# Chapter 1

# The role of modeling in systems biology

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The use of models in biology is at once both familiar and arcane. It is familiar because, as we shall argue, biologists presently and regularly use models as abstractions of reality: diagrams, laws, graphs, plots, relationships, chemical formulae and so on are all essentially models of some external reality that we are trying to describe and understand (Fig. 1.1). In the same way we use and speak of 'model organisms' such as baker's yeast or *Arabidopsis thaliana*, whose role lies in being similar to many organisms without being the same as any other one. Indeed, our theories and hypotheses about biological objects and systems are in one sense also just models. Yet the use of models is for most biologists arcane because familiarity with a subset of model types, especially quantitative mathematical models, has lain outside the mainstream during the last 50 years of the purposely reductionist and qualitative era of molecular biology. It is largely these types of model that are an integral part of the 'new' (and not-so-new) Systems Biology and on which much of the rest of this book concentrates. Since all such models are developed for some kind of a purpose, our role in part is to explain why this type of mathematical model is both useful and important, and will likely become part of the standard armory of successful biologists.

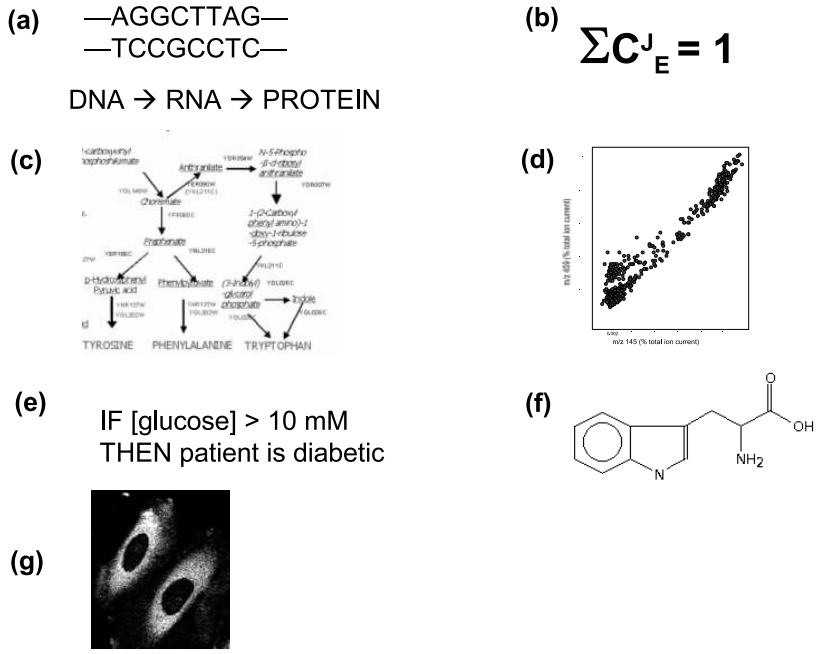



Figure 1.1: Models in biology. Although we shall be concentrating here on a subset of mathematical models, we would stress that the use of all sorts of models is entirely commonplace in biology – examples include (a) diagrams (here a sequence of bases and the 'central dogma'), (b) laws (the flux-control summation theorem of Metabolic Control Analysis), (c) graphs – in the mathematical sense of elements with nodes and edges (a biochemical pathway), (d) plots (covariation of 2 metabolites in a series of experiments), (e) relationships (a rule describing the use of the concentration of a metabolite in disease diagnosis), (f) chemical formulae (tryptophan), (g) images (of mammalian cells), etc.

## 1.1 Philosophical overview

> "When one admits that nothing is certain one must, I think, also admit that some things are much more nearly certain than others."
>
> Bertrand Russell, *"Am I an Atheist or an Agnostic?",* 1947

It is conventional to discriminate (as in Fig. 1.2) (a) the world of ideas, thoughts or other mental constructs and (b) the world of observations or data, and most scientists would recognize that they are linked in an iterative cycle, as drawn: we improve our mental picture of the world by carrying out experiments that produce data, and such data are used to inform the cogitations that feed into the next part of the right-hand arc, that designs and performs the next set of experiments as part of an experimental program. Such a cycle may be seen as a 'chicken and egg' cycle, but for any individual turn of the cycle there is a clear distinction between the two essential starting points (ideas or data). This also occurs in scientific funding circles – is the activity in question *ideas-* (i.e. *hypothesis-*) driven or is it *data*-driven? (Until recently, the latter, hypothesis-*generating* approach was usually treated rather scornfully.)

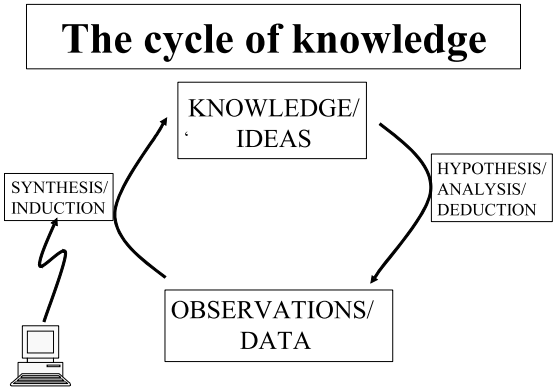



Figure 1.2: The iterative relationship between the world of ideas/hypotheses/thoughts and the world of data/observations. Note that these are linked in a cycle, in which one arc is not simply the reverse of the other (Kell, 2002; Kell, 2005; Kell & Welch, 1991).

