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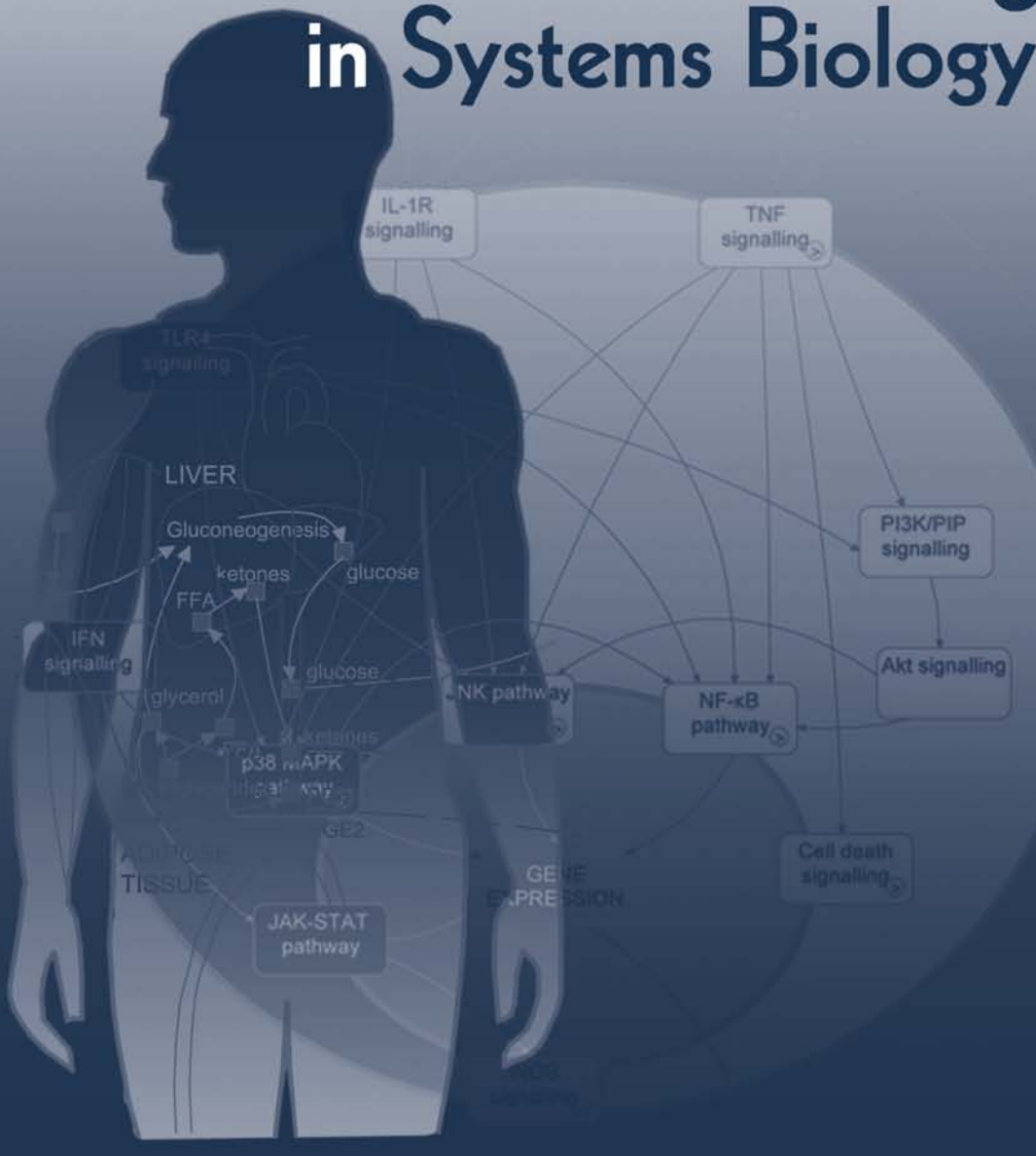

Oleg Demin and Igor Goryanin

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Kinetic Modelling in Systems Biology

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About the Authors

Oleg Demin is a Russian biophysicist who leads the Group of Kinetic Modeling of Complex Biochemical Systems at the Moscow State University. Oleg is also CSO of Institute for Systems Biology SPb.

