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)

Contents

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 cell 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 models 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 experimental measures—generally based on Western blot data—to simulated results. The figure 1.1 shows a set of interactions as well as parameters of a model of a signaling network. To create these computational models, two main tasks need to be accomplished.

TODO: rewords some things here. A model has parameters, finding the parameter values is more like a model calibration instead of a model creation.

The first task one must complete to create a model is to determine a set of interactions to consider in the ODE system. Searching for pathway maps on the Kyoto Encyclopedia of Genes and Genomes (KEGG) [KG00] is a good start. 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 even removing interactions. One might reason that we should use as many interactions as we can to get a better simulation, however, this usually implies in poor or computationally infeasible models because of two reasons: first, complex models will require more time in the numerical solution computation, which may be infeasible due to limited

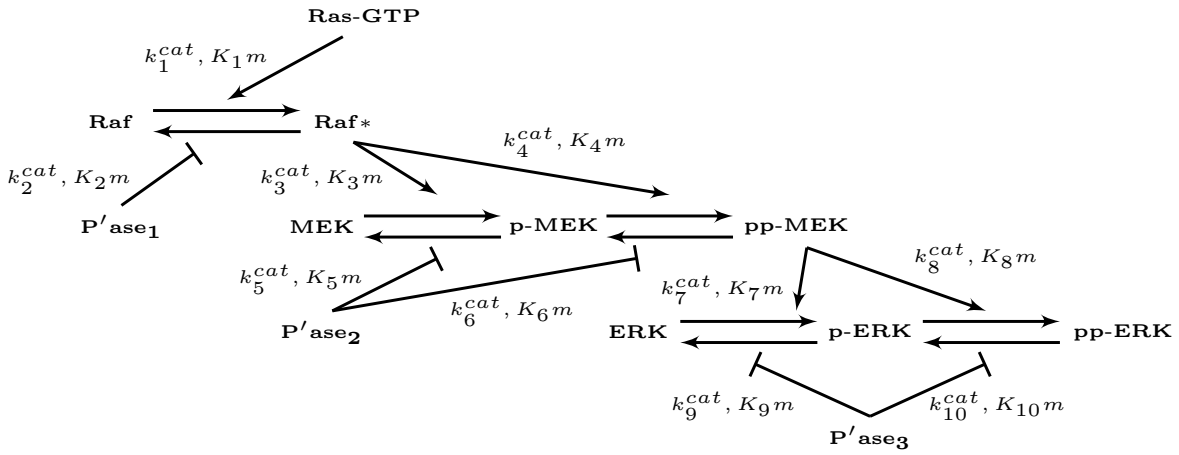


Figure 1.1: The above diagram show a hypothesis for a signaling pathway that flows through Raf-MEK-ERK cascade. Names in bold represent chemical species. Names in italic represent parameters of the ordinary differential equation of each interaction.Horizontal arrows represent phosphorylation when directed 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].

computational resources; and second, when considering many interactions, we are also placing many parameters (multiplying constants of the differential equations) on the model, and finding appropriate values for them becomes harder as we increase the number of parameters.

The second task to create a model is to find values for all the system parameters. There are two approaches for this task, one can either fetch values for these constants from the literature or one can find values that makes 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 can be used. For optimization, it is necessary to define a metric that can evaluate how close the parameters brings the model output to the experimental observation so that it is possible to search for the optimal parameter in the parameter space. Statistical inference, in the other hand, usually tries to maximize some likelihood function, which is defined to represent the probability of a model, with a set of parameter values, to reproduce the experimental observation.

After completing both tasks, however, as we mentioned before, we might still not have found a model 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 a set of interactions to the model topology in order to better simulate the signaling pathway of interest. This set of interactions is a subset of interactions from a database created by Lulu 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 the search space itself does not define models, but only the topology, i.e. the set of interactions, therefore, to consider the second task of creating computational models for signaling networks, 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 model and experimental 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 Lulu 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 is then considered as the cost of the model. The size of the search space is exactly 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 why Lulu Wu used 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 Lulu 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 Lulu 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 conduced with any kind of perturbation to the biological environment or to the data collected.

