Dynamics of biological systems: Role of systems biology in medical research

Article *in* Expert Review of Molecular Diagnostics · December 2006

DOI: 10.1586/14737159.6.6.891 · Source: PubMed

4 authors, including:

Heike Aßmus
Max-Delbrück-Centrum für Molekulare Medizin
14 PUBLICATIONS **466** CITATIONS

SEE PROFILE

Ralf Herwig
Max Planck Institute for Molecular Genetics
304 PUBLICATIONS **27,950** CITATIONS

SEE PROFILE

Olaf Wolkenhauer
University of Rostock
473 PUBLICATIONS **6,647** CITATIONS

SEE PROFILE

Dynamics of biological systems: role of systems biology in medical research

Heike E Assmus†, Ralf Herwig, Kwang-Hyun Cho and Olaf Wolkenhauer

Cellular systems are networks of interacting components that change with time in response to external and internal events. Studying the dynamic behavior of these networks is the basis for an understanding of cellular functions and disease mechanisms. Quantitative time-series data leading to meaningful models can improve our knowledge of human physiology in health and disease, and aid the search for earlier diagnoses, better therapies and a healthier life. The advent of systems biology is about to take the leap into clinical research and medical applications. This review emphasizes the importance of a dynamic view and understanding of cell function. We discuss the potential for computer-aided mathematical modeling of biological systems in medical research with examples from some of the major therapeutic areas: cancer, cardiovascular, diabetic and neurodegenerative medicine.

Expert Rev. Mol. Diagn. 6(6), 891–902 (2006)

CONTENTS

†*Author for correspondence*
University of Rostock, Systems Biology and Bioinformatics Group, Department of Computer Science, 18051 Rostock, Germany
Tel.: +49 381 498 7574
Fax: +49 381 498 7572
assmus@informatik.uni-rostock.de

KEYWORDS:
computer simulation, dynamic behavior, mathematical modeling, predictive medicine, systems biology

Dynamics of biological systems

A system is a set of inter-related and interacting objects. In biology, systems appear on all levels of organization: subcellular, cellular, tissue, organ, individual and population. Systems analysis studies the properties of dynamic processes, which suggests the definition of systems biology as a merger of (dynamic) systems theory and biology (for a more detailed discussion of the term's definition, see [1]). Therefore, systems biology signals a shift of focus from the identification, characterization and classification of components of cells or organisms towards the understanding of their dynamic interactions and the resulting functional activity.

The systems approach emphasizes the dynamic character of biological processes, including gene expression, metabolism and signaling. For example, cell differentiation is a nonlinear dynamic process; knowledge of the molecular components involved (e.g., receptors, cytokines and transcription factors) and static graphical representations (e.g., pathway maps) are only a first step. Whether or not a stem cell differentiates depends on the level and duration of stimuli, and other temporal properties of the system. A systems-theoretical analysis identifies cell differentiation as a bistable system and allows, through simulations, an optimized design of experiments.

All biological systems are characterized by dynamic changes (FIGURE 1). This behavior is what underlies development, adaptation to environmental changes and disease, and it is being created by the interactions of the system components. This implies that a shift of emphasis is needed: from a static view towards a dynamic view of biological systems. In recent years, new high-throughput technologies enabled the large-scale analysis of many of the cellular components. To name only a few, the protein expression pattern describes all of the proteins of a cell (also called the proteome) and a cellular mRNA profile describes all of its transcription products (also called the transcriptome). Similarly, the metabolome is the quantitative complement of all the low-molecular-weight molecules present in cells in a particular physiological or developmental state. These various '-omes' are inventories or descriptive lists of (isolated) parts, and the enormous quantity of data generated by the -omics disciplines represents at best a 'snapshot' or static description of the investigated

10.1586/14737159.6.6.891

ISSN 1473-7159

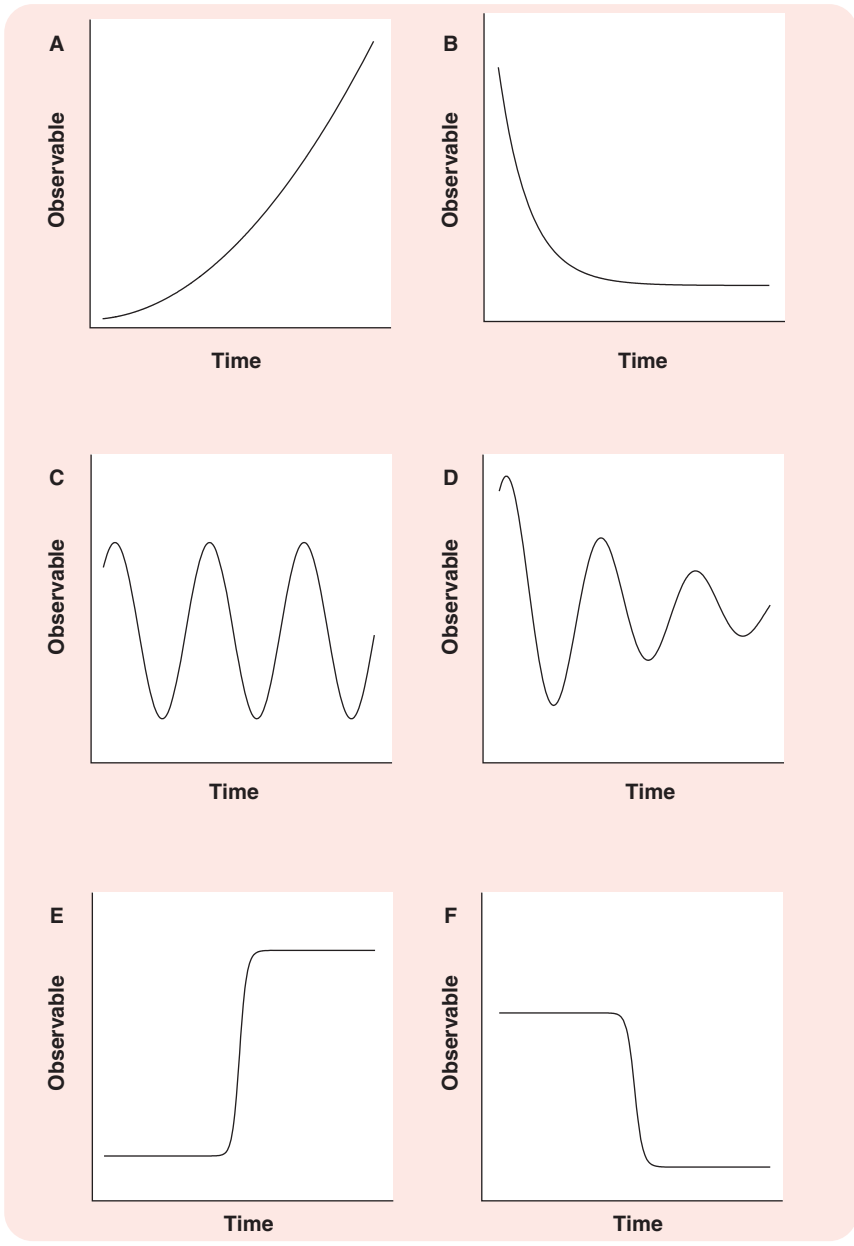

Figure 1. Idealizations of possible dynamical changes (motifs) of an observable in a biological system. An observable can be a physiological parameter or the concentration of a biochemical compound. (A) Growth, increase, accumulation; (B) Decay, decrease, depletion; (C) Sustained oscillation; (D) Damped oscillation; (E) Switch 'on'; (F) Switch 'off'.

system. A step towards the reconstruction of its dynamics is to record several such snapshots at different time points (note the subsequent section on data acquisition). In addition, the pairing of the systematic application of experimental perturbations with high-throughput data collection methods and the observation of the consequences of the former (i.e., the changes caused by the perturbations) can provide systematic insights [2–5].