From a philosophical point of view, then, the hypothetico-deductive analysis, in which an idea is the starting point (however muddled or wrongheaded

that idea may be), has been seen as much more secure, since deductive reasoning is sound in the sense that if an axiom is true (as it is supposed to be by definition) and the observation is true, we can conclude that the 'facts' are at least consistent with the idea. If the hypothesis is 'all swans are white' then the prediction is that a measurement of the whiteness of known swans will give a positive response. By contrast, it has been known since the time of Hume that inductive reasoning, by which we seek to generalize from examples ("swan A is white, swan B is white, swan C is white ... so I predict that all swans are white") is insecure – and a single black swan shows it. Nothing will ever change that, and the 'problem of induction' probably lies at the heart of Popper's insistence (see (Popper, 1992) and/or more readable commentators such as Medawar (Medawar, 1982)) that theories can only be disproved. Note of course that it is equally true for the hypothetico-deductive mode of reasoning that a single black swan will disprove the hypothesis. This said, the ability of scientists to ignore any number of ugly facts that would otherwise slay a beautiful hypothesis is well known (Gilbert & Mulkay, 1984), and in this sense – given that there are no genuinely secure axioms (Hofstadter, 1979; Nagel & Newman, 2002) – the deductive mode of reasoning is not truly much more secure than is induction.

Happily, there is emerging a more balanced view of the world. This recognizes that for working scientists the reductionist and ostensibly solely hypothesis-driven agenda has not been as fruitful as had been expected. In large measure in biology this realization has been driven by the recognition, following the systematic genome sequencing programs, that the existence, let alone the function, of many or most genes – even in well-worked model organisms – had not been recorded. This could be seen in part as a failure of the reductionist agenda. In addition there are many areas of scientific activity that have nothing to do with testing hypotheses but which are exceptionally important (Kell & Oliver, 2004); perhaps chief among these is the development of novel methods. In particular there are fields – functional genomics not least among them (Kell & King, 2000), although this is very true for many areas of medicine as well – that are data-rich but hypothesis-poor, and are best attacked using methods that are data-driven and thus essentially inductive (Kell & King, 2000).

A second feature that has emerged from a Popperian view of the world (or at least from his attempt to find a means that would allow one to discriminate 'science' from 'pseudo-science' (Medawar, 1982; Popper, 1992)) is the intellectual

significance of prediction: if your hypothesis makes an experimentally testable (and thus falsifiable) prediction it counts as 'science', and if the experimental prediction is consistent with the prediction then (confidence in) the 'correctness' of your hypothesis or world-view is bolstered (see also (Lipton, 2005)).

## 1.2 Historical context

The history of science demonstrates that both inductive and deductive reasoning occur at different stages in the development of ideas. In some cases, such as in the history of chemistry, a period of almost purely inductive reasoning (stamp-collecting and classification) is followed by the development of more powerful theories that seek to explain and predict many phenomena from more general principles. Often these theories are reductionist, that is to say, complicated phenomena that seem to elude coherent explanation are understood by some form of breaking down into constituent parts, the consideration of which yields the required explanation of the more complicated system. A prime example of the reductionist mode is the explanation of the macroscopic properties of solids, liquids and gases – such as their temperature, pressure and heat – by considering the average effect of a large number of microscopic interactions between particles, governed by Newtonian mechanics. For the first time, accurate, quantitative predictions with accompanying, plausible explanations were possible, and unified much of our basic understanding of the physical properties of matter.

The success of early reductionist models in physics, and later those in chemistry, led in 1847 to a program to analyze (biological) processes, such as urine secretion or nerve conduction, in physico-chemical terms proposed by Ludwig, Helmholtz, Brucke and du Bois-Reymond (Bynum et al., 1981). However, although reductionism has been successful in large part in the development of physics and chemistry, and to a great extent in acquiring the parts list for modern biology – consider the gene – the properties of many systems resist a reductionist explanation (Sole & Goodwin, 2000). This ultimate failure of reductionism in biology, as in other disciplines, is due to a number of factors, principal among them being the fact that biological systems are inherently *complex*.

