model evaluates quantitatively and qualitatively the dependence of model inferences and predictions on the assumed model structures. Failure to consider the impact

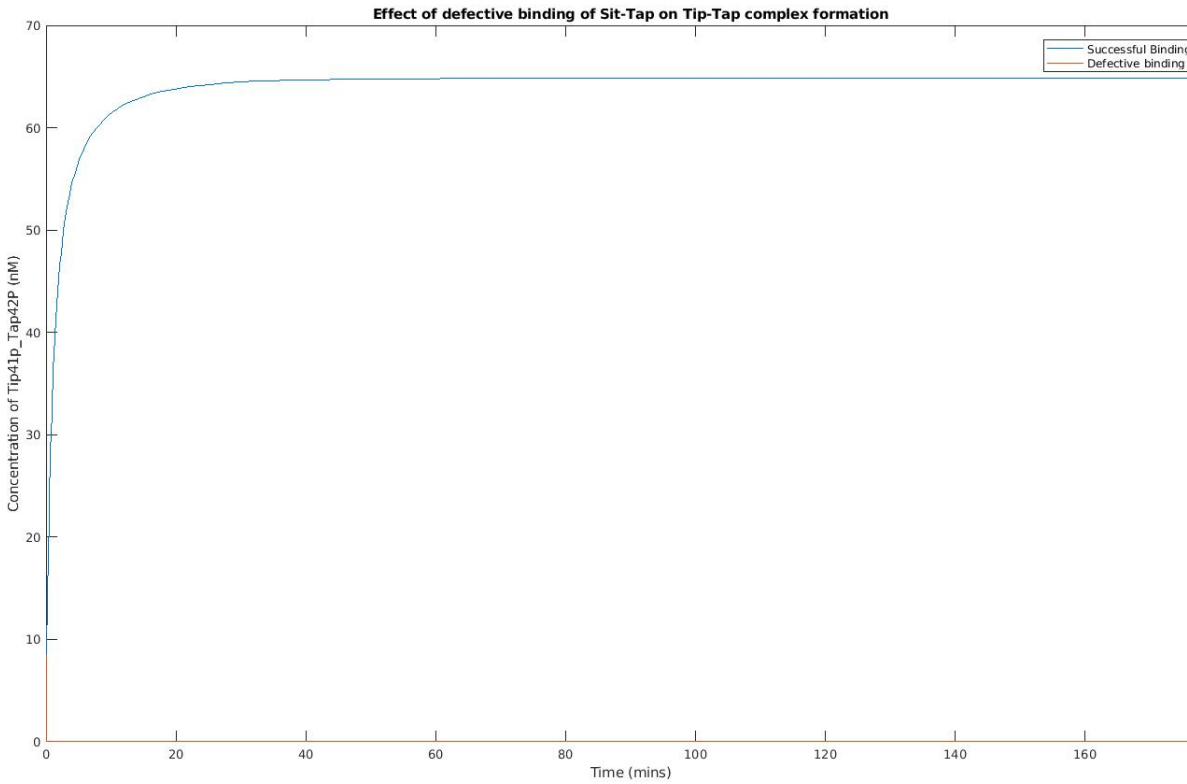


Figure 1: Model Determination and Uncertainty Quantification [2]

of structural uncertainty introduces biases into the analysis and potentially gives rise to misleading conclusions. There are many ways to assess the sensitivity of our inferences based on the model chosen [6]. This process is called **topological sensitivity analysis (TSA)**.

Another big issue that is not very widely addressed in the study of biological networks is uncertainty in the data that gives dubious results. The main reason behind it is the dearth of reliable parameter sets and data. In biology, we deal with two kinds of uncertainty namely, aleatoric and epistemic. Aleatoric uncertainty is caused due to the inherent randomness of a system like the gene expression noise or rolling of a die. Epistemic uncertainty arises due to the lack of knowledge of the system. We need knowledge in stochas-

tic processes to correct **aleatoric** uncertainity and one need to have a clear knowledge of statistics for resolving **epistemic** errors. Uncertainty can be quantified in various ways. One popular way is to **bootstrap** your observed data to generate estimates that will in turn give a distribution owing to the uncertainty of the model [6].

1.4 Ensemble Modelling

After determining the required data for a modelling problem, one needs to determine what kind of models are needed for determining the *truth* that can be inferred from the given data. *The estimation of truth will only be as good as the data that is used to do the estimation.* One of the techniques that is quite prevalent nowadays is ensemble modelling (EM) which relies on the structural diversity of the dynamic model in consideration where you define multiple models. However, despite it's various advantages, EM becomes computationally challenging for many complex biological systems. In this method, a core model is defined using experimental approaches including various biological hypotheses pertaining to that model and then various other derived models relying on systematically casting molecular hypotheses. One of the ways to determine the best model that I have come across till now is to represent the model as a network where each node represents each of the biological component and the edges will represent their interaction with other components and so this would give us the whole system of coupled ODEs and then one can proceed with TSA [6,7]. In order to determine the uncertainty associated with each model, one need to do a parameter sensitivity analysis which in turn, will define the model uncertainty.

1.5 Sensitivity Analyses

Sensitivity Analyses can be majorly quantified as $\frac{dK}{dP_i}$ where K is the component for which sensitivity is being tested and P_i is the parameter but that's only local sensitivity. For global sensitivity, we use the expectation operator to consider all the parameters for a particular biological component as $E(\frac{dK}{dP_i})$ [8]. In order to determine the global sensitivity, one has to consider all other aspects at steady state so that there can be seen observable perturbations if sensitivity exists.

1.6 Optimization

A modelling problem statement generally consists of solving an objective function that minimises the error between the observed and predicted values. These majorly fall under the category of optimization of distance with respect to the parameters (decision vectors). Various classes of optimization are there: local minima/maxima and global minima/maxima. Generaly, in biology, we look to optimize solutions using maximization of minimization of activity of a particular component. Majorly, we consider *growth* of an organism to be the objective function, that is to maximize growth would fetch us the best decision vectors for the champion set. Numerical analysis and linear algebra have contributed significantly to this field but there have been various optimization algorithms inspired from biology. A class of such algorithms called **genetic algorithms** which work on the principle of *survival of the fittest* have been used in PyGMO, a global optimisation toolbox as described in detail in [9].

1.7 Objectives

The objective of this study were:

- Verification of error generated by a model of choice with given experimental values
- Minimise the error even further for the same model, if possible through other optimisation algorithms (PyGMO)
- Determine how much data is required to make valid estimates
- Quantify uncertainty in parameter estimates based on error in the experimental data
- Do topological sensitivity analyses (global) for germane conclusions
- Introduce/validate hypotheses to find the importance of specific components

