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Abstract

The overall goal of the ESIGNET project is to study the computational properties of Cell Signaling Networks (CSN) by evolving them using methods from evolutionary computation, and to re-apply this understanding in developing new ways to model and predict real CSNs.

In this report we describe the current state of the art in the field. As this project is highly interdisciplinary, we present a thematic bibliography involving papers from different scientific fields: Biology, Mathematics, Computer Science and Engineering. The various distinguished themes relevant to our project are: Evolution of CSNs, Mathematical analysis of CSNs, Modelling CSNs: concept and example, Reverse engineering of biochemical networks, Synthetic Biology, Real CSNs and Verification of CSN behaviour.

An objective is to develop open-source software packages for scientific use. In this optic, we review the existing computing tools available that might be useful. Main publication resources and research groups concerned in the field are also presented. Finally a glossary of biological terms is given as a reference.

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1 Introduction

The workpackage WP3 (State of the Art) contains initial research activities for all ESIGNET group members to become familiar with the project. An exhaustive information retrieval characterises this part of the project. It includes literature search, finding appropriate web resources, setup of the technical infrastructure as well as studying publications in relevant fields. This report summarizes the retrieval results and enables first decisions with respect to milestones M3.1 and M3.2.

Since this report should correspond to deliverable D3.1, it is structured as follows: A bibliography (section 2) gives an overview about useful publications. To come along with the recent state of the project, papers are assigned to six main categories and subsequent subclasses in terms of a growing ontology. Section 3 lists appropriate online databases and tools. A description of existing software packages for creating, denotation, analysis, and simulation of cell signal networks (CSNs) can be found in section 4. Possible platforms for publication of project results and selected external research groups active in the field cover sections 5 and 6. A glossary of biological terms is attached in appendix A.

In fulfilment of deliverable D3.2, a representative webpage (www.esignet.net) was created that serves two functions: On the one hand, it provides a platform for public relations. On the other hand, it serves as a reference of the project. A protected members area and a project internal wiki support communication and information exchange between group members. Furthermore, it is helpful to discuss ideas and upcoming publications. This report can be seen as an current excerpt of the information available at the project webpages.

The work of the ESIGNET project addresses some of the needs highlighted in the work of the NEST project on Synthetic Biology. For a comprehensive review of this work see the European Commission research report on Synthetic Biology- Applying Engineering to Biology (EUR 21796. ISBN 92-894-9742-4)

2 Bibliography

2.1 Evolution of CSNs

- *Design of genetic networks with specified functions by evolution in Silico.*, P. Francois and V. Hakim. PNAS (2004) 101(2):580-585.

Abstract Recent studies have provided insights into the modular structure of genetic regulatory networks and emphasized the interest of quantitative functional descriptions. Here, to provide a priori knowledge of the structure of functional modules, we describe an evolutionary procedure in silico that creates small gene networks performing basic tasks. We used it to create networks functioning as bistable switches or oscillators. The obtained circuits provide a variety of functional designs, demonstrate the crucial role of posttranscriptional interactions, and highlight design principles also found in known biological networks. The procedure should prove helpful as a way to understand and create small functional modules with diverse functions as well as to analyze large networks.

- *Network thinking in ecology and evolution.*, S.R. Proulx and D.E.L Promislow and P.C. Phillips., Trends in Ecology and Evolution. (2005) 20(6): 345-354.

Abstract Although pairwise interactions have always had a key role in ecology and evolutionary biology, the recent increase in the amount and availability of biological data has

placed a new focus on the complex networks embedded in biological systems. The increased availability of computational tools to store and retrieve biological data has facilitated wide access to these data, not just by biologists but also by specialists from the social sciences, computer science, physics and mathematics. This fusion of interests has led to a burst of research on the properties and consequences of network structure in biological systems. Although traditional measures of network structure and function have started us off on the right foot, an important next step is to create biologically realistic models of network formation, evolution, and function. Here, we review recent applications of network thinking to the evolution of networks at the gene and protein level and to the dynamics and stability of communities. These studies have provided new insights into the organization and function of biological systems by applying existing techniques of network analysis. The current challenge is to recognize the commonalities in evolutionary and ecological applications of network thinking to create a predictive science of biological networks.

- *Emergent properties of networks of biological signaling pathways.*, U.S. Bhalla and R. Iyengar. *Science*, 283(5400):3817, 1999.