Although *complexity* is a phenomenon about which little agreement has been

reached, and certainly for which no all-encompassing measure has been established, the concept is understood to pertain to systems of interacting parts. Having many parts is not necessary: it is sufficient that they are coupled in some way, so that the state of one of them affects the state of one or more others. Often the interactions are *non-linear* so, unlike systems which can be modeled by considering averaged effects, it is not possible to reduce the system's behavior to the sum of its parts (Davey & Kell, 1996). Common interactions in these systems are feedback loops, in which, as the name suggests, information from the output of a system transformation is sent back to the input of the system. If the new input facilitates and accelerates the transformation in the same direction as the preceding output, they are positive feedback - their effects are cumulative. If the new data produce an output in the opposite direction to previous outputs, they are negative feedback — their effects stabilize the system. In the first case there is exponential growth or decline; in the second there is maintenance of the equilibrium. These loops have been studied in a variety of fields, including control engineering, cybernetics and economics. An understanding of them and their effects is central to building and understanding models of complex systems (Kell, 2004; Kell, 2005; Milo et al., 2002).

*Negative feedback* loops are typically responsible for regulation and they are obviously central to homeostasis in biological systems. In control engineering, such systems are conveniently described using Laplace transforms — a means of simplifying the combination and manipulation of ODEs, and closely related to the Fourier transform (Ogata, 2001); Laplace transforms for a large variety of different standard feedback loops are known and well-understood, though analysis and understanding of non-linear feedback remains difficult (see **Chapter ch11** for details). Classical negative feedback loops are considered to provide stability (as indeed they do when in simple systems in which the feedback is fast and effective), though we note that negative feedback systems incorporating delays can generate oscillations (e.g. (Nelson et al., 2004)).

*Positive feedback* is a rather less appreciated concept for most people and, until recently, it could be all but passed over in even a control engineer's education. This is perhaps because it is often equated with undesired instability in a system, so it is just seen as a nuisance; something which should be reduced as much as possible. However, positive feedback should not really be viewed in this way, particularly from a modeling perspective, because it is an important

factor in the dynamics of many complex systems and does lead to very familiar behavior. One very simple model system of positive feedback is the *Polya urn* (Arthur, 1963; Barabasi & Albert, 1999; Johnson & Kotz, 1977). In this, one begins with a large urn containing two balls, one red and one black. One of these is removed. It is then replaced in the urn, together with another ball of the same color. This process is repeated until the urn is filled up. The system exhibits a number of important characteristics with respect to the distribution of the two colors of balls in the full urn. In particular: early, essentially random events can have a very large effect on the outcome; there is a lock-in effect where later in the process, it becomes increasingly unlikely that the path of choices will shift from one to another (notice that this is in contrast to the "positive feedback causes instability" view); and accidental events early on do not cancel each other out. The Polya urn is a model for such things as genetic drift in evolution, preferential attachment in explaining the growth of scale-free networks (Barabasi & Albert, 1999), and the phenomenon whereby one of a variety of competing technologies (all but) takes over in a market where there is a tendency for purchasers to prefer the leading technology, despite equal, or even inferior, quality compared with the others (e.g. QWERTY keyboards, and Betamax versus VHS video). (See also (Goldberg, 2002; Kauffman et al., 2000) for the adoption of technologies as an evolutionary process.)

Positive feedback in a resource-limited environment also leads to familiar behavior. The fluctuations seen in stock prices, the variety of sizes of sandpiles, and cycles of population growth and collapse in food-chains all result from this kind of feedback. There is a tendency to reinforce the growth of a variable until it reaches a value that cannot be sustained. This leads to a crash which 'corrects' the value again, making way for another rise. Such cyclic behavior can be predictably periodic but in many cases the period of the cycle is *chaotic* – i.e. deterministic but essentially unpredictable. All chaotic systems involve non-linearity, and this is most frequently the result of some form of positive feedback, usually mixed with negative feedback (Glendinning, 1994; Tufillaro et al., 1992; Strogatz, 2000). Behavior involving oscillatory patterns may also be important in biological signaling (Lahav et al., 2004; Nelson et al., 2004), where the downstream detection may be in the frequency rather than the amplitude (i.e. simply concentration) domain (Kell, 2005).

All of this said, despite encouraging progress (e.g. (Tyson et al., 2003;

Wolf & Arkin, 2003; Yeger-Lotem et al., 2004)), we are far from having a full understanding of the behavior of concatenations of these simple motifs and loops. Thus, the Elowitz and Leibler oscillator (Elowitz & Leibler, 2000) is based solely on negative feedback loops but is unstable. However, this system could be made comparative stable and robust by incorporating positive feedback loops, which led to some interesting work by Ferrell on the cell cycle (Angeli et al., 2004; Pomerening et al., 2003).

It is now believed that most systems involving interacting elements have both chaotic and stable regions or phases, with islands of chaos existing within stable regions, and vice versa (for a biological example, see (Davey & Kell, 1996)). Chaotic behavior has now been observed even in the archetypal, clockwork system of planetary motion; whereas the eye at the heart of a storm is an example of stability occurring within a wildly unpredictable whole.