We propose on this project to create a new method for modifying models of signaling networks, based on the work of Lulu Wu, and including possible solutions to the three aspects mentioned on the last paragraph. To the first aspect, we propose to create a database that includes interactions informations gathered from KEGG and STRING, and also that includes reaction constants values, which can be fetched from SABIO-RK, Brenda [Sch04] or BioNumbers [Mil+09]. To the second aspect, we propose to create new search algorithms that are more general than SFS, and to test and compare these new algorithms we intend to use the featsel framework [Rei+17b]. For the last aspect, about the cost function, 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

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

where $p(D|\theta, M)$ is the likelihood of model M, with parameters θ, to reproduce the experimental data D; $p(\theta|M)$ is the prior probability of θ; and finally, $p(D|M)$ 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.

The method developed on this work for model selection will then be applied in biological models, mainly related to tumor cells, that are relevant for Center of Toxins, Immune-response and Cell Signaling (CeTICS).

1.1 Objectives

In this work we propose to create a software that applies a method, based on the work o Lulu Wu, that is able to select a model to reproduce some biological experiment. This method should be able to consult a vast database of interactions and modify a starting model by removing or adding those interactions in order to construct a model that approximates more closely the biological experiment. The construction of this database and how the method consults it is also part of this work. We also propose to validate the methodology with real cell data. To achieve these goals, we should complete the following tasks:

1. **Define a cost function for models**. We propose to use a Bayesian approach to implement an algorithm that allows us to estimate the value of $p(D|M)$, which should be used as the "score" of the model. To complete this activity we will use as reference the Bayesian inference-based modeling method (BIBm) [Xu+10] and also the software ABC-SysBio [Lie+14].

2. **Build a database of interactions**. This database should include interactions gathered from KEGG and STRING, and reaction rate constants of interactions, which are available on other databases such as SABIO-RK, BioModels and BioNumbers.

3. **Formulate the systematic modifications on the models as a feature selection search space**. Given the initial model, we should be able to identify this model as a node of the search space, and we also should be able perform valid jumps from one node to another, in other words, we should be able to perform valid modifications to the model.

4. **Define search algorithms on the feature selection problem**. Given that we successfully structured the modifications of the model as a Boolean lattice, we should define algorithms to determine how to traverse this space in order to find a model with the least possible cost in a reasonable amount of execution time.

5. **Test feature selection algorithms**. Using artificial and then real data, we should test if the methodology can select a model that is able to reproduce the behaviour measured on the signaling network.

6. **Apply the methodology on a real case**. Finally, with a tested implementation of the methodology, we should help researchers from CeTICS to identify cell signaling networks of cells that are relevant to their research.

1.2 Organization

Chapter 2

Fundamental Concepts

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. Bad functioning signaling pathways 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 can either penetrate the cell or bind to some specific receptor in the membrane. Once either of those events happen, the signal or 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 that participate on the pathway of interest.

2.2 Measurements of Proteins in Cell Signaling Pathways

Western blot is a laboratory technique that can indicate the amount of a specific protein that is present in a mixture. This technique show the presence of a protein in a mixture by "blotting" a membrane where the molecules of interest are located. We can superficially 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).

