

CHAPTER 4

MODELING AND NETWORK ORGANIZATION

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INTRODUCTION

The use of mathematical modeling and analysis of networks has a long history in biological research. Perhaps the best-known early example of insightful modeling is the work of Hodgkin and Huxley in 1952 describing how sodium and potassium ion channels could function together to produce the membrane action potential in neurons (Hodgkin and Huxley, 1952). For several decades, models and theory were mostly the domain of applied mathematicians, physical scientists and engineers, many of whom worked rather independently of experimental science and the work remained somewhat obscure and theoretical. With the broad availability of computers and IT infrastructure that has emerged in the last several decades, the use of modeling and theory in biological research has expanded greatly, as has the size of the models being developed.

Historically, much modeling was used to study and interpret what could be directly observed in the laboratory, namely, functions of cells, tissues, organs and organism physiology. In addition, starting with the work of Jacob and Monod there was a great deal of biochemical and genetic modeling. Enzymological modeling such as that by Garfinkel was mechanistically very detailed and chemically supported. On the other hand, the genetic network models were far more abstract and rarely related to data. However, it was recognized that in many cases that there was a common underlying mathematical framework to both. Techniques from non-linear dynamics, chemical engineering analyses (stoichiometric network analysis, etc.) were brought to bear. More abstract models of these processes were used to study the possible organization and optimalities of biological networks. Jim Bailey, Goodwin, and others wrote monographs on the topic. And of course, Turing himself made the first links from chemistry to development.

With the advent of molecular biology and ensuing capabilities in genomics, proteomics, and so forth, mathematical models are now being used extensively to study intracellular molecular networks such as kinase cascades and metabolic pathways, as well as gene regulatory networks. These intracellular molecular networks are a primary subject of network organization analysis, along with epidemiological networks, and structural networks such as lung airway, vascular, and neural network topologies. At the same time, modeling of multicellular networks with multiple intercellular interactions, and sometimes multiple anatomical scales (biochemicals, cells, tissues, organs), has continued and in the last 5–10 years the biological breadth and detail of such models has increased dramatically. This has been possible because of both the increased availability of data that informs both model structure and parameter values, as well as the availability of sufficient computational power with which to code and solve them.

The growing popularity of modeling in biological research is evident from the increasing number of public forums dedicated to or including it. (Akutsu, Miyano et al., 2000) The number of research conferences including or fully devoted to biological modeling has increased dramatically in the last five years and are too numerous to list here. As noted in the introductory chapter, new journals devoted to systems biology, including modeling and network organization, have been launched, including *In Silico* Biology, PLoS Computational Biology, IEE Proceedings Systems Biology, and Molecular Systems Biology.

Applications of Models

Modeling and analysis are uniquely suited to a wide variety of applications. Models can be used to test specific hypotheses, for instance, about how a system is structured or how it functions. They can be used to make predictions, which can then be tested with appropriate experiments. Models can be used in a more exploratory manner, for instance, to discover the types of properties that might emerge from integration of parts with specific properties in specified ways. While experiments can also be used to test hypotheses, make predictions and explore, mathematical models explicitly represent components and their interactions in a controllable, manipulable environment (the computer) which allows calculation of how these things change through time and space (if those are included) and observation of every element and relationship in the model. The analogous experimental measurements would frequently be difficult or impossible and sometimes unethical. In addition to these specific applications in research, models are also excellent aids to communication and teaching. In contrast to static words or pictures on a page, they provide an interactive method for demonstrating and exploring how the modeled system works in an easy-to-use computer environment.

What is a Mathematical Model?

A model is a set of *structured assertions* that specify the *interactions* among *entities* of a *network*. An example of a model that illustrates this definition is given in Figure 4.1. The entities in a model can be many different things, such as properties of specific biological elements (e.g., molecular concentration, cellular density, or organ volume) or specific physiologic characteristics (e.g., blood pressure, heart rate, or weight). Likewise the interactions that are specified among the entities can represent various processes such as molecular reactions, binding of a molecule to a cell-surface receptor, subsequent stimulation of that cell, etc. From a model one can calculate how the entities change over time and/or through space or their value at steady state.

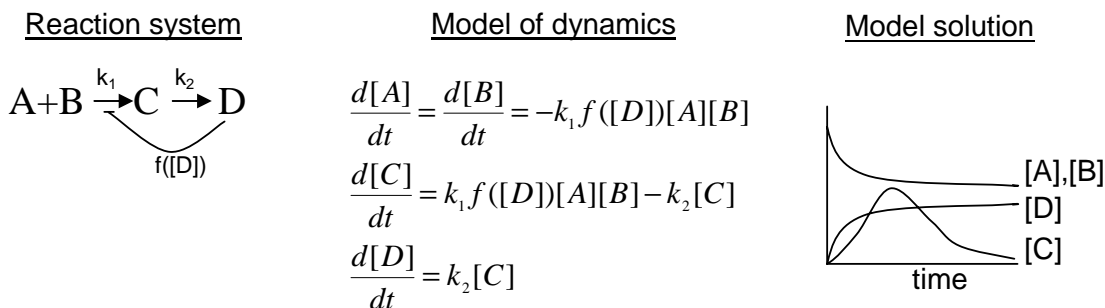


Figure 4.1. Schematic of a chemical reaction system where chemical species A and B react together to form C, which further changes into D, and D inhibits the first reaction. The equations of a mathematical model of that system using ordinary differential equations (ODEs) are shown along with a graph of a simulation of that model, assuming A and B start with equal, non-zero concentrations while C and D are initially zero. Brackets around a chemical name indicate concentration.

Mathematical models of biological systems can take many different forms, and the appropriate types of mathematical equations are highly dependent on the problem one is attempting to represent with the model. Many models utilize ordinary or partial differential equations to represent continuous, deterministic systems and partial differential equations if space or mechanics are involved. Various methods are used to include stochastic or probabilistic properties of the system including Langevin dynamics and the more physically rigorous chemical master equation. Discrete methods like particle/molecular dynamics, cellular automata and agent-based models are utilized where the actions of individual elements of a system, rather than the population behavior, is of interest.

What is Network Organization Analysis?

It is believed that biological networks are not randomly structured, but rather that various parts of the network have structures that provide specific functional units, and those structures can be found in multiple parts of the network. Although universal definitions are not agreed upon, these subunits with the same structure are often called motifs or modules, where the term motif is often used to indicate the smallest repeated unit and module to indicate a collection of units that form a “separable” functional group—that is, a

group of processes that together create some well-defined behavior that is a pure input/output function and is not otherwise affected by inclusion of the network—although those definitions are not universal. For example, Segal et al. (2003) define a module in the context of gene expression as a group of genes that tend to respond in a joint manner, i.e., through temporally coordinated gene expression. Wuchty et al. (2003) define a module in the context of network topology as a discrete group of interconnected elements that is abstracted from the topology of the network. Von Dassow et al. (2000) define a module functionally as a set of genes and their products which, as an emergent consequence of their interactions, perform some task nearly autonomously.

Figure 4.2 illustrates molecular networks and motifs. These smaller units are thought to derive from functional need of the organism as well as fundamental physical principles (e.g., thermodynamics). The analysis and theory of network organization focuses on discovering underlying principles and motifs of systems and networks. Many different approaches to network organization analysis are being used in biology and various reviews are available (Alm and Arkin, 2003; Barabasi and Oltvai, 2004; Itzkovitz and Alon, 2005).

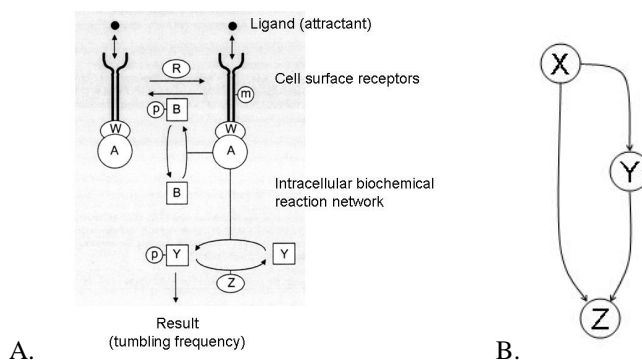


Figure 4.2. Example of (A) a molecular interaction network involved in bacterial chemotaxis adapted from Alon et al. (1999) and (B) a single motif, the feed-forward loop, frequently found within intracellular molecular networks.