Closely related to the vocabulary of complexity and of chaos theory is the slippery new (or not so new?) concept of *emergence* (Davies, 2004; Holland, 1998; Johnson, 2001; Kauffman, 2000; Morowitz, 2002). Emergence is generally taken to mean simply that the whole is more than (and maybe qualitatively different from) the sum of its parts, or that system-level characteristics are not easily derivable from the 'local' properties of their constituents. The label of *emergent phenomenon* is being applied more and more in biological processes at many different levels, from how proteins can fold to how whole ecosystems evolve over time. A central question that the use of the term "emergence" forces us to consider is whether it is only a convenient way of saying that the behavior of the whole system is difficult to understand in terms of basic laws and the initial conditions of the system elements (weak emergence), or whether, in contrast, the whole *cannot* be understood by the analysis of the parts, and current laws of physics, *even in principle* (strong emergence). The latter view would imply that high level phenomena are not reducible to physical laws (but may be consistent with them) (Davies, 2004). If this were true, then the modeling of (at least) some biological processes should not follow solely a bottom-up approach, hoping to go from simple laws to the desired phenomenon, but might eventually need us to posit high-level organizing principles and even downward causality. Such a world view is completely antithetical to materialism and remains as yet on the fringes of scientific thought.

In summary, reductionism has been highly successful in explaining some

macroscopic phenomena, purely in terms of the behavior of constituent parts. However, this was predicated (implicitly) on the assumption that there were few parts (e.g. the planets) and that their interactions were simple, or that there were many parts but their interactions could be neglected (e.g. molecules in a gas). However, the scope of a reductionist approach is limited because these assumptions are not true in many systems of interest (Kell & Welch, 1991; Sole & Goodwin, 2000). The advent of computers and computer simulations led to the insight that even relatively small systems of interacting parts (e.g. the Lorenz model) could exhibit very complex (even chaotic) behavior. Although the behavior may be deterministic, complex systems are hard to analyze using traditional mathematical and analytical methods. Prediction, control and understanding arise mainly from modeling these systems using iterated computer simulations. Biological systems, which are inherently complex, *must* be modeled and studied in this way if we are to continue to make strides in our understanding of these phenomena.

Some phenomena cannot be explained by averaged effects because of the presence of unstable or quasi-stable attractors that 'emerge' as the result of many non-linear interactions. Turbulence, weather systems, stock prices, the population sizes of organisms in a food-chain, and sand pile volumes are now classic examples of systems that are deemed 'complex' and which are explained today by 'emergence'. The exact initial conditions of some such systems can influence the long term behavior in ways that are entirely deterministic – but which are nevertheless impossible to determine in practice because of the impossibility of obtaining sufficiently precise measurements. Modeling these systems can, however, reveal such things as the boundaries of the regions of stability within which the accurate, long-term evolution of the system can be predicted, the conditions under which the system will become chaotic or enter oscillations – and even many useful parameters governing the limits of the chaotic behavior, albeit predicting tomorrow's gold price remains elusive. In biology, very many systems are inherently non-linear and quasi-stable. Almost all systems involving positive feedback are potentially chaotic, and these include food chains, foraging in ants, catalysis, and many more. The advent of computational approaches to complexity modeling has ushered in a new era in the mathematics of science, and now may enable the accurate prediction and understanding of large-scale biological systems for the first time.

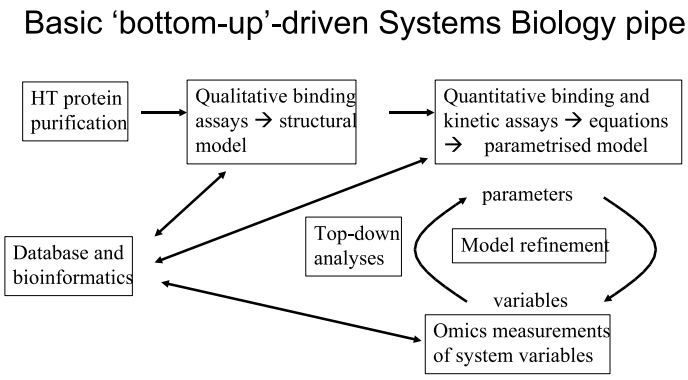
## 1.3 The purposes and implications of modeling

We take it as essentially axiomatic that the purposes of academic biological research are to allow us to understand more than we presently do about the behavior and workings of biological systems (see also (Klipp et al., 2005)) (and in due time to exploit that knowledge for agricultural, medical, commercial or other purposes). We consider that there are several main reasons why one would wish to make models of biological systems and processes, and we consider each in turn. In summary, they can all be characterized as variations of simulation and prediction. By simulation we mean the production of a mathematical or computational model of a system or subsystem that seeks to represent or reproduce some properties that that system displays. Although often portrayed as substantially different (though we consider that it is not) prediction involves the production of a similar type of mathematical model that simulates (and then predicts) the behavior of a system related to the 'starting' system described above. Clearly simulation and prediction are thus related to each other, and the important concept of generalization describes the ability of a model derived for one purpose to predict the properties of a related system under a separate set of conditions. Thus some of the broad reasons – indeed probably the main reasons – why one would wish to model a (biological) system include:

- Testing whether the model is accurate, in the sense that it reflects – or can be made to reflect – known experimental facts
- Analyzing the model to understand which parts of the system contribute most to some desired properties of interest
- Hypothesis generation and testing, allowing one rapidly to analyze the effects of manipulating experimental conditions in the model without having to perform complex and costly experiments (or to restrict the number that are performed)
- Testing what changes in the model would improve the consistency of its behavior with experimental observations

Our view of the basic 'bottom up' Systems Biology agenda is given in Fig. 1.3.