Oleg graduated (M.Sc.) in 1992 as a biophysicist from the Biophysical Department, Faculty of Biology, Moscow State University, and as an applied mathematician from the Faculty of Applied Mathematics and Cybernetics, Moscow State University, where he was developing approaches to quantitative description of biological systems. He obtained his Ph.D. in 1995 at Moscow State University. During this time he constructed kinetic model of mitochondria and developed an approach to describe regulatory properties of oscillatory biochemical systems.

In 1995–1999 Oleg worked as a visiting scientist in the Department of Microbial Physiology, Free University of Amsterdam, The Netherlands.

He joined the A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, in 1999, working firstly as research scientist (1999–2002), a senior research scientist (2002–2004) and later as a leader of the Group of Kinetic Modeling of Complex Biochemical Systems. Since 2004 Oleg has also been the CSO of the Institute for Systems Biology SPb, Russia. During this time, Oleg was working on development of various approaches of modelling techniques and their application to the optimization of biotechnological processes, pharmaceutical research and drug development.

Igor Goryanin is a Russian biophysicist who holds a Henrik Kascer Chair in computational systems biology at the University of Edinburgh and leads the Computational Systems Biology Group, School of Informatics. Igor is also director for the Edinburgh Centre for Bioinformatics and codirector for the Centre for Systems Biology at Edinburgh. Igor graduated (M.Sc.) in 1985 as an applied mathematician from the Computer

Science Department, Moscow Engineering Physical Institute (MEPHI), where he was developing numerical methods and algorithms for analysis of stiff ordinary differential equations. Igor Goryanin spent more than twelve years working in the Institute of Biophysics, Russian Academy of Science, and obtained his Ph.D. in 1995 at the same institute. During this time he developed DBSolve, a software for mathematical stimulation and analysis of the cellular metabolism and regulation (Igor is an author for DBSolve). From 1989 to 1995 he was also CEO and cofounder of Biobank Inc., Russia.

In 1995–1997 Igor worked as a visiting computer scientist at the Mathematics & Computer Science Division, Argonne National Laboratories. He joined GlaxoSmithKline (formerly known as GlaxoWellcome) in 1997, working firstly as senior bioinformatics analyst/scientist (1997–1999), a senior research bioinformatics scientist, group leader, project manager (1999–2001) and later as a head of cell simulations and pathway modelling (2001–2005). During his time at GlaxoSmithKline, Igor was working on application of modelling and informatics techniques for the pharmaceutical research and development and drugs manufacturing industry. The whole-cell modelling of organisms approach developed by Igor has been successfully used to improve drug R&D and manufacturing process in production plants; i.e., designing antimicrobial assays and antimicrobial drug targets identification, rational organism design, rational biomarker design and target prioritisation, and reconstructing cellular networks for cancers, metabolic and lipid disorders.

In 2005, Goryanin moved to Edinburgh to take the position of a Henrik Kascer Chair in computational systems biology. In 2006 Igor developed one of the first master's courses in computational systems biology in the U.K., which is currently taught at the University of Edinburgh.

Introduction

At the time of publication of this book, systems biology is in its adolescence. More than ten books on this subject have recently been published covering a wide spectrum of scientific endeavour. Until recently, even the definition of systems biology was vague, but this has now converged to the science that discovers how, at all levels of biological hierarchy, functionality emerges from the interactions between components of biological systems. Now, the big challenge is to develop a multi-year vision and strategy for the future. We consider kinetic modelling as one of the major pillars for the future development of systems biology.

This book, organized into nine chapters, provides an overview of the method of kinetic modelling in systems biology, including practical applications.

Chapter 1, an introduction, gives a historical overview of knowledge management and mathematical modelling in biology. It emphasizes the importance of computational techniques in reducing and guiding experimental design and data analysis.