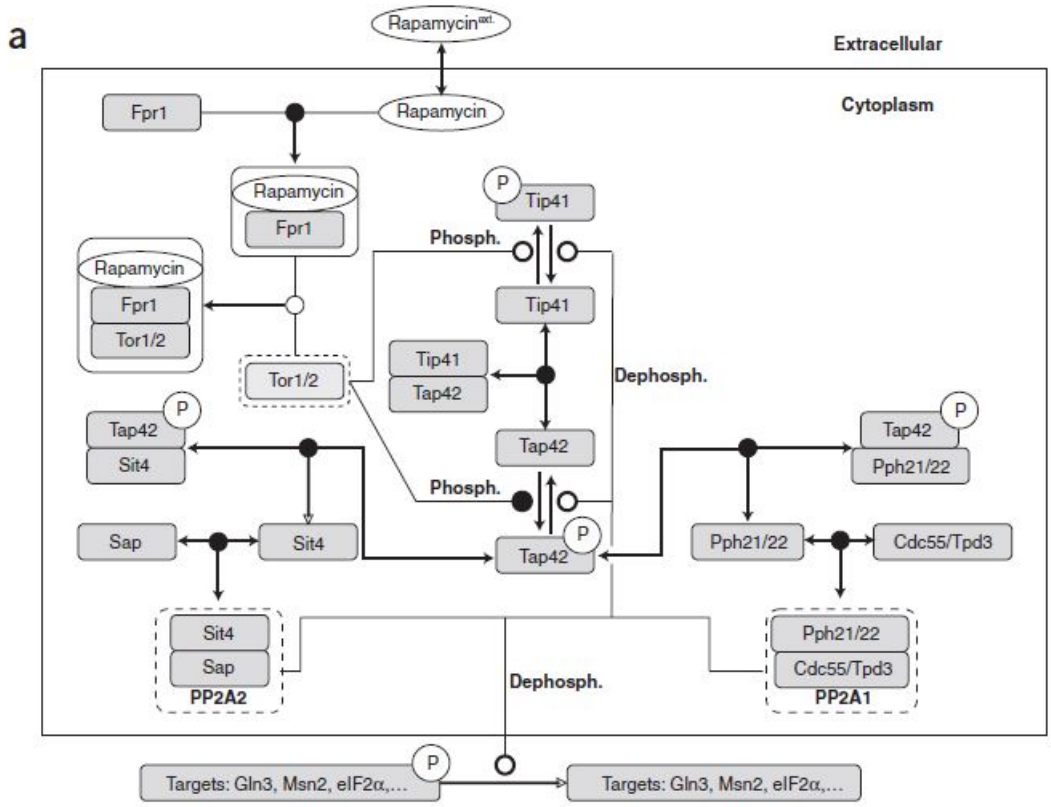


Figure 2: TOR pathway

2 Target-of-Rapamycin Pathway: An Ensemble Modelling Approach

TOR is a signalling pathway that responds to nutrient availability in the organism. A mechanistic model of the TOR pathway is shown in Fig. 2. As can be seen from Fig. 2, Tor1/2p are responsible for cellular response via modulation of PP2As. Active TOR kinases signal less nitrogen quality or addition of rapamycin (an anticancer agent) to inhibit Tor1/2p to activate PP2As [1].

The regulatory subunits bind with Pph21/22p or Sap proteins to form PP2As. This complex interplay is mediated by Tap42p and Tip41p. These species compete for binding with PP2As but the functionality of this bind is still largely unknown along with various other function of the system. This

study by *Keupfer et al, Nature Biotechnology (2007)*, does not represent a single model, but a group of models that can be further enhanced for more deductions [1,3].

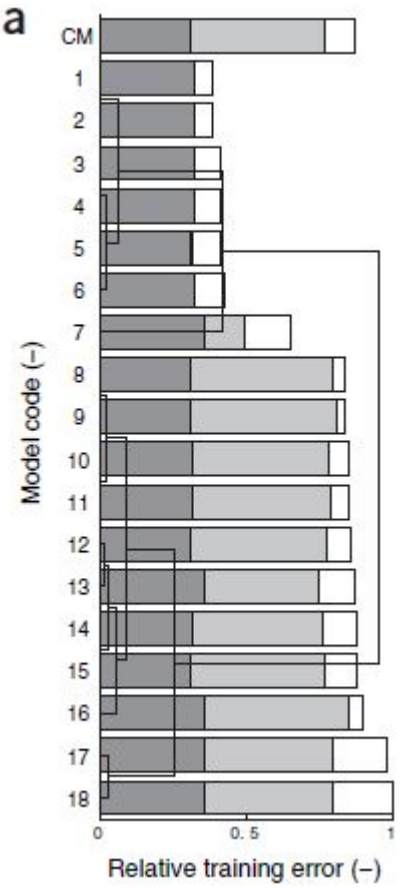


Figure 3: Error figure for various experiments in TOR pathway [1]; dark grey color represents experimental error for Tap42p^p dephosphorylation; light grey area represents experimental error for Tip41p-Tap42p complex formation and white represents experimental error for the rest of the experiments

NOTE: Notation for phosphorylation is the superscript p.

All the reactions happened according to law of mass action. *Kuepfer et al* tested for various hypotheses of the system as listed in Fig. 4. The first

1	Tip41p has two phosphorylation sites
2	Tap42p p-Pph21/22p forms an anti-phosphatase protecting phosphoproteins
3	Complex formation of Tap42p p and Tip41p
4	Complex formation of Tap42p p and Tip41p p
5	Pph21/22p is phosphorylated by Tor1/2p and dephosphorylated by PP2A1/2
6	Tap42pP-Pph21/22p acts as a phosphatase
7	Specific catalytic constants for dephosphorylation of Tip41pP by PP2A1/2
8	Tap42pP-Sit4p forms an anti-phosphatase that protects phosphorylated proteins
9	Tap42pP-Sit4p is a phosphatase
10	Sit4p is phosphorylated by Tor1/2p and dephosphorylated by PP2As
11	Tap42p has two phosphorylation sites
12	PP2A1/2 form with Sit4p and Pph21/22p bound to Tap42P and dephosphorylate it
13	Tap42pP bound to Sit4p or Pph21/22p can be dephosphorylated by PP2A1/2
14	Specific constants for dephosphorylation of Tap42pP by PP2A1 / PP2A2
15	Monomeric Sit4p is an active phosphatase for Tip41pP
16	Complex formation of Tap42p and Tip41pP
17	Complex formation of Tap42p and Pph21/22p
18	Complex formation of Tap42p and Sit4p
19	Tip41p can form a complex with Sap proteins

Figure 4: Experimental topological variants used in the core model

list of reactions of the core model developed for this study is in Appendix A for reference. The major fallacy in the system was that for all the studies they only had 17 experimental values for 11 experiments, which might not be able to define the system so well given the complexity associated with it.

The experimental setup was to introduce rapamycin at a point of time and observe its effects over various variants of the system. The detailed experimental conditions and values are listed in Supplementary material as reported by [1].

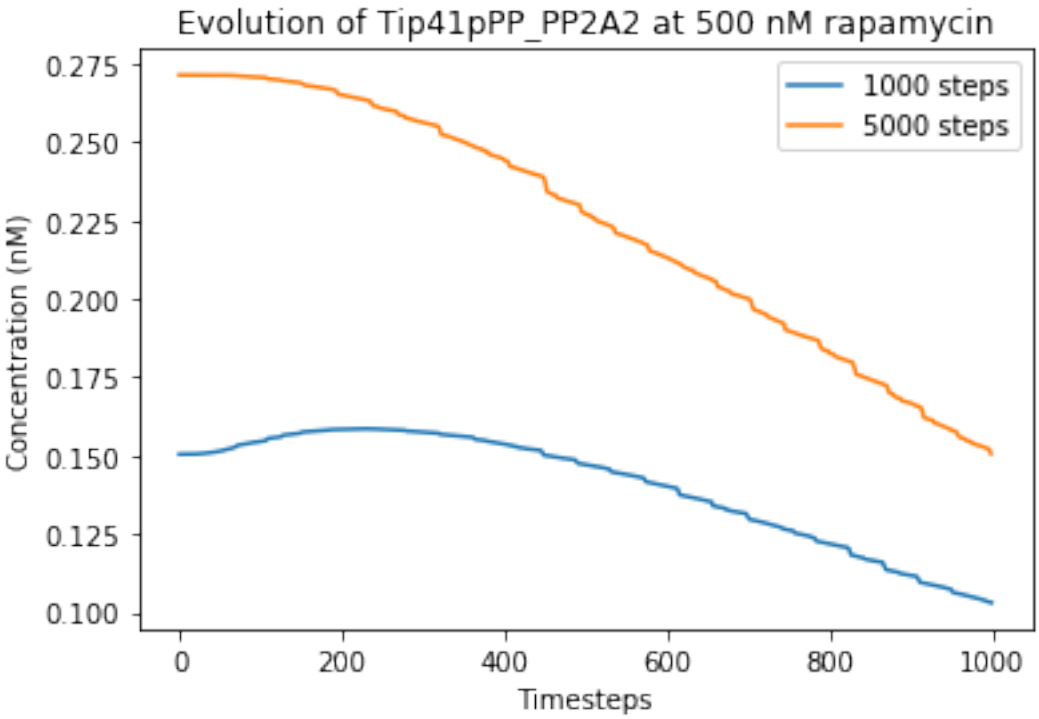


Figure 5: LSODA showing varying trend for same ODEs in python

3 ODE Solvers: A Comparative Case Study

ODE solvers while their objectives are same, that is, to solve the stiff or non-stiff ODE problems with equal efficiency, but they can function differently for a stiff system. Due to numerical complexity and varying efficiency as to how a package is written for different programming environments, there can be inconsistency in the outcome of these ODE solutions.