**(a)**




**(b)**

**'Bottom-up' Systems Biology pipeline (dry)**

1. Qualitative ('structural') model – who talks to whom as substrate, product or effector →
2. Quantitative model including 'real' or approximate equations describing individual steps →
3. Parametrisation of those equations →
4. Run the model and assess its most important parameters
5. Iteratively, with wet data, GOTO 1….

**(c)**

**Systems biology experiments (including the wet side)**

- Set up a well-defined system
- Effect systematic perturbations (genetic, environmental, chemical)
- Measure a time series of as many concentrations of variables, especially RNAs, proteins, metabolites (the 'omes) as possible
- Model the system and compare the experimental time series to those generated by the model
- Repeat iteratively

Figure 1.3: The role of modeling in the basic Systems Biology agenda, (a) stressing the 'bottom-up' element while showing the iterative and complementary top-down analyses. (b) The development of a model from qualitative (structural) to quantitative, and (c) its integration with ('wet') experimentation.

### 1.3.1 Testing whether the model is accurate, in the sense that it reflects – or can be made to reflect – known experimental facts

A significant milestone in a modeling program is the successful representation of the behavior of the 'real' system by a model. This does not of course mean that the model is accurate, but it does mean that it might be. Thus the dynamical behavior of variables such as concentrations and fluxes is governed by the parameters of the systems such as the equations describing the local properties and the parameters of those equations. This of itself is not sufficient, since generalized equations (e.g. power laws, polynomials, perceptrons with nonlinear properties) with no mechanistic or biological meaning can sometimes reproduce well the kinetic behavior of complex systems without giving the desired insight into the true constitution of the system.

Such models may also be used when one has no experimental data, with a view to establishing whether a particular design is sensible or whether a particular experiment is worth doing. In the former case, of engineering design, it is nowadays commonplace to design complex structures such as electronic circuits and chips, buildings, cars or aeroplanes entirely inside a computer before committing them to reality. Famously the Boeing 777 was designed entirely in silico before being tested first in a wind tunnel and then with a human pilot. It is especially this kind of attitude and experience in the various fields of engineering that differs form the current status of work in biology that is leading many to wish to bring numerical modeling into the biological mainstream. Another example is the development of 'virtual' screening, in which the ability of drugs to bind to proteins is tested in silico using structural models and appropriate force fields to calculate the free energy of binding to the target protein of interest of ligands in different conformations (Böhm & Schneider, 2000; Klebe, 2000; Langer & Hoffmann, 2001; Shen et al., 2003; Zanders et al., 2002), the most promising of which may then be synthesized and tested. The attraction of course is the enormous speed and favorable economics (and scalability) of the virtual over the actual 'wet' screen.

### 1.3.2 Analyzing the model to understand which parts of the system contribute most to some desired properties of interest

Having a model allows one to analyze it in a variety of ways, but a chief one is to establish those parts of the model that are most important for determining the behavior in which one is particularly interested. This is because simple inspection of models with complex (or even simple) feedback loops just does not allow one to understand them (Westerhoff & Kell, 1987). Techniques such as sensitivity analysis (see below) are designed for this, and thus indicate to the experimenter which parameters must be known with the highest precision and should be the focus of experimental endeavor. This is often the focus of so-called top-down analyses in which we seek to analyze systems in comparatively general or high-level terms, lumping together subsystems in order to make the systems easier to understand. The equivalent in pharmacophore screening is the QSAR (quantitative structure-activity relationship) type of analysis, from which one seeks to analyze those features of a candidate binding molecule that best account for successful binding, with a view to developing yet more selective binding agents.

### 1.3.3 Hypothesis generation and testing, allowing one to analyze the effects of manipulating experimental conditions in the model without having to perform complex and costly experiments

Related to the above is the ability to vary e.g. parameters of the model, and thereby establish combinations or areas of the models space that show particular properties in which one might be interested (Pritchard & Kell, 2002), and then to perform that small subset of possible experiments that it is predicted will show such 'interesting' behavior. An example here might be the analysis of which multiple modulations of enzymatic properties are best performed for the purposes of metabolic engineering (Cascante et al., 2002; Cornish-Bowden, 1999; Fell, 1998). We note also that when modeling can be applied effectively it is far cheaper than wet biology and as well as its use in metabolic engineering can reduce the reliance on *in vivo* animal/human experimentation (a factor of

significant importance in the pharmaceutical industry).