Understanding the dynamics of a biological system means that one understands how properties of the system emerge from the nonlinear interaction of its multiple components. The application of diverse quantitative methods can generate data to characterize complex networks of transcriptional and protein–protein interactions that follow a stimulation in biological systems, but the problem is to make sense of these data and to transform this molecular knowledge into a comprehension of complex phenomena in cells and at higher levels of structural organization (e.g., tissue or organ level). In systems biology, comprehensive datasets (not single genes or gene products) which describe interactions and relationships between many components are considered. By using computational and mathematical tools to provide meaningful models, systems biology goes beyond the analysis of large data sets by statistical methods. It stands for predictive understanding, enabling foresight of the consequences of external stimuli. In that, it differs from the classical bioinformatics and -omics approaches. However, it is not an alternative but complements them.

Systems biology approaches have been employed in the area of biotechnology [6], but it is about time now that they become more important in human biology and medicine. Through its history, systems biology is already linked with medical issues. It originates in part from physiology asking the question: how do the cell and body parts work together to provide function? Its origin is also, in part, from neuroscientists asking the question: how does consciousness arise from the interactions between neurons? Systems biology has a role to play in medical research and this review highlights current directions that are taken.

A burst of information regarding molecular entities of the human body was provided in the 1990s and, until today, by large-scale surveys and high-throughput data-collection projects. Examples include coding sequences [7,8], mRNA levels [2], enzymatic functions [9] and interactomics [10,11]. The catalog of elements (genes, mRNAs and proteins) is becoming more extensive as time passes. In order to make sense of this vast quantity of data, the challenge for systems biology now, at the beginning of the 21st Century, is to overcome the neglect of the dynamics that carry important functionality (e.g., the accumulation or depletion of a compound, oscillations, switches [on/off] and delays; see FIGURE 1 for an illustration of these dynamic motifs). Despite extensive data relating to the human body that is available now,

the connections between the molecular descriptions and the (emergent) systems phenomena (i.e., diseases, disorders or aging) are still mostly unclear.

Diseases arise from either genetic abnormalities, detrimental environmental factors (e.g., poor diet, infectious organisms and toxins), or a combination of these. Looking upon a disease as reflecting the operation of a perturbed network, modeling and simulating this network helps to identify key points for medical intervention. Computer-aided mathematical modeling can act as the missing link and fill the gap in our understanding of the dynamics of complex physiological processes. It can serve medical research by advancing the discovery and prioritization of drug targets or by eliminating toxic and ineffective compounds earlier [12,13]. In clinical research in particular, where a disease's etiology and potent drugs are known, understanding the disease's dynamics (onset, progression and control) can play a role in diagnosis, prognosis and prevention, as well as during treatment – namely by opening new ways of optimizing the administration of approved drugs.

The impact that systems biology starts to make and the contribution of modeling in different therapeutic areas, such as cancer research or immunology, will be examined in more detail in the last part of this review. In the following sections, some aspects of data acquisition, data integration and the pros and cons of some modeling approaches are discussed.

Data acquisition

Thanks to improving experimental technologies, it has become increasingly possible to tightly follow the concentrations of biochemical compounds in a cell and measure their changes. Most considerable, in this respect, are recent developments in real-time imaging (single cells or whole organs) and in system-wide measurements of the various -omes.

Microscopy has already been used to extract dynamic (time-series) data from the observation of living cells [14,15]. Real-time reconstruction has become possible through the combination of microscopy of living cells with automation technologies and with methods for image analysis (e.g., image or fluorescence correlation spectroscopy [16,17], or digital holography [18]).

Although some techniques (e.g., magnetic resonance imaging studies or metabolic profiling of biofluids) can already provide time-series data, most high-throughput technologies, such as DNA-, protein- or tissue microarrays [19], protein and metabolite profiling by mass spectrometry [20,21], or two-hybrid systems, which enable construction of maps of interactions among proteins [10,11], usually provide static pictures of the systems under investigation. For an anticipated dynamic outcome, namely time-series data, the setup of experiments has to be designed appropriately (i.e., permitting repetitions of measurements at certain time intervals and/or with the same sample or specimen). Furthermore, not only clean time-series data is of importance but also the choice of species to be measured and the kind and intensity of perturbation to be applied in an experiment [22]. Considering all this, a necessity for standard operating procedures has arisen in order to guarantee comparability of data attained by the still rather new -omics methods. The first successes of implementing standard operating procedures in systems biology have been reported [23,24].

The initial goal of the human genome sequence project was to obtain high-quality DNA sequence information from which all genes and their products could be predicted and excluded dynamic issues from its working definition [11]. Attempts are now underway to also define functional and regulatory elements, including their activity over time. Thus, although both the human genome sequence and the human interactome map provide useful biological insight by themselves, they should also be viewed as scaffold information from which systems-level models of gene or protein function can be derived.

Data integration

The information we can gain about a biological system appears, in practice, as an experimental observation, and research is restricted to the granularity and precision of the experimental techniques in use. Systems biology has evolved rapidly in the last few years, driven by the new high-throughput technologies. Data generated by these techniques are the basis for system-wide investigations. However, in order to validate such data in the system-wide hierarchical context, ranging from DNA to RNA to protein to interaction networks and further on to cells, organs and individuals, one needs to correlate and integrate such information. Thus, an important part of systems biology is data integration.

Data integration itself cannot explain the dynamic behavior of the biological system and cannot be a replacement for a mathematical model. However, it is extremely useful for increasing the information content of the individual experimental observation, enhancing the quality of the data and identifying relevant components in the model for the biological system. Both generation and analysis of genome, transcriptome and proteome data are becoming increasingly widespread and require merging for theoretical modeling.

On the lowest level of complexity, data integration defines common schemas for data storage, representation and transfer. This has already been established for particular experimental techniques, for example in the field of transcriptomics with Minimum Information About Microarray Experiment (MIAME) [23], in proteomics with Proteomics Experiment Data Repository (PEDRo) [25] and the Human Proteome Organisation's (HUPO) consortium [26]. On a more complex level, schemas have been defined for biological models and pathways, such as Systems Biology Markup Language [27] and CellML [28].

On a second level of complexity, data integration deals with query-based information retrieval, the connection of different data types (typically stored in different databases) and the visualization and presentation of the data. Here, for example, commercial applications such as the sequence retrieval system (SRS) are in use [29]. SRS provides a user interface that enables access to hundreds of biological databases. The EnsMart system, developed at the European Bioinformatics Institute, is an

advanced tool for data retrieval from database networks using a powerful query system [30]. Both systems allow a simple integration of additional resources and programs so that they are continuously growing.

Data integration on the next level of complexity consists of data correlation. This is a growing field as researchers combine information from multiple diverse data sets to learn about and explain natural processes [3,31]. For example, methods have been developed to integrate insights from transcriptome or proteome experiments with genome sequence annotations. The integration of data enables their explanation and analysis, for example, the comparison of gene expression patterns for orthologous genes or their evaluation in the light of conserved transcription factor binding sites in upstream regions of the corresponding gene sequences [32].

The highest level of data integration is the mapping of integrated experimental data from multiple sources onto networks in order to model interactions. These networks represent qualitative models for the biological system. For example, in a study of the galactose utilization pathway in yeast, several strains of yeast were employed, each with a different galactose gene knocked out, and changes were monitored in the levels of yeast genes using DNA arrays with the system in presence and in absence of galactose [3]. Together with known data, such as protein–protein and protein–DNA interactions, this enabled the authors to construct an entire physical interaction network of that pathway. Most of the network architecture is based on perturbation experiments and expression data. Several conclusions can be drawn from this and other studies: there appears to be a variety of small modules, similar to those found in engineering (feed-forward loops and single-input motifs). Current research attempts to classify such modules in a kind of lexicon for higher order functioning. By topological analysis, genes can be identified in these networks that may change fundamental properties of the system (e.g., hubs and articulation points) and give rise to suggestions for further perturbation experiments [33,34].