OVERVIEW OF WORLDWIDE EFFORTS

A wide variety of research efforts utilize biological modeling and network analysis throughout the U.S., Europe, Japan, and elsewhere. This report's summary of work in the different regions discusses modeling efforts in terms of several broad subjects, including intracellular gene regulatory and biochemical networks; cellular metabolism; receptor dynamics and cell function; multi-cellular/tissue/organ function; electrophysiology; organism development; and spatial organization and pattern formation. While somewhat arbitrary, these categories group problems that are frequently of common interest to a given set of researchers. Network organization research is discussed as a separate category, and the efforts of industry in these areas are discussed in a separate section below.

Biological Modeling and Network Organization Analysis in the United States

The use of models in biological research in the U.S. is extensive; a comprehensive review is beyond the scope of this chapter. Instead, representative studies that illustrate the variety of ongoing research are highlighted and referenced.

The subject of signaling networks and gene regulatory networks has garnered much attention in recent years, and strong efforts at representing and understanding specific networks using models have been put forth by researchers such as Ravi Iyengar (Mt. Sinai School of Medicine), John Tyson (University of Virginia), Adam Arkin (University of California-Berkeley) Peter Sorger (Massachusetts Institute of Technology) and Hamid Bolouri (Institute for Systems Biology). Arkin, working with Harley McAdams, John Ross and recently Michael Samoilov, pioneered the study of the importance of stochastic chemical processes in gene expression and signal transduction. He demonstrated how, from physical chemical first principles, gene expression in prokaryotes is expected to show bursts and erratic production of proteins. In later work he showed that the fundamental fluctuations in reaction rates can result in qualitatively different behaviors than that predicted by standard mass-action kinetic models. He has followed up the implications of this noise in the lysis/lysogeny

decision of λ -phage and type-1 pili phase variation among other systems and shown that the noise is a fundamental and biologically important part of the regulation of these systems. Van Oudenaarden and Elowitz have each separately followed-up this work with elegant theories and measurements of the effect in bacteria. Wolf and Arkin then recently showed under what conditions such non-genetic diversification mechanisms are part of an evolutionarily stable strategy.

Iyengar's research focuses on cellular signaling systems, utilizing close integration of experimental and theoretical methods, with emphasis on signaling associated with G-protein coupled receptors. For example, Iyengar and colleagues developed a model of the mitogen-activated protein kinase (MAPK)/protein kinase C (PKC) system, and in concert with targeted experimentation they demonstrated that this system can operate with one or two stable states and is therefore quite flexible in its ability to control cellular processes such as cell cycle (Bhalla, Ram, and Iyengar, 2002). Tyson and his collaborators have developed detailed models of the molecular control mechanisms of cell cycle in fission yeast, budding yeast, frog embryo and mammalian cells (Chen et al., 2004; Zwolak, Tyson, and Watson, 2005; Novak and Tyson, 2003; Svecizer, Tyson, and Novak, 2004). They have worked closely with experimentalists to compare the function of the budding yeast cell cycle model in 131 mutant cells, finding good agreement for 120 and disagreement for 11, in the process demonstrating specific areas where the biology is not well-understood (Chen et al., 2004).

Sorger applies experimental and computational approaches to the analysis of chromosome segregation, genomic stability and programmed cell death in yeast, mice and human cells. In collaboration with Doug Lauffenburger (MIT) and others, his apoptosis work has included the development of an experimentally-based 400 equation ODE model that can capture cell-type specific variation in the generation of survival signaling emanating from the epidermal growth factor (EGF) receptor (Schoeberl et al., 2003). Bolouri has utilized similar modeling as well as theoretical analysis to study gene regulatory networks, including those important for organism development and the immune system (Ramsey, Orrell, and Bolouri, 2005; Bolouri and Davidson, 2003).

One step up on the continuum of biological scale is cell behavior, frequently modeled in relationship to cell surface receptor dynamics as well as intracellular signaling networks. For example, Jennifer Linderman (University of Michigan) focuses on the dynamics of receptor binding and trafficking and how these influence cell response to endogenous and exogenous ligands (e.g., therapeutic drugs). She has used modeling to demonstrate how receptor desensitization and drug-induced signaling may be decoupled through alteration of drug properties (Woolf and Linderman, 2003). Doug Lauffenburger's (MIT) work has focused on deciphering how cells interpret ligand binding through the dynamics of receptor trafficking and signal transduction to result in a specific behavior, with primary focus on cell proliferation, chemotaxis and apoptosis. A recent study combining modeling of receptor trafficking leading to uptake and degradation of granulocyte-colony stimulating factor (G-CSF) by neutrophils with molecular modeling of receptor-ligand structure-function interactions predicted how amino acid substitutions in G-CSF could reduce uptake and thereby increase its half-life within the bloodstream when administered to neutropenic patients, such as cancer patients on chemotherapy (Sarkar, et al., 2002; Sarkar and Lauffenberger, 2003). Hans Othmer (University of Minnesota) has made significant contributions through modeling in a number of biological areas, for example in the chemokinesis and chemotaxis of both single bacteria and populations of bacteria (Albert, Chiu, and Othmer, 2004; Erban and Othmer, 2004). Jason Haugh (North Carolina State University) has used models of the platelet-derived growth factor (PDGF) receptor/PI3-kinase/Akt signaling system in relationship to cell survival to test alternative hypotheses about the dynamic behavior of ligand- receptor interactions. His group demonstrated that dimerization requires the association of two 1:1 ligand-receptor complexes as an initial step with possible formation of stable 1:2 complexes thereafter, rather than dimerization of two receptors by one ligand initially (Park, Schneider, and Haugh, 2003).

The area of metabolic networks and metabolic control within cells is a subset of both of the above but is specialized enough as a field to describe it separately. Because of its special status as an industrially important field, metabolic modeling and analysis was one of the earliest systems biological fields to emerge. The field rests on a foundation of chemical engineering and enzymology that matured in the 1950s and '60s. One of the earliest and most ambitious models was by Garfinkel and Hess in the early '60s and covered hundreds of reactions in the metabolism of Ehrlich ascites tumor cells. Few models of this scale and mechanistic detail have been attempted since. Whole theories have grown up around these and like models to understand the control of flux in these networks. In the early 1970s Heinrich and Rapoport and Kacser and

Burns developed Metabolic Control Analysis (MCA), which concerns the sensitivity of steady-state fluxes of metabolic networks to perturbations in enzyme concentrations and other parameters of the system.

Major U.S. contributors in this area include James Liao (University of California-Los Angeles), Gregory Stephanopoulos (Massachusetts Institute of Technology (MIT)) and Bernhard Palsson (University of California-San Diego), among others. Greg Stephanopoulos at MIT has been the leader at using MCA coupled to measurements of metabolites and fluxes to learn how to redistribute material flux in these networks towards desired end products of metabolism. Bernhard Palsson at UCSD has built on classical work in stoichiometric network analysis (SNA) and other flux-based chemical engineering analyses to develop an effective flavor of flux balance analysis that, given fairly conservative assumptions and a set of input nutrients, can predict the flux through the metabolic network that maximizes growth. He has used these analyses and metabolic reconstructions to predict under which conditions a cell will grow while producing a molecule of interest. He has also used such models and experiments to predict the effect of mutants on the growth mechanism and to derive so-called extreme-pathways that might represent the controllable fluxes in metabolism. Ideker, initially together with Lee Hood, has pioneered data-fusion on molecular interactions, gene expression measurements, and genetic perturbations to build more statistical models of the control of metabolic pathways. Liao's research focuses on mapping control circuitry of metabolism and re-engineering those circuits to provide new functionality within a cell. For example, Liao's group identified the stoichiometric limitation caused by the phosphotransferase system (PTS) in the production of various metabolites, and experimentally demonstrated a solution by overexpression of phosphoenopyruvate synthase to recycle pyruvate back to phosphoenopyruvate (Patnaik and Lao, 1994; Patnaik and Spitzer, 1995).

