

Identification of cell signaling pathways based on biochemical reaction kinetics repositories

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Abstract

Cell signaling pathways are composed of a set of biochemical reactions that are associated with signal transmission within the cell and its surroundings. Traditionally, these pathways are identified through statistical analyses on results from biological assays, in which involved chemical species are quantified. However, once generally it is measured only a few time points for a fraction of the chemical species, to effectively tackle this problem it is required to design and simulate functional dynamic models. Recently, it was introduced a method to design functional models, which is based on systematic modifications of an initial model through the inclusion of biochemical reactions, which in turn were obtained from the interactome repository KEGG. Nevertheless, this method presents some shortcomings that impair the estimated model; among them are the incompleteness of the information extracted from KEGG, the absence of rate constants, the usage of sub-optimal search algorithms and an unsatisfactory overfitting penalization. In this project, we propose a new methodology for identification of cell signaling pathways, which will make use of a myriad of public interactome and biochemical reaction kinetics repositories to deal with the incompleteness of a priori information. Moreover, we will use optimal algorithms for model selection, as well as more effective cost functions for overfitting penalization. The new methodology will be tested on artificial instances and also on cell signaling pathways identification in our case study, the Y1 mouse adrenocortical tumor cell line. (AU)

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Chapter 1

Introduction

Cell signaling pathways are cascades of chemical interactions that allow the communication between the cell environment and the cell itself. These pathways are also able to regulate many cell functions, including DNA replication, cell division and death. We can observe the functioning of signaling pathways as a mechanism that can conform the cell behavior with signals that come from the environment conditions in which the cell is placed. The studies of cell signaling pathways can lead to determining how cells can respond to different stimuli; for instance, with the studies of signaling pathways activated by a chemical species, one could determine how an unhealthy cell would respond to a drug containing this species.

It is possible to construct mathematical models to represent a set of chemical reactions and consequently a signaling network. One approach on the modeling of those interactions is based on the law of mass action. This law proposes that the rate of a chemical reaction is proportional to the product of reactants concentrations, i.e. we can calculate the concentration change rate of a species in an interaction by calculating the product of reactants concentrations, up to a multiplying constant. If we consider the set of interactions of a signaling pathway, we can then come up with a system of ordinary differential equations (ODEs) that can model the dynamics of the concentration of each chemical species from the pathway. Generally, these systems are complex and cumbersome, if not impossible, to be solved analytically, therefore we resort on computational tools that apply numerical methods to approximate solutions of these systems.

In this work, we are interested in computational models that can reproduce the behavior of signaling networks, comparing simulations generated by those models to experimental measures, generally based on Western blot data. The Figure 1.1 shows a set of interactions as well as parameters of a model of a signaling network. To create computational models that are able to simulate the behaviour of a signaling pathway, two main tasks need to be accomplished.

The first task one must complete to create a model is to determine a set of interactions that will be considered in the ODE system. Searching for pathway maps on the Kyoto Encyclopedia of Genes and Genomes (KEGG) [KG00] is a good start for this task. The KEGG PATHWAY Database provides manually drawn diagrams that represent signaling networks created with experimental evidences. However, it is possible that there is no pathway on KEGG that is able to correctly represent the biological experiment of interest; for those situations, it is necessary to modify the pathway by adding or removing interactions. One might reason that we should use as many interactions as we can to get a better simulation. Indeed, a model that consider more reactions is more general and might be able to reproduce different sets of experimental data. However, being more general may imply in poor or computationally infeasible models because of two reasons: first, complex models will require more time for a numerical solution computation, which may be infeasible due to limited computational resources; and second,



Figure 1.1: The above diagram show a hypothesis for a signaling pathway that flows through Raf-MEK-ERK cascade. Names in bold represent chemical species. Names in italic represent parameters of the ordinary differential equation of each interaction. Horizontal arrows represent phosphorylation when directed from left to right or dephosphorylation when directed in the opposite direction. Other arrows represent positive feedback if they are directed downwards or negative feedback otherwise. Original image of Marcelo S. Reis et al. (2017) [Rei+17a].

when considering many interactions, we are also considering many parameters (constants of the differential equations system), and finding appropriate values for them becomes harder as we increase the number of parameters.

The second task is to find values for all the model parameters. We can highlight two approaches for this task; one can either fetch values for these constants from the literature, or one can find values that make the model output approximate the experimental observations. For the first approach, repositories such as BioModels [LN+06] can be used; for the second approach, statistical and optimization methods are needed. For optimization, it is necessary to define a metric that can evaluate how close a set of parameters bring the simulation to the experimental observation. Only after that it is possible to search for the optimal parameter in the parameter space. Statistical inference, in the other hand, will usually try to maximize some likelihood function (find parameters that makes the data more likely to happen) on a more classical approach, while in a Bayesian approach the goal is usually to compute some posterior distribution for the model parameters (the probability of parameter values given the experimental observation).

After completing both tasks, however, as we mentioned before, we might still not have found a pair of model and parameter values that fairly approximates the biological experiment of interest. That could indicate that the set of chemical interactions chosen for the model is incomplete or has interactions that are not relevant for the biological experiment. Therefore, it is desirable to construct a systematic method of modifying the set of chemical reactions of the model in order to find a good set to represent the signaling network.

With the title “A method to modify molecular signaling networks through examination of interactome databases” [Wu15] Lulu Wu presented in her masters dissertation a methodology to systematically modify computational models of signaling networks to better simulate biological experiments. Starting with a model that does not approximate well the biological data, this methodology proposes to add to the model a set of chemical interactions that are relevant for the biochemical experiment, and consequently approximate the model simulation to experimental data. This set of interactions is a subset of interactions from a database created by Wu, joining

information from many static maps of signaling networks available on KEGG. The choice of this subset can be modeled as a combinatorial optimization problem, the feature selection problem, in which the search space is the set of all possible subsets of interactions (features) to be added. The cost function of this problem, however, is not as simple to define as the search space. Note that points from the search space do not fully define models, because there is still need to define parameter values to produce a simulation. Therefore, to analyze the quality of a model, the cost function must take into account the set of values for the model parameters. As an example, we could define the cost as the minimum distance between experimental and model measures considering all possibilities of parameter values; however, unfortunately, finding the minimizing parameter values is a hard problem.

Since this is a hard problem, the method presented by Wu implements a heuristic version of this cost function, moreover, the algorithm used to traverse the search space is also a heuristics. The cost function heuristics is based on a Simulated Annealing procedure that searches for a set of parameter values trying to minimize (as much as possible) the distance between model and experimental measures. The best found distance times -1 is then considered as the cost of the model. The size of the search space is the number of all possible subsets of interactions to be added, and this number grows exponentially on the number of interactions from the database. That explains the need of a heuristic to traverse the search space. This heuristic is based on the greedy algorithm called Sequential Forward Selection (SFS) [Whi71]. The heuristic implemented by Wu selects a fixed number of interactions from the database and then creates candidate models by adding to the current solution the respective interaction; then, after evaluating the cost function for each model, the algorithm moves to the best candidate.

The results presented on Wu's dissertation show that the method is useful when there are only a few differences between the starting model and a model that closely approximates the biological experiment. This limitation could be explained by the intrinsic difficulty of the problem, which demands fitting complex models with few experimental data; however, we would like to highlight three aspects of the work that contributes to its limitations. The first aspect is that the constructed database could be more nearly complete, adding information from other interactome databases, such as STRING [Szk+10], and also by adding information about model parameters, i.e. chemical reaction constants, that are available in other databases, e.g. the SABIO-RK [Wit+11] database. Second, the search algorithm used to modify the models can only add interactions, therefore, if the algorithm starts with (or add along the search) a spurious interaction on the topology, then the algorithm will not be able to "regret" that interaction even though there might be similar solutions without it with better fit. Third, the cost function does not include a proper penalization of complex models; the used penalization is based on a execution time limit on the simulated annealing procedure, implying on a random penalization for more complex models, which typically demand more execution time. Without a proper penalization, the algorithm is doomed to select overly complex models that, even with a good fit to the experimental data, are not likely to reproduce the same experiment conducted with any kind of perturbation to the biological environment or to the data collected.

We propose on this project to create and test a new method for modifying models of signaling networks, based on the work of Wu, and including a possible solution to the third aspect mentioned on the last paragraph. Although the first and second aspects have potential of improvements, we have not prioritized them and, because of the complexity of the third aspect, we decided that including the first and second aspect would excessively enlarge the scope of this work. Therefore, we limit our work to a few known cell signaling pathways, and we only include simple algorithms for traversing the search space. To the last aspect, which considers the cost function, as our major concern in this work, we intend to use Bayesian approaches to

rank models [VG07] based on the likelihood of them to reproduce the observed data; if we say M is a model with parameter space Θ and D is a set of observations, then we would like to estimate

$$p(\mathbf{D}|M) = \int_{\theta \in \Theta} p(\mathbf{D}|\theta, M)p(\theta|M)d\theta,$$

where $p(\mathbf{D}|\theta, M)$ is the likelihood of data \mathbf{D} , given that the model M with parameters θ are “correct”, which is the same as stating that this model and parameters determine the behaviour of the cell; $p(\theta|M)$ is the prior probability of θ ; and finally, $p(\mathbf{D}|M)$ is the probability of the data being generated by model M . This cost function has as an advantage the fact that models are not ranked using a single value for parameters, instead, the cost considers all possibilities of parameters, integrating over the parameter space. Another advantage of this cost function is that, since it is based on likelihoods of the model to reproduce data, overly complex models are automatically penalized.