From the experimental side, the dynamic outcome, taking the form of time-series data, needs to be anticipated in the setup of the experiments. Appropriate standard operating procedures need to be implemented and software tools for data analysis are required to be applied. From the theoretical side, for the generation of new hypotheses, the choice of a suitable modeling approach is crucial, as is the design of meaningful models.

Kinetic modeling

Experimental data and theoretical models must be integrated in order to understand the dynamics and enable the quantitative prediction of the behavior of a system. Knowledge is gained through an iterative cycle, where models based on measurements lead to new hypotheses that are subsequently validated with further experiments (FIGURE 2).

Kinetic models are necessary for quantitative modeling and simulation. They are usually based on qualitative or informal models and hold descriptions of the kinetics of all the interactions in them. The choice of modeling framework depends on the biological system in question, as well as on the problem to be addressed. There are two fundamentally distinct ways of describing any dynamic system: the stochastic description (with master equations) and the deterministic description (with differential equations) [35]. One difference in interpretation of the two is the negligence of random fluctuations in the deterministic description, which might be distorting when simulating biological systems at low molecular concentrations [36].

A common deterministic approach is to use systems of ordinary differential equations that describe biochemical reaction networks [37,38]. Other possible deterministic approaches are formal languages, cellular automata, Boolean or Petri nets [39,40]. The ever more complex and/or comprehensive models can be interrogated, analyzed and simulated with the help of computers and employing several different strategies from system's theory, such as: stoichiometric analysis, sensitivity analysis and detection of rate-limiting steps, control analysis and stability and bifurcation analysis, robustness (against parameter changes) and optimization. The design of models, as well as their interrogation and simulation, relies on biological databases and modeling software tools (see [40] for a comprehensive list).

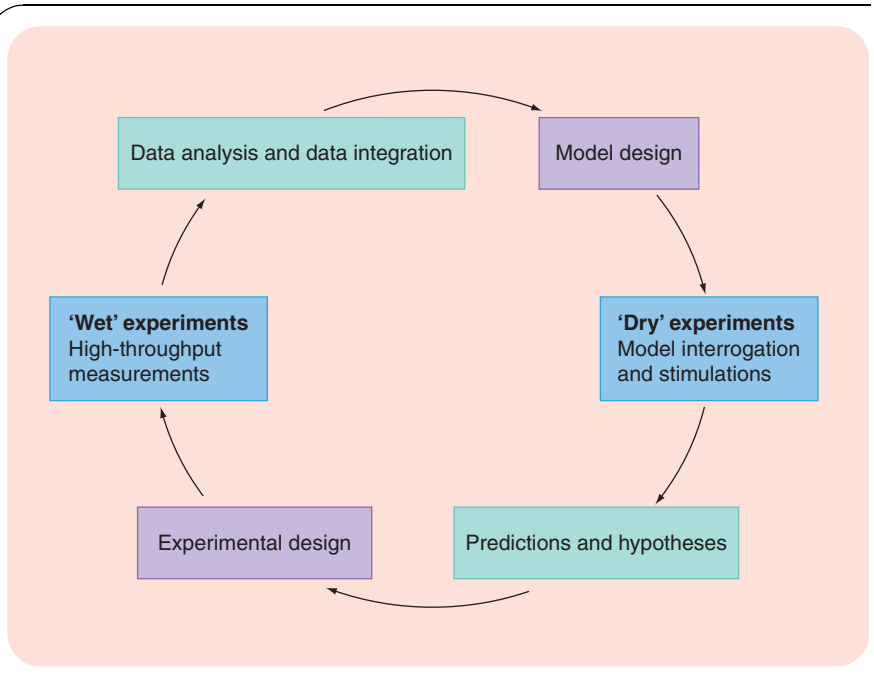

Figure 2. The iterative process of hypothesis forming and its experimental testing, as defined in the Scientific Method [100], is expanded in systems biology such that experiments become large-scale, genome-wide methods; whereas data integration and model simulation become manageable only with the aid of computers.

Modeling can be carried out at various levels of detail. There are different organizational levels of biological systems, all of which can be modeled within their level boundaries. A model can represent a cellular system, where it is concerned with a mechanism or network within a single cell [41], or it can represent a whole organ or an organ system. For cellular systems, there are numerous examples: models of transport mechanisms and membrane permeability; metabolic models (e.g., red blood cell metabolism [42,43] or mitochondrial energy metabolism [44]), models of cell-signaling pathways (e.g., mitogen-activated protein kinase signaling [45,46], Wnt signaling [47], Jak–Stat signaling [48], caspase activation [49]); or cell-cycle models [50]. Additionally, there are already clinically relevant computer models of intra- and intercellular signaling [51]. For the multicellular level, the most prominent example is the whole-heart model [52].

There are three major challenges concerning the integration of data and models. First, only a very limited number of time-series data sets are available. This will hopefully change within the next few years. Second, there is the problem of unsatisfactory comparability of data from different experiments and from different laboratories (e.g., owing to different conditions and cell lines). This is about to change with the introduction of standards for experimental protocols and conditions, which is underway. A general introduction and implementation of standard operating procedures will also prevent and minimize systematic errors and permit rational-error estimation. Third, models considering different organizational levels are a challenging task because the 10–12 order-of-magnitude span of time scales for events (e.g., ion channel gating 10^{-6} s, aging 10^{5} s) limits resolution. The different levels of organization and time scales that occur when considering the dynamics of biological systems are shown in FIGURE 3. Nevertheless, multiscale models, spanning more than one level, for example as envisioned in the Blue Brain Project [101] or Physiome Project [102], are the next undertakings.

In the first section, the importance of understanding the dynamics of biological systems has been discussed. The previous three sections provided a brief introduction into the means of achieving this understanding, via data collection and integration into suitable models. Finally, the following two sections focus on the role of understanding the dynamics of biological systems in medical research.

Role in medical research

Dynamic changes occur in both health and disease. In the former, the bodily functions vary within a certain acceptable state space (either owing to normal biological rhythms, such as the menstrual cycle or rapid eye movement cycle, or owing to the adjustments after a perturbation), whereas in the latter the bodily functions have perpetrated those boundaries considered to be normal. A principal goal of biomedical research is to understand disease mechanisms at the physiological level with the help of information derived from the cellular level. Models that describe even quite small systems (e.g., intracellular signal-transduction systems) provide predictive, quantitative

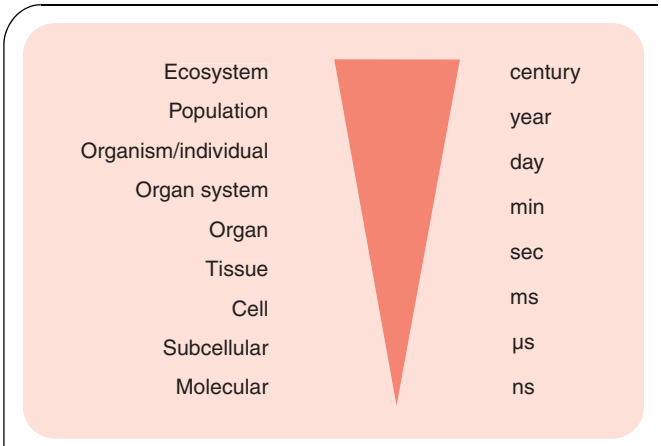

Figure 3. Levels of organization and time scales occurring in biological systems.

frameworks with the potential to aid drug-discovery efforts [51] or to guide therapy by suggesting points, perhaps in individual cell types of individual patients, for which small, perhaps multiple, simultaneous interventions might have great therapeutic value. The progress made in some therapeutic areas is presented in the following section but first the drug-discovery process and the therapy-framing instances of diagnosis, prognosis and prevention are addressed.

