## Darwin s invisible hand

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# CHAPTER 1

## General introduction

Cells are the fundamental units of life. They are also incredibly complex. Amongst many other things, cells need to take up nutrients from the environment and through a complex network of metabolic reactions produce energy and building blocks from these nutrients. Thousands of reactions occur simultaneously in a single cell. Each of these reactions is catalysed by an enzyme, which in itself is a highly complex molecular machine. Furthermore, cells self-replicate; the nutrients and energy derived from metabolism are used to build new biomolecules, a process carried out by other molecular machines, such as ribosomes. To persevere, cells need to respond appropriately to changing environments. For this, information about the environmental and intracellular state needs to be processed and integrated by signalling cascades. Eventually, a decision needs to be taken, which is then executed by gene regulatory networks. Additional levels of complexity arise from multicellular life, where cells need to cooperate and communicate for the organisms as a whole to function properly.

All these functions are carried out by a large number of different molecular machines. With the emergence of whole genome sequencing, we now have a fairly complete overview of the cellular "parts list" of an ever increasing number of organisms. However, a list of parts does not tell us anything about how these tasks are carried out. Functionality only arises as a result of the dynamic interactions between these molecules. The ultimate aim of systems biology is to elucidate how all these molecules work together to form a living cell that can eat, grow, replicate, make and execute decisions, and so on. The sheer complexity of such a cell can be both fascinating and intimidating to the researcher.

The title of this thesis refers to Adam Smith's idea of an "Invisible hand" and Darwin's theory of evolution through natural selection. The invisible hand is a metaphor for the idea that markets are auto-regulated through competition between

manufacturers, which leads to optimal allocation of production resources so that goods are produced as efficiently as possible.* Darwin's theory of evolution states that through natural selection the "fittest" individuals will eventually come to dominate the population because they produce more offspring that survives. In this thesis I will explore the consequences of the idea that billions of years of natural selection shaped cellular functioning so as to optimally allocate limited resources such as biosynthetic capacity, available nutrients, or membrane area. It is the hope that using such an ordering principles might facilitate the understanding of the complex system that is a living cell.

## 1.1 Evolutionary optimisation as an ordering principle

"Nothing in biology makes sense except in the light of evolution" is the title of a famous essay by Theodosius Dobzhansky [1]. In this essay, Dobzhansky argues that both the incredible diversity of species as well as the remarkable unity in the basic biochemistry of all life can only be the result of life that originated once and subsequently was subjected to billions of years of mutations and selection to generate incredible diversity. While this essay was originally aimed as a critique on creationism, nowadays this quote is often interpreted in a slightly different way. Knowing that all life forms are the outcome of billions of years of evolution can help us make sense out of seemingly hopelessly complex biological systems. If we know what function a certain biological system, e.g. a regulatory circuit, fulfils, we can make the conjecture that selection must have optimised this system for its task, at least to a certain extent. The task of the researcher is then to reverse engineer the underlying logic in the system. Perhaps, with knowledge of this logic, other systems that perform a similar task can then also be understood. The work presented in this thesis is carried out in the spirit of this philosophy.

The use of optimality principles has a long tradition in (evolutionary) biology [2], and there is a large and growing body of literature that employs it to understand biology on many different levels. For single enzymes, the Heinrich group extensively studied enzyme kinetic properties from a theoretical optimality perspective [3–5]. Their analysis is based on the assumption that there are constraints in the free energy landscape of reaction profiles, creating trade-offs between different kinetic parameters. Enzymes in central metabolism, which need to carry the bulk of all cellular fluxes, are on average 30 times faster than those in secondary metabolism. However, most are still far from the theoretically maximal diffusion limited rate [6]. This indicates that the more stringent the selection on efficiency, the closer an enzyme comes to optimality. Another example is kinetic proofreading by translating ribosomes, which entails a trade-off between accuracy of substrate selection and speed of translation. Ehrenberg and Kurland show that the translation rate is such that it optimises the growth rate [7].

*It is, of course, not at all clear if this is really the way a free market economy functions. But that is beyond the scope of this thesis.