The cell functions discussed above frequently occur within multicellular organisms or populations of single-celled organisms and therefore mathematical models have been utilized to investigate many biological functions that involve networks of cells, tissues and organs. Denise Kirschner (University of Michigan) has developed ODE and agent-based models of interacting populations of immune cells and infecting microbes to study infectious diseases including HIV/AIDS and tuberculosis. For example, Kirschner's model of *M. tuberculosis* infection in the human lung and draining lymph node was used to predict how different balances of key T cell and macrophage functions would lead to different patient outcomes such as active infection versus latency as well as the biologic functions that could be good targets for modulation by antibiotics (Marino and Kirschner, 2004). Alan Perelson (Los Alamos National Laboratories) has used models to study the dynamics of the T and B cells of the immune system and its response to infection and therapy for infection, for example the treatment of HIV and hepatitis (Gilchrist and Coombs, 2004; Dixit et al., 2004). Building on this model, Leor Weinberger, David Schaffer and Adam Arkin have begun to use such models as the foundation for the design of therapies for control of the onset of AIDS. They proposed an extension to Perelson's model that allowed the exploration of how to best engineer a conditionally-replicating viral gene therapy that would prevent AIDS but not HIV-1 infection. Another area with a long history of modeling is vascular structure and angiogenesis. Thomas Skalak (University of Virginia) has made significant contributions to the understanding of vascular remodeling regulation by mechanical stresses and wound repair using cellular automata models (Pierce, Van Gieson, and Skalak, 2004), while Rakesh Jain (Harvard University) has worked through a succession of ODE and partial differential equation (PDE) models in close conjunction with experimental work to understand the physicochemical drivers of tumor angiogenesis (Stoll et al., 2003; Ramanujan et al., 2000). Mechanical aspects of biological functions at both the macroscopic and microscopic levels are also subjects of modeling. The fluid mechanics of blood flow is a major subject of modeling. For example, Roger Kamm (MIT) is using finite element models of blood flow in the carotid artery in conjunction with magnetic resonance imaging (MRI) and histology to understand how the blood shear stress correlates with histologic markers in atherosclerotic plaques (Kaazempur-Mofrad et al., 2004).

Electrophysiology of cells and the organization of electrically active cells into tissues and organs is a major subfield spanning molecular networks, cell biology, and multicellular/tissue/organ systems. It has involved so much modeling activity that it deserves its own discussion. Electrophysiology may in fact be the biological area that has the most modeling associated with it, and the studies in this area are on average significantly more quantitative than in most other areas of biology. The electrophysiology of the cardiac myocyte and the organization of myocytes into the structure of the heart are the subject of much research both in the U.S. and internationally, oftentimes through international collaborations. In the U.S., Raimond Winslow (Johns Hopkins University (JHU)), Andrew McCulloch (UCSD), and Yoram Rudy (Washington University), among others, have developed various models of excitation-contraction coupling and its regulation in the cardiac

myocyte, as well as integrated models of multiple myocytes into heart tissue, single ventricles, and the whole heart (Winslow et al., 2000; Luo and Rudy, 1994 1; Luo and Rudy 1994 2; McCulloch, Hunter, and Smaill, 1992). Such modeling has been used to better understand how molecular and cellular behavior together with spatial organization determines normal heart function as well as arrhythmias, myocardial infarction, and other cardiac dysfunctions. Electrophysiology is also central to the function of the nervous system, and modeling is used extensively in this field to understand how various ion channels and pumps drive neural electrical conduction and transmission, as well as how networks of neurons function in organized tissues to result in observable physiology. As the field is quite vast, the reader is referred to several books with review chapters for a broader view of the field (Chow et al., 2005; Koch, 2004; Koch and Segev, 1998; Dayan and Abbott, 2001).

Pattern formation and spatial organization in biological systems involve significant modeling efforts. Many phenomena with important spatial organization aspects occur in organism development. George Oster (UC-Berkeley) has used models to, for example, demonstrate how waves and aggregation patterns in populations of microbes are driven by various characteristics of cell motility (Igoshin et al., 2004). Davidson has developed models to compare alternative hypotheses about the mechanism of the invagination of the sea urchin (Davidson et al., 1995); while Garrett Odell (University of Washington) has studied numerous morphogenesis problems, including how specific genetic control modules drive segment polarity in *Drosophila* (von Dassow, et al., 2000; von Dassow and Odell, 2002). James Murray (University of Washington) has contributed to the understanding of numerous spatial and patterning phenomena in biology including scarring, fingerprint formation, and skin patterning (Tranquillo and Murray, 1993; Cruywagen, Maini, and Murray, 1994). Like Odell, Stanislav Shvartsman (Princeton University) also combines modeling with genetics and cell biology to understand patterning (Pribyl, Muratov, and Shvartsman, 2003 1; Pribyl, Muratov, and Shvartsman, 2003 2).

Finally, it is worth noting that various aspects of whole organism physiology, such as metabolism and respiration have a long history of modeling. These models typically described the biological components and functions at the level of tissues, organs, and/or organ systems including physical properties and geometric features, which fell from favor with the advent of cellular and then molecular biology in the later part of the twentieth century. The work described above in multicellular/tissue/organ networks, which includes more molecular and cellular biology, could be considered the new physiologic modeling when it bridges to aspects of organism function.

Analysis of network organization is most frequently focused on the properties and organization of intracellular biochemical networks based on the large databases arising from genomics, transcriptomics, and proteomics, although the same principles are applicable to networks of cells or other biological elements. This is exemplified by the work of Albert-Laszlo Barabasi (Notre Dame) and colleagues analyzing the properties of many network types, from intracellular proteins to the internet (Yook, Jeong, and Barabasi, 2002; Barabasi and Bonabeau, 2003; Jeong et al., 2000). In studying the connectivity of elements in these networks, he has demonstrated that many such networks are “scale-free.” In scale-free networks the probability P of any node being linked to some number k of other nodes follows a power law distribution ($\log P(k)$ vs. $\log k$ is linear). Slightly different analyses of the properties of these networks lead to predictions of different variations of the scale-free architectures which have implications both for how they are controlled and how they arose evolutionarily. These studies attempt to link various topological properties of the network to properties such as the speed at which information can be communicated, which points in the network are most susceptible to failure, and how different network architectures are more or less robust. The architectural arguments are still somewhat phenomenological (such as noting that highly-connected proteins may have a higher chance of being essential, etc.), while more generic statistical theories come to diametrically opposed interpretations (Carlson and Doyle, 2002; Carlson and Doyle, 1999; Morohashi et al., 2002; Csete and Doyle, 2002; Kitano et al., 2004).

Motifs and modularity are another of the major areas of study of network organization. One of the seminal papers in this area was a mechanistic study of the robustness of exact adaptation in the *E. coli* chemotactic response by Naama Barkai (now at Weizmann Institute) and Stanislas Leibler (Rockefeller University). In this work they demonstrate how the architecture of the signal transduction network ensures exact adaptation of the response regulatory activity to a step of chemoattractant throughout a wide range of kinetic parameters for the underlying biochemical reactions. This robustness was subsequently shown to exist through

measurements of *E. coli* with differently expressed chemotactic pathway molecules by Uri Alon and Leibler. Tau MuYi and John Doyle showed that the engineering explanation for this was the existence of an integral feedback motif in the network. The search for “overrepresented” examples of these seemingly important control motifs has become a popular area of study facilitated by better quality databases of cellular networks and high-throughput datasets of molecular interaction. However, caution in the analysis of these motifs from the topological viewpoint is necessary. Elowitz and Leibler have shown experimentally that the same topology of a gene expression motif can yield very different dynamics depending on the exactly kinetic and thermodynamic parameters. This had been predicted theoretically for years but the experimental demonstration was powerful.

The linkage of motifs to evolutionary processes is only just beginning to be explored. The statistical overrepresentation of certain topologies of biochemical interactions is evocative and Chris Voigt, Denise Wolf and Adam Arkin have explored why certain topologies might be selected evolutionarily because of their dynamic flexibility in a study of the *Bacillus subtilis* sinIR operon and made a first attempt to understand the evolutionary selection on different parts of the motif by comparing the sequence of orthologous implementations of the motif in related bacteria. Rao and Arkin examined how small differences in the orthologous chemotaxis pathways in *E. coli* and *B. subtilis*, while having similar gross behavior, differed in the mechanisms of control and the resultant robustness of the network. These approaches to the quantitative analysis of cellular regulatory motifs and the linkage to the evolution of these pathways promised a more complete understanding of the design and architecture of cellular pathways.

Major Alliances, Collaborations and Institutions

It is noteworthy that in the last decade several large alliances and collaborations have been formed that have goals to understand biological systems as integrated systems (e.g., systems biology) and have included mathematical modeling as a central method for the research. Perhaps one of the oldest, a grassroots international effort with many U.S. contributors but without specific federal support, is the Physiome Project (Crampin et al., 2004), spearheaded by James Bassingthwaite (<http://www.physiome.org/>). The Physiome Project’s major long-range goal is to understand and describe the human organism, its physiology and pathophysiology quantitatively, and to use this understanding to improve human health.

