Summary

Cellular functioning is shaped by billions of years of natural selection. As a result, most subsystems within a cell are highly optimised for some particular task. Understanding what this task is, and how it should be executed to maximise fitness, helps in understanding why a cell functions the way it does. Or, as Theodosius Dobzhansky famously described it: "Nothing in biology makes sense except in the light of evolution". Indeed, the use of so-called optimality principles has proven fruitful in understanding cellular biology.

In this thesis, we use optimality principles to study unicellular organisms. The fitness of a unicellular organism is tightly coupled to the rate at which it can produce offspring, its growth rate. This growth rate is limited by external factors such as nutrient availability, and by internal resource constraints, such as the limited amount of proteins that fit into a cell and the total membrane area in which transporters can be placed. By assuming that the limited internal resources are used as efficiently as possible, we try to find the logic behind a number of well-established features of microorganisms, that have not yet been satisfactorily been explained**. These features involve the use of multiple transporter systems for the same nutrient (chapters 4 and 5), and the regulation of ribosome expression in *Escherichia coli* and presumably also other organisms (chapter 6). We mainly use simple kinetic models of cells or cellular subsystems to study these questions. In the general introduction (chapter 1) we elaborate on the role of optimal resource allocation principles in understanding cellular functioning, and on why simple kinetic models are the most suitable tool to investigate this role.

Before we can proceed to study any specific system, we need a clear idea of what fitness is in different (laboratory) settings, and what this implies for the optimal allocation of resources. This is the topic of chapters 2 and 3, in which we develop theory and formalisms about what is optimal under which circumstances. More concretely, how much of which proteins should the cell optimally make? In chapter 2 we

**The title of this thesis, 'Darwin's invisible hand' reflects this philosophy. It refers to Darwin's idea of natural selection, and to Adam Smith's metaphor of an invisible hand, in the form of competition between manufacturers, which leads to optimal allocation of production resources such that goods are produced as efficiently as possible.

study this for cells growing in a constant environment, the situation of exponential growth in batch. We provide a framework to untangle the benefits of making an enzyme in terms of its biochemical activity and the costs in terms of usage of precursors-metabolites, energy or biosynthetic resources. From this framework, general definitions of the cost and benefit of enzyme expression follow, as well as the notion of a fitness landscape. These definitions are placed in the context of metabolic control analysis. Next, we study selection and evolution in the more complex environment of the chemostat (chapter 5), a continuous culture device often used for prolonged cultivation of microorganisms. Through consumption of limiting nutrients and (potential) excretion of auto-inhibitory metabolites, there is a strong feedback of the organism on its own environment. In a chemostat the steady state growth rate is set externally through the rate at which the limiting nutrient enters the vessel, so growth rate maximisation as such is not the objective. However, selection is still mediated through transient growth rate differences. We investigate what this implies for the optimisation of metabolic strategies and enzyme concentrations. 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 the stable coexistence of multiple strains in a constant, homogeneous environment.

Microorganisms often have several distinct proteins that can carry out the same task. Which one of these is used typically depends on the environmental conditions. One recurring theme is the occurrence of both high and low affinity transporters, for the same substrate, in a single organism. A particularly striking example is glucose uptake in yeast; there are about 17 different glucose transporters, which strongly differ in their affinity. At low glucose concentrations, yeast employs high affinity transporters, but with increasing glucose concentrations it starts using low affinity transporters. So far, there is no satisfactory general explanation of what benefit these low affinity transporter confers. In chapter 4 we argue that they provide a benefit at saturating extracellular substrate concentrations by reducing the rate of substrate efflux, thereby enhancing the net uptake rate and allowing the cells to grow faster. Another recurring theme is the use of transport systems that require extracellular binding protein, while binding protein independent systems are also available. In binding protein dependent systems a substrate molecule first needs to bind to a binding protein, after which the substrate-binding protein-complex interacts with a transporter and the substrate is transported into the cell. These binding proteins are typically quite highly expressed, so they constitute a considerable cost, while the benefit they confer is not clear. In chapter 5 we argue that they enhance the uptake rate per transporter protein, in conditions when the extracellular substrate is scarce, by increasing the substrate-transporter encounter rate increases.

The final aspect of optimisation we study is how cells are capable of optimising their protein concentrations. In chapter 6, we show that the regulatory system underlying ribosome expression in *E. coli* allows it to attain the theoretically optimal protein levels in any particular environment. *E. coli* "measures" how many ribosomes are inactive; many inactive ribosomes implies an ribosomal overcapacity and leads to inhibition of the synthesis of new ribosomes. The signal that *E. coli* uses to measure

this is extremely sensitive to small deviations from optimality, making this system very robust.

All results from this thesis are derived from analysing (mathematical) models that describe the processes in question. In the general discussion (chapter 7) we critically (re-)examine the assumptions underlying these models, propose experiments that can test the hypotheses based derived from these models, and speculate about some generalisations.