The package LSODA in python showed varying result when same amount of rapamycin was injected into the CORE model for 2 different timesteps, showing incoherrency as shown in Fig. 5.

Different trends were seen for the same input of rapamycin in ode 23s and solve_ivp() solver in python.

But the trends were somewhat similar when compared for various components showing that we could use ivp solver, but with inaccuracies. These

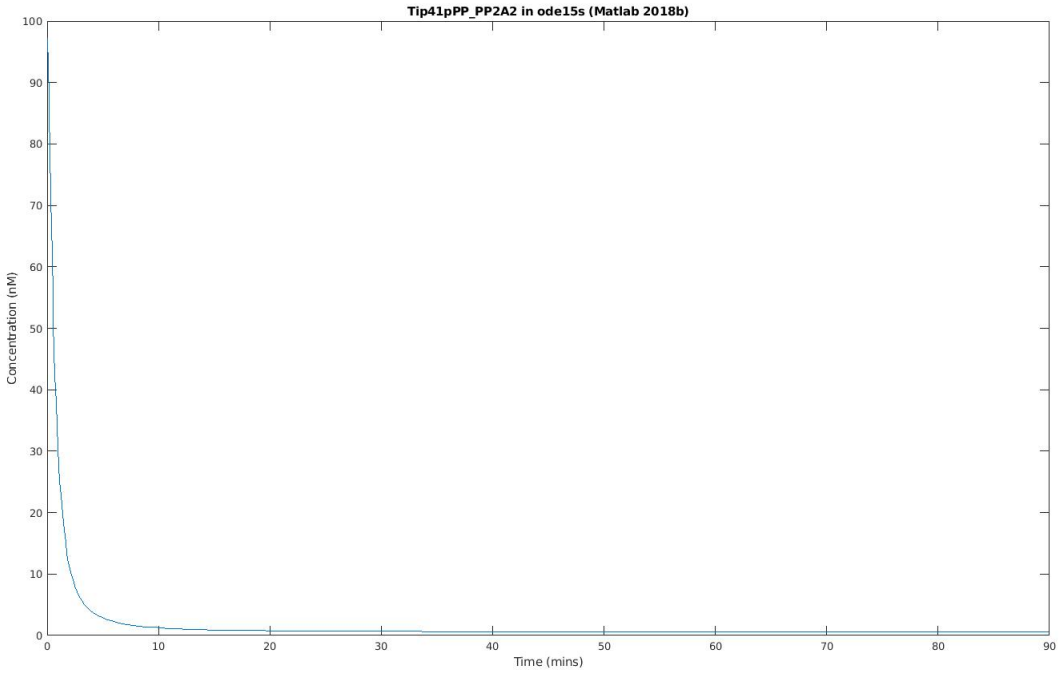


Figure 6: Tip-PP2A2 variation in Matlab (ode15s)

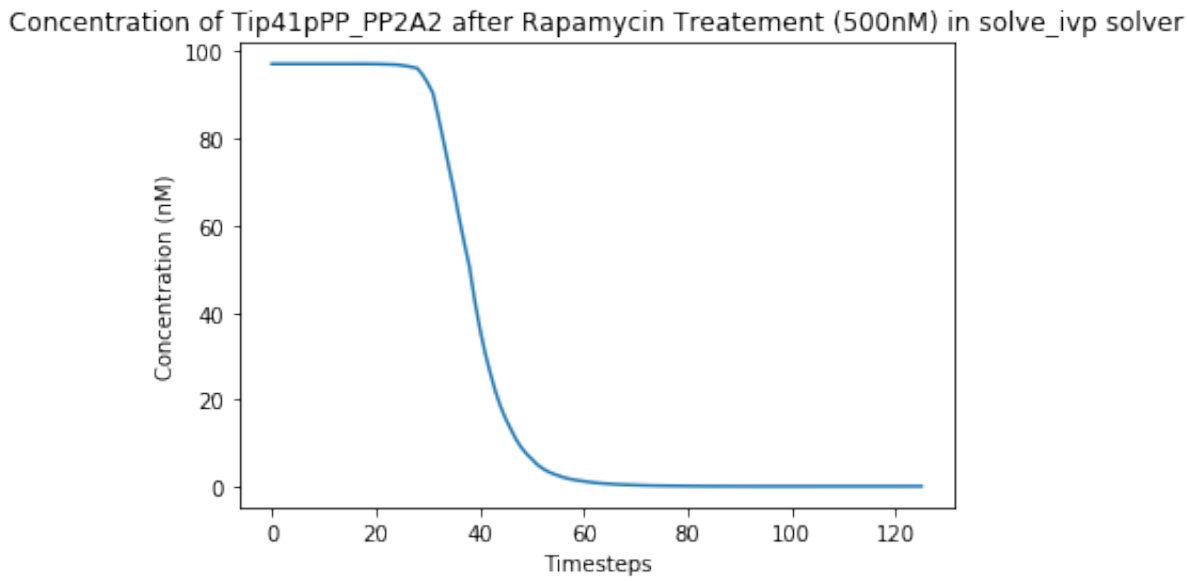


Figure 7: Tip-PP2A2 variation in python

inaccuracies did pile up to be a huge error in the later ODE solving of the system with varying hypotheses. Owing to this problem, I shifted to generate synthetic data from the core model via Matlab and use that for further optimization.

4 Model Optimization

TOR pathway is one of the most widely studied pathways in biology. It is generally based on the stress response of nitrogen intake. Nitrogen catabolite repression (NCR) are the genes whose expression is highly repressed when excess of nitrogen is present, and it gets derepressed when the TOR pathway has rapamycin introduced into it. The TOR pathway in itself has been well-defined for years, but there are various unintelligible hypotheses that are yet to be proven by experimental studies of this signalling pathway.

4.1 Approximate Bayesian Computing

I deployed approximate Bayesian computing to discern the correct pathway for a reaction in a toy model and stated its computational limitations on the TOR pathway [10,11]. In order to increase the computational speed of the process, I tried using parallel programming via ginSODA, a general purpose numerical integrator tool that distributes its calculations on a graphical processing unit.

4.2 5-state Toy Model

As shown in Fig. 8, there are 2 ways in which the given reaction can proceed from A to C. It would either be through B or through D. So, I simulated the model using all combinations of parameter sets that can define the system, by deleting a particular parameter. Even though, this approach is not correct biologically since deleting parameters only amounts to deleting one way of reaction [10]. Rather, one can delete a whole reaction set to bring down the number of computations.

Clearly, as one can see that over 10,000 simulations of the model, when parameters k_5, k_6 and k_7 are deleted gives the highest frequency reported for viability of that particular model with respect to the given dataset. One of the biggest drawbacks of this method is that it is **dependent on the choice of prior and threshold** for its values.