The Alliance for Cell Signaling (AfCS, www.signaling-gateway.org), directed by Alfred Gilman (University of Texas Southwestern Medical Center), is a multidisciplinary, multi-institutional research program to study the network properties of cellular signaling systems utilizing a well-organized system for obtaining cell samples and running experiments, databasing results, and integrating the knowledge into models. The AfCS was originally focused on B cells and muscle cells, but recently refocused on macrophages because of significant technical difficulties dealing with the first two. Approximately 50 investigators at 20 academic centers are involved in the AfCS, and it is funded by the National Institute of Health (NIH) and five major pharmaceutical companies. In 2003, the AfCS formed a partnership with Nature Publishing Group to create the Signaling Gateway (<http://www.signaling-gateway.org>), which provides signaling data and results from AfCS freely to interested parties. Another multi-institution initiative is the Cell Migration Consortium (<http://www.cellmigration.org/index.html>), which aims to accelerate progress in migration-related research by fostering multi-disciplinary research activities and producing novel reagents and information. The Consortium is comprised of over thirty investigators and collaborators from over 15 institutions, and includes a modeling initiative as one of its key thrusts.

An example of a major center in systems biology at a single institution is the Cell Decision Processes (CDP) Center at MIT (<http://csbi.mit.edu/research/projects/celldecision>), directed by Peter Sorger and funded by the National Institute of General Medical Sciences (NIGMS) for \$16 million over five years. The CDP Center research involves an interdisciplinary team of cell biologists, computer scientists and microsystems engineers tackling the systems biology of protein networks and signal transduction in mammalian cells, with particular focus on programmed cell death. CDP Center research is based on the hypothesis that understanding cell decision processes requires the development of network models that combine quantitative rigor with molecular detail. The resulting models are hybrids that contain highly specific representations of critical reactions and abstract representations of the system as a whole. Other systems biology centers supported by NIGMS include the Center for Quantitative Biology in Princeton; the Center for Cell Dynamics at the University of Washington; and the Bauer Center for Genomics research at Harvard.

The U.S. government also has a number of departments and centers associated with its research organizations that focus on systems biology and/or modeling of biological systems. These include the systems biology department at Pacific Northwest National Laboratory (<http://www.sysbio.org>), the physical biosciences division dedicated to quantitative biology at the Lawrence Berkeley National Laboratory, and the systems biology Genome to Life (GTL) projects sponsored by the Department of Energy.

Biological Modeling and Network Organization Analysis in Europe

Biological modeling in Europe has a long history, and is indicative of the wide variety of subjects addressed and techniques being utilized in the field. The specific sites that employ modeling of biology systems in their research that the panel visited in Europe and Japan are listed in Table 4.1. Here a number of studies that were notable for their innovation and contributions in the different categories listed above are described.

A particularly notable study of a biochemical network is the work of Ursula Klingmüller of the German Cancer Research Center in Heidelberg and a leader within the German Hepatocyte Project. She and her colleagues' work on the Jak-stat regulatory network has led to new insights about its structure and function. She utilized a combined modeling-experiment approach to distinguish between two alternative hypotheses about how signal transducer and activator of transcription (STAT), after phosphorylation by a Jak-activated receptor, could enter the nucleus and activate gene expression (Swameye et al., 2003). They used a novel procedure to compare mass-action kinetic models of the alternative hypotheses to experimental data to demonstrate that STAT5 must become unphosphorylated and recycled out of the nucleus and back in again rather than get trapped in the nucleus and be degraded. They then used the model to ask what intervention is most effective for increasing STAT5-p in the nucleus, the conventional view of increasing phosphorylation, or blocking nuclear export. The model predicted the latter, and they used experiments to demonstrate this was correct.

Intracellular metabolic networks and control are areas with significant attention in Europe. David Fell at Oxford Brookes University in England utilizes metabolic engineering approaches to study metabolism primarily in plants and bacteria, including threonine biosynthesis in *E. coli*, potato tuber metabolism (in collaboration with Advanced Cell Technologies in Cambridge), photosynthesis, and antibiotic production in actinomycete (Poolman, Assmus, and Fell, 2004; Schafer et al., 2004; Chassagnole et al., 2003; Thomas et al., 1997). In addition, he utilizes structural modeling, the deconstruction of large networks into smaller substructures, to predict pathways that are feasible from gene expression information and those capable of greatest metabolic yields. In one notable study, he predicted that there should be six categories of arid-environment plants in terms of crassulacean acid metabolism instead of only four as previously known. Recent recognition of a fifth and discovery of a sixth lends support to his prediction.

Hans Westerhoff of Free University in Amsterdam, Netherlands, is a leader within a large group of researchers in the Netherlands that utilize metabolic control analysis to study metabolic networks and regulation in yeast and bacteria as well as signal transduction. In related work, the focus of Reinhart Heinrich of Humboldt University, Berlin, is on dynamic models of metabolism and control of networks as well as other biological pathways. His approach involves modeling, with close verification by experiments, using methods from nonlinear dynamics and simulation, bifurcation theory, metabolic control analysis, stoichiometric network analysis, stochastic process theory, optimization and graph theory. In recent work with Mark Kirschner combining modeling and experimentation, he studied the effect of Wnt stimulation on β -catenin expression in *Xenopus* oocyte extracts, finding that depending on topology there were greater and lesser regions of stability of the G-protein signal transduction network based on both the number of kinases in the network and phosphatase activity (Lee et al., 2003). In other work, Heinrich has analyzed the evolution of networks by using the large metabolic maps in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (<http://www.genome.jp/kegg>), determining how such metabolism could be built a step at a time from finding critical routes from each metabolite to other necessary endpoints. Different substrates can make different numbers of primary metabolites through the known reaction network. For example, adenosine triphosphate (ATP) can make about 1,500 whereas glucose can only make around 50, suggesting that a great deal of the cell's metabolic network could have been elaborated from a smaller metabolism based only on transformations of ATP (Ebenhoh, Handorf, and Heirich, 2004).

Cell function and its intracellular regulation are the subjects of interest of a longstanding modeler in Europe, Albert Goldbeter at the University Libre de Bruxelles in Belgium, a university with a long tradition of

theoretical biology. Through his modeling work Goldbeter has contributed to the understanding of various dynamic cellular phenomena including regulation of Circadian rhythms and metabolic oscillations (Goldbeter et al., 2001; Leloup and Goldbeter, 2003; Goldbeter, 2002). Others in his department have contributed in the areas of dynamics of regulatory gene networks, calcium signaling, theoretical ecology and social insect behavior.

Cellular chemotaxis of both prokaryotes and eukaryotes is another subject with a significant history of modeling and analysis to help understand the molecular and physical mechanisms driving it. Dennis Bray, Cambridge University, has made significant contributions on chemotaxis of *E. coli* since the early 1990s. This is a particularly tractable system to study because only six or seven proteins are involved in its regulation. Bray's group has both deterministic and stochastic models they use to study excitation, adaptation, mutant phenotype, individuality, and the effect of architecture of intracellular space (Lipkow, Andrews, and Bray, 2005; Shimizu, Aksenov, and Bray, 2003; Bray and Bourret, 1995). Another European researcher modeling chemotaxis and cell motility is Wolfgang Alt (Bonn, Germany), although the panel did not visit his laboratory.

In the subfield of electrophysiology, Denis Noble of Oxford University has studied cardiac biology for more than forty years, collaborating with Raimond Winslow (U.S.), Peter Hunter (New Zealand), and Andrew McCullough (U.S.). Noble's research group tightly integrates experimental and modeling approaches in their study of how ionic currents drive cardiac myocyte function and how cellular function is integrated spatially and dynamically to drive the function of the whole heart (ten Tusscher et al., 2004; Garny et al., 2003; Markhasin et al., 2003; Noble, 2002). Over the years, his models have contributed to the understanding of energy conservation, the necessary stoichiometry of ion exchangers, and mechanisms of calcium balance in cardiac myocytes, and the implications of these for cardiac dysfunctions such as arrhythmia (see Noble, 2002, for review). The heart is arguably the organ most comprehensively modeled, in terms of biological detail (molecular, cellular, spatial organization, dynamic function). Noble notes that what has made this possible is that relevant experimental work has been ongoing for 40 years, providing a vast body of data and knowledge. The major regulators of the cell and tissue function (ion channels generally) are quite accessible to measurement, and the cell properties that contribute to many aspects of whole organ function are relatively few and aren't strongly dependent on vast intracellular signaling networks.