Abstract Many distinct signaling pathways allow the cell to receive, process, and respond to information. Often, components of different pathways interact, resulting in signaling networks. Biochemical signaling networks were constructed with experimentally obtained constants and analyzed by computational methods to understand their role in complex biological processes. These networks exhibit emergent properties such as integration of signals across multiple time scales, generation of distinct outputs depending on input strength and duration, and self-sustaining feedback loops. Feedback can result in bistable behavior with discrete steady-state activities, well-defined input thresholds for transition between states and prolonged signal output, and signal modulation in response to transient stimuli. These properties of signaling networks raise the possibility that information for "learned behavior" of biological systems may be stored within intracellular biochemical reactions that comprise signaling pathways.

- *Computer Simulated Evolution of a Network of Cell-Signaling Molecules.*, D. Bray and S. Lay, *Biophysical Journal* (1994) 66:972-977

Abstract We have trained a computer model of a simple cell-signaling pathway to give specified responses to a pulse of an extracellular ligand. The pathway consists of two initially identical membrane receptors, each of which relays the concentration of the ligand to the level of phosphorylation of an intracellular molecule. Application of random "mutational" changes to the rate constants of the pathway, followed by selection in favor of certain outputs, generates a variety of wave forms and dose-response curves. The phenotypic effect of mutations and the frequency of selection both affect the efficiency with which the pathway achieves its target. When the pathway is trained to give a maximal response at a specific concentration of the stimulating ligand, it gives a consistent pattern of changes in which the two receptors diverge, producing a high-affinity form with excitatory output and a low-affinity form with inhibitory output. We suggest that some high- and low-affinity forms of receptors found in present-day cells might have originated by a similar process.

- *Preliminary Studies on the In Silico Evolution of Biochemical Networks*, A. Deckard and H. Sauro, *ChemBioChem* 5:1423-1431, 2004.

Abstract Due to the variety and importance of roles performed by signalling networks, understanding their function and evolution is of great interest. Signalling networks allow organisms to process and react to changes in their internal and external environment. Current estimates suggest that two to three percent of all genomes code for proteins involved in signalling networks. The study of signalling networks is hindered by the complexities of the networks and difficulties in ascribing function to form. For example, a very complex dense network might comprise eighty or more densely connected proteins. In the majority of cases there is very little understanding of how these networks process signals. Unlike in electronics, where there is a broad practical and theoretical understanding of how to construct devices that can process almost any kind of signal, in biological signalling networks there is no equivalent theory. Part of the problem stems from the fact that in most cases it is unknown what particular signal processing circuits would look like in a biological form. This paper describes the evolutionary methods used to generate networks with particular signal- and computational-processing capabilities. The techniques involved are described, and the approach is illustrated by evolving computational circuits such as multiplication, radicals and logarithmic functions. The experiments also illustrate the evolution of modularity within biochemical reaction networks.

- *Do proteins learn to evolve? the hopfield network as a basis for the understanding of protein evolution.*, Leighton Pritchard and Mark J. Dufton. *Journal of Theoretical Biology*, 202(1):7786, January 2000.

Abstract Correlations between amino-acid residues can be observed in sets of aligned protein sequences, and the analysis of their statistical and evolutionary significance and distribution has been thoroughly investigated. In this paper, we present a model based on such covariations in protein sequences in which the pairs of residues that have mutual influence combine to produce a system analogous to a Hopfield neural network. The emergent properties of such a network, such as soft failure and the connection between network architecture and stored memory, have close parallels in known proteins. This model suggests that an explanation for observed characters of proteins such as the diminution of function by substitutions distant from the active site, the existence of protein folds (superfolds) that can perform several functions based on one architecture, and structural and functional resilience to destabilizing substitutions might derive from their inherent network-like structure. This model may also provide a basis for mapping the relationship between structure, function and evolutionary history of a protein family, and thus be a powerful tool for rational engineering. Copyright 2000 Academic Press.