### 1.3.4 Seeing what changes in the model would improve the consistency of its behavior with experimental observations

In a similar vein, we may have existing experimental data with which the model is inconsistent, and it is desirable to explore different models to see which changes to them might best reproduce the experimental data. In biology this might e.g. allow the experimenter to test for the presence of an interaction or kinetic property that might be proposed. In a more general or high-level sense, we may use such models to seek evidence that existing hypotheses are wrong, that the model is inadequate; that hidden variables need to be invoked (as in the Higgs Boson in particle physics, or the invocation of the existence of Pluto following the registration of anomalies in the orbit of Neptune), that existing data are inadequate, or that new theories are needed (e.g. the invention of the quantum theory to explain or at least get round the so-called 'ultraviolet catastrophe'). In kinetic modeling this is often the case with 'inverse problems' in which one is seeking to find a ('forward') model that best explains a time series of experimental data (see below).

## 1.4 Different kinds of model

Most of the kinds of systems that are likely to be of interest to readers of this book involve entities (metabolites, signaling molecules, etc.) that can be cast as 'nodes' interacting with each other via 'edges' representing reactions that may be catalyzed via other substances such as enzymes. These will also typical involve feedback loops in which some of the nodes interact directly with the edges. We refer to the basic constitution of this kind of representation as a 'structural model' (not, of course, to be confused with a similar term used in the bioinformatic modeling of e.g. protein molecular structures). A typical example of a structural model is shown in Fig. 1.4.

The classical modeling strategy in biology (and in engineering), the Ordinary Differential Equation (ODE) approach (discussed in **Chapter ch7**), contains three initial phases, and starts with this kind of structural model, in which

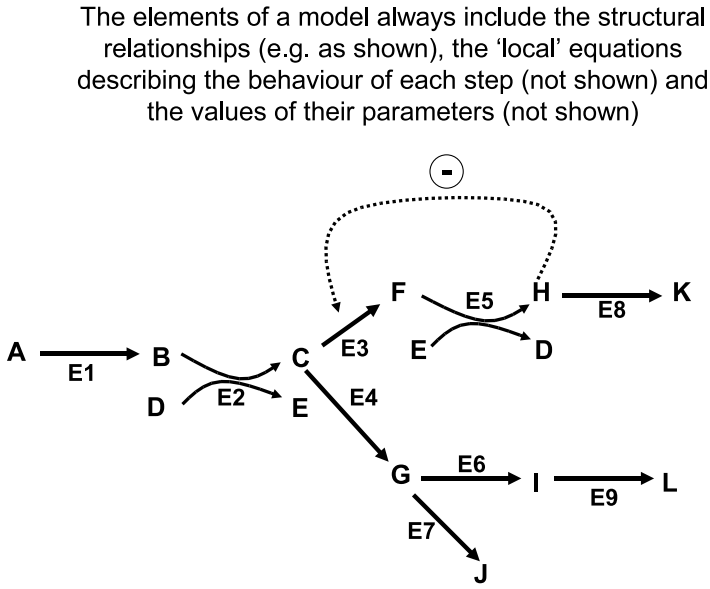



Figure 1.4: A 'structural model' of a simple network involving nine enzymes (E1 to E9), four 'external metabolites (A,J,K,L – whose concentration must be assumed to be 'fixed' if a steady state is to be attained), and eight 'internal' metabolites B,C,D,E,F,G,H,I. D and E are effectively cofactors and are part of a 'moiety-conserved cycle' (Hofmeyr et al., 1986) in that their sum is fixed and they cannot vary their concentrations independently of each other.

the reactions and effectors are known. The next level refers to the kinetic rate equations describing the 'local' properties of each edge (enzyme), for instance that relating the rate of the reaction catalyzed by say E1 to the concentrations of its substrates; a typical such equation (which assumes that the reaction is 'irreversible') is the Henri-Michaelis-Menten equation $v = V_{max}.[S]/([S] + K_m)$. The third level involves the parameterization of the model, in terms of providing values for the parameters (in this case $V_{max}$ and $K_m$. Armed with such knowledge, any number of software package can predict the time evolution of the variables (the concentrations and fluxes of the metabolites) until they may reach a steady state. This is done (internally) by recasting the system as a series of coupled ordinary differential equations which are then solved numerically. We refer to this type of operation as forward modeling, and provided that the structural model, equations, and values of the parameters are known,

it is comparatively easy to produce such models and compare them with an experimental reality. We have been involved with the simulator *Gepasi*, written by Pedro Mendes (Mendes, 1997; Mendes & Kell, 1998; Mendes & Kell, 2001), which allows one to do all of the above, and that in addition permits automated variation of the parameters with which to satisfy an objective function such as the attainment of a particular flux in the steady state (Mendes & Kell, 1998).