Modeling & simulation in drug discovery

The goal of drug discovery or pharmaceutical research is the cure or amelioration of diseases. It is confronted with the two central problems: the search for disease-related targets and the study of drug–protein interactions. The main issues associated with the discovery of effective new drugs are:

- Identification of a relevant drug target
- Identification of a drug that will appropriately perturb the target
- Recognition and avoidance of adverse properties of therapeutics
- Monitoring of clinical efficacy using surrogate markers
- Personalized approaches to disease treatment

The first three challenges must be met before commitment to clinical trials, since testing and efficacy screening (i.e., determination of the effectiveness of a drug and/or treatment) is the most costly and time-consuming step. For a rational drug design, especially concerning the first and third issues from above, systems biology offers powerful new approaches. These are stoichiometric analysis, sensitivity analysis and detection of rate-limiting steps, control analysis, stability and bifurcation analysis, robustness (against parameter changes) and optimization. In the past 10 years, considerable progress has been made with the use of computational approaches, in particular in the early stage of the drug discovery process. As a result, modeling and simulation becomes possible prior to any *in vivo* tests.

Completion of the human genome sequence provided access, in principle, to all protein primary sequences and, thus, to all potential drug targets [53]. Out of approximately

30,000–40,000 protein-coding genes, the OMIM database [103] lists over 3000 mutations with their phenotypes [54]. However, diseases are complicated processes, arising from a complex set of underlying genetic traits and environmental factors and it has since been recognized that novel therapies cannot be easily deduced from gene data only [55,56]. Mechanisms and intensity of interactions cannot be derived directly from the genome sequence. Postgenomic research must involve analyzing the dynamics of gene regulation [39].

Characterization of the metabolome, the thousands of products of metabolism derived from both internal and external sources, has already altered thinking about lead compound elaboration in drug discovery [57]. Prioritizing, or putting more emphasis on design and testing via simulation before fabrication, is a way to reduce the number of potential drug compounds usually produced by high-throughput drug screening and combinatorial-chemistry arrays to a smaller, more manageable number. Protein design technologies mature, enabling the setting of criteria and objectives for the biochemical and biophysical properties of the drug [13].

Pharmacokinetics, the study of the drug–organism interaction, in particular the investigation of absorption, distribution, metabolism, excretion and toxicological (ADME-Tox) processes, can also benefit from computational network analysis [12]. Pharmacokinetics aims at the recognition and, as far as possible, avoidance of adverse properties of therapeutics (such as the manifestation of deficiencies in absorption or the yielding of metabolites that have unfavorable side effects) by assessment of the possible side effects and pharmaceutical properties of the drug before its deployment in clinical trials.

From diagnosis to treatment to prevention

For diagnosis, a fast and confident detection of pathological changes in cells that indicate diseases, dysfunction or malignity is important and its continued improvement is desired. Diagnostic tools are based on comparison and thus rely on the identification and classification of differences between healthy and normal cells, and diseased and abnormal cells (or between normal physiological parameters and abnormal ones, respectively). Such differences are best found by screening methods (the subject of many reviews in this journal), which are predominantly comparative, and do not consider dynamics. Conversely, a better understanding of the dynamics of a disease process may contribute to an earlier diagnosis, since it permits not only predictions but also backtracing of a system's behavior. Simulations of an appropriate model can indicate conditions or physiological parameters that are significantly altered even before the current point-of-detection of a disease, thus allowing us to move the moment of possible diagnosis closer to disease onset. One example comes from diabetes research, where an observed impaired pulsatile release in early Type 2 diabetes suggests that monitoring insulin pulsatility can lead to an improvement of the present diagnostic method, which relies on two measurements at single time points only [58].

In treatment, the overall success rate of a therapy and the assessment of its risks and benefits are important. Owing to the heterogeneity of individuals (i.e., differences in disease susceptibility and disease characteristics due to inherited traits, circumstances of life and lifestyle choices), their response to one and the same therapy differs. This stratification is exploited, for example, in the identification of tumor susceptibility and the prediction of response to a therapeutic intervention by genetic characteristics: clinical response to gefitinib therapy depends on mutations in epidermal growth factor receptor [59,60]. Besides the appropriate choice of drug according to disease as well as patient, the best timing of administration (note the working example of cancer chronotherapy [61]) and optimal doses will become part of a sophisticated personalized therapy. There are already some examples of computer programs that have been validated and can now be applied to certain clinical and administrative problems: Archimedes, developed for diabetes modeling, predicts diabetes-related clinical outcomes [62]; Optimata's Virtual Cancer Patient Engine is to be used for personalizing cancer treatment and in drug development [63]; or the human cancer cell simulation DigitalCell from Gene Network Sciences [64]. In the future, computer programs that predict the dynamic response of individuals to a treatment can be consulted in order to deliver the right therapy (type of drug, best timing and dosage) to the appropriate patient.

Therapeutic areas

A therapeutic area is a medical domain or specialized sub-branch of medicine that deals with particular body systems, diseases or sectors of health. Not all areas can be considered herein, but for some of the therapeutic areas exemplary dynamic issues are discussed briefly. This section is by no means a comprehensive account, it only intends to supply some examples.

Oncology

Oncology includes the characterization, diagnosis and treatment of malignancies. The concerns for clinical oncologists are to detect cancer as early as possible, to identify effective anticancer agents, to apply them selectively (i.e., maximizing the concentration of agents in the cells of abnormal tissue while minimizing the effects on surrounding normal tissue) and to alleviate and ease the side effects of the chosen treatment.

Experimental oncology (by way of human genetics and molecular biology) is awash with data. Hundreds of genes that promote or enhance cancer have been identified [65] and multitudinous cancer-related alterations in the structure and function of macromolecules that control the cell cycle or other essential processes were stated [66]. There are (proto)oncogenes, tumor-suppressor genes and DNA-repair genes, among others [67].

Oncology is an exemplary field for the use of mathematical modeling. Cancer arises as a consequence of somatic evolution and evolutionary concepts, such as mutation and selection. This can be best described when formulated as mathematical equations [68]. Thus, the mathematical approach can be used to understand the process of cancer initiation and progression. Mathematical modeling of cancer growth is now beginning to take into account experimental

data and information over a wide range of spatial and temporal scales (genetic, intracellular, extracellular, cell–cell and cell–tissue interactions), resulting in multiscale models.

A specific example from the cellular or molecular level is the investigation of protein p53. The factor p53 has long been known as a tumor-suppressing gene and has been found to be mutated in more than 50% of all human tumors [69]. Dynamic modeling of the p53 network [70,71] is essential in the investigation of p53 involvement in cell-cycle regulation [72]. Another example is a mathematical model that explains the molecular response to imatinib and permits the calculation of the probability of developing imatinib-resistance mutations and estimates the time until detection of resistance [73]. Also on the cellular level, in the search for anticancer drugs, the development of reference models of both normal and tumor cells has been proposed [74]. Such models could be used to analyze the dynamic behavior in response to various stimuli.

On the multicellular level, besides mathematical modeling of tumorigenesis (e.g., colorectal tumorigenesis [74]), the dynamics of cancer progression, of tumor angiogenesis and vascularization, of solid tumor growth and invasion, are of interest [75]. Models for these must consider tumor heterogeneity, in the sense of different cell types of a tumor (stromal or vascular neoplastic), with differing gene expression [76].

A more clinical aspect is the challenge of directed delivery of drugs to abnormal cells only (e.g., of cytotoxins selective for tumor cells). This can be achieved by the utilization of tumor-induced acidification and mitochondrial hyperpolarization. Tumor-induced acidification results from altered metabolic patterns characteristically exhibited by malignant cells when compared with normal mammalian cells, namely an increased reliance on anaerobic metabolism of glucose to lactic acid even in the presence of abundant oxygen [77]. Mitochondrial hyperpolarization is a shared feature of many tumor cell lines [78], and explains the accumulation of molecules with a delocalized positive charge in the mitochondria of responsive cells. An understanding of these two principles of chemical uptake enables their combination and the application of the theory to the problem of targeting human tumor cells [79]. Another method of delivery of cytotoxic agents selectively to cancer cells is the development of monoclonal antibodies with improved tumor targeting [80].