4.3 ginSODA

ginSODA is a python-based API for GPU powered stiff ODE solving. It finds its application in various parallel programming problem statements [12]. In

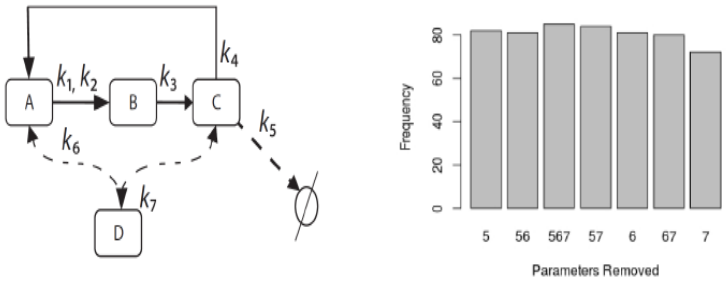


Figure 8: Proposed 2-reaction Mechanism and Barplot for Removed Parameters

the TOR pathway for the model that we chose based on the Fig. 2, we have 26 parameters, so basically it will have 2^{26} possible models that one can choose from. This is a huge number for approximate Bayesian computing, and is not feasible to implement on a generic desktop. As a test, I tried to check for RMSE values for the chosen model with the core model on ginSODA. ginSODA throws a **parsing error** for any number of parameters that are greater than 10. This parsing error is being currently looked into by my labmate, Vishnu who is working on parallel programming based problem statements. Even for 10 parameters, we got a high RMSE of about **51,000** over 10,000.

4.4 PyGMO

Considering the computational expense of ABC, I shifted my focus towards optmising the error using genetic algorithms. I first generated the error figure as shown in Fig. 3 below in Fig. 9 on the left hand side.

The figures are similar but not identical. The reason for this discrepancy is the inefficiency of ODE solving in python as discussed in Section 3.

I used PyGMO for global optimization here which has a repository of algorithms for efficient global optimization using parallel computing for faster operations [9]. PyGMO works on the principle of creating islands for parallel computing, and then passing the arguments for fitness function over a population of candidate population set, which would give the final champion set. Please note that PyGMO only works for minimization problems. The algorithm for PyGMO is surmised in Fig. 10

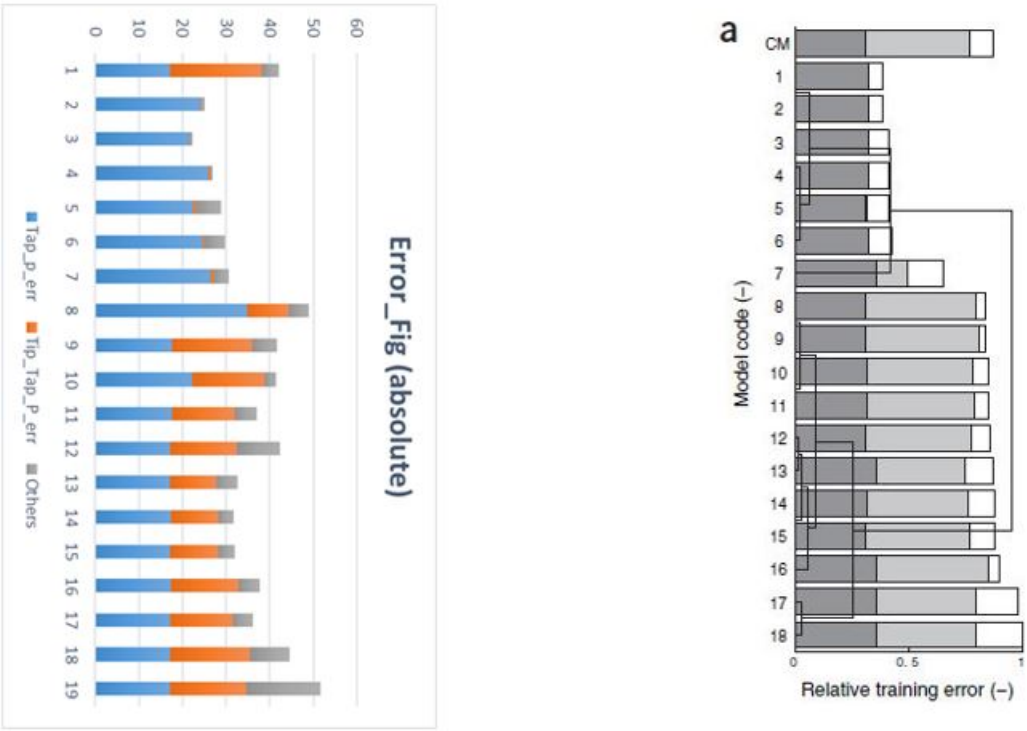


Figure 9: Comparison of original errors and generated errors

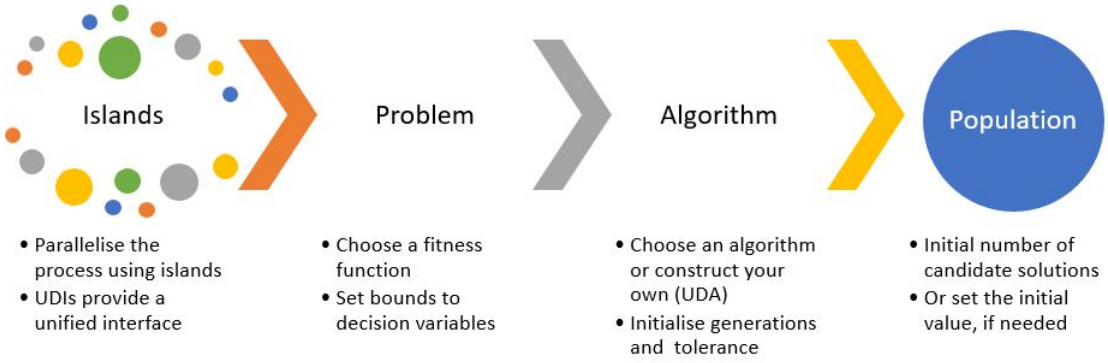


Figure 10: Workflow of PyGMO

4.5 PyGMO for optimisation of ODE outputs

I used the steps followed in Fig. 11 to optimise the error in Extended Model 1 for the TOR model, which showed the minimum relative error as shown in Fig. 3. This model was based on the hypotheses that **Tip41p has 2 phosphorylation sites**. The extended reaction set for this model are as follows:

$$[Tip41p^{\sim p}]+[Tor1/2p] \underset{K_{26}}{\overset{K_{25}}{\rightleftarrows}} [Tip41p^{\sim p}\cdot Tor1/2p]$$

$$[Tip41p^{\sim p}\cdot Tor1/2p] \xrightarrow{K_{27}} [Tip41p^{\sim p\sim p}]+[Tor1/2p]$$

$$[Tip41p^{\sim p\sim p}]+[PP2A1] \underset{K_{29}}{\overset{K_{28}}{\rightleftarrows}} [Tip41p^{\sim p\ \sim p}\cdot PP2A1]$$

$$[Tip41p^{\sim p\ \sim p}\cdot PP2A1] \xrightarrow{K_{30}} [Tip41p^{\sim p}]+[PP2A1]$$

$$[Tip41p^{\sim p\sim p}]+[PP2A2] \underset{K_{32}}{\overset{K_{31}}{\rightleftarrows}} [Tip41p^{\sim p\ \sim p}\cdot PP2A2]$$

$$[Tip41p^{\sim p\ \sim p}\cdot PP2A2] \xrightarrow{K_{33}} [Tip41p^{\sim p}]+[PP2A2]$$

Please not that double 'p' in superscript represents the second phosphorylation site for avoiding confusion.

I used PyGMO to optimize the error given the synthetic data generated for the extended model which had an error of 42.32. I used various evolutionary algorithms and other global optimisers as shown in Fig. 12

Clearly the simple evolutionary algorithm gave the best error with 3.28. The synthetic data was generated only for the time points mentioned in Supplementary material as mentioned by [1], but is this the correct way to go about this? In the next section, I have tried to quantify uncertainty for a parameter set and checked how much data is required to make cogent conclusions.