In such cases, however, the experimental data that are most readily available do not include the parameters at all, and are simply measurements of the (time-dependent) variables, of which fluxes and concentrations are the most common (see **Chapter ch9**). Comparison of the data with the forward model is much more difficult, as we have to solve an 'inverse modeling', 'reverse engineering' or 'system identification' (Ljung, 1999) problem (discussed in **Chapter ch10**). Direct solution of such problems is essentially impossible, as they are normally hugely underdetermined and do not have an analytical solution. The normal approach is thus an iterative one in which a candidate set of parameters is proposed, the system run in the forward direction, and on the basis of some metric of closeness to the desired output a new set of parameters is tested. Eventually (assuming that the structural model and the equations are adequate), a satisfactory set of parameter, and hence solutions, will be found (see Table 1.1). These methods are much more computer-intensive than those required for simple forward modeling, as potentially many thousands or even millions of candidate models must be tested. Modern approaches to inverse modeling use approaches from heuristic optimization (Corne et al., 1999) to search the model space efficiently. Recent advances in multiobjective optimization (Fonseca & Fleming, 1996) are particularly promising in this regard, since the quality of a model can usually be evaluated only by considering several, often conflicting criteria. Evolutionary computation approaches (Deb, 2001) allow exploration of the Pareto front, that is the different trade-offs (e.g. between model simplicity and accuracy) that can be achieved, enabling the modeler to make more informed choices about preferred solutions.

We note, however, that there are a number of other modeling strategies and issues that may lead one to wish to choose different types of model from that described. First, the ODE model assumes that compartments are well stirred and that the concentrations of the participants are sufficiently great as to permit fluctuations to be ignored. If this is not the case then stochastic simulations (SS)

Table 1.1: 10 Steps in (Inverse) Modeling.

| | |
|---|---|
| 1. | Get acquainted with the target system to be modeled |
| 2. | Identify important variable(s) that changes over time |
| 3. | Identify other key variables and their interconnections |
| 4. | Decide what to measure and collect data |
| 5. | Decide on the form of model and its architecture |
| 6. | Construct a model by specifying all parameters. Run the model forward and measure behavior. |
| 7. | Compare model with measurements. If model is improving return to 6. If model is not improving and not satisfactory, return to 3, 4, and 5. |
| 8. | Perform sensitivity analysis. Return to 6 and 7 if necessary. |
| 9. | Test the impact of control policies, initial conditions, etc. |
| 10. | Use multicriteria decision-making (MCDM) to analyze policy tradeoffs. |

are required (Andrews & Bray, 2004) (which are topics of **Chapter ch8** and **Chapter 15**). If flow of substances between many contiguous compartments is involved, and knowledge of the spatial dynamics is required (as is common in computational fluid dynamics), Partial Differential Equations (PDEs) are necessary. SS and PDE models are again much more computationally intensive, although in the latter case the designation of a smaller subset of representative compartments may be effective (Mendes & Kell, 2001).

If the equations and parameters are absent, it may prove fruitful to use qualitative models (Hunt et al., 1993), in which only the direction of change (and maybe rate of change) is recorded, in an attempt to constrain the otherwise huge search space of possible structural models (see **Chapter ch5**). Similarly models may invoke discrete or continuous time, they may be macro or micro, and models may be at a single level (e.g. metabolism, signaling) or at multiple levels (in which the concentrations of metabolites affect gene expression and *vice versa* (ter Kuile & Westerhoff, 2001). Models may be top-down (involving large 'blocks') or bottom-up (based on elementary reactions), and analyses beneficially use both strategies (Fig. 1.3). Thus a 'middle-out' strategy is preferred by some authors (Noble, 2003) (see **Chapter ch14**). Table 1.2 sets out some of

the issues in terms of choices which the modeler may face in deciding which type of model may be best for particular purposes and on the basis of the available amount of knowledge of the system.

Table 1.2: Different types of model, presented as choices facing the experimenter when deciding which strategy or strategies may be most appropriate for a given problem.

| ***Dimension or Feature*** | ***Possible choices*** | ***Comments*** |
|---|---|---|
| Stochastic or deterministic | Stochastic: Monte Carlo methods, or statistical distributions<br>Deterministic: equations, eg ODEs | Phenomena are not of themselves either stochastic or deterministic; large-scale, linear systems can be modeled deterministically, while a stochastic model is often more appropriate when nonlinearity is present. |
| Discrete versus continuous (in time) | Discrete: Discrete event simulation e.g. Markov chains, cellular automata, Boolean networks.<br>Continuous: rate equations | Discrete time is favored when variables only change when specific 'events' occur, e.g. modeling queues. Continuous time is favored when variables are in constant flux. |
| Macroscopic versus microscopic | Model individual 'particles' in a system and compute averaged effects, or just model the average effects themselves, e.g. concentration, temperature, etc. | Are the individual particles or subsystems important to the evolution of the system, or is it enough to approximate them by statistical moments or ensemble averages? |
| Hierarchical versus multilevel | Fully modular networks versus loosely-coupled processes. | Can some processes/variables in the system be hidden inside modules or objects that interact with other modules, or do all the variables interact, potentially? This relates to reductionism versus holism. |

Table 1.2: Different types of model, presented as choices facing the experimenter when deciding which strategy or strategies may be most appropriate for a given problem.