Chapter 2 introduces the basic biological cellular network concepts (DNA, genes, proteins, regulatory mechanisms) in the context of cellular functioning. Different types of visual categories and graphical standards are presented. It describes the process of pathway reconstruction in detail (static modelling).

Chapter 3 presents the Edinburgh Pathway Editor (EPE) software package with the main concepts explained in detail. The EPE is extensively used throughout the book for pathway visualization and illustration.

In chapter 4 we present the process of construction and verification of kinetic models. We discuss the basic principles of kinetic model construction, development of a system of ordinary differential equations describing the dynamics, and derivation of the rate law for biochemical reactions.

We go on to describe the verification of kinetic model using *in vitro* and *in vivo* experimental data.

Chapter 5 is an introduction to DBSolve. The main features and user interface are described and examples are provided, from simple pathway to complex behaviour oscillation, chaos and bifurcations.

In chapter 6 ('Kinetic Modelling of Enzymes and Transporters') we discuss the basic principles of modeling of individual enzymes and transporters. To demonstrate how different types of experimental data can be incorporated into a kinetic model, we present kinetic models of adenine nucleotide translocator from mitochondria and of the following *Escherichia coli* enzymes: histidinol dehydrogenase, imidazologlycerol-phosphate synthetase, isocitrate dehydrogenase, isocitrate dehydrogenase kinase/phosphatase, phosphofructokinase-1 and galactosidase. Development of kinetic models of these enzymes and transporters illustrates the basic principles of kinetic description of enzymatic reactions; namely, how kinetic data measured under different conditions (pH, temperature and others) can be combined to construct a quantitative description predicting the kinetic behaviour of the enzyme and transporter under any set of conditions.

In chapter 7 ('Pathways Kinetic Modelling') we give a detailed explanation of how to construct kinetic models of intracellular systems (metabolic, signalling or gene regulatory pathways) on the basis of models of individual enzymes. We present kinetic models of the mitochondrial Krebs cycle and the *Escherichia coli* branched-chain amino acid biosynthesis to illustrate this approach.

Chapter 8 ('Kinetic Models of Organelles') illustrates how the principles of kinetic modelling described in detail in previous chapters can be applied to collect all the available information on energy metabolism of whole organelles such as mitochondria, to construct a kinetic model and predict response of the organelle to changes in external conditions. We present here a kinetic model describing the functioning of oxidative phosphorylation in mitochondria respiring on succinate. In the framework of the model we have simulated not only biochemical reactions but reactions coupled to production and consumption of electric potential difference as well as transport processes.

Over many years, the KM approach has been successfully applied for different problems in biotechnology and biomedicine. In chapter 9 ('Applications of Kinetic Modelling'), we present several example applications, ranging from drug safety mechanisms, in which we analyse the hepatotoxic effect of salicylate, to multiple target identification analysis for

Mycobacterium tuberculosis and optimization of *E. coli* amino acid biosynthesis for isoleucine and valine production.

On the companion CD, the Edinburgh Pathway Editor v 1.0 installation can be found, with pathway diagrams from this book. Pathway diagrams are available in several graphical formats, including *pwz* files for reuse. The full DBsolve 7 installation with examples is also included. All models from this book are available on CD in DBsolve SLV and SBML formats.* So, the reader can repeat all *in silico* simulations presented in the book as a learning exercise and use the models as templates for further modelling in research and practical applications.

Turning to acknowledgements, firstly, we thank Brendan Hamill and Luna de Ferrari for extensive help in the preparation of this book. Special credit is due to Alexander Mazein for his pathway diagrams.

We thank members of our groups and, especially, acknowledge the contributions of Anatoly Sorokin, Hongwu Ma, Galina Lebedeva, Ekaterina Mogilevskaya, Nail Gizzatkulov, Evgeniy Metelkin, Kirill Peskov, Aleksey Goltsov and Tatiana Plyusnina.