Ernst Dieter Gilles and his associates at the Max Planck Institute for Dynamics of Complex Technical Systems in Magdeburg, Germany have focused on aspects of model validation and model-based experimental design (Kremling, 2004), areas with a long history in engineering and physical sciences but less in biological modeling. Gilles' department closely integrates experimental and modeling work for the purpose of improving the understanding of biological phenomena and identifying solutions for medical problems, particular drug target identification. The methods employed included detailed mathematical modeling, model validation and iterations (via design of experiment) for hypothesis testing, system-theoretic analysis of properties including robustness, and decomposition and model reduction. The particular biological areas of research include signal transduction and regulation in bacterial cells and eukaryotes, metabolic network structure, and computer-aided modeling and analysis of cellular systems (Stelling and Gilles, 2004; Schmid et al., 2004; Stelling et al., 2004). Gilles has noted that their initial attempts at collaborations with biologists were not effective because their engineering approaches were not appreciated by the biologists. They have become more effective over the years by refining their modeling efforts, becoming more visual and including lower-level biological details. Their work has made important contributions in the extraction of design principles from the robustness analysis of the circadian clock (Stelling, Gilles, and Doyle, 2004), and the use of identifiability tools to guide experimental design for modeling of cell cycles. An important conclusion of the latter work was that perturbations were more important than additional measurements in formulating an optimal experiment for model identification.

Large-Scale Alliances and Collaborations

Several large-scale systems biology projects in Europe include modeling as a cornerstone. One launched in January 2004 is the Hepatocyte Project in Germany, funded by the German Federal Ministry of Education and Research (BMBF) in its "Systems for Life—Systems Biology" initiative. The thematic focus and structure for the Hepatocyte Project was shaped starting in 2001 by an expert panel of about 80 scientists through four workshops. The goal to understand the mechanisms of behavior of hepatocytes was ultimately selected because of the hepatocyte's central function in metabolism, their central role in the uptake and

conversion of drugs and thereby their interest to industry, and their ability to regenerate. The panel expected this research to have high impact on problems in pharmacology and pathophysiology, despite the challenges involved in the complex biology of hepatocytes, the difficulty in their handling and cultivation, and need to create a bioinformatic and modeling framework to organize information about them. The Hepatocyte Project has an interdisciplinary competence network linking bioscience with computer science, mathematics and engineering sciences. Two sub-projects (Project A is detoxification and dedifferentiation in hepatocytes and Project B is regeneration of hepatocytes) rest on two technological platforms, cell biology and modeling. The project has 25 participating groups, and funding of €14 million is provided over three years beginning in 2004.

Another large consortium project entitled “Biosimulation: A New Tool in Drug Development” (http://chaos.fys.dtu.dk/biosim/Beskrivelse_af_BioSim.html) was announced in December, 2004 by The European Commission and the Technical University of Denmark (DTU) to support the growing importance of modeling for biomedical research, and pharmaceutical development in particular. The project is funded under the European Union's Sixth Framework Program for Research at the level of €10.7 million over five years. The project's aim is to strengthen Europe's competitiveness within drug development by bringing together the leading European biosimulation experts in a scientific network and promoting collaboration across disciplinary boundaries as well as between industry, regulatory authorities, and academia. The network will focus on the development of professional, physiologically based models that can help the pharmaceutical industry develop safe and effective drugs at significantly lower costs. It was motivated by the recognition that academic institutions in Europe have significant expertise in biological modeling, and several groups are individually at the research front in their specific areas, but the research is strongly fragmented and the industry itself has relatively few qualified experts in the field. Coordinated by the DTU, the network comprises approximately 100 researchers from 25 universities/research centres, nine small or medium-sized enterprises, the medicines agencies of Denmark, Spain, the Netherlands and Sweden, and one large pharmaceutical company, Novo Nordisk.

Biological Modeling and Network Organization Analysis in Japan

Modeling of biological systems is quite evident in Japan, although from the sites the panel visited it appears to be less extensively utilized than in the U.S. and Europe. The sites with modeling components of their research that the panel visited are included in Table 4.1 and a few of those are highlighted here.

The work of Satoru Miyano focuses on estimating gene networks from genome-wide biological data, as well as software tools for bioinformatics and modeling (described below) and pathway database projects. Miyano is the current president of the Japanese Society for Bioinformatics, and the editor-in-chief of the newly established IEEE Transactions on Computational Biology and Bioinformatics. This group has developed hybrid functional Petri net methods for gene network inference (Akutsu, Miyano, and Kuhara, 2000; Doi et al., 2004) and their current Gene Network Inference Method (G.NET) can yield the optimal gene network for twenty genes (on a Sun Fire 15K 100 CPU machine) in one day. As a test case they used the method to discover the function of the oral antifungal griseofulvin and predict new targets related to it. Specifically, they measured gene expression as a function of exposure, and used a Boolean network approach using the drug as a virtual gene to predict genes that were directly affected by the drug. Then they made predictions of related components in the pathway that would also be effective if manipulated (Savoie et al., 2003). This work was done in collaboration with a small company with compounds related to griseofulvin.

A well-known modeling group in Japan is that of Hiraoki Kitano of the Symbiotic Systems Project associated with the Systems Biology Institute (SBI). The basic goal of the project is to develop and apply new technology and computational tools to understand dynamical phenomenon in cellular systems. Areas of research include the theory of the robustness of cellular networks, signal transduction in yeast and mammals (collaborating with the Alpha Project and the Alliance for Cellular Signaling), respiratory oscillations in yeast, and calcium oscillations in mammalian cells (including collaborations with the Karolinska Institute) (Kitano et al., 2004; Kitano, 2004; Yi, Kitano and Simon, 2004). A significant focus of Kitano's work has been the development of modeling software and markup languages to encode and share models as described below, as well as advanced hardware platforms for simulations.

Table 4.1
European and Japanese Sites Utilizing Modeling Visited by Panel

Institution	Location	Principal Investigator(s)	Research Subjects
Department of Physiology, Oxford University	Oxford, England	Denis Noble, Peter Kohl, Ming Lei	Cardiac biology; respiratory biology
Department of Anatomy, Cambridge University	Cambridge, England	Dennis Bray	Chemotaxis in <i>E. coli</i>
University of Sheffield (multiple departments)	Sheffield, England	Chris Cannings, Richard Clayton, Steve Dower, Mike Holcombe, Nick Monk, Eva Qvarnstrom, Francis Ratnieks, Rod Smallwood, Phillip Wright, Will Zimmerman	Cell organization in tissues; system organization in insect societies; protein-protein interaction networks; inflammatory mediator signaling; agent-based models; toll receptor signaling; cell signaling and network pattern formation; ventricular fibrillation
Mathematics Institute, University of Warwick	Coventry, England	Andrew Millar, Nigel Burroughs, Jim Beynon, Greg Challis	Network regulation; transmembrane protein transport; Circadian clock; immunology; population genetics
Centre for Mathematical Biology/Mathematical Institute, Oxford University	Oxford, England	Philip Maini (Director), Jon Chapman, Chris Scofield, Jotun Hein	Nutrient and drug delivery to tissue with application to cancer; cardiovascular biology; cell cycle; model integration methods; population genetics and genomics; wound healing
School of Biological and Molecular Sciences, Oxford Brookes University	Oxford, England	David Fell	Metabolic networks; cell cycle; I κ B regulation
Centre for Mathematics in the Life Sciences and Experimental Biology, University College London	London, England	Anne Warner (Director), Anthony Finkelstein, Jonathan Ashmore, Robert Seymour	Liver metabolism; software and methods to integrate across biological scales
Computational Systems Neurobiology program, European Bioinformatics Institute	Hinxton, England	Nicolas LeNovere	Topology and dynamics of neuronal cell signaling pathways; dopamine signaling
German Cancer Research Center/Hepatocyte Project	Heidelberg, Germany	Otmar Wiestler, Siegfried Neumann, Ursula Klingmüller, Willi Jager, Wolfgang Driever, Matthias Reuss, Eric Karsenti, Jens Doutheil, Jan Hengsteler, Sven Sahle	Hepatocyte Project; signaling pathways; structural and functional genomics; cancer risk factors and prevention; tumor immunology; innovative diagnostics and therapy
Max Planck Institute for Dynamics of Complex Technical Systems	Magdeburg, Germany	Ernst Dieter Gilles, Jorg Stelling	Model validation and experiment design; signal transduction and regulation; computer-aided modeling and analysis of cellular systems