- *Optimal Stoichiometric Designs of ATP-producing Systems as Determined by an Evolutionary Algorithm*, A. Stephani et al., *J. theor. Biol.* 199:45–61, 1999

Abstract The design of metabolic pathways is thought to be the result of an optimization process such that the structure of contemporary metabolic routes maximizes a particular objective function. Recently, it has been shown that some essential stoichiometric properties of glycolysis can be explained on the basis of the requirement for a high ATP production rate. Because the number of stoichiometrically feasible designs increases strongly with the number of reactions involved, a systematic analysis of all the possibilities turns out to be inaccessible beyond a certain system size. We present, therefore, an alternative approach to compute in a more efficient way the optimal design of glycolysis interacting with an external ATP-consuming reaction. The algorithm is based on the laws of

evolution by natural selection, and may be viewed as a particular version of evolutionary algorithms. The following conclusions are derived: (a) evolutionary algorithms are very useful search strategies in determining optimal stoichiometries of metabolic pathways. (b) Essential topological features of the glycolytic network may be explained on the basis of flux optimization. (c) There is a strong interrelation between the optimal stoichiometries and the thermodynamic and kinetic properties of the participating reactions. (d) Some subsequences of reactions in optimal pathways are strongly conserved at variation of system parameters, which may be understood by applying principles of metabolic control analysis.

- *Design of genetic networks with specified functions by evolution in silico*, P. Francois and V. Hakim, PNAS 101(2):580-585, 2004

Abstract Recent studies have provided insights into the modular structure of genetic regulatory networks and emphasized the interest of quantitative functional descriptions. Here, to provide a priori knowledge of the structure of functional modules, we describe an evolutionary procedure in silico that creates small gene networks performing basic tasks. We used it to create networks functioning as bistable switches or oscillators. The obtained circuits provide a variety of functional designs, demonstrate the crucial role of posttranscriptional interactions, and highlight design principles also found in known biological networks. The procedure should prove helpful as a way to understand and create small functional modules with diverse functions as well as to analyze large networks.

- *Computer Simulated Evolution of a Network of Cell-Signaling Molecules*, D. Bray and S. Lay, Biophys. J. 66: 972–977, 1994

Abstract We have trained a computer model of a simple cell-signaling pathway to give specified responses to a pulse of an extracellular ligand. The pathway consists of two initially identical membrane receptors, each of which relays the concentration of the ligand to the level of phosphorylation of an intracellular molecule. Application of random "mutational" changes to the rate constants of the pathway, followed by selection in favor of certain outputs, generates a variety of wave forms and dose-response curves. The phenotypic effect of mutations and the frequency of selection both affect the efficiency with which the pathway achieves its target. When the pathway is trained to give a maximal response at a specific concentration of the stimulating ligand, it gives a consistent pattern of changes in which the two receptors diverge, producing a high-affinity form with excitatory output and a low-affinity form with inhibitory output. We suggest that some high- and low-affinity forms of receptors found in present-day cells might have originated by a similar process.

- *Spontaneous evolution of modularity and network motifs*, N. Kashtan and U. Alon, PNAS 102(39):13773–13778, 2005

Abstract Biological networks have an inherent simplicity: they are modular with a design that can be separated into units that perform almost independently. Furthermore, they show reuse of recurring patterns termed network motifs. Little is known about the evolutionary origin of these properties. Current models of biological evolution typically produce networks that are highly nonmodular and lack understandable motifs. Here, we suggest a possible explanation for the origin of modularity and network motifs in biology. We use standard evolutionary algorithms to evolve networks. A key feature in this study is evolution under an environment (evolutionary goal) that changes in a modular fashion. That

is, we repeatedly switch between several goals, each made of a different combination of subgoals. We find that such "modularly varying goals" lead to the spontaneous evolution of modular network structure and network motifs. The resulting networks rapidly evolve to satisfy each of the different goals. Such switching between related goals may represent biological evolution in a changing environment that requires different combinations of a set of basic biological functions. The present study may shed light on the evolutionary forces that promote structural simplicity in biological networks and offers ways to improve the evolutionary design of engineered systems.

- *Evolving Control Metabolism for a Robot*, J. Ziegler and W. Banzhaf, *Artificial Life* vol. 7(2):171–190, 2001

Abstract This article demonstrates a new method of programming artificial chemistries. It uses the emerging capabilities of the system's dynamics for information-processing purposes. By evolution of metabolisms that act as control programs for a small robot one achieves the adaptation of the internal metabolic pathways as well as the selection of the most relevant available exteroceptors. The underlying artificial chemistry evolves efficient information-processing pathways with most benefit for the desired task, robot navigation. The results show certain relations to such biological systems as motile bacteria.