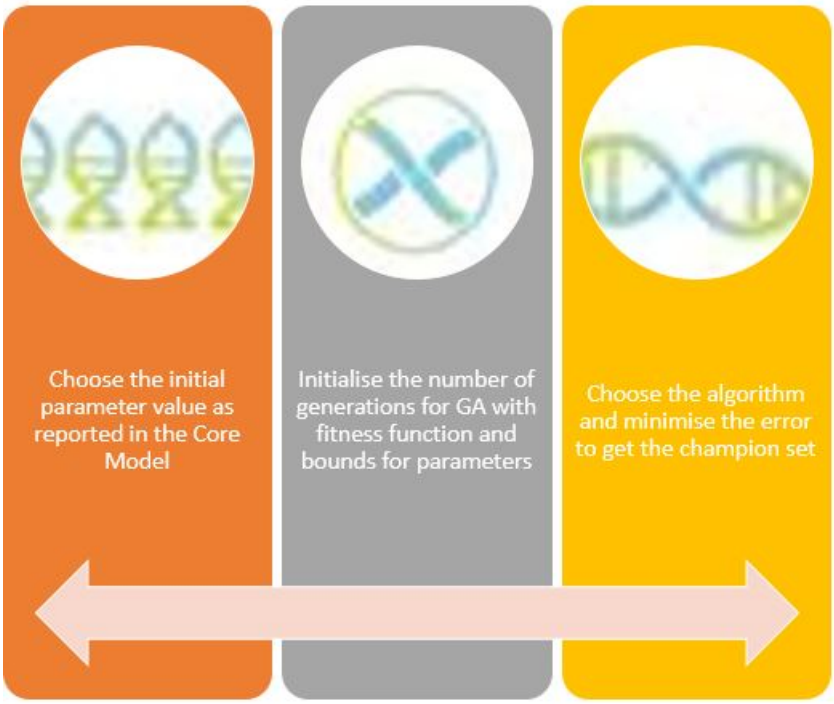


Figure 11: Workflow of PyGMO

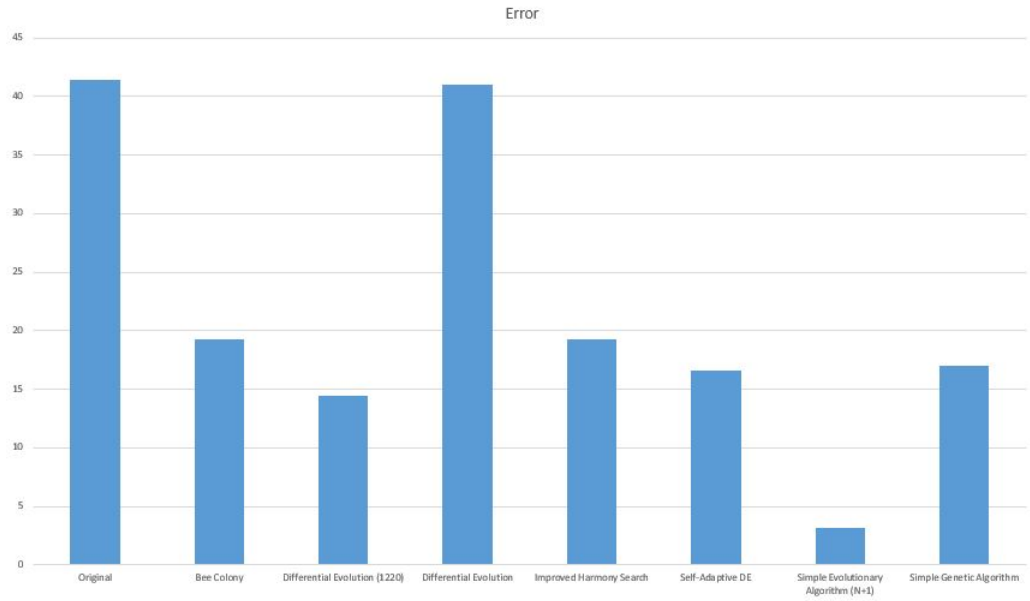


Figure 12: Error for extended model via different algorithms from PyGMO

5 Uncertainty Quantification and Sensitivity Analyses

I first developed a heatmap of sensitivity analyses taking into account the global sensitivity formula discussed in Section 1.5 as shown in Fig. 13 below. This was done at the steady state of 180th minute for all components.

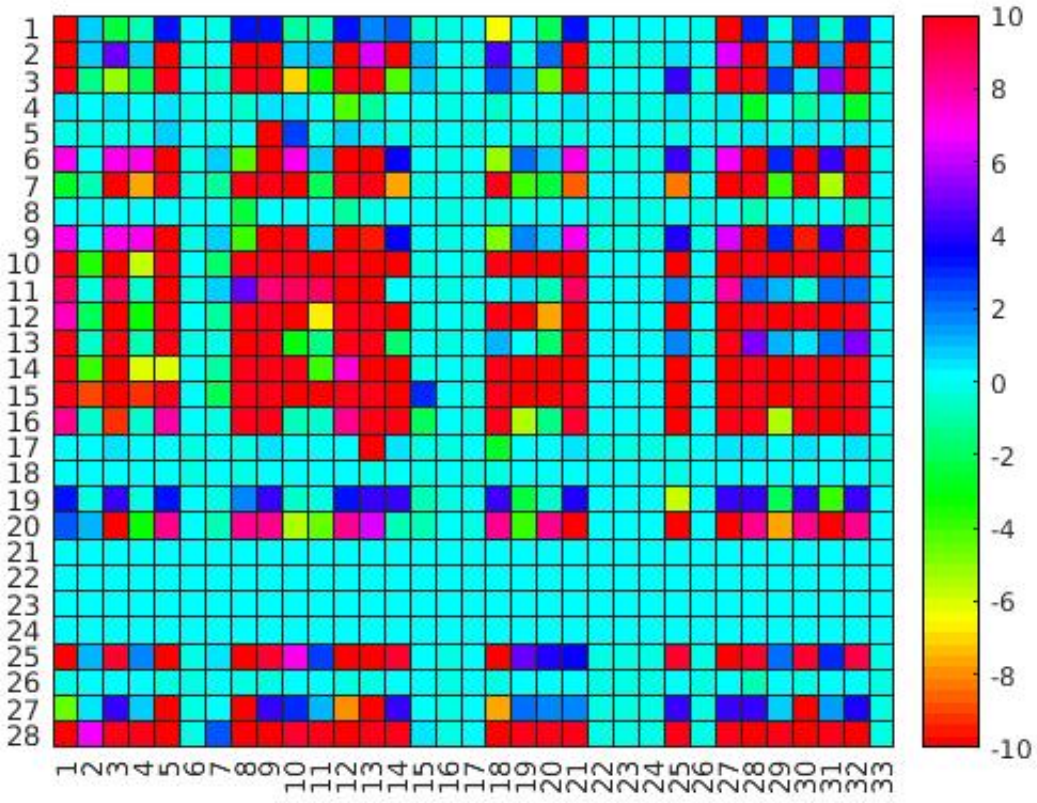


Figure 13: Heatmap of Global Sensitivity Analyses of various parameters with respect to each metabolite in Extended Model 1

I then tried to discern the amount of data needed to make the uncertainty in parameter estimates as less as possible. I followed the workflow mentioned

in Fig. 16.