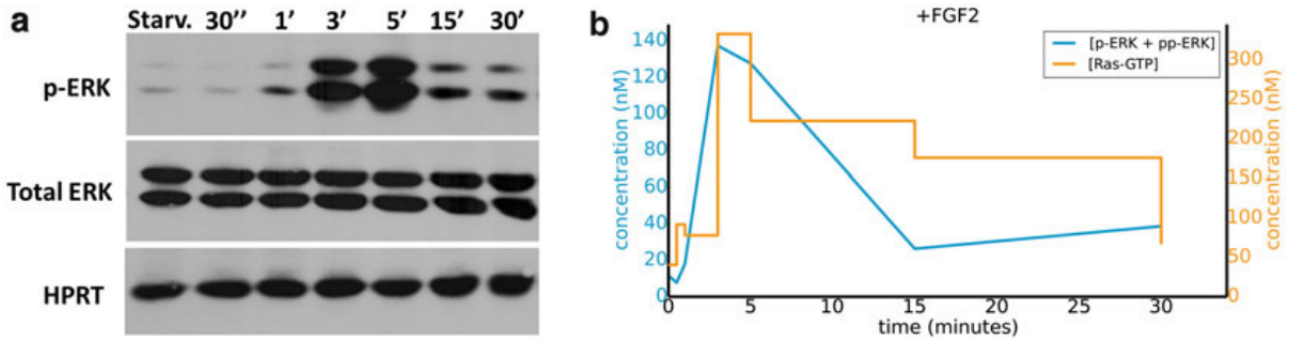


Figure 2.1: Figure **a** *shows time-course measurements of ERK, phosphorylated ERK and hypoxanthine-guanine phosphoribosyltransferase (HPRT). HPRT is a "loading" protein, that means that its concentration is fairly the same through the experiment, and therefore it is used as a normalizing factor to 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 models for this signaling networks that is able to reproduce experimental data, then it is possible to 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. 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:

$$\mathrm{A} \longrightarrow \mathrm{B}.$$

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

$$k_1[\mathrm{A}],$$

where k_1 is some constant and [A] is the concentration of A.

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:

$$\mathrm{C} + \mathrm{D} \longrightarrow \mathrm{F}.$$

The reaction rate of this reaction is:

$$k_2[\mathrm{C}][\mathrm{D}],$$

where k_2 is a 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 though 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:

$$\mathrm{E} + \mathrm{S} \underset{k_r}{\overset{k_f}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_{cat}} \mathrm{E} + \mathrm{P}, \tag{2.1}$$

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:

$$\mathrm{E} + \mathrm{S} \xrightarrow{k_f} \mathrm{ES} \tag{2.2a}$$

$$\mathrm{ES} \xrightarrow{k_r} \mathrm{E} + \mathrm{S} \tag{2.2b}$$

$$\mathrm{ES} \xrightarrow{k_{cat}} \mathrm{E} + \mathrm{P} \tag{2.2c}$$

and they have, respectively, reaction rates of:

$$k_f[\mathrm{E}][\mathrm{S}]$$
$$k_r[\mathrm{ES}]$$
$$k_{cat}[\mathrm{ES}].$$

TODO: Maybe I should stop this subsection here and start another where I explain what comes next as "Dynamic Modeling of a System of Reactions"

Now, to determine a model of the concentration dynamics of every chemical species involved in reaction 2.1, we will write a system of ordinary differential equations. To do so, for every species we should 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:

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

Repeating this procedure for every other species of the enzymatic reaction induces the desired system of ordinary differential equations:

$$\frac{d[\mathrm{E}]}{dt} = -k_f[\mathrm{E}][\mathrm{S}] + (k_r + k_{cat})[\mathrm{ES}] \tag{2.4a}$$
$$\frac{d[\mathrm{S}]}{dt} = -k_f[\mathrm{E}][\mathrm{S}] + k_r[\mathrm{ES}] \tag{2.4b}$$
$$\frac{d[\mathrm{ES}]}{dt} = k_f[\mathrm{E}][\mathrm{S}] - (k_r + k_{cat})[\mathrm{ES}] \tag{2.4c}$$
$$\frac{d[\mathrm{P}]}{dt} = k_{cat}[\mathrm{ES}]. \tag{2.4d}$$

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.