Cancer is caused by genetic changes in tumor cells, which lead to the aberrant expression of genes involved in regulating signal transduction, metabolism and other biological processes. For many cancer types, the crucial tumor-initiating events are well known. This has opened the way for novel specific therapeutic strategies aiming at inhibiting aberrantly overexpressed proteins or interfering with cellular processes such that proteins with low expression become activated. However, the processes involved in tumor formation and progression are not fully understood since detailed quantitative strategies are lacking. Disease-specific alterations of these pathways have thus been subject to intensive modeling studies. An example of such processes is the epidermal growth factor receptor (EGFR) pathway [81]. Members of the EGFR family are the primary targets for modern drugs. For example, overexpressed EGFR2 protein (ERBB2) is the basis for the highly successful Herceptin® therapy in breast cancer patients. Novel drugs such as Iressa™ and Tarceva™ are highly efficient in inhibiting the EGFR1 cell-surface receptor in several types of tumors, especially lung cancer, which still has the highest death toll among all cancer types. This is due to the fact that EGFR1 is overexpressed in the lung cancer tissues of most patients who are suffering from nonsmall cell lung cancer. Initial aspects of this pathway are described in computer models [82,83]. A comprehensive pathway map that covers more than 200 reactions has been published recently [84].

Cancer has now been recognized as a multifactorial disease that can greatly benefit from the systems biology approach [85].

Immunology & infectious diseases

The therapeutic area of immunology comprises a wide range of medical fields, from autoimmune diseases to inflammation and transplantation medicine, to name but a few. Contributions to the field through mathematical modeling of dynamics are, for example, the concepts of optimization of the immunization coverage of a population by booster or pulse vaccination [86], T-cell vaccination against autoimmune diseases [87] and a better understanding of the phenomenon of immunotherapy of allergies [88]. These are all results from the rather traditional field of theoretical immunology (more examples are reviewed in [89]).

More recent subjects are the modeling of HIV infections (e.g., population dynamics of the virus within an infected individual and evolution of drug resistance in response to therapy [90]) or modeling of control strategies of respiratory pathogens (e.g., quantitative insight into the heterogeneity of severe acute respiratory syndrome [SARS] outbreaks worldwide with implications for the assessment of public health strategies [91]).

Another study investigated acute systematic inflammation in humans [92]; clinical perturbation was monitored by measuring the gene expression in blood leukocytes for 24 h after administration of the immune stimulus, endotoxin. The overwhelming diversity of possible genome-wide interactions and gene-expression patterns limit effective learning from experimental data alone and a network analyses was employed that then revealed that the systemic inflammatory response includes widespread suppression at the transcriptional level of mitochondrial energy production and protein-synthesis machinery [92].

Other therapeutic areas

In the field of nutritional and metabolic diseases, modeling of pathways has been applied to better understand disease-state physiology in a variety of subcellular, cellular and organ systems, including mitochondria, cancerous cells, liver and heart. Significant accomplishments that were made using metabolic engineering (i.e., methods to model complex metabolic pathways and techniques to manipulate these pathways for a desired metabolic outcome) show the potential of applying both

metabolic modeling and pathway manipulation [93]. Metabolic pathway engineering has also been used to generate cells with novel biochemical functions for therapeutic use (specific examples in the areas of glycosylation engineering and dopamine replacement therapy are provided in [93]).

In the Cardiome project, a multiscale model is envisioned that augments Denis Noble's detailed cardiac model with the addition of regional blood flows, substrate uptake and metabolism, energy production and utilization in serving contraction and ionic balances [52].

Several neurodegenerative diseases, such as Alzheimer, Parkinson's or Huntington's diseases, are cumulative or progressive. An understanding of the dynamics of these complex diseases could help to develop strategies to halt them at the stage they have reached at detection or to prevent them entirely. The tetanus of the subthalamic nucleus in Parkinson's disease [94], where the function of the system is recovered by inhibiting an inhibitory loop, is one of the first applications of systems thinking to medical treatment.

An example from the area of regenerative medicine, which is concerned with wound and bone healing and the improvement of implants, is a mathematical model that explains the interactions between osteoblasts and osteoclasts [95]. The model is able to simulate metabolic bone diseases, such as estrogen deficiency, vitamin D deficiency, senescence and glucocorticoid excess. Furthermore, it allows testing and evaluation of possible routes for therapeutic interventions.

Expert commentary

A systemic view of human physiology is necessary to advance our knowledge of the workings of the human body and, with this, our prospects to treat or heal its failures or injuries. Understanding dynamic behavior through the use of computer models with predictive power will play an increasingly important role in medical research.

Most diagnostic methods are based on comparison and do not consider dynamic changes directly, but it is possible that indirectly, through an understanding of the dynamics of the onset and course of a disease or cancer, an earlier diagnosis could become possible. Understanding the dynamics means not only the ability to foresee the consequences of a perturbation (thus, prediction of the response), but also to be able to backtrace and thus define earlier detection points.

Medical research is just beginning to pursue quantitative methods to consolidate the vast body of data and integrate rapidly accumulating new information in the different fields. Cancer and complex chronic diseases, such as diabetes, will be among the beneficiaries of a dynamic and systemic view of the human body [85,96]. Examples from ongoing research in oncology, immunology, infectious diseases and neurodegenerative diseases given in this review show how systems biology can contribute to the understanding of complex disease processes. They should attenuate reservations that the systems approach must still show its usefulness.

In our view, systems biology will inevitably change the rules that govern the selection and development of new therapeutics and will catalyze the development of personalized, predictive and preventive medicine in the next decade. In the future, it will add to and advance medical research, by aiding drug discovery and by testing of treatments on computer models, which may lead to a decrease in the frequent failure rate of clinical decision-making and contributes to an improvement of the success rates of drug discovery programs. A better understanding of the dynamics of complex diseases and healing processes will improve disease stratification, enable individualized therapy and, ultimately, preventive drugs.

Five-year view

In data acquisition, not more capacity but better comparability of experiments and measurements is the order of the day. This request calls for the improvement of the standardization of experiments through standard operating procedures. Measurements undertaken in this manner will also combat the lack of experimental data from which biological parameters (of crucial importance for kinetic models) could be estimated [24,97]. Furthermore, besides aiming for more and comparable time-series data, the planning and design of experiments will also improve through the development of realistic experimental planning tools [22] that help to optimize the choice of the species to be measured and of the correct perturbation to be applied, so that the generated data are suitable to further the modeling process.

An advanced ability to measure biological parameters together with increases in biological understanding and ccomputational methods and computer power, make it possible to foresee the construction of useful and predictive simulations of cellular processes. By integrating a number of models into one 'virtual cell', the aim is to understand cellular systems on the basis of the characteristics of their components [98,99]. Several virtual cell projects have taken up this challenge (e.g., the E-cell [104], the Silicon Cell Initiative [105,106], the Virtual Cell [107] and HepatoSys [108]).

On the next higher level, the construction of useful and predictive simulations not only of cellular processes but also of whole organs or even of a whole organism is underway; see, for example, the biomedical simulations on SimTK [109] or the Virtual Human [102] that claims to be the successor of the Human Genome Project.

Acknowledgements

Heike Assmus is supported by the German Federal Ministry of Education and Research (BMBF) grant 01GR0475 as part of the National Genome Research Network (NGFN-2). The responsibility for the content lies with the authors. We thank the referees for their comments and apologize to the many investigators whose important studies could not be cited directly here owing to space limitations.

Key issues

- Time-dependent behavior is observed at all organizational levels of biological systems.
- Experimental methods for its detection and appropriate standard operating procedures improve rapidly. Systems biology supports a rational design of experiments.
- Mathematical modeling enables simulation of the observed dynamic behavior and leads to a better understanding of such dynamics.
- Modeling allows quantitative predictions (i.e., to foresee the consequences of perturbations and external stimuli). Understanding is tantamount to prediction.
- Models for simulation can be of cellular processes (e.g., cell signaling), whole organs (e.g., heart physiology and tumor growth) or individuals (e.g., drug metabolism and virtual patient).
- The progress made through studying dynamics is exemplified for a few therapeutic areas, oncology, immunology and diabetes.
- Systems biology contributes to the understanding of complex disease processes, and will lead to practical innovations in drug discovery, diagnostics and personalized medicine.