| ***Dimension or Feature*** | ***Possible choices*** | ***Comments*** |
|---|---|---|
| Fully quantitative versus partially quantitative versus qualitative | Qualitative: direction of change modeled only, or on/off states (Boolean network)<br>Partially quantitative: fuzzy models<br>Fully quantitative: ODEs, PDEs, microscopic particle models | Reducing the quantitative accuracy of the model can reduce complexity greatly and many phenomena may still be modeled adequately. |
| Predictive versus exploratory / explanatory | Predictive: specify every variable that could affect outcome<br>Exploratory: only consider some variables of interest | If a model is being used for precise prediction or forecasting of a future event, all variables need to be considered. The exploratory approach can be less precise but should be more flexible, e.g. allowing different control policies to be tested. |
| Estimating rare events versus typical behavior | Rare events: Importance sampling | Estimation of rare events, such as apoptosis times in cells? Is time-consuming if standard Monte Carlo simulation is used. Importance sampling can be used to 'speed up' the simulation. |
| Lumped or spatially segregated | Are compartments e.g. cells to be treated as homogeneous or differentiated/ heterogeneous? | If heterogeneous it may be necessary to use the computationally intensive partial differential equation, though other solutions are possible (Mendes & Kell, 2001) |

## 1.5 Sensitivity Analysis

*-Sensitivity analysis for modelers?*

*-Would you go to an orthopaedist who didn't use X-ray?*

Jean-Marie Furbringer

Sensitivity analysis (Saltelli et al., 2000) represents a cornerstone in our analysis of complex systems. It asks the generalized question 'what is the effect of changing something (a parameter P) in the model on the behavior of some variable element M of the model?'. To avoid the magnitude of the answer depending on the units used we use fractional changes $\Delta$P and observe their effects via fractional changes ($\Delta$M) in M. Thus the generalized sensitivity is ($\Delta$M/M)/($\Delta$P/P) and in the limit of small changes (where the sensitivity is then independent of the size of $\Delta$P) the sensitivity is (dM/M)/(dP/P) = d lnM/ dlnP. The sensitivities are thus conceptually and numerically the same as the control coefficients of Metabolic Control Analysis (see (Fell, 1996; Heinrich & Schuster, 1996; Kell & Westerhoff, 1986)).

Reasons for doing sensitivity analysis include the ability to determine:

1. if a model resembles the system or process under study
2. factors that may contribute to output variability and so need the most consideration
3. the model parameters that can be eliminated if one wishes to simplify the model wihtout altering its behavior grossly
4. the region in the space of input variables for which model variation is maximum
5. the optimal region for use in a calibration study
6. if and which group of factors interact with each other.

A basic prescription for perform sensitivity analysis (adapted from (Saltelli et al., 2000)) is:

1. Identify the purpose of the model and determine which variables should concern the analysis

2. Assign ranges of variation to each input variable
3. Generate an input vector matrix through an appropriate design (DoE)
4. Evaluate the model, thus creating an output distribution or response.
5. Assess the influence of each variable or group of variables e.g. using correlation/regression, Bayesian inference (**Chapter ch4**), machine learning or other.

Two examples from our recent work illustrate some of these issues. In the first, (Nelson et al., 2004; Ihekwaba et al., 2004), we studied a refined version of a model (Hoffmann et al., 2002) of the NF-$\kappa$B pathway. This contained 64 reactions with their attendant parameters, but sensitivity analysis showed that only 8-9 of them exerted significant influence on the dynamics of the nuclear concentration of NF-$\kappa$B in this system, and that each of these reactions involved free I$\kappa$B$\alpha$ and free IKK. An entirely different study (White & Kell, 2004) asked whether comparative genomics and experimental data could be used to rank candidate gene products in terms of their utility as antimicrobial drug targets. The contribution of each of the sub-metrics (such as essentiality, or existence only in pathogens and not hosts or commensals) to the overall metric was analyzed by sensitivity analysis using 3 different weighting functions, with the 'top 3 targets' – which were quite different from those of traditional antibiotics – being similar in all cases. This gave much confidence in the robustness of the conclusions drawn.

## 1.6 Concluding remarks

The purpose of this chapter was to give an overview of some of the reasons for seeking to model complex cellular biological systems, and this we trust that we have done. We have also given a very brief overview of some of the methods, but we have not dwelt in detail on: their differences, the question of which modeling strategies to exploit in particular cases, the problems of overdetermination (where many models can fit the same data) and of model choice (which model one might then prefer and why), nor on available models (e.g. at http://www.biomodels.net/) and model exchange using e.g. the Systems Biology Markup Language (http://www.sbml.org) (Finney & Hucka, 2003; Hucka

et al., 2003; Shapiro et al., 2004) or others (Lloyd et al., 2004). These issues are all covered well in the other chapters of this book.

Finally, we note here that despite the many positive advantages of the modeling approach, biologists are generally less comfortable with, and confident in, models (and even theories) than are practitioners in some other fields where this is more of a core activity, e.g. physics or engineering. Indeed, when Einstein was once informed that an experimental result disagreed with his theory of relativity, he famously and correctly remarked "Well, then, the experiment is wrong!" It is our hope that trust will grow, not only from a growing number of successful modeling endeavors, but also from a greater and clearer communication of models enabled by new technologies such as Web services and the SBML.

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