A basic the 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:

$$[\mathrm{E}_0] = [\mathrm{E}] + [\mathrm{ES}].$$

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

$$\frac{d[\mathrm{ES}]}{dt} = k_f([\mathrm{E}_0] - [\mathrm{ES}])[\mathrm{S}] - (k_r + k_{cat})[\mathrm{ES}]. \tag{2.5}$$

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. $\frac{d[\mathrm{ES}]}{dt} = 0$, and it was first proposed in 1925 by Briggs and Haldane [BH25]. Generally, this assumption is applicable whenever $[\mathrm{S}] \gg [\mathrm{E}]$. TODO: and we can apply it on this work because... Applying this assumption together with the mass conservation assumption on the equation 2.5, we get:

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

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:

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

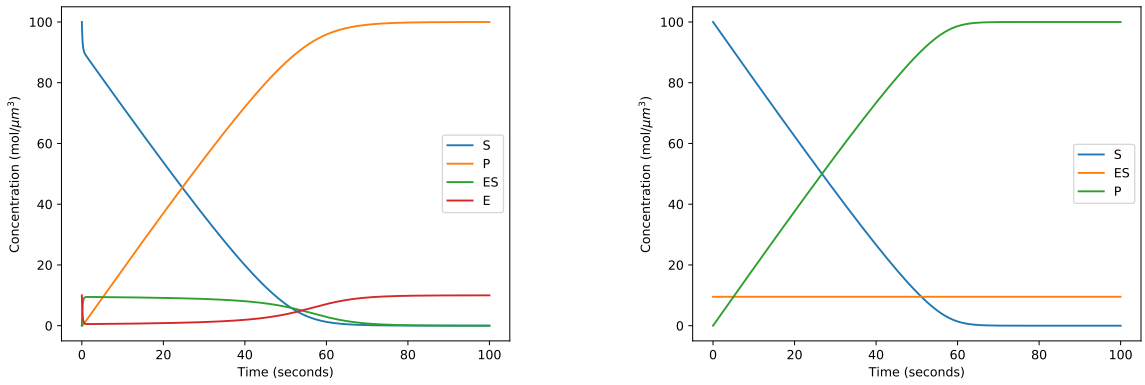
And finally, if we apply mass conservation to the substrate, we will get the following equation:

$$[\mathrm{S}_0] = [\mathrm{S}] + [\mathrm{ES}] + [\mathrm{P}],$$

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

$$\frac{d[\mathrm{S}]}{dt} = -\frac{d[\mathrm{P}]}{dt}. \tag{2.7}$$

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 system 2.4 that had four equations and three parameters to a new model that has only two equation and two parameters (k_{cat} and K_m). Figure 2.2 shows a comparison between the complete and Michaelis-Menten models of enzymatic reactions.



(a) (b)

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/$(\mu m)^3$ for the enzyme (E); 100 molecules/$(\mu m)^3$ is used for the substrate (S); and 0 molecules/$(\mu m)^3$ 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 $(\mu m)^3 (molecules * s)^{-1}$ for k_f; 0.1 (s^{-1}) for k_r; 0.2 (s^{-1}) for k_{cat}; and, following the Michalis-Menten model, 5 molecules/$(\mu m)^3$ 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 behaviour of the cell 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, and a set of measurements, commonly Western blot data. 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 this 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 and approximate 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 treats 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 and penalize 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 makes 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. We can write this scoring function as:

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

where M is the model, Θ is the parameter space, Θ' is the subset of the parameter space where the search for the best parameter values was conduced, D is the experimental measurement, 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 algorithm to search for parameter values that minimizes the distance between simulation and experiment; however, the 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 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:

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

where D is the observed data, M is the model and Θ is the parameter space. The function $p(\theta|M)$ is the prior probability function of the parameter θ on model M and the function $p(D|M, \theta)$ is the likelihood of observing the data 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 Aproximate Bayesian Computation (ABC), to estimate the probability $p(M|D)$ 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 is not as many works on the literature as there is 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:

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

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

$$q_0(\theta) = p(\theta|M),$$

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

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

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

As we mentioned before, the likelihood function $p(D|M,\theta)$ may be very hard to calculate if not impossible. For those cases it is possible to use parameter inference approaches that are 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 θ^* from a proposal distribution; then, a simulation, $\phi(M,\theta^*)$ of the model using the candidate parameters is produced; if, for some distance function d, is true that $d(\phi(M,\theta^*),D) < \epsilon$, then we accept θ^* 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 of 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 it 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 directly the target function, because all it is needed to perform the sampling is a function that is proportional to the target.