At the level of cellular decision making by regulatory networks, optimality principles are also often supplemented with insights from control theory. For instance, in chemotaxis, *Escherichia coli* implements a control strategy known as integral control to attain robust perfect adaptation [8], allowing for a response to concentration gradients rather than absolute concentrations [9]. Similarly, it implements a fold-change detection strategy to be able to respond to the shape of an input, rather than the absolute intensity [10]. Chemotactic sensitivity is ultimately limited by the stochastic fluctuations in the concentration of the chemoattractant. The chemotactic sensitivity of *E. coli* is close to this theoretical limit [11]. Another example of a biological implementation of integral control is the *Saccharomyces cerevisiae* response to hyper-osmotic shock [12]. The heat shock response of *E. coli* consists of a number of feedback and feed-forward loops that allow for a response that is both robust and fast in responding to environmental heat disturbances, and at the same time minimises the energy expenditure of the response [13]. Network motifs are recurring patterns of connections between gene regulatory elements. An appealing hypothesis is that different motifs are actually optimised to perform different functions, such as responding to particular fluctuations in external signals. The very complex wiring diagram of gene regulatory networks is indeed, to a large extent, composed of such network motifs [14].

At a whole cell level, Molenaar *et al.* used a course-grained self-replicator model of microbial growth to explain a number of well known cellular physiological responses from the perspective of growth rate optimisation [15]. This could for instance explain the inverse relation between cell size and growth rate, and the tendency of fast growing cells to switch to a low yield metabolic strategy. Other support for the idea of using optimality principles on the whole cell level is the fact that *E. coli* underwent evolutionary adaptation to achieve a flux distribution that was predicted to be optimal for growth yield by a genome-scale constraint-based metabolic model [16]. Similarly, Schuetz *et al.* showed that flux states could have evolved as a result of trade-off between two objectives: being optimal in a particular condition and minimal adjustment between conditions [17].

It is important to note that postulating optimality principles to understand cellular functioning does not necessarily imply that the system as such is optimal for the task and no improvements can be made. For instance, when two or more systems can carry out the same task, one might be better suited for a particular situation, and the other for a different situation, while neither are necessarily absolutely optimal in any of two situations. In this thesis I will discuss two such examples: the uptake of nutrients by high- and low affinity, and by binding protein dependent- and independent transporters.

## Growth rate optimisation as a resource allocation problem

One problem with the notion of "fitness" optimisation is it that is highly non-trivial what fitness actually is. In other words, what exactly is selected for, and what is the "objective function" of an organism or system?

As opposed to multicellular organisms, every cell division of a unicellular organism gives rise to a new individual. Due to this tight coupling of growth and reproduction, growth rate maximisation is a major contributor to unicellular fitness. A cell that grows faster than its neighbouring cells will *exponentially* outgrow them, and thus achieve a much higher number of offspring cells. This might, in turn, increase the chances of survival, *e.g.*, under periods of stress. There are, of course, many other aspects to microbial fitness. For instance, there appears to be a trade-off between biomass yield and growth rate, and when lactic acid bacteria were evolved in an environment that selects for yield, the growth rate was observed to decrease [18].

Microbial growth rate is, by definition, the rate of biomass formation *per unit biomass*. This implies that biomass should be used as efficiently as possible. Components that contribute little or nothing to cell growth do require investment in biosynthetic resources and, as such, reduce the self-replication rate. In other words, more biomass needs to be synthesised for each new cell to be made. Consistent with this notion is the observation of an inverse relation between stress resistance and growth rate in *S. cerevisiae* [19]. Furthermore, biosynthetic resources such as amino-acids, energy in the form of ATP and, mainly, translational machinery, are limited. Since the main cost of protein expression has been shown to be in the process of making that protein [20], synthesis of a particular protein goes at the expense of other proteins.

It is worth emphasising that this is not only about *which* proteins should be made, but also *how much* of each. Above a certain concentration, increasing the concentration of an enzyme typically contributes little to increasing the flux through the pathway that enzyme is part of, because other reactions will become limiting [21, 22]. Hence, the cell needs to solve a resource allocation problem: How much of what component needs to made in order to achieve the highest growth rate permitted by the prevailing environmental conditions? Due to their high abundance, this is an especially stringent question for metabolic and biosynthetic proteins. Indeed, several studies have shown that both under- and over-expression of metabolic enzymes reduce the growth rate [22–25]. The same holds true for under- or over-expression of ribosomes [26]. This indicates that protein levels are likely optimised.