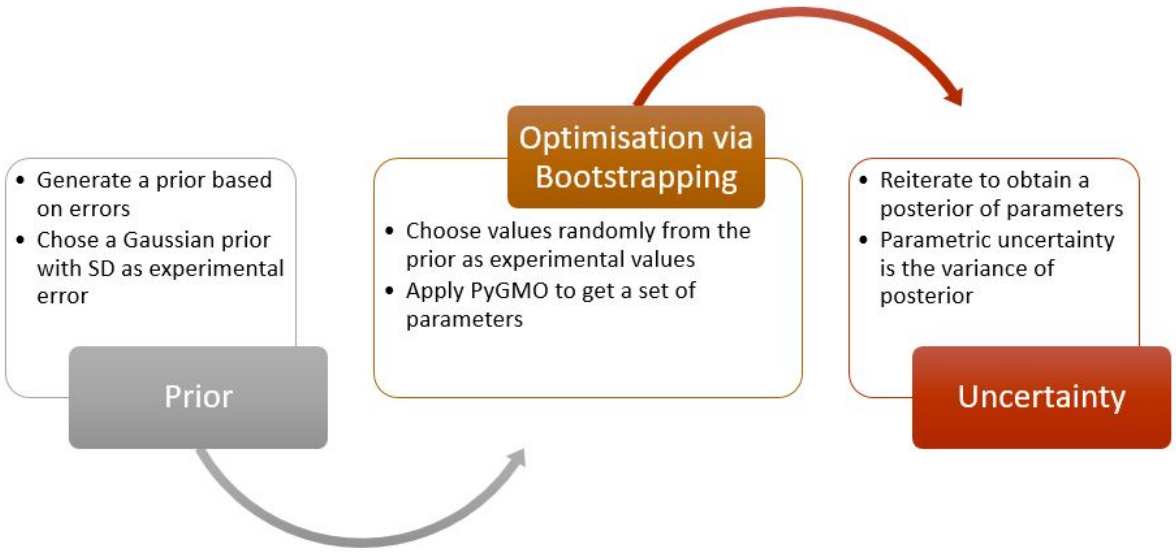


Figure 14: Workflow of Uncertainty quantification

The uncertainty quantification followed a method of bootstrapping from a box with replacements [13]. In this case, I used the reported error in supplementary material as the standard deviation plot and plotted a gaussian curve for each data point. From this curve, I picked random values as data samples which were then iteratively used to obtain the distribution of parameter set via PyGMO optimisation algorithms. For instance, the boxplot for parameter set obtained from 750 data points equally spaced over 1-180 minute is shown in Fig. 15 below.

The above figure was generated for 67, 500 and 1500 time points also and the mean of variance of all estimates was taken as the final uncertainty value for the model.

The graph should ideally die down to 0 as the uncertainty becomes 0. It does not happen because after a point of time data points cannot account for all the uncertainty generated in parameters. Uncertainty might be coming from how we sampled our synthetic data from gaussian curve or the model structure itself.

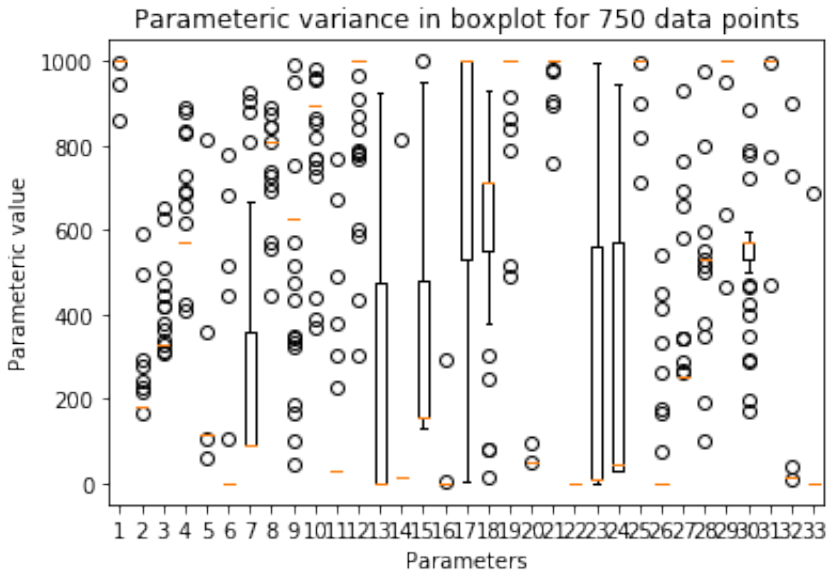


Figure 15: Boxplot of parameters for 750 data points equally spaced between 0-180 minutes

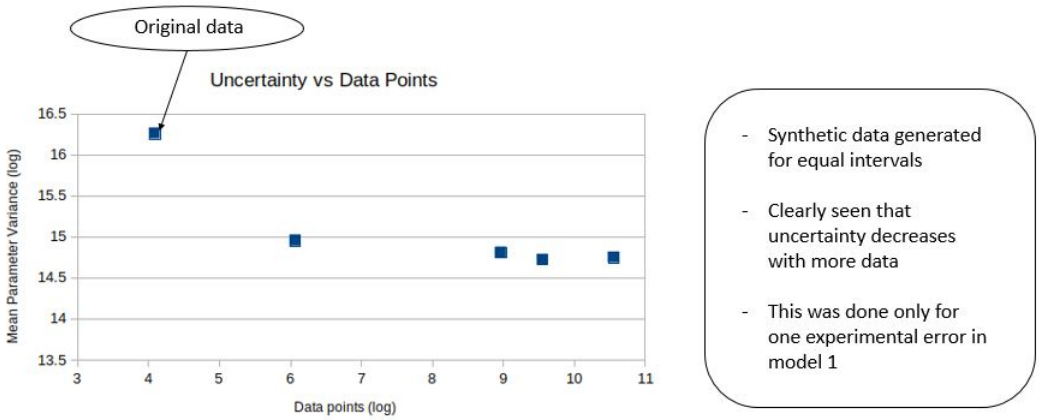


Figure 16: Uncertainty (log scale) vs number of time points (log scale)

6 Experimental Data Generation and Hypotheses Validation for TOR Pathway

We determined the effect of uncertainty associated with experimental data in the previous chapter and observed the sensitivity effects that are present based on $E(\frac{dM}{dP_i})$ signifying global sensitivity. There have been a lot of experimental hypotheses that have not been confirmed *in silico*. In this chapter, we have tried verify their existence and effect on the TOR pathway. Inn this way, we explore different topologies that have biological significance also, rather than only mathematical.

6.1 Mutant forms of Tap42 that are defective in binding to Sit4 block many major effects of TOR signalling

In *Rhode et al, Molecular and Cell Biology (2004)*, they have mentioned the role of PP2A-related phosphatase along with Sit4, together with its regulatory subunit Tap42 mediates several TOR pathway signalling processes. The central idea and working mechanism is based on the working of Tip41-Tap42 complex formation, or rather the degree to which Tip41 and Tap42 interact with each other.

As can be seen from Fig. 2, one can see that their interaction sits right in the middle of TOR pathway and is integral for the formation and activation of various other species.

Pertaining to that idea, we try to see the concentration of Tip-Tap complex after rapamycin action and disruption of Tap42-Sit complex as seen in Fig. 17.

This not only kills the activity of Tap-Tip complex but also desensitizes the system as seen in the heatmap of sensitivity analyses after disruption of Tap-Sit complex occurs as shown in Fig. 18.