- *Directed evolution of a genetic circuit*, Y. Yokobayashi, R. Weiss, F.H. Arnold, *PNAS* 99(26):16587–16591, 2002

Abstract The construction of artificial networks of transcriptional control elements in living cells represents a new frontier for biological engineering. However, biological circuit engineers will have to confront their inability to predict the precise behavior of even the most simple synthetic networks, a serious shortcoming and challenge for the design and construction of more sophisticated genetic circuitry in the future. We propose a combined rational and evolutionary design strategy for constructing genetic regulatory circuits, an approach that allows the engineer to fine-tune the biochemical parameters of the networks experimentally *in vivo*. By applying directed evolution to genes comprising a simple genetic circuit, we demonstrate that a nonfunctional circuit containing improperly matched components can evolve rapidly into a functional one. In the process, we generated a library of genetic devices with a range of behaviors that can be used to construct more complex circuits.

- *Tinkering, and Emergence in Complex Networks*, R.V. Sole, R.F. Cancho, S. Valverde, and J.M. Montoya, *Complexity* 8(1), 2003

Abstract Complex biological networks have very different origins than technologic ones. The latter involve extensive design and, as engineered structures, include a high level of optimization. The former involve (in principle) contingency and structural constraints, with new structures being incorporated through tinkering with previously evolved modules or units. However, the observation of the topological features of different biological nets suggests that nature can have a limited repertoire of "attractors" that essentially optimize communication under some basic constraints of cost and architecture or that allow the biological nets to reach a high degree of homeostasis. Conversely, the topological features exhibited by some technology graphs indicate that tinkering and internal constraints play a key role, in spite of the "designed" nature of these structures. Previous scenarios suggested to explain the overall trends of evolution are re-analyzed in light of topological patterns.

- *Evolution in Complex Systems*, D.G. Green, Complexity 2(1), 1995

Abstract Phase changes play a central role in the adaptation and self-organisation of many complex systems. Specifically I propose that external factors can cause a temporary phase change in the connectivity of a system. Different processes (selection and variation) predominate in each phase. The "chaotic edge" associated with the phase change may be an important source of variety in biological systems. The mechanism outlined here seems to occur in several kinds of biological systems, including species evolution and landscape ecology. Applications to evolutionary programming include parallel genetic algorithms that emulate population genetics on a landscape.

2.2 Mathematical Analysis of CSNs

- *Signaling in small subcellular volumes. I. Stochastic and diffusion effects on individual pathways.*, Bhalla, U.S., Biophysical journal, 87, 2, 2004, 733-744.

Abstract Many cellular signaling events occur in small subcellular volumes and involve low-abundance molecular species. This context introduces two major differences from mass-action analyses of nondiffusive signaling. First, reactions involving small numbers of molecules occur in a probabilistic manner which introduces scatter in chemical activities. Second, the timescale of diffusion of molecules between subcellular compartments and the rest of the cell is comparable to the timescale of many chemical reactions, altering the dynamics and outcomes of signaling reactions. This study examines both these effects on information flow through four protein kinase regulatory pathways. The analysis uses Monte Carlo simulations in a subcellular volume diffusively coupled to a bulk cellular volume. Diffusion constants and the volume of the subcellular compartment are systematically varied to account for a range of cellular conditions. Each pathway is characterized in terms of the probabilistic scatter in active kinase levels as a measure of "noise" on the pathway output. Under the conditions reported here, most signaling outcomes in a volume below one femtoliter are severely degraded. Diffusion and subcellular compartmentalization influence the signaling chemistry to give a diversity of signaling outcomes. These outcomes may include washout of the signal, reinforcement of signals, and conversion of steady responses to transients.

- *Signaling in small subcellular volumes. II. Stochastic and diffusion effects on synaptic,* Bhalla, U.S., Biophysical journal, 87, 2, 2004, p745-753

Abstract The synaptic signaling network is capable of sophisticated cellular computations. These include the ability to respond selectively to different patterns of input, and to sustain changes in response over long periods. The small volume of the synapse complicates the analysis of signaling because the chemical environment is strongly affected by diffusion and stochasticity. This study is based on an updated version of a previously proposed synaptic signaling circuit (Bhalla and Iyengar, 1999) and analyzes three network computation properties in small volumes: bistability, thresholding, and pattern selectivity. Simulations show that although there are diffusive regimes in which bistability may persist, chemical noise at small volumes overwhelms bistability. In the deterministic situation, the network exhibits a sharp threshold for transition between lower and upper stable states. This transition is broadened and individual runs partition between lower and upper states, when stochasticity is considered. The third network property, pattern selectivity, is severely degraded at synaptic volumes. However, there are regimes in which a process similar to

stochastic resonance operates and amplifies pattern selectivity. These results imply that simple scaling of signaling conditions to femtoliter volumes is unlikely, and microenvironments, such as reaction complex formation, may be essential for reliable small-volume signaling.