References

Papers of special note have been highlighted as:
• of interest
•• of considerable interest

1 Wolkenhauer O, Mesarovic M. Feedback dynamics and cell function: why systems biology is called systems biology. *Mol. BioSystems* 1(1), 14–16 (2005).

•• View is emphasized that systems dynamics and the organizing principles of complex biological phenomena give rise to the functioning and function of cells. Also proposes an agenda for systems biology.

2 Iyer VR, Eisen MB, Ross DT *et al.* The transcriptional program in the response of human fibroblasts to serum. *Science* 283(5398), 83–87 (1999).

• Describes temporal changes in 8600 human genes monitored during a physiological response and offers some interpretation for observed patterns.

3 Ideker T, Thorsson V, Ranish JA *et al.* Integrated genomic and proteomic analyses of a systematically perturbed metabolic network. *Science* 292(5518), 929–934 (2001).

4 Kholodenko BN, Kiyatkin A, Bruggeman FJ, Sontag E, Westerhoff HV, Hoek JB. Untangling the wires: a strategy to trace functional interactions in signaling and gene networks. *Proc. Natl Acad. Sci. USA* 99(20), 12841–12846 (2002).

5 Nicholson JK, Holmes E, Lindon JC, Wilson ID. The challenges of modeling mammalian biocomplexity. *Nat. Biotechnol.* 22(10), 1268–1274 (2004).

• Focusing on metabolism, the authors argue that it is important to measure system responses of stimuli (drug or disease) through time as this can show both the development of a lesion with multiple organ effects occurring at different times and also the recovery process.

6 Westerhoff HV, Palsson O. The evolution of molecular biology into systems biology. *Nat. Biotechnol.* 22(10), 1249–1252 (2004).

7 International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. *Nature* 409(6822), 860–921 (2001).

8 Venter CJ, Adams MD, Myers EW *et al.* The sequence of the human genome. *Science* 291(5507), 1304–1351 (2001).

9 Schomburg I, Chang A, Hofmann O *et al.* BRENDA: a resource for enzyme data and metabolic information. *Trends Biochem Sci.* 27(1), 54–56 (2002).

10 Stelzl U, Worm U, Lalowski M *et al.* A human protein–protein interaction network: a resource for annotating the proteome. *Cell* 122(6), 957–968 (2005).

11 Rual JF, Venkatesan K, Hao T *et al.* Towards a proteome-scale map of the human protein–protein interaction network. *Nature* 437(7062), 1173–1178 (2005).

12 Somogyi R, Greller LD. The dynamics of molecular networks: applications to therapeutic discovery. *Drug Discov. Today* 6(24), 1267–1277 (2001).

•• Description of how models that capture different aspects of network dynamics can be generated and applied in disease pathways identification, drug screening, diagnostics and individualized therapy.

13 Rao BM, Lauffenburger DA, Wittrup KD. Integrating cell-level kinetic modeling into the design of engineered protein therapeutics. *Nat. Biotechnol.* 23(2), 191–194 (2005).

•• Outlines the application of kinetic modeling in the drug discovery process and the use of models in defining biophysical design objectives for the engineering of proteins.

14 Nelson G, Wilde GJC, Spiller DG *et al.* Dynamic analysis of STAT6 signalling in living cells. *FEBS Lett.* 532(1–2), 188–192 (2002).

15 Nelson DE, Ihekwaba AEC, Elliott M *et al.* Oscillations in NF-κB signaling control the dynamics of gene expression. *Science* 306(5696), 704–708 (2004).

16 Schwille P, Haupts U, Maiti S, Webb WW. Molecular dynamics in living cells observed by fluorescence correlation spectroscopy with one- and two-photon excitation. *Biophys. J.* 77(4), 2251–2265 (1999).

17 Digman MA, Brown CM, Sengupta P, Wiseman PW, Horwitz AR, Gratton E. Measuring fast dynamics in solutions and cells with a laser scanning microscope. *Biophys. J.* 89(2), 1317–1327 (2005).

18 Xu WB, Jericho MH, Meinertzhagen IA, Kreuzer HJ. Digital in-line holography for biological applications. *Proc. Natl Acad. Sci. USA* 98(20), 11301–11305 (2001).

19 Ewis AA, Zhelev Z, Bakalova R *et al.* A history of microarrays in biomedicine. *Expert Rev. Mol. Diagn.* 5(3), 315–328 (2005).

20 Goodacre R, Vaidyanathan S, Dunn WB, Harrigan GG, Kell DB. Metabolomics by numbers: acquiring and understanding global metabolite data. *Trends Biotechnol.* 22(5), 245–252 (2004).

21 Fernie AR, Trethewey RN, Krotzky AJ, Willmitzer L. Innovation – metabolite profiling: from diagnostics to systems biology. *Nature Rev. Mol. Cell Biol.* 5(9), 763–769 (2004).

22 Gadkar KG, Varner J, Doyle III FJ. Model identification of signal transduction networks from data using a state regulator problem. *IEE Proc. Systems Biology* 2(1), 17–30 (2005).

23 Brazma A, Hingamp P, Quackenbush J *et al.* Minimum information about a microarray experiment (MIAME) – toward standards for microarray data. *Nat. Genet.* 29, 365–371 (2001).

• Standard for recording and reporting microarray-based gene expression data, Minimum Information About Microarray Experiment (MIAME), has since facilitated the establishment of databases and public repositories and enabled the development of data analysis tools.

24 Faller D, Klingmüller U, Timmer J. Simulation methods for optimal experimental design in systems biology. *Simulation* 79(12), 717–725 (2003).

• Describes the introduction and successful implementation of standard operating procedures for the design of quantitative dynamic experiments, using the MAP-kinase pathway as an example.

25 Taylor CF, Paton NW, Garwood KL *et al.* A systematic approach to modeling, capturing, and disseminating proteomics experimental data. *Nat. Biotechnol.* 21(3), 247–254 (2003).

26 Hermjakob H, Montecchi-Palazzi L, Bader G *et al.* The HUPO PSI's molecular interaction format – a community standard for the representation of protein interaction data. *Nat. Biotechnol.* 22(10), 177–183 (2004).

27 Hucka M, Finney A, Sauro HM *et al.* The systems biology markup language (SBML): a medium for representation and exchange of biochemical network models. *Bioinformatics* 19(4), 524–531 (2003).

• Introduction to the Systems Biology Markup Language (SBML).

28 Lloyd CM, Halstead MD and Nielsen PF. CellML: its future, present and past. *Prog. Biophys. Mol. Biol.* 85, 433–450 (2004).

29 Etzold T, Ulyanov A, Argos P. SRS: information retrieval system for molecular biology data banks. *Methods Enzymol.* 266, 114–128 (1996).

30 Kasprzyk A, Keefe D, Smedley D *et al.* EnsMart: a generic system for fast and flexible access to biological data. *Genome Res.* 14(1), 160–169 (2004).

31 Gitton Y, Dahmane N, Baik S *et al.* A gene expression map of human chromosome 21 orthologues in the mouse. *Nature* 420(6915), 586–590 (2002).

32 Tavazoie S, Hughes JD, Campbell MJ, Cho RJ, Church GM. Systematic determination of genetic network architecture. *Nat. Genet.* 22(3), 281–285 (1999).

33 Hwang D. A data integration methodology for systems biology. *Proc. Natl Acad. Sci. USA* 102(48), 17296–17301 (2005).

34 Hwang D. A data integration methodology for systems biology: experimental verification. *Proc. Natl Acad. Sci. USA* 102(48), 17302–17307 (2005).

35 Wolkenhauer O, Ullah M, Kolch W, Cho KH. Modeling and simulation of intracellular dynamics: Choosing an appropriate framework. *IEEE Trans. Nanobioscience* 3(3), 200–207 (2004).