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We hope that this book will help the reader to understand the kinetic modelling approach, apply it to solve real-life problems, and give the opportunity to think about future challenges.

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* These Models are accompanied with files of experimental data (in DAT format) and files with dynamic visualisation of modeling results (in XML and PLT formats).

CHAPTER 1

Systems Biology, Biological Knowledge and Kinetic Modelling

Nowadays, systems biology is extending to cover almost all biological sciences, from cellular molecular biology (Westerhoff 2003), to whole organ and organism-level biomedical studies (Kitano 2007). An excellent review (Westerhoff and Palsson 2004, has been published on convergence between molecular biology and formal understanding (mathematical modelling) of biological systems as a whole. The authors claimed that the process started in the middle of the twentieth century when methods of molecular biology and theoretical generalization emerged. In our opinion, the history of systems biology (or knowledge-based interdisciplinary systems thinking) started much earlier.

The current view on biology as knowledge acquisition about complex biological systems goes back several centuries to the time when Antony van Leeuwenhoek (1632–1723) (figure 1.1) was able to observe behaviour of living organisms and cells.

More than three hundred years ago, van Leeuwenhoek made many discoveries using his new tool, the microscope. He discovered infusoria, then bacteria, and, two years later, spermatozoa.

In his letter of June 12 1716 to the Royal Society (van Leeuwenhoek 1979), he wrote '[M]y work, which I've done for a long time, was not pursued in order to gain the praise I now enjoy, but chiefly from a craving

FIGURE 1.1 Antony van Leeuwenhoek.

after knowledge, which I notice resides in me more than in most other men. And wherewithal, whenever I found out anything remarkable, I have thought it my duty to put down my discovery on paper, so that all ingenious people might be informed thereof.'

Unfortunately, there was no online publishing, nor were there computers or even scientific journals at that time (figure 1.2).

Antony could only publish his research achievements and inventions in his letters; they were later translated into English by the editor of the

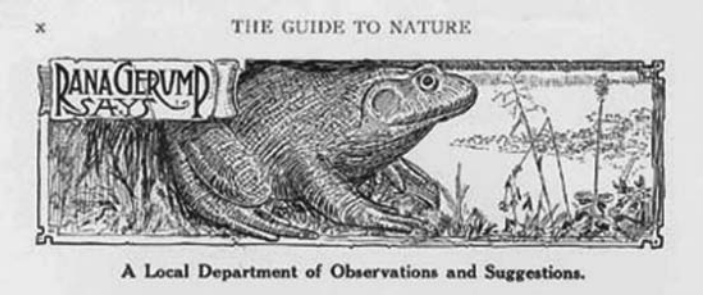

FIGURE 1.2 First issue of *Nature*.

Philosophical Transactions of the Royal Society of London (van Leewenhoek, 1979), Robert Hooke, who was also the author of *Micrographia* and who introduced the term 'cell'.

It is significant that, in 2008, the same journal is available through on-line publishing. The new worldwide scientific information facilitates exchanges. Free on-line publishing is expanding. To share biological knowledge is not only to have access to the text (letters, manuscripts, ideas)—there are images as well. Three hundred years ago these had to be drawn by hand, but today, thanks to digital photography, images and movies can be directly stored on the computer and shared worldwide (e.g., YouTube).

The problem is that biological knowledge is not only text or images. There is a large amount of quantitative data available from modern high-throughput 'omics' technologies. These data should be shared, analyzed and knowledge extracted and managed to provide new insights in biology.

The collection of quantitative biological data in systems biology started almost a century ago when Michaelis and Menten (figure 1.3) published their paper on enzyme kinetic mechanisms (Michaelis and Menten 1913). They showed that the rate of the enzyme reaction is hyperbolic and dependent on the concentration of substrate (figure 1.4 and figure 1.5).