1.1 Objectives

In this work we propose to implement a methodology that allows us to solve the problem of identification of cell signaling pathways as a feature selection problem, using a Bayesian approach for the cost function. This cost function should be able to rank models according to the likelihood of the experimental data being generated by them. After the definition and construction of such cost function, we intend to build small examples of model selection of cell signaling pathways, and run simple searches on their search spaces, using our chosen cost function. Finally, these runs should allow us to get a glance on the surface that the chosen cost function induces over the search space of the model selection problem. To achieve these goals, we should accomplish the following tasks

1. **Study state of the art Bayesian algorithms for signaling network model selection.** We have chosen two methodologies to study: the first, a work of Liepe et al. [Lie+14], uses an Approximate Bayesian Computation approach for ranking models; the second, a work of Xu et al. [Xu+10], uses an estimative of the marginal likelihood $p(\mathbf{D}|M)$ to rank models.
2. **Implementation and testing of cost functions.** We propose to compare both the cost functions used by Liepe and Xu. To test the cost function used by Liepe, we should use the software ABC-SMC, and to test the cost function used by Xu, we should implement our own software, SigNetMS. After the preparation of instances, we should test the performance of both software.
3. **Preparation of instances of the identification of cell signaling network problem.** After comparing both cost functions, we intend to construct instances to test our feature selection approach on identification of cell signaling network. These instances must be composed by a base model, a small database of candidate reactions, and a set of measurements to which our candidate models should fit.
4. **Traversal of the search space.** We propose to perform walks and runs of simple search algorithms over the search space of candidate models for the instances we created. These runs will provide us information about the surface that the used cost function induces over the search space. This type of information shall be useful for defining new search algorithms for the identification of cell signaling pathways.

1.2 Organization

TODO

- *Chapter 2 (Fundamental Concepts)*: we will give more details about the identification of signaling pathway problem. We will show how to obtain experimental data for signaling pathways and how can chain of chemical reactions can be modeled as a system of differential equations. We close the chapter presenting briefly state of the art methods of model ranking and also the Metropolis-Hastings algorithm, which is a useful tool for methods of model ranking.
- *Chapter 3 (Model Selection Methods)*: we will present two methodologies that are the state of the art in model selection. Both methods are Bayesian approaches, where the first is based on the estimation of marginal likelihoods and the second is based on Approximate Bayesian Computation.

Chapter 2

Fundamental Concepts

In this section we provide the concepts that are fundamental to understand the biological and computational problems, methodologies and results that we will present in this work. We start this chapter presenting what is a cell signaling pathway and how can one take measures to identify its activity on the cell. Later we present how it is possible to represent chemical interactions as differential equations, and how that allows one to model a cell signaling pathway with a system of ordinary differential equations. Then, we present more formally the problem we are trying to solve on this project, the identification of cell signaling pathways, as well as the state of the art methods of model ranking. Finally, we present the basics of posterior distribution sampling, which is a useful tool when working with Bayesian approaches, such as the ones used on this project to rank models.

2.1 Cell Signaling Pathways

Cell signaling pathways are part of the complex cell communication system, and it allows the cell to perceive the conditions of the environment in which it is placed and change its behaviour accordingly. Signaling pathways participate in the regulation of many cell functions, including development, division and cell death [Han17]. The dynamic of a signaling pathway can also be related to diseases, as in many cases of cancer.

The signal perceived by a cell can come from cells that are close (including the same cell that produced the signal), as in synapses, or it can travel long distances in the organism, as in hormones. When a signal reaches a cell, it binds to a specific receptor in the membrane, and once that happens, the receptor can trigger a sequence of chemical interactions that can include change of conformation of proteins, activation or inactivation of proteins, and change of concentration of chemical species in the cell. Ultimately, this chain of chemical reactions caused by the signal can alter the behaviour of the cell, what is called signal transduction.

Since signaling pathways participate in many of the cell functions, and are also related to diseases, it is important to study those structures in order to get a better understanding of the cell mechanisms and diseases. One approach on the study of the cell signaling pathways is to measure the concentration change of proteins and how they interact to produce those changes.

2.2 Measurements of Proteins in Cell Signaling Pathways

Western blot [TSG79] is a laboratory technique that can indicate the amount of a specific protein that is present in a mixture. This technique shows the presence of a protein in a mixture by “blotting” a membrane where the molecules of interest are located. We can summarize the procedure in the following steps: first a mixture containing a sample of cells of interest must be created; second, proteins from the mixture should be fixed on the blotting membrane; third, an antibody should bind to the target protein molecules; and finally, a method for highlighting the bound antibody should be applied. An image of the resulting membrane can then be analyzed with computer programs to quantify the relative concentration (with respect to some other protein, usually a control protein that has fairly the same concentration during the whole experiment) of the protein of interest.

By repeating this procedure in different times it is possible to create time-course observations of proteins throughout the biological experiment. With this tool, a researcher can choose a set of relevant proteins from a signaling network and gain knowledge about the dynamics of such chemical species during the experiment. For instance, in a signaling network experiment in which it is desired to understand how the change of concentrations of a species at the beginning of the pathway changes the concentration of some species at the end of the cascade, then measurements of both are relevant to understand the biological experiment. Figure 2.1 presents an example of time-course Western blot for an experiment where it is desirable to understand how extracellular signal-regulated kinase (ERK) is activated (phosphorylated) as a function of levels of Rat sarcoma bound to guanosine triphosphate (Ras-GTP).



Figure 2.1: Figure **a** shows time-course measurements of ERK, phosphorylated ERK (p-ERK) and hypoxanthine-guanine phosphoribosyltransferase (HPRT). HPRT is a “loading” protein, that means that its concentration is fairly the same through the experiment, and therefore it is used as a normalizing factor to total ERK concentration. Figure **b** shows values of phosphorylated ERK that are obtained after processing Figure **a**. Original image of Marcelo S. Reis et al. (2017) [Rei+17a].

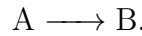
These measurements alone do not always provide means for researchers to understand a cell signaling pathway experiment. However, if we create a computational model for this signaling networks that is able to reproduce experimental data, then we might use this model as a summary of the signaling network, which can provide to researchers evidences of the biological phenomena.

2.3 Dynamic Modeling of Cell Signaling Pathways

One approach onto modeling cell signaling pathways is to model the dynamics of the concentrations of chemical species involved. This can be accomplished when using the law of mass action [VV10]. This law states that, in an elementary reaction, the speed (or rate) of a chemical reaction is proportional to the product of the concentration of all reactants. An elementary reaction is a reaction in which there is no participation or need of an intermediate reaction to describe the first in a molecular level. In practice, it is more common to see two types of elementary reactions, they are first or second order reactions.

2.3.1 Modeling Elementary Reaction Rates

A first order reaction is composed of one reactant only. Suppose A is the only reactant and B is the only product of a reaction, then we can write this reaction as:

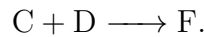


The reaction rate of this reaction, according to the law of mass action, is

$$k_1[A],$$

where k_1 is some constant and $[A]$ is the concentration of A. It is import to note that the constant k_1 is a rate coefficient of the reaction and, therefore, it can only assume positive values.

A second order reaction is composed of two reactants. Suppose C and D are both and the only reactants and F is the product of a reaction, then we can write this reaction as:



The reaction rate of this reaction is

$$k_2[C][D],$$

where k_2 is a (positive) constant and $[C]$ and $[D]$ are the concentrations of C and D, respectively.

Using these two laws to calculate the speed of reactions, we are able to describe how the concentration of chemical species in a system change through time using differential equations. To illustrate this and future concepts of this section, we are going to consider a minimal system composed of a simple enzymatic reaction:



where E is an enzyme, S is a substrate, ES is the enzyme-substrate complex, and P is the product.

Each arrow in Equation 2.1 represents one elementary reaction, and the names over or under arrows represent reaction rate constants. All three reactions can be represented by the equations:



and they have, respectively, reaction rates of:

$$\begin{aligned} & k_f[E][S] \\ & k_r[ES] \\ & k_{cat}[ES]. \end{aligned}$$

Now, to determine a model of the concentration dynamics for Reaction 2.1, we will write a system of ordinary differential equations. To do so, we should take every chemical species and calculate its concentration change rate based on the rate of each reaction that it participates. For instance, the enzyme E is a reactant on Reaction 2.2a and is also a product on reactions 2.2b and 2.2c, then we consider that E changes its concentration over time (t) according to the differential equation:

$$\frac{d[E]}{dt} = -k_f[E][S] + (k_r + k_{cat})[ES] \quad (2.3)$$

Note that we are adding reaction rates in which the species is a product and we are subtracting reaction rates in which the species is a reactant. Repeating this procedure for every other species of the enzymatic reaction leads to the following system of ordinary differential equations:

$$\frac{d[E]}{dt} = -k_f[E][S] + (k_r + k_{cat})[ES] \quad (2.4a)$$

$$\frac{d[S]}{dt} = -k_f[E][S] + k_r[ES] \quad (2.4b)$$

$$\frac{d[ES]}{dt} = k_f[E][S] - (k_r + k_{cat})[ES] \quad (2.4c)$$

$$\frac{d[P]}{dt} = k_{cat}[ES]. \quad (2.4d)$$

2.3.2 Simplification of Dynamic Models

The System 2.4 can be simplified if we apply properties of enzymatic reactions together with algebraic simplifications. We will show then how to derive the quasi-steady-state Michaelis-Menten model for enzymatic reactions. With the correct assumptions, this model is able to reproduce the behaviour of an enzymatic reaction without considering the intermediate enzyme-substrate complex, leading to simpler models.