Exactly which enzyme concentrations are optimal depends on the cellular environment. For instance, depending on limitations in the medium, *E. coli* redirects its biosynthetic resources to catabolic genes under carbon limitation, anabolic genes under amino-acid limitation, and ribosomal genes when translation in inhibited, respectively [27, 28]. Also in *E. coli*, Lac-operon expression was observed to evolve to different external lactose concentration dependent optimal levels within 500 generations [23]. When cells are stressed, resources need to be allocated to processes that help overcome that stress. For instance, when cells are subjected to a heat shock, so called heat shock proteins are synthesised in order to prevent denaturation of proteins. The investment in these heat shock proteins can be substantial (up to 25 % of total protein [29]), and would place a burden on the cell in the absence of the stress. Indeed, cells posses mechanisms to swiftly and adequately respond to a heat stress, but also ensure that no resources are wasted on heat shock proteins in the absence of the stress

[13].

There are other constraints besides those in biosynthetic resources. One example might be the cellular membrane, which has a limited total surface area. Nearly all interactions of the cell with its environment, such as nutrient uptake and sensing, require membrane-bound proteins. Thus, membrane area can also be a limiting resource. This makes investments in membrane bound proteins, such as nutrient transporters, more costly. It requires an investment both in biosynthetic resources and in membrane area. It has for instance been suggested that membrane occupation constraints can explain the shift between different modes of ATP production [30]. I will discuss the use of different transporter systems for the same nutrient under different conditions form the perspective of resource allocation principles in this thesis.

## 1.2 The role of theory and mathematical modelling in biology

It is completely infeasible to make intuitive sense out of the behaviour of complex dynamic systems such as cells. It is thus unavoidable to use at least some form of mathematical modelling if we are to understand cellular functioning. Indeed, one of the hallmarks of systems biology is the iterative cycle of using models to interpret experimental data, and based on that analysis make predictions and propose new experiments to validate or falsify the predictions, allowing for further model refinement and new predictions, and so on [31]. In this thesis, I will use mechanistic modelling tools and other methods of theoretical analysis. No new data is presented here. Rather, I will try to use insight from these analyses to (re)interpret a number of well known microbiological phenomena, and where possible suggest experiments that might test our interpretations.

Robert M. May discussed the various roles of mathematics in biology [32], based on an analogue with the classic sequence of Brahe, Kepler, Newton in elucidating the laws of gravitation: Facts are observed, then patterns are found in the data that give it coherence, and finally fundamental laws are formulated that explain these patterns. This thesis mainly deals with the last stage, although it is understood that in biology laws are hardly ever as fundamental as in physics (the theory of evolution being a notable exception). In this stage, May argues, various conjectures can be made about the underlying mechanisms to explain the patterns. Mathematical modelling can then be used to explore the consequences of these conjectures. Such an "exploration of possible worlds" is useless when done in vaguely verbal terms. Hence, "The virtue of mathematics in such a context is that it forces clarity and precision upon the conjecture, thus enabling meaningful comparison between the consequences of basic assumptions and the empirical facts. Here mathematics is seen in its quintessence: no more, but also no less, than a way of thinking clear" [32]. Or, as Haldane (supposedly) put it in somewhat less diplomatic terms: "An ounce of algebra is worth a ton of verbal argument" [33].

A wide variety of modelling techniques is being used in systems biology. Which technique is most suitable depends on the kind of question being asked. In this thesis, I will mainly study processes related to metabolism, and how specific steady state fluxes can be maximised. (A specific flux refers to the number of chemical conversions per unit of time per unit of protein. Steady state refers to the state where metabolite concentrations remain constant while there still is a flux through the network.) For this purpose, ordinary differential equation (ODE) based models are the most suitable type of models. These describe the time evolution of concentrations of molecules given some initial conditions. Often (although by no means always) they will tend to reach a steady state that is independent of the initial conditions. (Exceptions are e.g., oscillatory systems, which do not reach a steady state, or systems that contain a bistability, where the steady state depends on the initial conditions.) Importantly, these models explicitly take the enzyme-kinetics of the reactions being modelled into account. This allows to relate the attained flux to the properties of the enzymes and their expression levels. Since ODE models are deterministic and describe the amount of molecules as a (continuous-valued) concentration, they are suitable for situations where the number of molecules of the species considered are large enough for stochastic fluctuations to become irrelevant. Typically this is considered to be above 100-1000 molecules per chemical species. One caveat is that stochastic fluctuations in a low abundant species might propagate to species which are much more abundant.