DEPENDENCE OF ENZYME REACTION RATE ON THE SUBSTRATE CONCENTRATION

At that time it was very difficult to analyze and understand nonlinear behavior (it is something of a challenge even today), so the idea was to plot the data on a double reciprocal plot (figure 1.6). In this case the dependence becomes linear, and the intersection (by using a ruler) allows calculation of the $V_{\max}$, and K_m values. This technique is easy to understand. The data can be compared, stored and published. Linear regression procedures or other methods of elementary statistical analysis could be applied to calculate deviations and standard errors.

Afterwards, biologists and medical researchers introduced many more constants such as K_d, K_i, K_a, IC_{50} and similar constants, but all of them essentially have the same nature: some biological parameters that can be measured in reproducible experiments and then compared and published. At approximately the same time, we see the development of journals in which scientists started to publish their reports and articles.

FIGURE 1.3 Leonor Michaelis and Maud Menten.

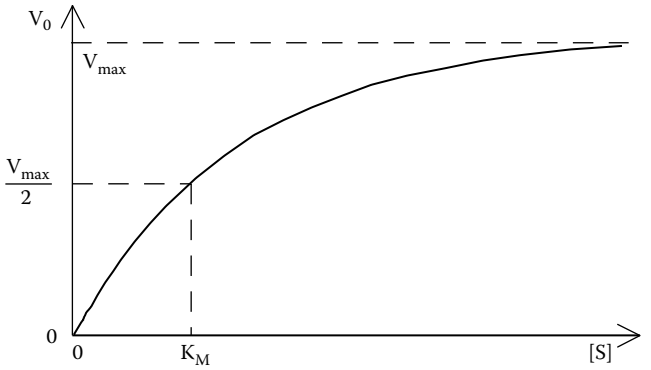

FIGURE 1.4 Michaelis–Menten curve.

Now, we could say it is our duty to convert all biological knowledge to a computer-readable form for analysis, comparison and hypothesis generation for new biological experiments.

In modern science, all enzymes, pathways and whole-cell models are created, analysed and simulated using computers. These analyses gave rise to enzyme kinetics, metabolic control analysis and pathway modelling and are now part of the field of systems biology. Simultaneous experimental (*in vitro* measurements), theoretical (ODE, regression analysis) and informatic (peer review journals, libraries) achievements were all required for scientific progress.

Another type of biological data emerged in the 1950s from the famous work of Watson and Crick (figure 1.7) on the elucidation of the structure of DNA (Watson and Crick 1953). The subsequent development of automated DNA sequencers and the generation of huge amount of sequence data would not have been possible without computers and databases.

Simultaneous new experimental (ABI), theoretical (DNA, sequence alignment algorithms) and informatics (databases) techniques were required to enable this progress. These gave rise to the new sciences of bioinformatics and computational biology and became the foundations for systems biology studies.

When the human genome was first sequenced, it was clear that publications in traditional paper journals were not enough to share the new biological knowledge on sequences. To cope with

$$v = \frac{V_{max}[S]}{[S] + K_m}$$

FIGURE 1.5 Michaelis-Menten equation.

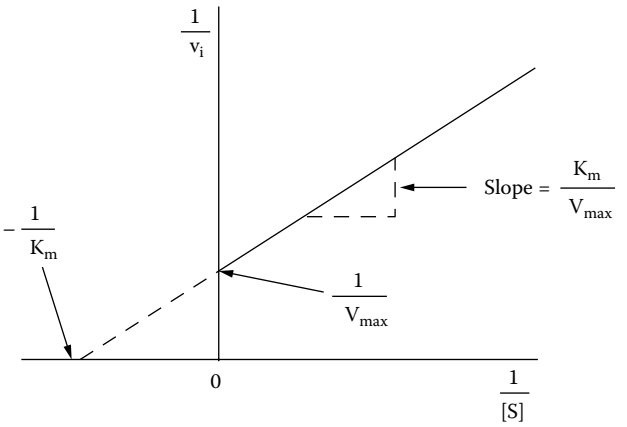

FIGURE 1.6 Double reciprocal Michaelis–Menten graph.

the problem, a number of sequence databases appeared, initially on CD, later with remote access via the Web. We are now experiencing the next phase of the distribution and sharing of biological data and knowledge, with thousands of sequenced organisms stored on the World Wide Web for users to access, search, retrieve and compare. Traditional paper publications no longer serve the purpose of biological data exchange or knowledge transfer. They can now be considered as an advertisement or an award for the teams who generate new biological data and knowledge. It is obvious now that the knowledge generation could not have been achieved without the World Wide Web (figure 1.8). Other new technologies are further expanding the social interaction of the World Wide Web for science. Examples of these are wikis

FIGURE 1.7 James D. Watson and Francis Crick.