A basic principle we need to apply to our system in order to derive the Michaelis-Menten model is the principle of mass conservation. This principle is valid if we assume that the reactions 2.1 are isolated, meaning that the chemicals on these reactions are not involved in other reactions at the same time. Applying this principle to the enzyme chemical, produces the following equation:

$$[E_0] = [E] + [ES].$$

If we apply this equation to the derivative of the concentration of ES, we will get the following equation:

$$\frac{d[ES]}{dt} = k_f([E_0] - [ES])[S] - (k_r + k_{cat})[ES]. \quad (2.5)$$

One more assumption is necessary to derive the simplification. This assumption states that the concentration of substrate-enzyme complex does not change over time, i.e. $\frac{d[ES]}{dt} = 0$, and it was first proposed in 1925 by Briggs and Haldane [BH25]. Generally, this assumption is

applicable whenever $[S] \gg [E]$. If we apply this assumption together with the mass conservation assumption on the Equation 2.5, we get:

$$[ES](k_r + k_{cat}) = k_f([E]_0 - [ES])[S],$$

$$[ES] = \frac{[E]_0[S]}{K_m + [S]},$$

in which $K_m = \frac{k_{cat} + k_r}{k_f}$ is known as Michaelis constant. Considering this, we can rewrite the rate of $[P]$ as:

$$\frac{d[P]}{dt} = k_{cat} \frac{[E]_0[S]}{K_m + [S]}. \quad (2.6)$$

And finally, if we apply mass conservation to the substrate, we will get the following equation:

$$[S_0] = [S] + [ES] + [P],$$

then, we can differentiate this equation on t and use the quasi-steady-state assumption ($\frac{d[ES]}{dt} = 0$) to obtain:

$$\frac{d[S]}{dt} = -\frac{d[P]}{dt}. \quad (2.7)$$

Now, with equations 2.6 and 2.7 we are able to reproduce the dynamics of the substrate and product of the enzymatic reaction. Therefore, using the Michaelis-Menten model, we could simplify the System2.4 that had four equations and three parameters to a new model that has only two equations and two parameters (k_{cat} and K_m). Figure 2.2 shows a comparison between the dynamics of the complete and Michaelis-Menten models of enzymatic reactions.



Figure 2.2: An example of the dynamics produced by two models of enzymatic reactions. The Figure 2.2(a) presents the dynamics of the model 2.4 and Figure 2.2(b) presents the dynamics of the Michaelis-Menten simplification to the same model. For this simulations, it is necessary to define initial concentrations of the chemical species involved, and it is used: 10 molecules/ $(\mu\text{m})^3$ for the enzyme (E); 100 molecules/ $(\mu\text{m})^3$ is used for the substrate (S); and 0 molecules/ $(\mu\text{m})^3$ is used for the other species. In addition to this, it is also necessary to define model parameter values, and it is used: 0.06 $(\mu\text{m})^3(\text{molecules}\cdot\text{s})^{-1}$ for k_f ; 0.1 (s^{-1}) for k_r ; 0.2 (s^{-1}) for k_{cat} ; and, following the Michaelis-Menten model, 5 molecules/ $(\mu\text{m})^3$ is used for K_m .

2.4 Identification of Cell Signaling Pathways

Identification of signaling pathways is the problem of finding the components of a signaling pathway and how they interact in order to reproduce a cell behaviour that has been previously

measured experimentally. The input of this problem is usually a description of the biological experiment, containing previous information about the signaling pathway, such as known reactions and parameters, and a set of measurements, commonly Western blot data, which is the only type of measurement we consider in this work. The output to this problem is then composed of a set of interactions that are actively controlling the behaviour of interest of the cell, and also the set of parameter values that should be used on these interactions to create a model that approximates the experimental observations; it is possible to output a single value for each parameter, as it was presented in [Wu15], or output information about these values, using a posterior (to the experimental data) distribution, as it was presented in [Lie+14] and [Xu+10].

Two main tasks must be completed to produce such output. The first task is to find candidate topologies for the pathway model, i.e. different set of interactions that are relevant to the pathway of interest. The second task is to rank those models according to their ability to simulate the pathway, approximating the experimental measurements.

The second task is also known as the model selection problem and even though it is a broad area, there are works on the literature that treat specifically the problem in a biochemical context. Solutions to this problem should be able to choose model candidates according to their ability to reproduce observed data, penalizing overly complex models to avoid overfitting.

One approach on setting the score of a model is to search for the set of parameter values that make the model measurements the closest to the experimental data, and then define a distance between these two measurements; then it is possible to use this distance, plus a penalty for complexity, to create a ranking of the models. Note that a penalty function is needed because complex and more general models can overfit, producing models that cannot represent the same signaling network with small perturbations to biological experiment. We can write this scoring function as:

$$score_1(M) = -\min_{\{\theta \in \Theta' \subset \Theta\}} dist(\phi(M, \theta), \mathbf{D}) + R(M),$$

where M is the model, Θ is the parameter space for model M , Θ' is the subset of the parameter space where the search for the best parameter values was conducted, \mathbf{D} is a list of experiment measurement (each measurement is a time-course observation of the biological phenomena of interest), ϕ is a function that determines the simulated measurement on the model, and R is a regularization function that penalizes model complexity. This approach was implemented on the work of Lulu Wu [Wu15], using a Simulated Annealing procedure to search for parameter values that minimize the distance between simulation and experiment. The Simulated Annealing procedure used is available on the software SigNetSim (Signaling Network Simulator), which is based on the work of Chu on [CDR99]. However, Wu's methodology showed limitations when testing the ability to reconstruct models from experiments, and this could be related to the used penalization term. In fact, choosing a good regularization function is crucial to the performance of this methodology.

Another approach to setting the model score is to consider the model parameters as random variables and then marginalize the probability of the model and parameters to reproduce the observed data, i.e. estimate (because calculating is usually hard) the marginal probability:

$$p(\mathbf{D}|M) = \int_{\Theta} p(\mathbf{D}|M, \theta)p(\theta|M)d\theta, \quad (2.8)$$

where \mathbf{D} is the observed data, M is the model and Θ is the parameter space for model M . The function $p(\theta|M)$ is the prior probability function of the parameter θ on model M . The function $p(\mathbf{D}|M, \theta)$ is the likelihood of observing the data \mathbf{D} when simulating the model M using parameters θ . Sometimes, however, the likelihood function is unknown or computationally intractable; for these cases, it is possible to use an alternative Bayesian approach, called

Approximate Bayesian Computation (ABC), to estimate the probability $p(M|\mathbf{D})$ and use it as a ranking score [Ton+09].

For the first task of identification of signaling pathways we described (the creation of model candidates), there are not as many works on the literature as there are for the second task. Commonly, researchers must resort on their own knowledge on the biological experiment and consult interactome maps available on repositories such as KEGG and BioModels to construct manually the hypothesis of models for a signaling pathway. That enlightens the importance to create a methodology that systematically creates candidate models of signaling networks, as we propose on this project.

2.5 State of the Art in Selection of Biochemical Models

The state of the art in biochemical model selection is based on Bayesian inference. The Bayesian approaches provide the benefits of ranking models with statistical formalism, and automatically penalizes overly complex models. More than that, through the prior distribution of the parameters, the researcher is able to input prior knowledge about interactions constants; this type of information can facilitate the parameter inference of models since it tends to concentrate the search.

Bayesian approaches consider that model parameters are random variables, instead of fixed unknown constants. We can argue that this modeling is fair to the reality in biochemical processes because interactions constants can vary depending on the cell conditions. Therefore, in a biological experiment in which there might be perturbations to the cell environment, it should be more adequate to rank models integrating the models score over a probability space of parameters instead of fitting the model to data using a single point of the parameter space. We will now present the basic concept of two methods that use this idea for model selection, the Annealing-Melting Integration (AMI) [VG07] and Approximate Bayesian Computation (ABC) [Ton+09].