• By comparing the mathematical bases of the two popular conceptual frameworks, stochastic simulation and rate equation models, the authors explore the question in which conceptual framework one should investigate intracellular dynamics.

36 Rao CV, Wolf DM, Arkin AP. Control, exploitation and tolerance of intracellular noise. *Nature* 420(6912), 231–237 (2002).

37 Tyson JJ, Chen KC, Novak B. Sniffers, buzzers, toggles and blinkers: dynamics of regulatory and signaling pathways in the cell. *Curr. Opin. Cell Biol.* 15(2), 221–231 (2003).

•• Comprehensive overview of signal-response elements, the basic building blocks of dynamic behavior.

38 Wolkenhauer O, Ullah M, Wellstead P, Cho KH. The dynamic systems approach to control and regulation of intracellular networks. *FEBS Lett.* 579(8), 1846–1853 (2005).

39 de Jong H. Modeling and simulation of genetic regulatory systems: A literature review. *J. Comp. Biol.* 9(1), 67–103 (2002).

• Review of formalisms that have been employed in mathematical biology and bioinformatics to describe genetic regulatory systems (in particular directed graphs, Bayesian networks, Boolean networks and their generalizations, ordinary and partial differential equations, qualitative differential equations, stochastic equations and rule-based formalisms), and of the usage of these formalisms in the simulation of the behavior of actual regulatory systems.

40 Klipp E, Herwig R, Kowald A, Wierling C, Lehrach H. *Systems Biology in Practice. Concepts, Implementation and Application.* Wiley-VCH, Weinheim, Germany (2005).

• In this very recent textbook, the first one focusing on systems biology, current strategies of computational modeling and data mining are presented.

41 Arkin AP. Synthetic cell biology. *Curr. Opin. Biotechnol.* 12(6), 638–644 (2001).

42 Schuster R, Jacobasch G, Holzhutter HG. Mathematical-modeling of metabolic pathways affected by an enzyme deficiency - energy and redox metabolism of glucose-6-phosphate-dehydrogenase-deficient erythrocytes. *Eur. J. Biochem.* 182(3), 605–612 (1989).

• Early example of relating mathematical modeling to medical problems.

43 Jamshidi N, Wiback SJ, Palsson BO. *In silico* model-driven assessment of the effects of single nucleotide polymorphisms (SNPs) on human red blood cell metabolism. *Genome Res.* 12(11), 1687–1692 (2002).

44 Cortassa S, Aon MA, Marbán E, Winslow RL, O'Rourke B. An integrated model of cardiac mitochondrial energy metabolism and calcium dynamics. *Biophys. J.* 84(4), 2734–2755 (2003).

45 Kholodenko BN. Negative feedback and ultrasensitivity can bring about oscillations in the mitogen-activated protein kinase cascades. *Eur. J. Biochem.* 267(6), 1583–1588 (2000).

46 Hornberg JJ, Binder B, Bruggeman FJ, Schoeberl B, Heinrich R, Westerhoff HV. Control of MAPK signalling: from complexity to what really matters. *Oncogene* 24(36), 5533–5542 (2005).

47 Lee E, Salic A, Krueger R, Heinrich R, Kirschner MW. The roles of APC and axin derived from experimental and theoretical analysis of the *Wnt* pathway. *PLoS Biology* 1(1), 116–132 (2003).

48 Swameye I, Müller TG, Timmer J, Sandra O, Klingmüller U. Identification of nucleocytoplasmic cycling as a remote sensor in cellular signaling by databased modeling. *Proc. Natl Acad. Sci. USA* 100(3), 1028–1033 (2003).

49 Fussenegger M, Bailey JE, Varner J. A mathematical model of caspase function in apoptosis. *Nat. Biotechnol.* 18(7), 768–774 (2000).

50 Tyson JJ, Novak B. Regulation of the eukaryotic cell cycle: molecular antagonism, hysteresis, and irreversible transitions. *J. Theoret. Biol.* 210(2), 249–263 (2001).

51 Rajasethupathy P, Vayttaden SJ, Bhalla US. Systems modeling: a pathway to drug discovery. *Curr. Opin. Chem. Biol.* 9(4), 400–406 (2005).

52 Noble D. Modeling the heart – from genes to cells to the whole organ. *Science* 295(5560), 1678–1682 (2002).

53 Hood L, Perlmutter RM. The impact of systems approaches on biological problems in drug discovery. *Nat. Biotechnol.* 22(10), 1215–1217 (2004).

54 Hamosh A, Scott AF, Amberger J, Bocchini C, Valle D, McKusick VA. Online Mendelian Inheritance in man (OMIM), a knowledgebase of human genes and genetic disorders. *Nucleic Acids Res.* 30(1), 52–55 (2002).

55 Butcher EC, Berg EL, Kunkel EC. Systems biology in drug discovery. *Nat. Biotechnol.* 22(10), 1253–1259 (2004).

56 Fishman MC, Porter JA. Pharmaceuticals – a new grammar for drug discovery. *Nature* 437(7058), 491–493 (2005).

57 Nicholson JK, Connelly J, Lindon JC, Holmes E. Metabonomics: a platform for studying drug toxicity and gene function. *Nat. Rev. Drug Discov.* 1(2), 153–161 (2002).

58 Porksen N, Hollingdal M, Juhl C, Butler P, Veldhuis JD, Schmitz O. Pulsatile insulin secretion: detection, regulation, and role in diabetes. *Diabetes* 51(Suppl.), S245–S254 (2002).

59 Lynch TJ, Bell DW, Sordella R *et al.* Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N. Engl. J. Med.* 350(21), 2129–2139 (2004).

60 Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S *et al.* EGFR mutations in lung cancer: Correlation with clinical response to gefitinib therapy. *Science* 304(5676), 1497–1500 (2004).

61 Mormont MC, Levi F. Cancer chronotherapy: principles, applications, and perspectives. *Cancer* 97(1), 155–169 (2003).

62 Eddy DM, Schlessinger L. Archimedes – a trial-validated model of diabetes. *Diabetes Care* 26(11), 3093–3101 (2003).

63 Agur Z, Arakelyan L, Belilty G *et al.* Application of the virtual cancer patient engine (VCPE) for improving oncological treatment design. *J. Clin. Oncol.* 22(14), 50S 692 Suppl. (2004).

64 Christopher R, Dhiman A, Fox J, *et al.* Data-driven computer simulation of human cancer cell. *Ann. NY Acad. Sci.* 1020, 132–153 (2004).

65 Futreal PA, Coin L, Marshall M *et al.* A census of human cancer genes. *Nat. Rev. Cancer* 4(3), 177–183 (2004).

66 Balmain A, Gray J, Ponder B. The genetics and genomics of cancer. *Nat. Genet.* 33, 238–244 (2003).

67 Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. *Nat. Med.* 10(8), 789–799 (2004).

68 Michor F, Iwasa Y, Nowak MA. Dynamics of cancer progression. *Nat. Rev. Cancer* 4(3), 197–205 (2004).

69 May P, May E. Twenty years of p53 research: structural and functional aspects of the p53 protein. *Oncogene* 18(53), 7621–7636 (1999).

70 Aguda BD. A quantitative analysis of the kinetics of the G(2) DNA damage checkpoint system. *Proc. Natl Acad. Sci. USA* 96(20), 11352–11357 (1999).

71 Bar-Or RL, Maya R, Segel LA, Alon U, Levine AJ, Oren M. Generation of oscillations by the p53-*Mdm2* feedback loop: a theoretical and experimental study. *Proc. Natl Acad. Sci. USA* 97(21), 11250–11255 (2000).

•• Mathematical model of the regulation of *p53* tumor suppressor activity through its transcriptional target mdm2 predicts, as a stress response, oscillations in p53 and Mdm2 protein levels. Such oscillations (that may allow cells to repair their DNA without risking the irreversible consequences of continuous excessive p53 activation) indeed occur on exposure of various cell types to ionizing radiation.