Table 4.1
European and Japanese Sites Utilizing Modeling Visited by Panel

Institution	Location	Principal Investigator(s)	Research Subjects
Collaborative Research Center for Theoretical Biology, Humboldt University	Berlin, Germany	Reinhart Heinrich, Hanspeter Herzel, Peter Hammerstein, Hermann-Georg Holzhutter	Metabolic control; biological dynamics; Ras signaling; Circadian clock; Huntington's disease; Hepatocyte Project
Department of Vertebrate Genomics, Max Planck Institute for Molecular Genetics	Berlin, Germany	Hans Lehrach, Edda Klipp, Silke Sperling	Yeast stress response and mitochondrial damage; Downs syndrome; cardiac development;
University Libre de Bruxelles	Brussels, Belgium	Albert Goldbeter	Biological dynamics, Circadian rhythms, cell cycle
Vrije Universiteit (Free University)	Amsterdam, Netherlands	Hans Westerhoff, Jurgen Haanstra, Frank Bruggemann, Jorrit Hornberg	Metabolic control analysis, network based drug design, Silicon Cell toolkit, signal transduction
Delft University	Delft, Netherlands	Wouter van Winden	Metabolic control
Cell/Biodynamics Simulation Project, Kyoto University	Kyoto, Japan	Akinori Noma (Director), Tetsuya Matsuda, Nobuaki Sarai	Cardiac biology; biosimulation software development
Symbiotic Systems Project, Systems Biology Institute	Tokyo, Japan	Hiraoki Kitano	Modeling technology; model standards technology; robustness of cellular networks; yeast signaling
Human Genome Center, Institute of Medical Science, University of Tokyo	Tokyo, Japan	Satoru Miyano	Gene network inference from genome-wide data; yeast networks; pathway databases; software for bioinformatics and simulation
Department of Computational Biology, Graduate School of Frontier Sciences, University of Tokyo	Tokyo, Japan	Shinichi Morishita, Takashi Ito	Functional genomics and signaling in budding yeast; mammal epigenomics; computational approaches to Omics
RIKEN Yokohama Institute	Yokohama City, Japan	Akihiko Konogaya, Mariko Hatakeyama, Shuji Kotani	ErbB-mediated signal transduction; yeast cell cycle; reaction-diffusion systems; Grid computing; cell cycle modeling
Institute for Advanced Biosciences, Keio University Tsuruoka Campus	Yamagata, Japan	Masaru Tomita, Hirotsada Mori	E-CELL project
Bioinformatics Center, Institute for Chemical Research, Kyoto University	Kyoto, Japan	Minoru Kanehisa	KEGG project; dynamic metabolic models

Modeling of electrophysiological phenomena, with flavors of intracellular signaling, cell behaviors, and multicellular/tissue/organ categories, is the work of Akinoi Noma at Kyoto University. Noma has had a distinguished career in experimental cardiac physiology and electrophysiology and only added modeling to his research methods in the past several years. His Cell/Biodynamics Simulation Project is focused on developing and applying models of cardiac myocytes and their integrated function in the heart, closely related to work by Noble described above. Their novel contributions include the addition of intracellular biochemical mechanisms such as ATP utilization, redox state balance, and pH (Matsuoka et al., 2004). In contrast, most myocyte models have concentrated on cell surface molecular entities and behaviors, specifically the ion channels, pumps and action potentials. This is a National Leading Project for Cooperation between Industry and Academia sponsored by Ministry of Education, Culture, Sports, Science and Technology (MEXT) on a five-year grant, which requires industry involvement and work that is relevant to economic growth. Dr. Noma has involved seven pharmaceutical companies to date, including Nippon Shinyaku, Shionogi, Sumitomo Chemicals, Tanabe, Sankyo, Takeda, and Mitsubishi Well Pharma, as well as researchers at Kyoto and Keio Universities in Japan and others in Poland and Korea. Four of the collaborating companies have placed a full-time employee in the Dr. Noma's group in Kyoto, while the others send visiting researchers for short visits periodically. This project is also a major developer of modeling/simulation tools, as described in infrastructure below.

In the area of network organization analysis, the work of Masanori Arita (University of Tokyo) has demonstrated how structural information of metabolites is important for computing biochemical pathways and understanding the network properties of those pathways (Arita, 2004).

INTEREST AND INVOLVEMENT OF INDUSTRY

The interest and involvement of industry in systems biology efforts that include modeling and network organization work are significant although hardly ubiquitous, and quite variable between regions. The industry of concentration is pharmaceutical and biotechnology, although applications in the nutraceutical, agricultural supply/chemical and bioprocess industries also exist. Here activities within the industry as well as the relationship between industry and academia are summarized.

R&D Using Modeling in Industry

Companies using mathematical models of biological systems are treating them essentially as *in silico* laboratories that complement the experimental laboratories. As described in the introduction, models are particularly suited to tracking the states of and relationships among numerous elements through time and space, especially for large, complex systems as found in biology and medicine. Applications of systems biology modeling within the pharmaceutical industry vary from drug target identification to clinical trial design and analysis. Models of intracellular biochemical networks are typically utilized to investigate how modulation of that network, by agonizing or antagonizing network components, affect the associated cell function. The direct relationship of that cell function to a particular disease is disease-specific, for instance proliferation of a cancer cell is directly related to disease outcome (tumor size, say), whereas modulation of an inflammatory cell within the asthmatic airways still requires extrapolation to relevant clinical endpoints. Models of whole organs in which a disease is isolated, like the heart for cardiac arrest or arrhythmia, or of multicellular/tissue/organ systems that include relevant clinical endpoints, bridge cellular function to relevant clinical outcomes much as an experimental animal model would.

Given that drug targets are nearly always molecular, modeling within the pharmaceutical industry is most frequently focused at the level of intracellular biochemical networks. Several companies in the U.S. that are focused on development and use of such intracellular network models include Gene Network Sciences (U.S.), Merrimack Pharmaceuticals (U.S.), Genomatica (U.S.) and Physiomics (U.K.). Gene Network Sciences uses inference modeling and mechanistic simulation of intracellular biochemical and gene networks related to cell cycle to do research on cancer and its treatment (Christopher et al., 2004). More recent directions include cardiac electrophysiology. Physiomics also focuses on modeling cell cycle control. EGF receptor dynamics and downstream signaling associated with various cancers are the subject of modeling at Merrimack Pharmaceuticals. In contrast, Genomatica utilizes models of microbial and yeast cell metabolism to improve bioproduction of chemicals and proteins, among other applications.

Multicellular/tissue/organ network function has also attracted the application to pharmaceutical development. Entelos, Inc. (U.S.) utilizes dynamic ODE models of the biological systems involved in specific diseases to evaluate drug targets, select lead compounds, predict biomarkers, and design clinical trials. Current areas of work include model representations of human diseases (asthma, hematopoiesis, obesity, rheumatoid arthritis, and type 2 diabetes), and animal models of human disease (type 1 diabetes).

Larger pharmaceutical companies that utilize biological systems modeling in some of their R&D activities include Pfizer, AstraZeneca, Hoffman-La Roche, Johnson & Johnson Pharmaceutical Research Division, GlaxoSmithKline, Novartis, Organon, and Bayer. This list is probably not exhaustive. In some of these companies the inclusion of modeling is quite extensive, while in many it is often confined within a single therapeutic area or a single group that works with multiple experimentalists.

There seem to be fewer companies primarily devoted to using modeling as a primary R&D method in other areas of the world. In addition to Physiomics mentioned above, another modeling-focused company is Optimata in Israel, which utilizes modeling to optimize drug dosing and schedules, in particular for cancer treatment.

While companies are notably less forthcoming than academic researchers with making their models and research results public, there is emerging evidence that modeling is significantly impacting pharmaceutical development. For example, Entelos and Organon recently made public that they are engaging in collaborative drug development focused on three novel targets that were identified using Entelos Rheumatoid Arthritis PhysioLab® platform (<http://www.entelos.com/news/pressArchive/press62.html>). Johnson & Johnson Pharmaceutical Research and Development has also disclosed that simulations of a type 2 diabetes drug with Entelos Metabolism PhysioLab platform enabled them to reduce the patient recruitment requirements by 60% and trial duration by 40%, as compared to the originally proposed trial protocol (Trimmer et al., 2005). Optimata has also made public that they are utilizing their simulation methods to create individualized treatment protocols for breast cancer patients in a clinical trial at the Nottingham City Hospital Trust, although results are not yet available for the study (http://www.export.gov.il/Eng/_Articles/Article.asp?CategoryID=464&ArticleID=1017). An example of results from a large pharmaceutical company is Hoffman-La Roche's use of modeling of a treatment for hepatitis C. They used modeling and simulation to account for a variety of factors in different patient populations such as genotype of virus and weight of the patient. The results were important for the approval of the drug in both Europe and the U.S. (McGee, 2005).