FIGURE 1.8 Tim Berners-Lee, father of the World Wide Web.

(wiki means 'quick' in Hawaiian; Wiki Wiki Web) that allow users to easily create, edit and link Web pages, just by typing. Wikis are one of the main staples of the Web 2.0 or 'editable' web. The prime example is Wikipedia (Wikipedia: The free encyclopedia 2008), an online encyclopaedia written collaboratively that has made the wiki software familiar to 2.5 million contributors. Wikis provide an easy way to coordinate multi-partner projects in biology and to share information between wet and dry lab scientists in systems biology. Wikis save time in administration and in science, fostering a positive relation with data and documentation, which feels owned and hence curated by all stakeholders.

It is also clear that contemporary biological knowledge requires integration of different data sets: from 'omics' experiments to imaging, from computational models to simulated data, from theoretical mechanistic understanding to informatics tools. For this new type of knowledge integration about living systems we believe the modern computer science approaches should be married with mechanistic understanding of the functioning of biological systems (kinetic modelling).

Indeed, modern biology is closely associated with computer science (as are other scientific disciplines like physics and chemistry). Although biology is now growing out of its infancy, modern experimental equipment still does not allow direct observation of all biological samples, and so inferred models become an essential part of biological sciences. As we have mentioned earlier, the process of conversion of biological knowledge (figure 1.9 and figure 1.10) to models started from the Watson and Crick (1953) double-helix model

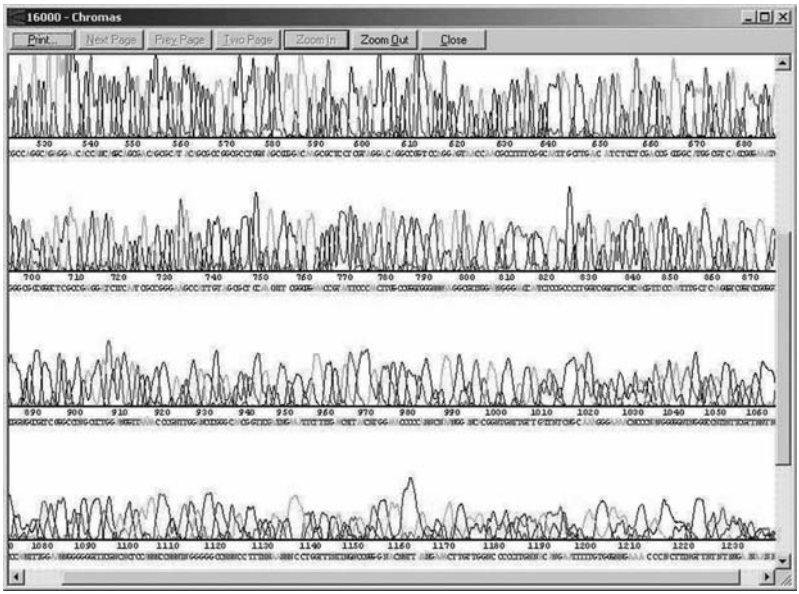

FIGURE 1.9 Output from first generation of DNA sequencing machines.

of DNA and then continued when bioinformaticians developed 3D models of proteins (Meyer 1997, figure 1.11) and later models of biological pathways and networks (Reich and Selkov 1981, figure 1.12). Modern systems of integrative biology depend heavily on the use of models of biological processes.

WHAT ARE THE MODEL LIMITATIONS? OR, IN OTHER WORDS, WHAT CAN BE MODELLED?