The Annealing-Melting Integration is a method that estimates the integral 2.8 using concepts of thermodynamics. With thermodynamic integration, it is possible to write the logarithm of this integral as:

$$\ln p(\mathbf{D}|M) = \int_0^1 \mathbb{E}_{q_\beta(\boldsymbol{\theta})} [\ln p(\mathbf{D}|M, \boldsymbol{\theta})] d\beta \quad (2.9)$$

where $p(\mathbf{D}|M, \boldsymbol{\theta})$ is the likelihood function (the probability of observing the data \mathbf{D} when M is the correct model, with parameters $\boldsymbol{\theta}$); and $\mathbb{E}_{q_\beta(\boldsymbol{\theta})}$ is an expectation taken over the probability space of $q_\beta(\boldsymbol{\theta}) \propto p(\mathbf{D}|M, \boldsymbol{\theta})^\beta p(\boldsymbol{\theta}|M)$. The variable β works in the integral as a temperature term, determining the probability functions $q_\beta(\boldsymbol{\theta})$, $\beta \in [0, 1]$; note that when $\beta = 0$ then

$$q_0(\boldsymbol{\theta}) = p(\boldsymbol{\theta}|M),$$

the prior distribution of the parameters; also, and when $\beta = 1$ then

$$q_1(\boldsymbol{\theta}) = \frac{p(\mathbf{D}, \boldsymbol{\theta}|M)}{\int_{\Theta} p(\mathbf{D}, \boldsymbol{\theta}|M)} = \frac{p(\boldsymbol{\theta}|\mathbf{D}, M)p(\mathbf{D}|M)}{p(\mathbf{D}|M)} = p(\boldsymbol{\theta}|\mathbf{D}, M),$$

the posterior distribution of parameters. Therefore, the integral 3.6 takes the expected value of the likelihood function of \mathbf{D} over a sequence of probability distributions that is a “bridge of distributions” connecting the prior and posterior distributions of parameters. Calculating this integral is usually infeasible, hence in practice it is needed to estimate this integral using samples of a finite number of tempered distributions, $q_\beta(\boldsymbol{\theta})$.

As we mentioned before, the likelihood function $p(\mathbf{D}|M, \boldsymbol{\theta})$ may be very hard to calculate if not impossible. For those cases it is possible to use a parameter inference approach that is likelihood-free, called Approximate Bayesian Computation (ABC). This method has the goal of producing a sample of parameters that brings the model simulations close to the observed data. A generic ABC algorithm starts proposing a candidate parameter $\boldsymbol{\theta}^*$ from a proposal distribution; then, a simulation, $\phi(M, \boldsymbol{\theta}^*)$ of the model using the candidate parameters is produced; if, for some distance function d , it is true that $d(\phi(M, \boldsymbol{\theta}^*), \mathbf{D}) < \epsilon$, then we accept $\boldsymbol{\theta}^*$ as part of the sample. If ϵ is sufficiently small, then the produced samples approximates well the posterior distribution. To use ABC methods for model selection it is enough to add a model indicator parameter to the parameter array, then it is possible to extract model distribution from the accepted parameters.

2.6 Metropolis-Hastings to Generate Samples

Both methods for model selection, based on ABC or using thermodynamics integration, need to generate samples of probability distributions. Generating samples of distributions is simple for many well known distributions, however, for some other distributions this task may not be as simple. Metropolis-Hastings algorithms are capable of generating a sample that has some probability distribution p , which is called the target distribution. In fact, the method can be used even when it is not possible to access the target function directly; for those cases, a distribution that is proportional to the target is necessary.

Being able to generate a sample of a target distribution without accessing the probability function itself is useful for our applications in Bayesian model ranking. Consider that we need to create a sample of the parameters posterior distribution $p(\boldsymbol{\theta}|M, \mathbf{D})$. Calculating this probability function is very hard because

$$p(\boldsymbol{\theta}|M, \mathbf{D}) = \frac{p(\mathbf{D}|\boldsymbol{\theta}, M)p(\boldsymbol{\theta}|M)}{p(\mathbf{D}|M)},$$

and this equation has the term $p(\mathbf{D}|M)$, which is only known (through some estimation) at the end of the model ranking. However, if we can access the likelihood function $p(\mathbf{D}|\boldsymbol{\theta}, M)$ and the prior $p(\boldsymbol{\theta}|M)$, then we can calculate the product of these two, which is proportional to the posterior distribution (since $p(\mathbf{D}|M)$ is only a constant because it does not depend on $\boldsymbol{\theta}$). Therefore, using the likelihood and the prior distributions together with a Metropolis-Hastings algorithm provides a mean to generate a sample of the posterior.

A generic Metropolis-Hastings algorithm that creates a sample of a target distribution $p(\lambda)$ proceeds as follows:

1. Choose some starting point λ_0 for which $p(\lambda_0)$ is not zero. Also set $t = 1$.
2. Sample a candidate point λ^* from a proposal (or jumping) distribution with probability $J_t(\lambda^*|\lambda^{t-1})$.

3. Calculate the ratio:

$$r = \frac{p(\lambda^*)J_t(\lambda^{t-1}|\lambda^*)}{p(\lambda^{t-1})J_t(\lambda^*|\lambda^{t-1})} \quad (2.10)$$

4. With probability $\min(1, r)$ set $\theta^t = \theta^*$ and set $\theta^t = \theta^{t-1}$ otherwise.
5. Increase t by one and, if not reached limit number of iterations, go back to step 2.

Note that if the target $p(\lambda)$ is not available, and rather another function $q(\lambda) = \frac{1}{c}p(\lambda)$ is available, then the ratio 2.10 can be calculated as:

$$r = \frac{p(\lambda^*)J_t(\lambda^{t-1}|\lambda^*)}{p(\lambda^{t-1})J_t(\lambda^*|\lambda^{t-1})} = \frac{(q(\lambda^*)c)J_t(\lambda^{t-1}|\lambda^*)}{(q(\lambda^{t-1})c)J_t(\lambda^*|\lambda^{t-1})} = \frac{q(\lambda^*)J_t(\lambda^{t-1}|\lambda^*)}{q(\lambda^{t-1})J_t(\lambda^*|\lambda^{t-1})}$$

More than that, if the proposal distribution is symmetric ($J_t(\lambda_a|\lambda_b) = J_t(\lambda_b|\lambda_a)$ for all λ_a, λ_b and t), the produced algorithm is called Metropolis algorithm and has the ratio $r = p(\lambda^*)/p(\lambda^{t-1})$.

Different implementations of the Metropolis-Hastings algorithm are possible. The possible changes include the choice of starting point, the choice of proposal distributions and number of iterations. As an example, some algorithms are adaptive in the sense that they can change the proposal distribution according to the acceptance rate of proposed points [Gel+13]. These types of algorithms are handy when we are not sure of how spread the proposal distribution should be: with larger variance, we are likely to stay in a local minima for a longer time, rejecting most of the proposed jumps; in the other hand, if the proposal distribution has small variance, then the algorithm needs more time to explore the parameter space.

Chapter 3

Model Selection Methods

In this chapter we will define the model selection problem in the context of this work and we will also present two state of the art methodologies that can be used to rank models, both of them are Bayesian approaches. These methods create estimatives of $p(\mathbf{D}|M)$ or $p(M|\mathbf{D})$ and use these values as model scores.

The first approach is to estimate the marginal likelihood of the data \mathbf{D} being reproduced by a model M , $p(\mathbf{D}|M)$. To use this method, it is necessary to define a likelihood function, $p(\mathbf{D}|M, \boldsymbol{\theta})$, and prior distributions of model parameters $p(\boldsymbol{\theta}|M)$. The estimation of this probability is done by taking samples of tempered posteriors (to experimental data) of model parameters. These tempered posterior distributions construct a bridge of distributions connecting the prior and posterior parameter distributions, allowing an estimation of the marginal likelihood.

Another method is to use Approximate Bayesian Computation. ABC methods are used to estimate posterior (also to experimental data) distributions of parameters. On this method, a sequence of populations of parameters is created, and for each generation, the distance between experimental data and data simulated by the population is decreased, leading to a population that tends to be closer to a posterior distribution. In our case, we add to $\boldsymbol{\theta}$ a model indicator parameter M , and then we create a sample of $p(\boldsymbol{\theta}, M|\mathbf{D})$. If we marginalize this sample, we can calculate an estimative of the probability $p(M|\mathbf{D})$. ABC methods allow simpler algorithms, and they also allow selecting models without the need of a likelihood function, although defining the prior distribution of parameters is still needed.

3.1 Elements of model selection

The model selection problem we consider in this work consists in the selection of a biochemical model that can best simulate the dynamics of concentrations of chemical species, measured previously on a biological experiment. The models we consider are mathematically represented by a system of ordinary differential equations that contains parameters related to reaction rates and experimental errors. We can list three main entities of this problem: the set of models, the parameter space of each model, and the biological experimental data. We shall then define how we represent these entities through this text.

We indicate a model with a random variable that assume natural values, $M : \Omega \rightarrow \mathbb{N}$. We consider that a model is not only bound to a set of chemical reactions and its mathematical representation, but it is also bound to initial concentrations of chemical species and a prior distribution of model parameters, which we represent as $p(\boldsymbol{\theta}|M)$.