Relationships between Academia and Industry in Different Regions

Close relationships between industry and academia were particularly obvious in Japan in comparison to the U.S. and Europe. Many Japanese academic researchers stated that the government research funding agencies strongly encouraged collaborations with industry and the transfer of technologies to industrial concerns, either through the start up of new companies or to established companies. In numerous laboratories there was active involvement in the research by industry staff in residence.

In contrast, in Europe the relationship of industry to academic research was at the level of a few collaborations (without exchanging personnel) and the encouragement by funding agencies to transfer findings and technology developments to industry. The recent large collaborative projects in Germany and the EU described above were both influenced by the interests of the pharmaceutical industry, although in the Hepatocyte Project there is no actual industry involvement. The EU Biosimulation project is too new to know how involved the corporate partners will become beyond providing funding.

In the United States, academic-industry collaborations are common, although it is uncommon for industry personnel to spend time in an academic lab. The reverse is probably more likely. It has become the norm for universities in the U.S. to patent research findings and then license them to companies or for the inventors to start new companies to commercialize the inventions.

INFRASTRUCTURE SUPPORTING MODELING AND NETWORK ORGANIZATION ANALYSIS

Throughout the U.S., Europe and Japan, the study panel found significant efforts devoted to the development of software platforms in which to build and simulate mathematical models of biology (and sometimes more general) systems. While variations exist among these platforms, the panel concluded that significant replication of numerous features and capabilities among them also exist. A table listing a number of

modeling and simulation platforms focused on or frequently used for modeling of biological system networks is given in Table 4.2 along with some descriptive information and web sites at which the reader can learn more. Additional lists on the web can be found on the Systems Biology Markup Language (SBML) web site (<http://sbml.org>) for software packages that are compatible with SBML and the Bio-SPICE web site (<https://users.biospice.org/tools.php>) for those that are part of the Bio-SPICE project.

Table 4.2
Software for Modeling and Simulation of Biological Systems

Software Platform	Main Applications	Developer or Main Contact	Availability to Others¹	Web site
Bio-SPICE	Collection of many software packages with many applications—see web site	DARPA-sponsored consortium; Sri Kumar, program manager	Source and binary download via web site	https://users.biospice.org/home.php
Virtual Cell	ODEs with multiple compartments; PDEs	Leslie Leow, U.S.	Use through internet	http://www.vcell.org
Teranode Design Suite		Teranode Corp.	Commercial; reduced price for academic use	http://www.teranode.com
MATLAB & SimuLink	General math simulation tool	The MathWorks	Commercial; reduced price for academic use	http://www.mathworks.com
Mathematica	General math equation solver and simulation	Wolfram Research, Inc.	Commercial; reduced price for academic use	http://www.wolfram.com
YAGNS (Yet Another Gene Network Simulator)	Biochemical reaction network simulator (ODEs)	RIKEN Yokohama, Japan	Access via web upon request	http://big.gsc.riken.jp/big/Research/Cellular_Knowledge_Modeling_Team/Folder.2004-01-15.5608/Folder.2004-01-15.5713/Document.2004-01-15.3211
Genomic Object Net / Cell Illustrator	Biological pathway modeling and simulation based on hybrid functional Petri net (HFPN) and XML	Gene Networks Inc., Japan	Contact company	http://www.genomicobject.net/member3
Cell Designer	Structured diagram editor for drawing gene-regulatory and biochemical networks; simulation by linking to other packages	Hiraoki Kitano, Japan	Download via web site	http://www.celldesigner.org
SimBio / DynaBioS®	Cell electrophysiology and Finite element modeling of electrophysiologic tissue (heart)	Akinori Noma, Japan	Download via web site	http://www.sim-bio.org

Table 4.2
Software for Modeling and Simulation of Biological Systems

Software Platform	Main Applications	Developer or Main Contact	Availability to Others¹	Web site
JDesigner/Jarnac	Biochemical network layout tool and simulation package (ODEs)	Systems Biology Workbench project, Japan and U.S.	Download via web site	http://www.sys-bio.org
ProMoT/DIVA	Object oriented and equations based modeling tool for simulation; differential and algebraic equations	Martin Ginkel, Germany	Download via web site	http://www.mpi-magdeburg.mpg.de/de/research/projects/1002/comp_bio/promot
GENESIS/Kinetikit	Graphical simulation environment focused on signaling networks	Sharat Vayttaden and Upinder Bhalla, India	Download via web site	http://stke.sciencemag.org/cgi/content/full/sigtrans;2004/219/p14/DC1
Copasi	Complex pathway simulator (not biology specific)	Pedro Mendes, U.S. and Ursula Kummer, Germany	Download via web site	http://www.copasi.org
Cellerator (a Mathematica package)	Mathematica package for automatic equation generation and simulation for signaling networks and networks of cells	Bruce Shapiro, Eric Mjolsness, U.S.	Download via web site	http://www.cellerator.info
BioNetGen	Cell signaling networks based on interactions of individual molecules	Michael Blinov, James Faeder, William Hlavacek, U.S.	Download via web site	http://cellsignaling.lanl.gov/bionetgen
E-Cell	Object-oriented software suite for modeling, simulation, and analysis of large scale complex systems	Masaru Tomita, Japan	Download via web site	http://www.e-cell.org
JigCell	Modeling of biochemical reaction pathways	Virginia Tech	Download via web site	http://jigcell.biol.vt.edu
MCell	Monte Carlo simulator of cellular microphysiology	Thomas Bartol, Jr., and Joel Stiles, U.S.	Download via web site	http://www.mcell.psc.edu
COR (Cellular Open Resource)	Cell electrophysiology	Denis Noble, U.K.	To be available via web site	http://cor.physiol.ox.ac.uk

¹ Terms of availability frequently differ depending on the expected use of the software (e.g., non-commercial or commercial) and may require licenses for other software used in the package.

Reasons given by researchers for developing new platforms included the need to have faster simulation capabilities, improved usability, and features specific to the biologic system being modeled by the

developer's research group. Usability issues were mentioned with particular attention to making models and simulation more accessible to non-expert users, although technical usage was also mentioned. The latter includes such things as ease of specifying model equations (e.g., specifying equations by drawing structured diagrams rather than typing them), input and modification of parameter values, specification of protocols to simulate, and storage/retrieval of simulation specifications and results. Features desired were typically specific mathematical methods such as those to handle stochastic processes or finite element algorithms, as well as analytical methods such as parameter optimization or sensitivity analysis.

Some modeling software is available commercially, the most commonly used (at least in the U.S.) general purpose numerical computing platform being MatLab by The MathWorks, followed perhaps by Mathematica (Wolfram Research). Matlab and Mathematica both have announced special tools for systems biology. More specialized commercial software includes Berkeley Madonna, and a number of pharmacokinetic simulation packages. Benefits of such commercial platforms are their formal quality assurance/quality control (QA/QC) processes as well as formal means for reporting bugs and requesting new features. The disadvantage is that they are not free, although the cost of most commercial packages tends to be nominal for academic use. Many of the mentioned packages are not specialized for biological systems, are not well linked to biological databases and data sources, and are not user-friendly for the biological user. For the many platforms being developed noncommercially within research groups, the most common method of dissemination (when available) is via the group's web site. The advantage of development within research groups is that the software features and user interface can be closely guided by end-users within the group. Some disadvantages, however, are that QA/QC procedures for such software are frequently unclear and likely absent in many cases and "services" to users outside the developer's group, such as ways to report bugs and request new features, are not always clear and response by developers not assured.

The fact that so many new packages are in development strongly suggests that those available, commercial or otherwise, are not fully meeting significant needs of the biological modeling community. While no one piece of software will meet the needs of all modeling efforts, the panel believes that the community would be well-served by a national or international resource devoted to making broadly applicable platforms widely and freely available, as well as supporting maintenance and expansion. Such a resource should reduce the ongoing proliferation of somewhat duplicative and quite expensive software development. The Bio-SPICE program funded by Defense Advanced Research Projects Agency (DARPA) for the last three years is an example of such a program although it focused primarily on initial development for an open-source infrastructure for integrating such software. The funding for this project ends in 2005 and currently there are no allocated resources from DARPA or elsewhere to continue funding to support dissemination of the resulting software or its continued development.