It is possible to model (and make predictions) only for biological systems that we can observe with or without the help of experimental techniques. So models are always following this path in the development of new experimental techniques: visualisation, then understanding, and finally modelling. Models can be of several types, granularity and scope, but all of them should have predictive power. We can now observe a diverse range of biological objects: organs, tissues, cells, organelles, proteins, lipids, carbohydrates and small molecules. Modern imaging devices have a wide range: from MRI, CT and ultrasound scanning (10^{-3} m), to MicroCT (10^{-5} m), to confocal microscopy (10^{-6} m), to electron microscopy (10^{-9} m) to X-ray crystallography (10^{-10} m). Image data provide a good foundation to model different space granularity. Biological processes have different time spans: from microseconds for signal transduction processes, to minutes for

FIGURE 1.10 The cover of *Nature* magazine, in which the first draft of the human genome was published.

metabolic reactions, to hours for gene regulations and circadian rhythms, to days for development and years for life span and disease. Diverse data could serve as a foundation for models different in time resolution.

In the past, to make a discovery, sometimes it was sufficient to provide images with explanation; now it is not enough. To understand biological systems we need quantitative measurements in time. It is important to measure concentration, location and states of other biological objects in time and in space. In this book we will try to describe the current state-of-the-art in understanding of biological networks and how all these diverse experimental data could be integrated to understand biology better by kinetic modelling.

First, we need to think about a catalogue (list) of entities involved in biological processes. What types of objects are known in biology? Traditionally, intracellular components (like cellular organelles) are visible

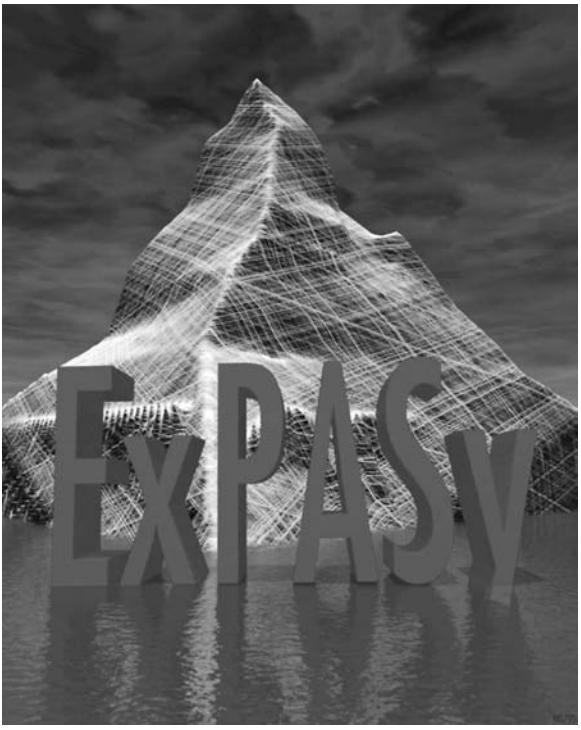

FIGURE 1.11 The Expasy logo. One of the first web-based sequence databases.

using microscopes and involved in the structural organisation of the cell. Organelles serve different biological purposes, but from the kinetic modelling perspective we could consider them simply as different compartments. In some cases we need to include organelle geometry to achieve spatial modelling.

We treat entities as biological when they are not only involved in biological processes but are directly encoded by the genome (i.e., DNA, RNA, small RNAs, peptides derived from proteins by cleavage, proteins, protein complexes, cellular organelles).