Since we are using a Bayesian approach, we consider that model parameters is a random

vector $\boldsymbol{\theta}|M : \Omega \rightarrow \mathbb{R}_+^n$, where d equals to the number of parameters in M plus one, so we can also represent one parameter related to experimental error. This parameter represents the standard deviation of errors in the observation of the experiment. It is important to note that all parameters are positive, since reaction rates and standard deviations can only be positive. That is an important information to choose a prior distribution for model parameters.

The experimental data is a vector of values that describe the dynamics of some measurement made on the biological phenomena of interest. We represent data as $\mathbf{D} \in \mathbb{R}^d$. As we described in Section 2.2, these measurements are often created with Western Blot experiments, and the values of \mathbf{D} usually represent the ratio of concentrations of proteins.

3.2 Model ranking using Marginal Likelihood

The marginal likelihood of an experiment measurement \mathbf{D} being reproduced by a model M , $p(\mathbf{D}|M)$, can be used as a model ranking metric as it determines which model makes the experimental observations more likely to happen. Before defining how to calculate the marginal likelihood, we must define some likelihood function. To understand our chosen likelihood function $p(\mathbf{D}|M, \boldsymbol{\theta})$, we must understand that conditioning the observation to a model and a set of parameters means that, in the probability space from which \mathbf{D} is taken, the model M is the “real” model and it has the parameter values of $\boldsymbol{\theta}$; i.e. the model M with parameters $\boldsymbol{\theta}$ controls the behaviour of the system from which \mathbf{D} was observed. Now, after understanding in which probability space we are, we can assume that:

$$p(\mathbf{D}|M, \boldsymbol{\theta}) = p_{\mathcal{N}(\bar{0}, \Sigma)}(\phi(M, \boldsymbol{\theta}) - \mathbf{D}), \quad (3.1)$$

where $\phi(M, \boldsymbol{\theta}) \in \mathbb{R}^d$ is the experimental measurement on the simulation generated by the model M with parameters $\boldsymbol{\theta}$, and $p_{\mathcal{N}(\bar{0}, \Sigma)}(\cdot)$ is the probability density function of a Multivariate Normal random variable with mean $\bar{0}$ and covariance matrix Σ . Note that we are considering that simulations of a model are deterministic, as ϕ is function, and therefore the randomness of the model arises from observation experimental error. We consider this random error follows a Gaussian distribution, similarly to Xu and Vyshemirsky [Xu+10][VG07].

As it is done in the work of Xu et al. (2010), we can consider that the observation error is independent for each time step [Xu+10], therefore we can simplify 3.1 to:

$$p(\mathbf{D}|M, \boldsymbol{\theta}) = \prod_{i=1}^d p_{\mathcal{N}(0, \sigma^2)}(\phi_i(M, \boldsymbol{\theta}) - D_i). \quad (3.2)$$

The σ^2 used in Equation 3.2 is also a parameter of the model, and as we explained on the last section, this parameter is incorporated into $\boldsymbol{\theta}$, which means that, for some $k \in \{1, \dots, n\}$, $\theta_k = \sigma^2$.

Now that we defined the likelihood function, we can write the marginal likelihood. As the name suggests, this value comes from the marginalization of the likelihood function, over the parameter space. We can write as:

$$p(\mathbf{D}|M) = \int_{\Theta} p(\mathbf{D}|M, \boldsymbol{\theta}) p(\boldsymbol{\theta}|M) d\boldsymbol{\theta}. \quad (3.3)$$

However, calculating this integral analytically is only possible in very special cases and, usually, it would depend on knowing models for the distributions associated to these probability functions, which is generally not possible in our case.

Even though this integral is very hard to be calculated, there are methods that allow us to estimate its value. A straight forward method to estimate this integral value is using Importance Sampling Estimators [NR93]. This method uses the Monte Carlo integral estimation method that can estimate integrals of the form $\int g(\lambda)p(\lambda)d\lambda$ using the estimator:

$$\hat{I} = \sum_{i=1}^m w_i g(\lambda_i) / \sum_{i=1}^m w_i,$$

where $w_i = p(\lambda)/p^*(\lambda)$, and $p^*(\cdot)$ is known as the importance sampling function. If we set $\lambda = \boldsymbol{\theta}|M$ and use the prior ($p(\boldsymbol{\theta}|M)$) or the posterior ($p(\boldsymbol{\theta}|M, \mathbf{D})$) as importance sampling functions, then we would get respectively the estimators:

$$\begin{aligned} & \frac{1}{m} \sum_{i=1}^m p(\mathbf{D}|M, \boldsymbol{\theta}^{(i)}) \quad (\text{with } \boldsymbol{\theta}^{(i)} \sim p(\boldsymbol{\theta}|M)), \\ & \left(\frac{1}{m} \sum_{i=1}^m p(\mathbf{D}|M, \boldsymbol{\theta}^{(i)})^{-1} \right)^{-1} \quad (\text{with } \boldsymbol{\theta}^{(i)} \sim p(\boldsymbol{\theta}|M, \mathbf{D})). \end{aligned}$$

However, as showed by Vyshemirsky et al. (2007), these estimators might produce very large variances and may not perform well for biochemical model selection applications. Hence, new methods with ideas of thermodynamics were proposed. These methods are based on rewriting the marginal likelihood equation using intermediate distributions of parameters between the prior and posterior distributions [FP08].

3.2.1 Thermodynamic Integration for Marginal Likelihood

The Thermodynamic Integration is a method that proposes to rewrite the Integral 3.3 using ideas of thermodynamics, providing new estimators for the marginal likelihood. This method is able to rewrite the marginal likelihood integral in a way that it marginalizes the likelihood through many intermediate probability spaces of parameters, bridging the prior and posterior distributions of parameters. These distributions are also called tempered distributions or power posteriors [FP08].

Given a parameter prior distribution $p(\boldsymbol{\theta}|M)$ and a posterior distribution $p(\boldsymbol{\theta}|\mathbf{D}, M)$, then we define a power posterior distribution with “temperature” β as:

$$p_\beta(\boldsymbol{\theta}) = \frac{p(\mathbf{D}|\boldsymbol{\theta}, M)^\beta p(\boldsymbol{\theta}|M)}{z(\beta)},$$

where

$$z(\beta) = \int_{\Theta} p(\mathbf{D}|\boldsymbol{\theta}, M)^\beta p(\boldsymbol{\theta}|M) d\boldsymbol{\theta}.$$

Note that when $\beta = 0$, then $p_{\beta=0} = p(\boldsymbol{\theta}|M)$, the prior distribution of the parameters; also, when $\beta = 1$, then

$$p_{\beta=1}(\boldsymbol{\theta}) = \frac{p(\mathbf{D}|\boldsymbol{\theta}, M)p(\boldsymbol{\theta}|M)}{z(\beta)} = \frac{p(\mathbf{D}, \boldsymbol{\theta}|M)}{\int_{\Theta} p(\mathbf{D}, \boldsymbol{\theta}|M) d\boldsymbol{\theta}} = \frac{p(\boldsymbol{\theta}|\mathbf{D}, M)p(\mathbf{D}|M)}{p(\mathbf{D}|M)} = p(\boldsymbol{\theta}|\mathbf{D}, M),$$

the posterior distributions of the parameters. Therefore, if we vary the value of β from 0 to 1 we can create a path of probability distributions that connect the prior and posterior distributions.

We will explain now, how this sequence of distributions are applied to calculate the marginal likelihood.

Consider the derivative of $\ln z(\beta)$.

$$\begin{aligned}
\frac{d}{d\beta} \ln z(\beta) &= \frac{1}{z(\beta)} \frac{d}{d\beta} z(\beta) \\
&= \frac{1}{z(\beta)} \frac{d}{d\beta} \int_{\Theta} p(\mathbf{D}|\boldsymbol{\theta}, M)^\beta p(\boldsymbol{\theta}|M) d\boldsymbol{\theta} \\
\text{(using that } \frac{d}{dx} c^x &= c^x \ln c) \\
&= \frac{1}{z(\beta)} \int_{\Theta} p(\mathbf{D}|\boldsymbol{\theta}, M)^\beta p(\boldsymbol{\theta}|M) \ln p(\mathbf{D}|\boldsymbol{\theta}, M) d\boldsymbol{\theta} \\
&= \int_{\Theta} \frac{p(\mathbf{D}|\boldsymbol{\theta}, M)^\beta p(\boldsymbol{\theta}|M)}{z(\beta)} \ln p(\mathbf{D}|\boldsymbol{\theta}, M) d\boldsymbol{\theta} \\
&= \int_{\Theta} p_{\beta}(\boldsymbol{\theta}) \ln p(\mathbf{D}|\boldsymbol{\theta}, M) d\boldsymbol{\theta} \\
&= \mathbb{E}_{p_{\beta}(\boldsymbol{\theta})} [\ln p(\mathbf{D}|\boldsymbol{\theta}, M)].
\end{aligned} \tag{3.4}$$

And it is not hard to see that:

$$\begin{aligned}
z(0) &= \int_{\Theta} p(\boldsymbol{\theta}|M) d\boldsymbol{\theta} = 1 \\
z(1) &= \int_{\Theta} p(\mathbf{D}, \boldsymbol{\theta}|M) d\boldsymbol{\theta} = p(\mathbf{D}|M)
\end{aligned} \tag{3.5}$$

Using equations 3.5 and Equality 3.4 we can write that:

$$\begin{aligned}
\int_0^1 \mathbb{E}_{p_{\beta}(\boldsymbol{\theta})} [\ln p(\mathbf{D}|\boldsymbol{\theta}, M)] d\beta &= \int_0^1 \frac{d}{d\beta} \ln z(\beta) d\beta \\
&= \left[\ln z(\beta) \right]_0^1 \\
&= \ln p(\mathbf{D}|M).
\end{aligned} \tag{3.6}$$

Then we have written an expression for the logarithm of the marginal likelihood. This expression is still hard to be calculated analytically, however from this equation we will be able to find estimators for the logarithm of the marginal likelihood, and consequently for the likelihood. To calculate these estimators, we will need to generate samples for a series of power posteriors of parameters, and this will be explained in the next section.