Sharing of Models

Historically models have been shared through literature publication. As modeling has become a more common way to do biologic research and the models themselves have become larger, this method has become less satisfactory to many and a strong desire to more easily share models in electronic form between research groups has grown. While some researchers simply want to use or modify published models without having to re-generate computer code to do so, others also want to integrate others' models with their own to create models of larger biological systems.

The main difficulty in sharing models electronically is that models are encoded for specific software and hardware platforms that aren't universally compatible with software or hardware in other labs. To alleviate this problem, two main international efforts are underway to better enable model sharing, namely, the development of markup languages that encode mathematical equations typically used in models of biological systems. For encoding models, Systems Biology Markup Language (SBML; <http://sbml.org>) (Hucka et al., 2003) and CellML (Cell Markup Language; www.cellml.org) are two major languages under development. The idea is to create a language analogous to HTML (hypertext markup language), the common encoding language for the web. As long as one has an HTML "decoder" on one's computer, e.g., a browser such as Microsoft Internet Explorer, then one can interpret the text, pictures, etc. encoded in an HTML file and the same file can be viewed on all computers. SBML is focused on language to encode math that describes biochemical reaction networks while CellML is focused on describing cellular components and compartments as well as biochemical reaction networks. SBML began in Japan and at CalTech and has since become an international collaboration effort with funding from the U.S., Japan and the U.K. Development

efforts on CellML are based in New Zealand. At least one other biological modeling-focused markup language is being developed by Satoru Miyano's group in Japan, labeled Cell System Markup Language, although it does not appear to be publicly available yet.

Part of the effort associated with both SBML and CellML is the development of a public repository of models online in the markup language (see <http://sbml.org/models.html> and <http://www.cellml.org/examples/repository/index.html>, respectively). Authors of models are encouraged to deposit a version of their model on the web site, available for download by others. Another such repository just launched in April, 2005, is Biomodels.net (<http://www.biomodels.net/>), which is supported by multiple organizations from several countries. Numerous individual labs also provide either models for download or for simulation over the Internet via their web sites.

While none of these markup languages are as yet accepted as the standard, researchers (at least in the U.S.) generally acknowledge SBML as being the most frequently used. Certain U.S. funding agencies, including the National Science Foundation (NSF), have taken the position that models developed with their funding must be made publicly available in SBML. The panel is not aware of such a policy at other agencies or nonprofit funding groups in the U.S. or by funding agencies in other countries. Journals generally do not yet require that authors provide electronic versions of their models to the readership in any form.

NEEDS AND RECOMMENDATIONS

In this study the panel has recognized several needs and deficits of the modeling efforts in systems biology and makes several recommendations to alleviate these. First and foremost, the panel notes the need for substantially greater and more widespread integration of modeling and experimental programs. Currently the majority of modeling and experimental efforts on a given subject are performed somewhat or completely remotely from each other. This decreases the benefit of both to each other, and slows progress in developing new understanding in many fields. Data from diverse laboratories and with diverse protocols are often found to be difficult to compare when placed in model context implying both that models can help ensure consistency among datasets thus preventing spurious conclusions about the significance of a particular observation and that data quality control is even more important than model quality control. While it may seem from the highlighted examples above that modeling work is commonly integrated with experimentation, the examples were selected in part because they demonstrated how well-integrated programs provided unique insights into the subjects of study. The reason that such tight integration is promoted by the panel is because models can't be developed or tested without experimental data, and the experimentation that provides the necessary data is often not obvious without the model guiding its design. The experiment-model iteration paradigm that is most productive is illustrated in Figure 4.3.

Closely related to the need for experimental-modeling integration within research endeavors is a need for improved means of comparing experimental data and modeling results. Many software platforms for modeling currently don't support easy representation of experimental data, for instance, so both the data and modeling results have to be exported to a third software to allow the comparison.

Another major need is for a means of disseminating and maintaining good cheap or free software appropriate to modeling of various problems nationwide and internationally. The proliferation of modeling software platforms was discussed above. It is the panel's conclusion that many of these platforms are duplicative and the time and cost being expended to develop all of them could be better used to make some of them more widely available as well as to maintain and expand those platforms.

The panel also agrees with the widely stated desire of researchers to be able to more easily share their models with one another in electronic form. The panel therefore supports the efforts of the groups developing SBML and CellML, however, these are efforts funded by several grant agencies, that funding is not guaranteed, and no one is required to use either of these (or other translational languages), so their future, and the possible future of improved ease of sharing models, is not assured.

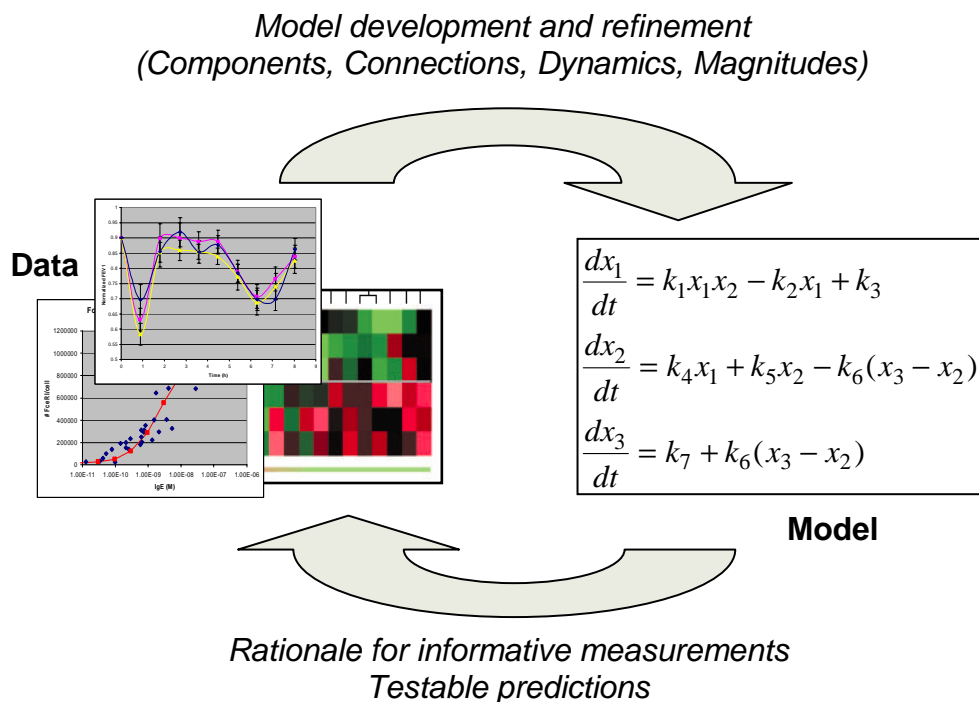


Figure 4.3. With the purpose of increasing understanding of a biological system function, one needs a set of data to develop the first model. That first model can then be simulated or analyzed to pinpoint uncertainties that are important to the system and then recommend a new set of experiments to measure relevant quantities to reduce those uncertainties. The new data can be used to revise the model. The model at various stages of iteration can also be used to test hypotheses about the system's function or means of modulating that function, and interesting predictions from those tests can then also be verified (or refuted) experimentally.

SUMMARY OF KEY FINDINGS

This chapter described the state of systems biology research involving modeling and network organization analysis. A number of key findings can be summarized. Modeling and network organization analysis efforts are utilized in many areas of biological study and in all countries visited, but are definitely not ubiquitous throughout biological and biomedical research. The panel found that research efforts that closely integrated modeling with experimental work were the most productive in terms of driving new understanding of a biological system. Related to this, the panel concluded that a substantial increase in the number of efforts using model-based experimental design is needed to attain the most informative data, which leads to maximally useful models. An implication of this is that having large data generation centers to globally profile molecular abundances or activities might not provide the ideal substrate for gaining a mechanistic/causal understanding of how cells transform genotype into phenotype. Data generation and models that integrate, follow implications of, and make testable assertions about the causal basis of that data need to be strongly linked. In addition, better tools for model-experiment comparison would be helpful. Significant resources are being invested in the development of modeling and simulation software worldwide, and at least some duplication of effort is apparent. Sharing of models between researchers remains a challenge but is being addressed by the development of several markup languages. Finally, the involvement and interest of industry in use of modeling in biology is significant although, again, not ubiquitous.

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