72 Kitano H. Cancer as a robust system: implications for anticancer therapy. *Nat. Rev. Cancer* 4(3), 227–235 (2004).

73 Michor F, Hughes TP, Iwasa Y *et al.* Dynamics of chronic myeloid leukaemia. *Nature* 435(7046), 1267–1270 (2005).

•• Model provides quantitative insights into the *in vivo* kinetics of a human cancer.

74 Michor F, Iwasa Y, Rajagopalan H, Lengauer C, Nowak MA. Linear model of colon cancer initiation. *Cell Cycle* 3(3), 358–362 (2004).

• Colorectal tumorigenesis was studied with a mathematical model, in which a linear flow from stem cells to differentiated cells to apoptosis was assumed. Simulations of the consequences of mutations in different cell types imply a role of early emergence of chromosomal instability in colorectal tumorigenesis.

75 Komarova NL. Mathematical modeling of tumorigenesis: mission possible. *Curr. Opin. Oncol.* 17(1), 39–43 (2005).

•• Reviews recent advances (since July 2003) in the fast-growing research area of mathematical modeling of tumorigenesis, also discusses possible implications for the field of cancer biology in general.

76 Greller LD, Tobin FL, Poste G. Tumor heterogeneity and progression: conceptual foundations for modeling. *Invasion Metastasis* 16(4–5), 177–208 (1996).

77 Gatenby RA, Gawlinski ET. The glycolytic phenotype in carcinogenesis and tumor invasion. *Cancer Res.* 63(14), 3847–3854 (2003).

78 Fantin VR, Berardi MJ, Scorrano L, Korsmeyer SJ, Leder P. A novel mitochondriotoxic small molecule that selectively inhibits tumor cell growth. *Cancer Cell* 2(1), 29–42 (2002).

79 Trapp S, Horobin RW. A predictive model for the selective accumulation of chemicals in tumor cells. *Eur. Biophys. J.* 34(7), 958–966 (2005).

80 Adams GP, Weiner LM. Monoclonal antibody therapy of cancer. *Nat. Biotechnol.* 23(9), 1147–1157 (2005).

• Overview of monoclonal antibody therapy of cancer.

81 Hynes NE, Lane HA. ERBB receptors and cancer: the complexity of targeted inhibitors. *Nat. Rev. Cancer* 5, 341–354 (2005).

82 Kholodenko BN, Demin OV, Moehren G, Hoek JB. Quantification of short term signaling by the epidermal growth factor receptor. *J. Biol. Chem.* 274(42), 30169–30181 (1999).

83 Resat H, Ewald JA, Dixon DA, Wiley HS. An integrated model of epidermal growth factor receptor trafficking and signal transduction. *Biophys. J.* 85(2), 730–743 (2003).

84 Oda K, Matsuoka Y, Funahashi A, Kitano H. A comprehensive pathway map of epidermal growth factor receptor signaling. *Mol. Syst. Biol.* 1, E1–E17 (2005).

85 Hornberg JJ, Bruggeman FJ, Westerhoff HV, Lankelma J. Cancer: a systems biology disease. *Biosystems* 83(2–3), 81–90 (2006).

86 Agur Z, Cojocaru L, Mazor G, Anderson RM, Danon YL. Pulse mass measles vaccination across age cohorts. *Proc. Natl Acad. Sci. USA* 90(24), 11698–11702 (1993).

87 Segel LA, Jager E, Elias D, Cohen IR. A quantitative model of autoimmune-disease and T-cell vaccination: does more mean less? *Immunol. Today* 16(2), 80–84 (1995).

88 Fishman MA, Segel LA. Modeling immunotherapy for allergy. *Bull. Math Biol.* 58(6), 1099–1121 (1996).

89 Segel LA. Multiple attractors in immunology: theory and experiment. *Biophys. Chem.* 72(1), 223–230 (1998).

• Selective survey that discusses the relative merits of various modeling approaches in immunology, and also assesses the ability of the different models to contribute to deeper biological understanding.

90 Bretscher MT, Althaus CL, Muller V, Bonhoeffer S. Recombination in HIV and the evolution of drug resistance: for better or for worse? *Bioessays* 26(2), 180–188 (2004).

91 Meyers LA, Pourbohloul B, Newman MEJ, Skowronski DM, Brunham RC. Network theory and SARS: predicting outbreak diversity. *J. Theoret. Biol.* 232(1), 71–81 (2005).

92 Calvano SE, Xiao WZ, Richards DR *et al*. A network-based analysis of systemic inflammation in humans. *Nature* 437(7061), 1032–1037 (2005).

93 Yarmush ML, Banta S. Metabolic engineering: advances in the modeling and intervention in health and disease. *Ann. Rev. Biomed. Eng.* 5, 349–381 (2003).

•• Documents the potential of applying both metabolic modeling and pathway manipulation, and describes pertinent advances in the field of diabetes research.

94 Chevalier G, Deniau JM. Disinhibition as a basic process in the expression of striatal functions. *Trends Neurosci.* 13(7), 277–280 (1990).

95 Lemaire V, Tobin FL, Greller LD, Cho CR, Suva LJ. Modeling the interactions between osteoblast and osteoclast activities in bone remodeling. *J. Theoret. Biol.* 229(3), 293–309 (2004).

96 Kitano H, Oda K, Kimura T *et al*. Metabolic syndrome and robustness tradeoffs. *Diabetes* 53(Suppl.), S6–S15 (2004).

97 Schilling M, Maiwald T, Bohl S *et al*. Quantitative data generation for systems biology – the impact of randomisation, calibrators and normalisers. *IEE Proc. Systems Biology* 152(4), 193–200 (2005).

• Authors show how a limiting lack of reliable quantitative data can be overcome by data correction and standardization, taking the technique of immunoblotting as example.

98 Tomita M. Whole-cell simulation: a grand challenge of the 21st century. *Trends Biotechnol.* 19(6), 205–210 (2001).

99 Snoep JL. The Silicon Cell initiative: working towards a detailed kinetic description at the cellular level. *Curr. Opin. Biotech.* 16(3), 336–343 (2005).

100 Bunge MA. *Scientific Research.* Springer, Berlin, Germany (1967).

Websites

101 Blue Brain Project - Modeling the Mammalian Brain
www.bluebrainproject.epfl.ch

102 IUPS Physiome project
www.physiome.org.nz

103 Online Mendelian Inheritance in Man™ database
www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM

104 E-Cell Project
www.e-cell.org

105 The Silicon Cells
www.siliconcell.net

106 JWS Online - Online Cellular Systems Modelling
www.jjj.bio.vu.nl

107 Virtual Cell Modeling and Simulation Framework
www.nrcam.uchc.edu

108 HepatoSys - Systems Biology of Regenerating Hepatocytes
www.fdm.uni-freiburg.de/SysBio

109 SimTK: physics-based models and software
www.simtk.org

Affiliations

- *Heike E Assmus, PhD*
Researcher, University of Rostock, Systems Biology and Bioinformatics Group, Department of Computer Science, 18051 Rostock, Germany
Tel.: +49 381 498 7574
Fax: +49 381 498 7572
assmus@informatik.uni-rostock.de
- *Ralf Herwig, PhD*
Group Leader, Max-Planck-Institute for Molecular Genetics, Bioinformatics Group, 14195 Berlin-Dahlem, Germany
Tel.: +49 30 8413 1265
Fax: +49 30 8413 1388
herwig@molgen.mpg.de
- *Kwang-Hyun Cho, PhD*
Professor, Seoul National University, College of Medicine, Jongno-gu, Seoul 110-799, Republic of Korea, and Bio-MAX Institute, Seoul National University, Gwanak-gu, Seoul 151-818, Republic of Korea
Tel.: +82 2 887 2650
Fax: +82 2 887 2692
ckh-sb@snu.ac.kr
- *Olaf Wolkenhauer, PhD*
Professor, University of Rostock, Systems Biology and Bioinformatics Group, Department of Computer Science, 18051 Rostock, Germany
Tel.: +49 381 498 7570
Fax: +49 381 498 7572
ow@informatik.uni-rostock.de