One drawback of ODE type models is that they require input of many parameters, which makes them unsuitable to study metabolism on a genome wide scale. Another type of model that is often used to study metabolic networks are so called constrained-based models. The basic idea behind these models is to assume that all metabolic reactions are in steady state, and calculate which flux distributions are consistent with this assumption [34]. Because they do not take enzyme kinetics into account, they are computationally cheap and do not require knowledge of the kinetic parameters of the enzymes involved. This has the advantage that they can be used to model metabolism on a genome-wide scale. However, this is simultaneously their biggest disadvantage. Because they do not take enzyme kinetics into account, they also do not naturally take enzyme capacity as a limiting resource into account. As such, they are not very suitable to study the effect of enzyme expression on specific flux or growth rate. Efforts are being made to incorporate such effects into constraint-based models (e.g., by limiting or minimising the (absolute) sum of all fluxes [35, 36]). However, the problem remains that without explicitly modelling kinetics, it is impossible to assess what the intracellular concentrations of substrates, products and other effectors of the enzymes are, and thus how much of a certain enzyme is needed to carry a certain flux. As an alternative, combinations of kinetic and constraint-based models are currently being developed. For instance, Wortel *et al.* showed that maximal specific flux, as calculated by a kinetic model, must always be a so-called elementary flux mode (EFM), which is a minimal route through a (genome scale) metabolic network [37]. Some of the work in this thesis will follow up on this observation, and as such can be relevant for constraint-based models.

## 1.3 Simplicity and the use of minimal models

When using models to study biological processes, one needs to make a important decision regarding the desired level of detail of the model. What an optimal level of detail is strongly depends on what the model is developed for and what one expects from it. This can be exemplified by the stereotype of an engineer and a physicist. The engineer has the goal to rationally manipulate a cell, for instance for biotechnological or medical purposes. For this, he or she requires a model as a tool to simulate the system that is to be manipulated and to predict the effect of a perturbation that might be applied. One then requires an as faithful as possible *in silico* replica of that biological system. Needless to say, this is a very relevant endeavour. However, there is a number of pitfalls to this approach, which I will discuss below. The physicist, on the other hand, aims to understand the principles underlying the functioning of the system. While this might appear contradictory to the notion that cells are extremely complex, this actually requires an as simple as possible model. Below, I will discuss why this is the case, but the bottom line has been nicely summarised by Blagoev *et al.*, paraphrasing Philip W. Anderson's Nobel prize address [38]: "the job of a theorist is to get at the crux of the system by ignoring details and yet to find a testable consequence of the resulting simple picture".

At first glance, a more detailed description of reality (i.e. a detailed model) might appear more useful than a simplified view. But there is a number of reasons why this is not generally true. The first is pragmatic. Detailed models often have many parameters, and the vast majority of these parameters is usually unknown. Fitting the model parameters then requires vast amounts of high quality data, which are often neither available nor feasible to generate. And even if these data are available, fitting the model parameters to the data remains a formidable task.

There are, however, more fundamental reasons to aim for simplicity rather than detail. Two closely related aspects are that simple models are easier to understand, and that the underlying assumptions are much clearer. Arguably, a more easily understandable model leads to deeper insight. If a model is very large and complex, and it produces a certain result, it is often still unclear what exactly the requirements are for that result. Furthermore, large models unavoidably have many, often implicit, assumptions underlying them. It is therefore hard to assess under what conditions the model will break down. On the other hand, if a simple model is able to describe a certain data-set, one can remove or alter parts of the model in order to break it. This gives insight in which aspects of the system are critical for its functioning, i.e., it allows identification of the dominant effects. It also potentially allows for even further model reduction.

This also works the other way around. If a model cannot be fitted to a data-set, or if a prediction is wrong, at least one of the underlying assumptions must not hold. (Often, this will be the absence of a certain interaction in the model that is present in the cell). Such a negative result can be very insightful, for it points to insufficient understanding of the system under study. However, if a model is very complex, it is difficult to assess whether the model failure is really due to a lack of understanding,

or due to a failure in e.g., the parameter optimisation procedure. Finally, and perhaps most importantly, models with many free parameters fitted to insufficient data run the risk of being overfitted. As a result, many qualitatively different models can in principle explain the same data, and the models become uninformative. They neither allow for rejection nor for verification of an hypothesis. To quote Pauli, such a model is "not even wrong".