3.2.2 Estimation of the Marginal Likelihood

There are multiple approaches on estimating the Integral 3.6 and, usually it is necessary to find samples of $p_{\beta_t}(\boldsymbol{\theta}|M, \mathbf{D})$ for a sequence of values of β_t that vary from zero to one [Xu+10; VG07; FP08]. The methods that create such samples can take different approaches on the choice of the sequence $\beta_1 < \beta_2 < \dots < \beta_T$, on the Metropolis-Hastings (MH) algorithms used, and finally on the estimator.

We are now going to show one possible methodology to estimate parameter values. First, we assume that there is a chosen sequence of $\beta_1 < \beta_2 < \dots < \beta_T$, so we can explain how to generate samples of power posterior distributions with these values of β . After that we will explain how to choose the sequence of β values and, finally, present two possible estimators for $p(\mathbf{D}|M)$.

Power posteriors sampling

Given a sequence of β values, the method we present to sample from the power posteriors has three different steps, and all of them use Metropolis-Hastings algorithms, similarly to what is done by Xu et al. (2010). For each of the T β values, the first two phases are run independently, generating T chains that are samples of the power posteriors. Then, on the third phase, each chain continues to grow, but not independently, because two random chains will have their last accepted points swapped. This exchange leads to a mixture of chains of samples of different power posteriors; this approach is called Populational Monte Carlo Markov Chain (Populational MCMC) [FP08].

The first step of sampling is run independently for each temperature and we call it **naive burn-in**. The Metropolis-Hastings performed on this step is started by taking a sample from the parameter priors. For each step, the proposal distribution has to generate positive values and the covariance matrix should be a diagonal covariance matrix, i.e. parameters are proposed independently. This covariance matrix is updated after every predefined number of iterations to adapt the proposal distribution according to the current sample. This type of MCMC algorithm, that updates its rules according to its trace is also called adaptive MCMC algorithm. This update is performed as proposed in Gelman et al. (2013):

- if the acceptance rate of parameter points in the last iterations is greater than 0.44, then increase the variance of the jump;
- if the acceptance rate is lower than 0.23, then decrease the variance of the jump.

This rule is a golden rule for Metropolis-Hastings calibration since the work of Roberts et al. [RGG97]. The rationale behind this rule is that chains generated with small variance tend to need more steps to cover the space, while chains generated with larger variance tend to reject more proposed parameters, staying at the same point of the chain for many iterations.

Given that we are taking a sample from a power posterior with temperature β_t , the target function is $p_{\beta_t}(\boldsymbol{\theta})$. Hence, if the current point is $\boldsymbol{\theta}^{(t,i)}$, the probability of accepting a proposed parameter $\boldsymbol{\theta}^* \sim J_{(t,i)}(\boldsymbol{\theta}^*|\boldsymbol{\theta}^{(t,i)})$, is $\min(1, r)$ with

$$r = \frac{p_{\beta_t}(\boldsymbol{\theta}^*)}{p_{\beta_t}(\boldsymbol{\theta}^{(t,i)})} \frac{J_{(t,i)}(\boldsymbol{\theta}^{(t,i)}|\boldsymbol{\theta}^*)}{J_{(t,i)}(\boldsymbol{\theta}^*|\boldsymbol{\theta}^{(t,i)})},$$

with $J_{(t,i)}$ being the proposal jump distribution on iteration i on chain of power posterior β_t . Since we do not have direct access to the $p_t(\boldsymbol{\theta}|M, \mathbf{D})$ distribution, and considering the definition of power posterior we presented in the beginning of the chapter, that $p_{\beta_t}(\boldsymbol{\theta}) \propto p(\mathbf{D}|M, \boldsymbol{\theta})^{\beta_t} p(\boldsymbol{\theta}|M)$, we can rewrite this equation as:

$$r = \frac{p(\mathbf{D}|M, \boldsymbol{\theta}^*)^{\beta_t}}{p(\mathbf{D}|M, \boldsymbol{\theta}^{(t,i)})^{\beta_t}} \frac{p(\boldsymbol{\theta}^*|M)}{p(\boldsymbol{\theta}^{(t,i)}|M)} \frac{J_{(t,i)}(\boldsymbol{\theta}^{(t,i)}|\boldsymbol{\theta}^*)}{J_{(t,i)}(\boldsymbol{\theta}^*|\boldsymbol{\theta}^{(t,i)})}. \quad (3.7)$$

After a pre-determined number of iterations, we stop the naive burn-in to move to the next sampling algorithm. By the end of the naive burn-in, we expect to have generated a sample that nearly approximates a sample of the posterior distribution. However, the sampled points might still be very correlated. To reduce this correlation and improve the posterior approximation, we will need the next two steps of sampling. We call the sampling algorithm that follows naive burn-in as **posterior shaped burn-in**.

The posterior shaped burn-in has this name because the used proposal distribution has a covariance matrix similar to an estimative of the covariance of the posterior distribution. To

create this estimative, we use, for each chain, the current sample of the posterior generated. On the first iteration of posterior shaped burn-in, we estimate the covariance of the posterior using the last half points sampled in the naive burn-in (we discard the first half of the chain because we assume that these points can have very low probability on the posterior distribution). For the second and further iterations of the posterior shaped burn-in, we estimate the covariance of the posterior considering the second half of sampled points of the first iterations plus all the points accepted after that.

The proposal distribution chosen on the posterior shaped burn-in must be multivariate with a covariance that is similar to the one estimated from the current sample of the posterior, differently from the naive burn-in, in which pairs of parameters (θ_i, θ_j) , $i, j \in \{1, \dots, n\}$, $i \neq j$, were sampled independently. Similarly to the first sampling phase, we also sample each chain (for each β_t value) separately, and we also scale the covariance matrix according to the number of accepted parameters (as the golden rule for MCMC suggests). The probability of accepting a proposed parameter on this sampling phase is the same as it was on the first phase, as described by Equation 3.7.

By the end of the posterior shaped burn-in it is expected that the created sample is more likely to be a sample of the posterior distribution. However, there might still exist big correlations between sampled points of the same chain. To solve this problem, we need the third and last sampling phase: the **Populational Monte Carlo Markov Chain**. This algorithm continues the chains for each value of β_t , and after each step, a couple of chains have their last sampled value exchanged. This procedure mixes the chains and reduces correlation of sampled points.

At the begin of the Populational MCMC phase, we will have T chains of sampled parameters, containing the selected parameters of the first and second steps. For each of these chains there is a covariance matrix that was being used to determine the covariance of the proposal distribution of the respective temperature. On the third phase, we fix all of these matrices and, for each iteration, two steps are performed. The first step is to iterate all chains, using the respective fixed covariance matrix, identically to how it was done in the posterior shaped burn-in. The second step consists in choosing two different chains β_i and β_j , with $i \neq j$ and $i, j \in \{1, \dots, T\}$, and exchange the last sampled parameters of these chains with a certain probability. The value of i is chosen randomly and uniformly over $\{1, \dots, T\}$. Once i is chosen, the second chain, of temperature β_j , is chosen following a Discrete Laplacian distribution given i , with probability function $p(j|i) \propto e^{|i-j|/2}$. This algorithm, as we described, was proposed by Friel et al. (2008) and can be summarized in the following steps:

1. For each temperature β_t , $t \in \{1, \dots, T\}$, update the t -th chain using MCMC with a proposal distribution that has the same covariance matrix used on the last iteration of the same chain, on the last sampling phase.
2. Choose uniformly i from $\{1, \dots, T\}$. Then, choose j with probability density function $p(j|i) \propto e^{|i-j|/2}$. Finally, swap the last sampled points of the chains of power posteriors β_i and β_j , $\theta^{(i,k)}$ and $\theta^{(j,k)}$, with probability $\min\{1, r\}$, where:

$$r = \frac{p_{\beta_i}(\theta^{(j,k)}) p_{\beta_j}(\theta^{(i,k)}) p(j|i)}{p_{\beta_i}(\theta^{(i,k)}) p_{\beta_j}(\theta^{(j,k)}) p(i|j)}$$

which can be simplified to:

$$r = \left[\frac{p(D|\theta^{(j,k)})}{p(D|\theta^{(i,k)})} \right]^{\beta_i} \left[\frac{p(D|\theta^{(i,k)})}{p(D|\theta^{(j,k)})} \right]^{\beta_j} \frac{p(j|i)}{p(i|j)}$$

After finishing the third step of sampling, the samples obtained on the first two phases are discarded and only the samples from the last phase are used to estimate the marginal likelihood.