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 $p(\theta|M, D)$. Calculating this probability function is very hard because

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

and this equation has the term $p(D|M)$, which is only known (by some estimation) at the end of the model ranking; however, if we can access the likelihood function $p(D|\theta, M)$ and the prior $p(\theta|M)$ than the product of these two is proportional to the posterior distribution (since $p(D|M)$ is only a constant because it does not depend on θ), and therefore they are enough 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:
$$r = \frac{p(\lambda^*)J_t(\lambda^{t-1}|\lambda^*)}{p(\lambda^{t-1})J_t(\lambda^*|\lambda^{t-1})} \tag{2.10}$$
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 $q(\lambda) = cp(\lambda)$ 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, 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].

Chapter 3

Model Selection

Simple description of the content of this chapter.

3.1 Model ranking using Marginal Likelihood

The marginal likelihood of an experiment measurement D being reproduced by a model M, $p(D|M)$, 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 what the likelihood function is. To calculate the likelihood $p(D|M,\theta)$, we must understand that conditioning the observation to the model and parameters means that in the probability space from which D is taken, the model M is the "real" model and it has the parameter values of θ; i.e. the model M with parameters θ controls the behaviour of the system from which D was observed. Then, assuming that the observations have a Gaussian error, and that they are taken in a time series of m time steps, we can define the likelihood as:

$$p(D|M,\theta) = p_{\mathcal{N}_{(\vec{0},\Sigma)}}(\phi(M,\theta) - D), \tag{3.1}$$

where $\phi(M,\theta) \in \mathbb{R}^m$ is the experimental measurement on the simulation generated by the model M with parameters θ, and $p_{\mathcal{N}_{(\vec{0},\Sigma)}}(\cdot)$ is the probability density function of a Multivariate Normal variable with mean $\vec{0}$ and covariance matrix Σ. As a matter of fact, as it is done in the work of Xu et al., we can consider that the observation error is independent for each time step [Xu+10], therefore we can simplify 3.1 to:

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

The σ^2 used in equation 3.2 is also a parameter of the model, which means that, for some k, $\theta_k = \sigma^2$.

Now that we defined the likelihood function, we can write the marginal likelihood as:

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

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 $\lambda = \theta|M$ and use the prior $(p(\theta|M))$ or the posterior $(p(\theta|M, D))$ as importance sampling functions, then we would get respectively the estimators:

$$\frac{1}{m}\sum_{i=1}^{m} p(D|M, \theta^{(i)}) \quad (\text{with } \theta^{(i)} \sim p(\theta|M)),$$

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

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].

3.1.1 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 $p(\theta|M)$ and a posterior distribution $p(\theta|D, M)$, then we define a power posterior distribution with temperature β as:

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

where

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

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

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

the posterior distributions of the parameters.

Now, let we consider the derivative of $\ln z(\beta)$.

$$\begin{aligned}\frac{d}{d\beta}\ln z(\beta) &= \frac{1}{z(\beta)}\frac{d}{d\beta}z(\beta) \\ &= \frac{1}{z(\beta)}\frac{d}{d\beta}\int_\Theta p(D|\theta, M)^\beta p(\theta|M)d\theta\end{aligned}$$

(using that $\frac{d}{dx}c^x = c^x \ln c$)

$$\begin{aligned}
&= \frac{1}{z(\beta)} \int_\Theta p(D|\theta, M)^\beta p(\theta|M) \ln p(D|\theta, M) d\theta \\
&= \int_\Theta \frac{p(D|\theta, M)^\beta p(\theta|M)}{z(\beta)} \ln p(D|\theta, M) d\theta \\
&= \int_\Theta p_\beta(\theta) \ln p(D|\theta, M) d\theta \\
&= \mathbb{E}_{p_\beta(\theta)}[\ln p(D|\theta, M)]. \qquad (3.4)
\end{aligned}$$

And it is not hard to see that:

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

Using equations 3.5 and equality 3.4 we can write that:

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

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 this estimators, we will need to generate samples for a series of power posteriors of parameters, and this will be explained in the next section.