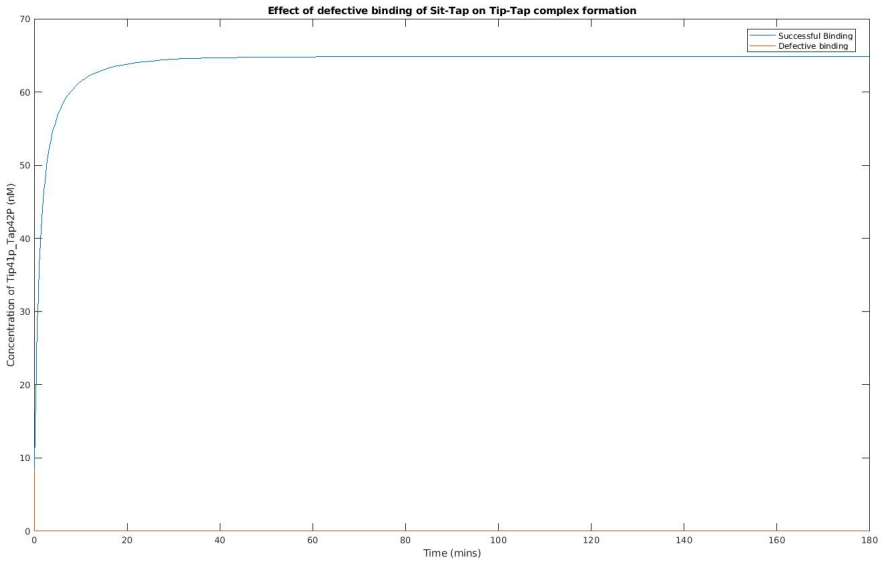


Figure 17: Disruption of Tap-Sit complex leads to inactivity of Tip-Tap complex

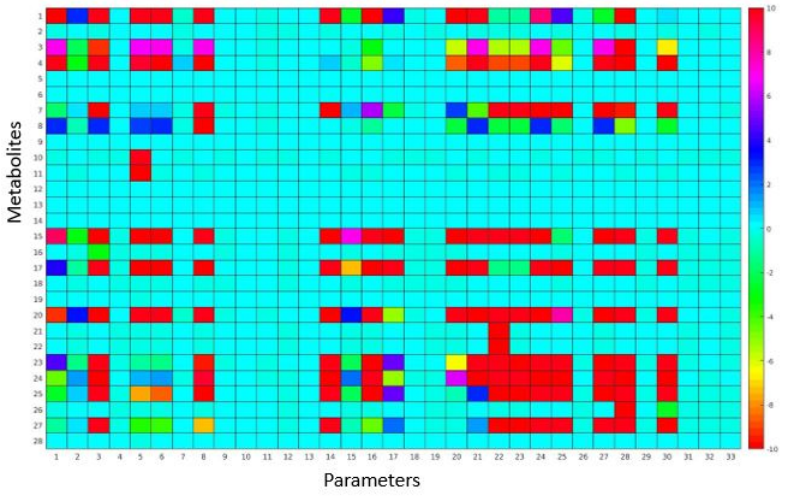


Figure 18: Sensitivity analyses after Tap-Sit complex disruption

6.2 PP2As compete for binding with complexes

PP2As are phosphatases which are formed by the catalytic activity of Sit4p and Sap protein complexes. These phosphatases compete with each other to bind to certain complexes as seen in [3]. I disrupted the formation of PP2A2 by making the constants K11 and K5 involved in the formation of PP2A2 to almost 0 and then checked the binding of PP2A1 with complexes. Fig. 19 shows an example of binding of Tor-PP2A1 complex.

The plot on the left hand side shows absolute degradation of Tor-PP2A1 while PP2A2 is present, but reaches a steady state value much larger than 0 in plot on the right hand side.

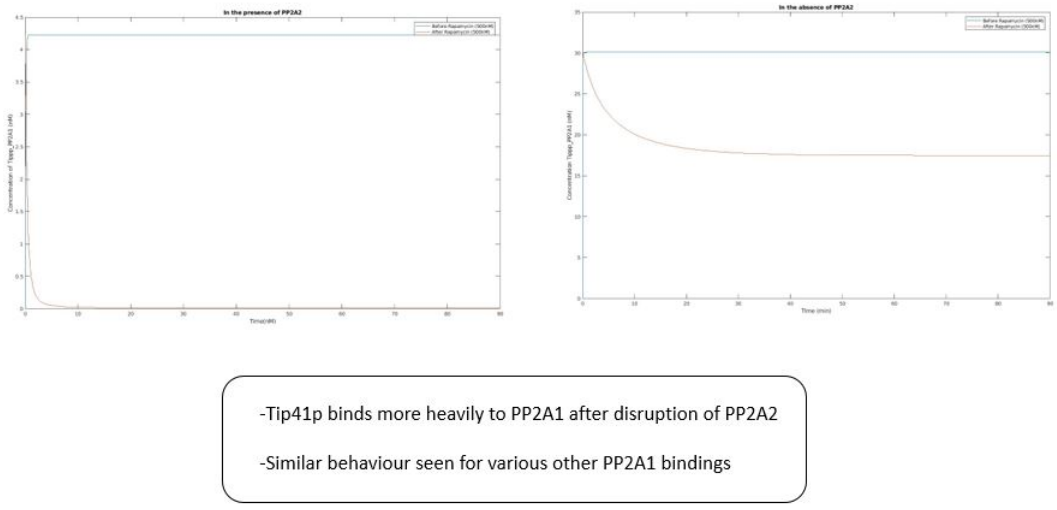
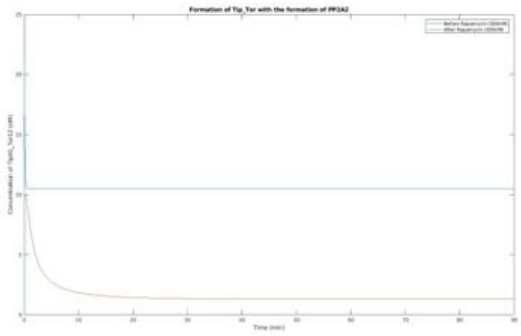


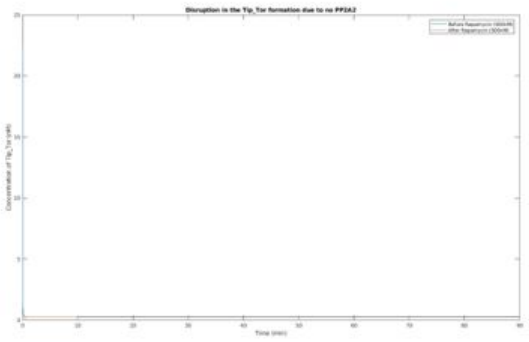
Figure 19: Tor-PP2A1 binding is enhanced when PP2A2 formation is disrupted

6.3 PP2A2 formation and activity is crucial to the TOR signalling pathway

As an extension of the previous hypotheses, I checked how crucial of a role does PP2A2 play in the overall system. After making the constants K5 and K11 to be 0, the activity of Tip phosphorylation by Tor1/2 kinase dies down

to 0 in presence of rapamycin [14]. The Tor1/2p-Tip complex is central to the application of stress response to nutrient deficiency. Fig. 20 shows the effect of PP2A1/2 absence on the Tor-Tip complex formation.





-Tip-Tor activity dies down in the absence of formation of PP2A2 (or PP2A1) by Sit4p and Sap proteins

-Affects the whole system also as the signalling is no longer dependent on the parametric values

Figure 20: Tor1/2-Tip complex disrupted in the absence of PP2A complexes

Furthermore, this disruption densensitizes the entire system as seen in the heatmap below in Fig. 21. The same effect was seen for other complexes also like Pph21/22p, Sapp and Tap-Sit complex formation.

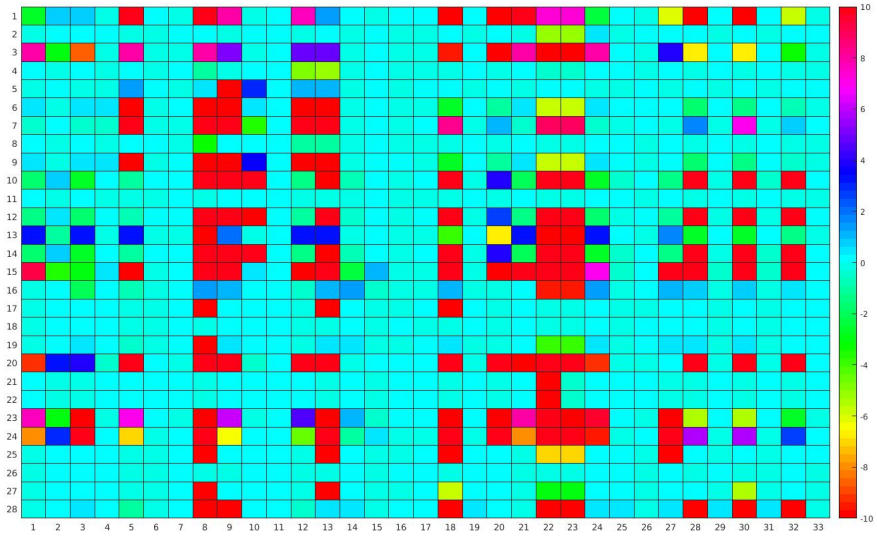


Figure 21: Desensitized TOR pathway in the absence of PP2A2/PP2A1

7 Results and Conclusions

I was able to formulate hypotheses based on the biology of the system and validated them *in silico* through disruption of reactions and their extent of completion. I also found the dis-functioning due to stiffness among Python ODE solvers which are probably due to numerical methods, and can be studied in future. I developed a work flow of PyGMO to solve ODEs for parameter estimation and other optimization-based problems. Quantification of uncertainty was done based on the error in experimental values and shown that more the data, lesser will be the uncertainty in the model.