Estimators of the Marginal Likelihood

The sampling procedure explained on the Section 3.2.2 produces samples of the power posteriors $p_{\beta_1}(\boldsymbol{\theta}), \dots, p_{\beta_T}(\boldsymbol{\theta})$, and these samples can be used to estimate logarithm of the marginal likelihood:

$$\ln p(\mathbf{D}|M) = \int_0^1 \mathbb{E}_{p_\beta(\boldsymbol{\theta})} [\ln p(\mathbf{D}|\boldsymbol{\theta}, M)] d\beta. \quad (3.8)$$

This can be achieved using both numerical integration or creating an unbiased estimator. The choice of either approach of estimation will imply in a method for choosing the sequence of temperatures β_1, \dots, β_T .

The method proposed by Friel et al. (2008), uses a numerical integration method to estimate the Integral 3.8. Given that T power posteriors, $0 = \beta_1 < \beta_2 < \dots < \beta_T = 1$, were selected and samples of its respective distributions posteriors were generated, then using trapezoidal rule over the β values allows us to estimate the integral as:

$$\log p(\mathbf{D}|M) \approx \sum_{t=0}^{T-1} (\beta_{t+1} - \beta_t) \frac{\mathbb{E}_{p_{\beta_{t+1}}(\boldsymbol{\theta})} [\log p(\mathbf{D}|M, \boldsymbol{\theta})] + \mathbb{E}_{p_{\beta_t}(\boldsymbol{\theta})} [\log p(\mathbf{D}|M, \boldsymbol{\theta})]}{2}$$

and if the sample of power posterior of temperature β_t has L_t parameter points, then we can rewrite this equation as:

$$\log p(\mathbf{D}|M) \approx \sum_{t=0}^{T-1} (\beta_{t+1} - \beta_t) \frac{\frac{1}{L_{t+1}} \sum_{i=1}^{L_{t+1}} \log p(\mathbf{D}|M, \boldsymbol{\theta}^{(t+1,i)}) + \frac{1}{L_t} \sum_{i=1}^{L_t} \log p(\mathbf{D}|M, \boldsymbol{\theta}^{(t,i)})}{2} \quad (3.9)$$

According to the work of Friel et al. (2008), a good temperature schedule for β_1, \dots, β_T that can be used in this approach is:

$$\beta_t = \left(\frac{t-1}{T-1} \right)^c,$$

with $t \in 1, \dots, T$; with better results achieved when T is between 20 and 100 and c is between 3 and 5.

Another method, proposed by Xu et al. (2010), considers that β can be treated as a random variable, and therefore we can rewrite Integral 3.8 as:

$$\mathbb{E}_{p(\beta)} \left[\frac{\mathbb{E}_{p_\beta(\boldsymbol{\theta})} [\ln p(\mathbf{D}|\boldsymbol{\theta}, M)]}{p(\beta)} \right] \quad (3.10)$$

The author uses this ideas to derive the following estimator. First, the interval $[0, 1]$ is discretized into $S-1$ disjoint intervals $\Delta\beta_i = [t_{i+1}, t_i]$ such that $\sum_{i=1}^{S-1} (t_{i+1} - t_i) = 1$. Then, for each interval $\Delta\beta_i$, T_i temperatures are taken randomly from the uniform distribution on the interval $[t_{i+1}, t_i]$. Finally, the estimator of the logarithm of the marginal likelihood is given by:

$$\sum_{s=1}^{S-1} \frac{|\Delta\beta_k|}{T_k} \sum_{i=1}^{M_k} \log p(\mathbf{D}|M, \boldsymbol{\theta}^{(\beta_{k,i})}) \quad (3.11)$$

where $\beta_{k,i}$ is the i -th sampled temperature from the interval $\Delta\beta_k$; $\boldsymbol{\theta}^{(\beta_{k,i})}$ is a parameter sampled from the power posterior $p_{\beta_{k,i}}(\boldsymbol{\theta})$; and $|\Delta\beta_k| = t_{k+1} - t_k$. However, Xu et al. do not provide information about the discretization method of the interval $[0, 1]$.

3.3 Approximate Bayesian Computation

Approximate Bayesian Computation (ABC) is an approach that allows generating samples from the posterior distribution without accessing the likelihood function. Adding a model indicator parameter to parameters being sampled also allows us to create an approximate sample of the posterior $p(\boldsymbol{\theta}, M|\mathbf{D})$, and from this sample it is possible to estimate $p(M|\mathbf{D})$, which can be used as a model ranking metric. The main idea of ABC methods is to generate a sample from posterior by generating parameter points that, when plugged into a model, generates simulations that differ from the experimental measurements at most by some small ϵ .

A generic ABC method that generates a sample of the posterior $p(\boldsymbol{\theta}, M|\mathbf{D})$ is composed of the following steps:

1. Sample a parameter candidate $(\boldsymbol{\theta}^*, M^*)$ from some proposal distribution.
2. Simulate the model M^* with parameter values $\boldsymbol{\theta}^*$, generating simulated measurements $\phi(M^*, \boldsymbol{\theta}^*) = D^*$.
3. Calculate, for some distance function d , the value of $d(D^*, D)$. If $d(D^*, D) < \epsilon$ for some previously specified ϵ , then add $(\boldsymbol{\theta}^*, M^*)$ to the sample.
4. Repeat until some iteration limit.

The simplest ABC algorithm is the ABC Rejection, and it goes very similarly to the generic algorithm we just presented, with the specification that on step 1 the proposal distribution is the prior distribution. The output of this algorithm is a sample of the distribution $p(\boldsymbol{\theta}, M|d(\phi(M, \boldsymbol{\theta}), \mathbf{D}) \leq \epsilon)$. When $\epsilon \rightarrow \infty$ this is then a sample of the prior distribution, and as $\epsilon \rightarrow 0$ then this sample tends to be a sample of the posterior distribution [Pri+99]. This algorithm, however, does not perform well when the posterior distribution is very different from the prior distribution. For that reason, new ABC methods using Monte Carlo Markov Chains were created [Mar+03]. The ABC MCMC method proposed by Marjoram et al. (2003) produces a Markov Chain whose stationary distribution is $p(\boldsymbol{\theta}, M|d(\phi(M, \boldsymbol{\theta}), \mathbf{D}) \leq \epsilon)$. Nonetheless, this algorithm might still suffer from correlation in samples or even get stuck in regions of local peaks of probability. For that reason, the ABC sequential Monte Carlo (ABC SMC) method was proposed [Ton+09].

The ABC SMC method creates a sequence of samples with the goal of getting closer to a posterior sample in each step. Let us simplify the notation by saying that $d(\phi(M, \boldsymbol{\theta}), \mathbf{D})$ is just $\rho_{M, \boldsymbol{\theta}}$. From a predefined sequence $\epsilon_1, \dots, \epsilon_T$ the algorithm generates a sequence of samples that represents the distributions $p(\boldsymbol{\theta}, M|\rho_{M, \boldsymbol{\theta}} \leq \epsilon_1), p(\boldsymbol{\theta}, M|\rho_{M, \boldsymbol{\theta}} \leq \epsilon_2), \dots, p(\boldsymbol{\theta}, M|\rho_{M, \boldsymbol{\theta}} \leq \epsilon_T)$. At the first generation, a sample of $p(\boldsymbol{\theta}, M|\rho_{M, \boldsymbol{\theta}} \leq \epsilon_1)$ is created by proposing points from the prior distribution. Then, for next generations the candidates to the sample are proposed based on the points of the last generation and their weight, plus some noise determined by a perturbation Kernel; the weight of a point $(\boldsymbol{\theta}^*, M^*)$ on generation t is an estimative of $p(\boldsymbol{\theta} = \boldsymbol{\theta}^*, M = M^*|\rho_{M, \boldsymbol{\theta}} \leq \epsilon_t)$. The pseudo-code 1 presents the ABC SMC algorithm.

The ABC SMC algorithm is implemented in Python language in a software called ABC-SysBio [Lie+14].

ABC SMC (\mathcal{M}, D)

```

1: Define the sequence  $\epsilon_1, \dots, \epsilon_T$ .
2: Define  $N$ , the sample size for each generation.
3: Sample  $\{(\theta^{(1,1)}, M^{(1,1)}), (\theta^{(1,2)}, M^{(1,2)}), \dots, (\theta^{(1,N)}, M^{(1,N)})\}$  from  $p(\theta, M|D)$ .
4: Set  $w^{(1,i)} = 1, \forall i \in 1, \dots, N$ .
5: for  $t \in \{1, \dots, T\}$  do
6:    $i \leftarrow 1$ 
7:   while  $i \leq N$  do
8:     Sample  $M^*$  from  $p(M|D)$ , the model prior.
9:     Sample  $(\theta^{(t-1,k)}, M^*)$  from the last generation with weight  $w^{(t-1,k)}$ .
10:    Create  $(\theta^*, M^*)$  by perturbing  $\theta^{(t-1,k)}$ ;  $\theta^* \propto K^t(\theta|\theta^{(t-1,k)})$ .
11:    if  $p(\theta^*|M^*) = 0$  then
12:      Continue to next iteration.
13:    end if
14:     $D^* \leftarrow \phi(M^*, \theta^*)$ 
15:    if  $d(D^*, D) \leq \epsilon$  then
16:       $i \leftarrow i + 1$ 
17:       $(\theta^{(t,i)}, M^{(t,i)}) \leftarrow (\theta^*, M^*)$ 
18:    end if
19:  end while
20:  Calculate the weights of the population:  $w^{(t,i)} = \frac{p(\theta^{(t,i)}|M^{(t,i)})}{\sum_{j=1}^N w^{(t-1,j)} p_{K^t}(\theta^{(t-1,j)}|\theta^{(t,i)})}$ 
21: end for
22: return

```

Algorithm 1: Pseudo-code of ABC SMC.