In practice, models of different levels of complexity can often complement each other. A good example of this is a recent study by van Heerden *et al.* [39]. This study shows that due to a bistability in the yeast glycolytic pathway, when exposed to a glucose pulse a subpopulation of cells will enter an unviable state whereas other cells will continue growing. A minimalistic core model was required to elucidate the underlying mechanism of this bistability. Subsequently, a more detailed model allowed to make quantitative predictions about the effect of certain perturbations to the subpopulation that survived. Thus, a detailed model allowed for the experimental validation of a principle uncovered by a minimal model. More generally, coming back to the stereotypes of the engineer and the physicist, one might argue that these are two complementary approaches. In order to be able to manipulate a cell in a rational manner, one first needs a solid understanding of the underlying principles before one can delve into the details. On the other hand, trying to rationally manipulate a cell would be the ultimate test of our understanding of the underlying principles.

## 1.4 Outline of the thesis

There are several aspects to the idea of using optimisation of resource allocation to study biological systems. Three of these aspects are covered in this thesis. The first is the question of what "fitness" actually is in different (laboratory) settings, and what this implies for the optimal allocation of resources. This is the topic of the first two chapters, which deal with developing theory and formalisms about what is optimal under which circumstances. More concretely, which enzymes should be invested in to what extend?

In chapter 2, I study what the objective of growth rate maximisation implies for enzyme concentrations of cells growing in batch. I provide a framework to untangle the benefits of making an enzyme in terms of biochemical activity and the cost in terms of usage of precursors-metabolites, energy or biosynthetic resources. I propose general definitions of this cost and benefit and the notion of a fitness landscape. These definitions are placed in the context of metabolic control analysis.

In chapter 3, I study selection and evolution in the more complex environment of the chemostat. Through consumption of limiting nutrients and (potential) excretion of auto-inhibitory metabolites, there is a strong feedback of the organism on its own environment. Since the steady state growth rate is set externally, growth rate maximisation as such is not the objective. However, selection is still mediated through (transient) growth rate differences. I investigate what this implies for the optimisation of metabolic strategies and enzyme levels. Interestingly, while there are large

differences between growth in a chemostat and in batch, there are also strong similarities between the characteristics of optimal strategies in both environments. However, contrary to the situation in batch, in a chemostat the feedback between growth and environment can lead to negative frequency dependent selection and hence to coexistence (or even speciation) in a constant, homogeneous environment.

Often, multiple proteins or multi-protein systems can perform the same biological task. The second aspect of optimisation discussed in this thesis is which of these systems are optimal under what circumstances. I focus on transporter proteins, since the limited amount of membrane surface area might enhance the selection pressure for their efficient use.

Particularly striking is glucose uptake in yeast; there are about 17 yeast glucose transporters, which strongly differ in their affinity for the substrate. More generally, the occurrence of both high and low affinity transport systems in a single organism are ubiquitous in nature. So far, there is no satisfactory general explanation of what the benefit of a low affinity transporter is. In chapter 4, I argue that low affinity transporters provide a benefit at saturating extracellular substrate concentrations because they reduce the amount of substrate efflux, thereby enhancing the net uptake rate.

Also ubiquitous are binding protein dependent nutrient uptake systems. In these systems a substrate molecule first binds to an extracellular binding protein before translocation across the membrane. Since these binding proteins can be quite highly expressed, they constitute a considerable cost, while the benefit that they confer is not yet clear. In chapter 5, I argue that they can enhance the uptake rate per transporter protein by simultaneously increasing the number of binding sites and the effective substrate concentration.

The third aspect of optimisation I study is the question of how cells are actually capable of optimising their protein levels given a particular environmental condition. In chapter 6, using the expression of ribosomes in *E. coli* as a case study, I investigate what kind of regulatory strategy allows cells to robustly attain the theoretically optimal protein levels in a particular environment.

Finally, in chapter 7, I conclude with a general discussion in which I elaborate on the role of general principles in systems biology.