8 Future Work

I was able to find a few hypotheses that gave cogent conclusions *in silico*, but these studies have to be verified via experimental values also. The idea of quantifying uncertainty was not valid to a large extent since I did not account for uncertainty rising from various other factors like human error, or systematic error which need to be accounted for. ODE solver incoherence is an issue which limits the use of this study since similar behaviour is not seen among Matlab and Python. Probably, shifting the platform to Mathematica might help which give analytic solution to the ODE problem also. Speedup using parallel computing via ginSODA and cupSODA is also an aspect that was not exploited in this study but can be considered for future applications.

9 APPENDIX A

List of reactions that were used to define the core TOR model. The notation is standard one, that is double arrows signify reversibility of the reaction and the direction in which the reaction is happening.

Furthermore, super-scripted P is used to signify the phosphorylation of the particular species. All reactions followed the mass action kinetics and their treatment with Rapamycin is seen in last three reactions. [Add more lines]

$$[Tap42p]+[Tor1/2p] \underset{K_2}{\overset{K_1}{\rightleftarrows}} [Tap42p{\bullet}Tor1/2p]$$

$$[Tap42p{\bullet}Tor1/2p] \xrightarrow{K_3} [Tap42p^{\sim P}]+[Tor1/2p]$$

$$[Tap42p^{\sim P}]+[Pph21/22p] \underset{K_5}{\overset{K_4}{\rightleftarrows}} [Tap42p^{\sim P}{\bullet}Pph21/22p]$$

$$[Tap42p^{\sim P}]+[PP2A1] \underset{K_7}{\overset{K_6}{\rightleftarrows}} [Tap42p^{\sim P}{\bullet}PP2A1]$$

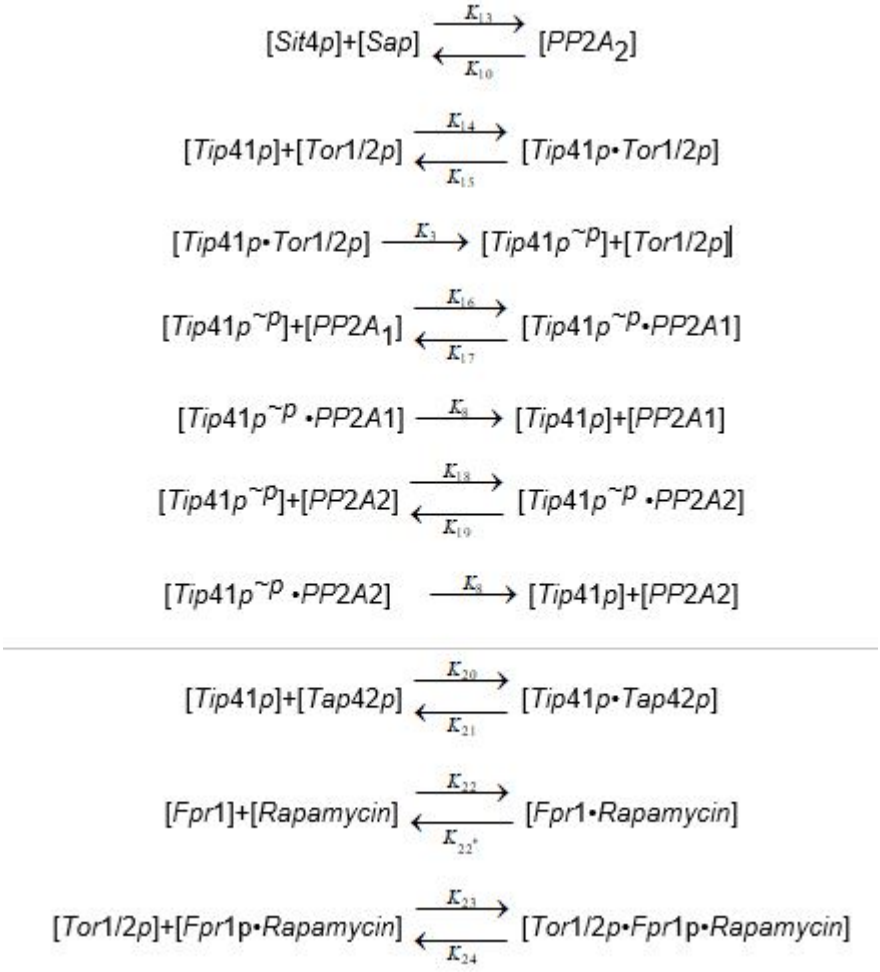
$$[Tap42p^{\sim P}{\bullet}PP2A1] \xrightarrow{K_8} [Tap42p]+[PP2A1]$$

$$[Cdc55p/Tpd3p]+[Pph21/22p] \underset{K_{10}}{\overset{K_9}{\rightleftarrows}} [PP2A_1]$$

$$[Tap42p^{\sim P}]+[Sit4p] \underset{K_5}{\overset{K_{11}}{\rightleftarrows}} [Tap42p^{\sim P}{\bullet}Sit4p]$$

$$[Tap42p^{\sim P}]+[PP2A_2] \underset{K_7}{\overset{K_{12}}{\rightleftarrows}} [Tap42p^{\sim P}{\bullet}PP2A_2]$$

$$[Tap42p^{\sim P}{\bullet}PP2A_2] \xrightarrow{K_8} [Tap42p]+[PP2A_2]$$



10 Supplementary Material

Strain	0	15	30	60	180
Wild type	100%	49%	29%	12%	15%
cd55/tpd3 mutant	260%	NA	NA	NA	235%

Table 1: Relative degree of Tap42p phosphorylation in wild type and cd55/tpd3 mutants before and after addition of 500 nM rapamycin. Values were reported to be 20% accurate.

Strain	0	30
wild type	100%	543%
sit4 mutant	102%	138%

Table 2: Tap42p-Tip41p complex formation in wild type and sit4 before and after addition of 109 nM rapamycin

Complex	Reference values and standard errors	
Tap42p-Pph21/22p	10% Tap42p; delta=0.5	2% Pph21/22p; delta=1
Tap42-Sit4p	10% Tap42p; delta=0.5	5% Sit4p; delta=1
Sit4p-Sapp	60%Sit4p; delta=1	15% Sapp; delta=0.83

Table 3: Amount of different protein complexes relative to overall concentration

All these experiments were done by the contributors in [1]. The experimental setup for TOR pathway was based on 11 experiments with varying error in those setups. Tor2p concentration was not determined, assumed a 25% higher concentration than that of Tor1p. It was concluded that the Tip41p concentration exceeds that of Tap42p by about three-fold with a deviation of 50%.

Relative complex concentrations were assumed to be accurate within 50%. For results that were too low, the upper bounds were allowed a deviation of upto 100%. 40% error was recorded for Tap42p-Pph21/22p after addition of 109nM rapamycin. Tip41p had an estimated error of about 10%.

Strain	0	30	60
WT	100%	40%	40%
tip41 mutant	112%	82%	82%

Table 4: Tap42p-Sit4p complex formation in wild type and tip41p before and after addition of 109 nM rapamycin.Obtained via western blot

Protein	Molar Concentration (nM)
Tor1/2p	57
Tap42p	86
Pph21/22p	425
Cdc55/tpd33p	376
Tip41p	250
Fpr1p	1892
Sit4p	173
Sapp	489

Table 5: Concentration of proteins in TOR; taken as initial value for ODEs

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