Chapter 4

Development of SigNetMS, a Software for Model Ranking

In this chapter we present the development process and implementation details of the SigNetMS software, which stands for **S**ignaling **N**etwork **M**odel **S**election. This software is capable of producing an estimative of the marginal likelihood of a model given experimental data, $p(\mathbf{D}|M)$.

4.1 The SigNetMS Software

SigNetMS is a Python program that can be used as a tool for model selection. The source code is available on Github¹ and it is open source, under the GNU General Public License.

This program expects as the input: a signaling pathway model, represented by a Systems Biology Markup Language (SBML) [Huc+03b] file, with the definition of reactions, kinetic laws and initial concentrations of chemical species; an Extensible Markup Language (XML) file with experimental data, including time series measurements of the biological phenomena of interest; another XML file with definitions of prior distributions of reaction rate constants; and, finally, a set of parameter values that determine the sampling process of model parameters. There are also optional parameters on SigNetMS, used to control random number generator seeds, number of execution threads, and verbose runs.

The output of the program is composed by an estimative of the marginal likelihood of the model, given experimental data, $p(\mathbf{D}|M)$ and a list of parameter values $(\theta^1, \theta^2, \dots, \theta^l)$ that represent a sample of the distribution $p(\boldsymbol{\theta}|M, \mathbf{D})$. If one simulates the model M with parameter values from the sample, we expect that, the higher the marginal likelihood, the closer the simulation is to the experimental data.

To calculate this estimative, we use the ideas of Thermodynamic Integration we presented on section 3.2. Before implementing SigNetMS we also considered using another software, called BioBayes [VG08], that has a very similar approach to produce an estimative of the marginal likelihood. However, we did not proceed to use this software because there was only a graphical user interface version of the software, which made the process of running instances and collecting results cumbersome. We have also tried contacting the authors to obtain the source code, however they could not provide us that.

¹<https://github.com/gustavoem/SigNetMS>

4.2 Creating an estimative of the marginal likelihood

To create estimates of the marginal likelihood using the Thermodynamic Integration approach, we need to define a likelihood function $p(\mathbf{D}|M, \boldsymbol{\theta})$ and also samples power posterior distributions $p_\beta(\boldsymbol{\theta})$ for a sequence of values of β . On SigNetMS, we use the trapezoidal rule to approximate the marginal likelihood, given by the equation 3.9. Inspired by the work of Friel et al., we discretize the interval $[0, 1]$ of power posteriors using the $\beta_i = \left(\frac{i-1}{T-1}\right)^c$, where $T = 20$, $c = 4$ and $i \in \{1, 2, \dots, T\}$.

4.2.1 Implementing the likelihood function

The likelihood function we used is the same as we defined in equation 3.1:

$$p(\mathbf{D}|M, \boldsymbol{\theta}) = p_{\mathcal{N}(\bar{\mathbf{0}}, \Sigma)}(\phi(M, \boldsymbol{\theta}) - \mathbf{D}),$$

where \mathbf{D} is the experimental measurement, M is the model, $\boldsymbol{\theta}$ is a set of parameter values, Σ is the variance of the error of experimental observations, and, finally, ϕ is a function that calculates an approximation of the results of the experiment that produced \mathbf{D} , applied to the simulated environment of model M with parameter values $\boldsymbol{\theta}$. This simulation of the model is created by deriving a system of ordinary differential equations, and then numerically integrating this system, over the time steps defined by the experimental measurements. To reproduce the same experiment that generated \mathbf{D} , SigNetMS expects that the XML file containing experimental data also contains a mathematical representation of which quantity was measured, in terms of concentrations of chemical species of the system.

The numerical integration of the system is alone a hard problem, and therefore, we used third-party software to produce such integrations. The most popular software available for this problem conduce iterative algorithms that, step by step, aproximate the state of the system for a time interval. It is important to know that some instances of the problem can be stiff, meaning that they may make the integration algorithm be unstable, since it may need consecutive iterations in really small steps. Since we did not have time to go in details of when such cases occur, we chose third-party softwares that can adapt the used algorithm according to the stiffness of the instance.

After the implementation of the function ϕ , most of the work to implement the likelihood function is done. The remaining work is to calculate the value of the probability density function $p_{\mathcal{N}(\bar{\mathbf{0}}, \Sigma)}$ and that can easily be accomplished using statistical packages such as SciPy [Vir+20].

4.2.2 Sampling parameters from power-posteriors

After implementing the likelihood function, we can move to the creation of samples of power posteriors. The methodology we used to generate such samples is identical to the one we presented on section 3.2.2. And for this reason, we divided the sampling process in three phases: naive burn-in, adaptive burn-in and Populational MCMC; all of them are types of a Metropolis-Hastings procedure. The number of iterations of each phase is determined by SigNetMS's arguments, and each phase has a different scheme to determine the proposal distribution.

On the first phase, we start the sample of every chain (for each value of β) with a random draw from the prior distribution of parameters. Before the first iteration, we also create an estimative of the variance of the logarithm of each model parameter, independently. These estimates compose the first covariance matrix of the jumping distribution; we use a diagonal matrix where the diagonal elements are set as the estimated variance of the logarithm scaled

sample of the associated parameter. This matrix is rescaled according to the acceptance rate, as described in section 3.2.2, after a number of iterations that is defined in one of the arguments of SigNetMS. For each iteration, we determine that the jumping distribution is a multivariate log-normal $(\boldsymbol{\mu}, \Sigma)$ distribution, with covariance matrix as explained before, and with $\boldsymbol{\mu} = \log_e(\boldsymbol{\theta}^t)$, where $\boldsymbol{\theta}^t$ is the current sample point, i.e., we take a sample \mathbf{X} of the multivariate normal $\mathcal{N}(\boldsymbol{\mu}, \Sigma)$ and then we set our sampled value as $\mathbf{Y} = \exp(\mathbf{X})$, which is a standard procedure to produce samples of lognormal distributions.

On the second phase, the posterior shaped burn-in phase, we also use a lognormal as the proposal distribution, however the covariance matrix is not diagonal. Half of the sample produced in the first phase is discarded, and for each step, we calculate the covariance of the log-scaled current sample, producing the matrix used as the covariance matrix for the proposal distribution. Similarly to the first phase, the proposal distribution also uses $\boldsymbol{\mu} = \log_e(\boldsymbol{\theta}^t)$. It is important to remember that up to the end of this sampling phase, each power posterior sample is created independently.

The third and last phase, we perform a Populational Monte Carlo Markov Chain. In this procedure, we iterate each chain of power posterior samples using the same algorithm of the second phase (except we do not update the covariance matrix anymore), followed by an exchange the last sampled points on two random selected power posteriors. At the end of this phase, we discard parameters sampled on previous phases and we set the actual sample as all the parameters sampled in this phase.

4.3 Fast integration of system of differential equations

The numerical integration of a system of ordinary differential equations is one of the most computationally expensive processes for the estimation of the marginal likelihood. Besides being computationally expensive, the integration is also performed numerous times; for each calculation of the likelihood function, one integration is necessary. Note that the likelihood function is evaluated for all proposed jumps in the sampling, for each of the power posterior distributions. Even in basic experiments, we may evaluate this function hundreds of thousands times. This is the main reason why our first implementation of SigNetMS did not cope even with toy models. Therefore, we needed to add optimizations to our software before experimenting with more complex instances.

Since most of the computational time spent by SigNetMS is concentrated in the generation of samples of power posteriors, we decided to focus optimizations in two different aspects of the sampling. The first aspect, is to reduce the required time to integrate a model, exploring different approaches to represent the model; the second aspect, is to take advantage of the independence of the sampling of two different power posteriors, in the first two phases of sampling.

4.3.1 Optimizing the integration of a system of ordinary differential equations

The first consideration that was taken into account when optimizing the integration of the model was to use different algorithms, because, in general, algorithms prepared for non-stiff instances have better performance. However, because of the different nature of instances of the problem, we could not choose between algorithms prepared for stiff or non-stiff instances, and then we chose to continue using the `odeint` integrator, offered by SciPy, which can adapt

according to the stiffness of the instance. The integrator `odeint` is actually a wrapper to the `LSODA` integrator, which part of the Fortran package called `ODEPACK` [Hin82] language.

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