3.1.2 Estimation of the Marginal Likelihood

To estimate the integral 3.6 we need to generate samples of a sequence of power posterior distributions.

3.2 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 $p(\theta, M|D)$, and from this sample it is possible to estimate $p(M|D)$, 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 dist from the experimental measurements at most by some small ϵ.

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

1. Sample a parameter candidate (θ^*, M^*) from some proposal distribution.

2. Simulate the model M^* with parameter values θ^*, generating simulated measurements $\phi(M^*, \theta^*) = D*$.

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 (θ^*, 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 $p(\theta, M|d(\phi(M, \theta), D) \leq \epsilon)$; when $\epsilon \to \infty$ this is then a sample of the prior distribution, and as $\epsilon \to 0$ 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 $p(\theta, M|d(\phi(M, \theta), D) \leq \epsilon)$. 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 sequantial 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 we simplify the notation by saying that $d(\phi(M, \theta), D)$ is just $\rho_{M,\theta}$. From a predefined sequence $\epsilon_1, \ldots, \epsilon_T$ the algorithm generates a sequence of samples that represents the distributions $p(\theta, M|\rho_{M,\theta} \leq \epsilon_1), p(\theta, M|\rho_{M,\theta} \leq \epsilon_2), \ldots, p(\theta, M|\rho_{M,\theta} \leq \epsilon_T)$. At the first generation, a sample of $p(\theta, M|\rho_{M,\theta} \leq \epsilon_1)$ 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 (θ^*, M^*) on generation t is a estimative of $p(\theta = \theta^*, M = M^*|\rho_{M,\theta} \leq \epsilon_t)$. The pseudo-code 1 presents the ABC SMC algorithm.

ABC SMC $(\mathcal{M}, D)$

Define the sequence $\epsilon_1, \ldots, \epsilon_T$.
Define N, the sample size for each generation.
Sample $\{(\theta^{(1,1)}, M^{(1,1)}), (\theta^{(1,2)}, M^{(1,2)}), \ldots, (\theta^{(1,N)}, M^{(1,N)})\}$ from $p(\theta, M|D)$.
Set $w^{(1,i)} = 1, \forall i \in 1, \ldots, N$.
for $t \in \{1, \ldots, T\}$ **do**
    $i \leftarrow 1$
    while $i \leq N$ **do**
        Sample M^* from $p(M|D)$.
        Sample $(\theta^{(t-1,k)}, M^*)$ from the last generation with weight $w^{(t-1,k)}$.
        Create (θ^*, M^*) by perturbing $\theta^{(t-1,k)}$; $\theta^* \propto K^t(\theta|\theta^{(t-1,k)})$
        if $p(\theta^*|M^*) = 0$ **then**
            Continue to next iteration.
        end if
        $D* \leftarrow \phi(M^*, \theta^*)$
        if $d(D^*, D) \leq \epsilon$ **then**
            $i \leftarrow i + 1$
            $(\theta^{(t,i)}, M^{(t,i)}) \leftarrow (\theta^*, M^*)$
        end if
    end while
    Calculate the weights of each parameter of the population:

$$w^{(t,i)} = \frac{p(\theta^{(t,i)}|M^{(t,i)})}{\sum_{j=1}^{N} w^{(t-1,j)} p_{K^t}(\theta^{(t-1,j)}, \theta^{(t,i)})}$$

end for
return

Algorithm 1: Pseudo-code of ABC SMC.

Chapter 4

Conclusion

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