To make the picture complete we include nonbiological entities that are involved in biological processes in living organisms. We call these molecular entities 'small molecules'. The term 'small molecule' refers to any constitutionally or isotopically distinct atom, molecule, ion, ion pair, radical, radical ion, complex, conformer, etc., identifiable as a separately distinguishable chemical entity. The small molecules in question are either

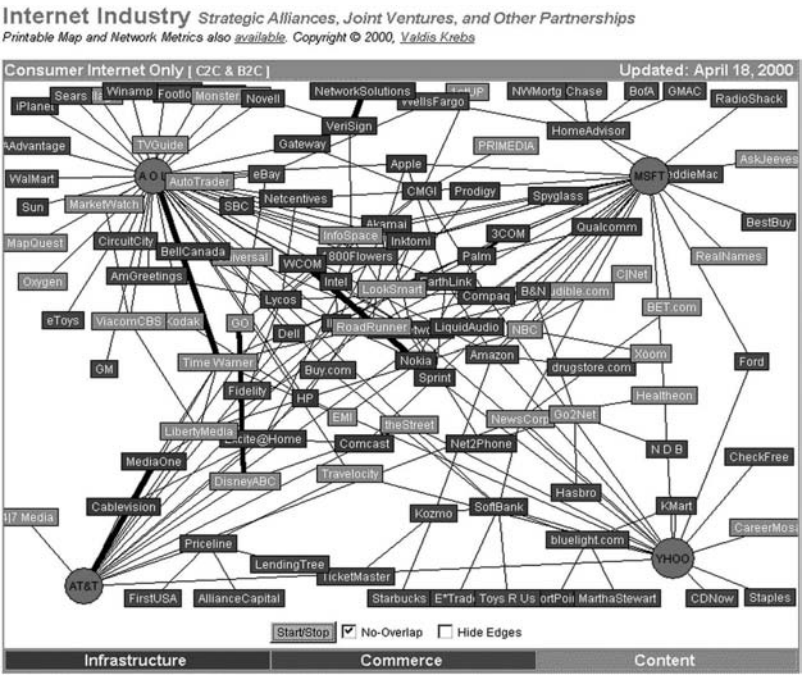

FIGURE 1.12 Networks of computers in the Internet have common properties with cellular networks.

products of nature or synthetic products (xenobiotics) used to intervene in the processes of living organisms (like drugs). For further details, see Chapter 2 and the References for links to biological ontologies, controlled vocabularies and chemical databases.

Having defined biological entities, we can now try to classify biological processes for kinetic modelling purposes. The essential distinguishing feature of all biological living (or alive) organisms is permanent change.

All changes have particular nonlinear characteristics that provide sustainability of life, cycles of reproduction, development and proliferation (the 'cycles of life'). In cell or organism death there are changes like constant degradation and decay. We cannot classify these changes as biological processes because there is no intrinsic control (compared with processes like apoptosis). So, all entities are always changing, even if the resulting changes could become zero. These changes are caused by dynamic interactions between entities.

We consider any two entities as connected if, for any length of time, there is a physical or chemical relationship connecting them.

All these temporal genotypic and phenotypic changes can be accounted for by kinetic models. In the following chapters we describe how the kinetic modelling approach could be applied to diverse activities such as biological information integration, model creation, generation of new hypotheses and, most importantly, for real life applications.

CHAPTER 2

Cellular Networks Reconstruction and Static Modelling

PATHWAY RECONSTRUCTION

The first step in kinetic modelling is to develop a static model of the biological system. This is called 'pathway reconstruction'; i.e., to find out information about all players: cellular proteins, enzymes, small molecules, transcription factors, and all known interactions between them. Nonenzymatic spontaneous processes are usually included. The resulting network (i.e., a directed bond graph) should include all interactions connecting all known entities. A proper cellular network should only contain interconnected entities. Every biological entity should have a source and sink or at least participate in one of the reactions. Disconnected fragments, resulting from incomplete knowledge, could, optionally, be considered as part of the one whole cellular network. Cellular network reconstruction can be performed in two different ways: by annotation and integration of knowledge on biochemical reactions from the literature and databases ('literature-derived network') or by annotating genomes ('genome-derived network'). The best reconstruction is obtained by comparing and combining the two approaches. Before the genome sequencing era, the process of pathway reconstruction, or static model building, was very time consuming and laborious. One had to read all papers related to a particular pathway; identify the biological entities involved in the pathway, substrates and