- *Spontaneous separation of bi-stable biochemical systems into spatial domains of opposite phases*, Elf, J. and Ehrenberg, M., *Syst. Biol.*, 1, 2, 2004, p230-236

Abstract Bi-stable chemical systems are the basic building blocks for intracellular memory and cell fate decision circuits. These circuits are built from molecules, which are present at low copy numbers and are slowly diffusing in complex intracellular geometries. The stochastic reaction-diffusion kinetics of a double-negative feedback system and a MAPK phosphorylation-dephosphorylation system is analysed with Monte-Carlo simulations of the reaction-diffusion master equation. The results show the geometry of intracellular reaction compartments to be important both for the duration and the locality of biochemical memory. Rules for when the systems lose global hysteresis by spontaneous separation into spatial domains in opposite phases are formulated in terms of geometrical constraints, diffusion rates and attractor escape times. The analysis is facilitated by a new efficient algorithm for exact sampling of the Markov process corresponding to the reaction-diffusion master equation.

- *An amplified sensitivity arising from covalent modification in biological systems*, Goldbeter, A. and Koshland, D. E., *Proc. Natl. Acad. Sci. USA*, 78, 11, p6840-6844

Abstract The transient and steady-state behavior of a reversible covalent modification system is examined. When the modifying enzymes operate outside the region of first-order kinetics, small percentage changes in the concentration of the effector controlling either of the modifying enzymes can give much larger percentage changes in the amount of modified protein. This amplification of the response to a stimulus can provide additional sensitivity in biological control, equivalent to that of allosteric proteins with high Hill coefficients.

- *Cell-signalling dynamics in time and space*, Kholodenko, B.N. *Nature reviews Molecular cell biology*, vol 7:3, 2006,

Abstract The specificity of cellular responses to receptor stimulation is encoded by the spatial and temporal dynamics of downstream signalling networks. Temporal dynamics are coupled to spatial gradients of signalling activities, which guide pivotal intracellular processes and tightly regulate signal propagation across a cell. Computational models provide insights into the complex relationships between the stimuli and the cellular responses, and reveal the mechanisms that are responsible for signal amplification, noise reduction and generation of discontinuous bistable dynamics or oscillations.

- *Signaling switches and bistability arising from multisite phosphorylation in protein kinase cascades*, Markevich, N.I. and Hoek, J.B. and Kholodenko B.N., *The Journal of cell biology*, vol 164:3, 2004, p353-359

Abstract Mitogen-activated protein kinase (MAPK) cascades can operate as bistable switches residing in either of two different stable states. MAPK cascades are often embedded in positive feedback loops, which are considered to be a prerequisite for bistable behavior. Here we demonstrate that in the absence of any imposed feedback regulation, bistability and hysteresis can arise solely from a distributive kinetic mechanism of the

two-site MAPK phosphorylation and dephosphorylation. Importantly, the reported kinetic properties of the kinase (MEK) and phosphatase (MKP3) of extracellular signal-regulated kinase (ERK) fulfill the essential requirements for generating a bistable switch at a single MAPK cascade level. Likewise, a cycle where multisite phosphorylations are performed by different kinases, but dephosphorylation reactions are catalyzed by the same phosphatase, can also exhibit bistability and hysteresis. Hence, bistability induced by multisite covalent modification may be a widespread mechanism of the control of protein activity.

- *The Computational Versatility of Proteomic Signaling Networks*, Sauro, Herbert M., Current Proteomics, vol 1, 2004, p67-81

Abstract Almost all proteomic signaling networks in prokaryotes and eukaryotes are based on the simple phosphorylation/dephosphorylation cycle; from this simple unit it is possible to construct a huge variety of control and computational circuits, both analog and digital. With the characterization of many signaling networks, researchers are turning to address the question of how a particular physiological response can be understood in terms of the proteins that make up the network; this is one of the central questions in Systems Biology. In this article I wish to summarize the great versatility of the basic protein cycle as a means to construct complex functional behaviors including the central role that feedback plays in determining the properties of protein based networks.

- *Quantitative analysis of signaling networks*, Sauro, H. M and Kholodenko, B. N., Progress in biophysics and molecular biology, vol 86:1, 2004, p5-43

Abstract The response of biological cells to environmental change is coordinated by protein-based signaling networks. These networks are to be found in both prokaryotes and eukaryotes. In eukaryotes, the signaling networks can be highly complex, some networks comprising of 60 or more proteins. The fundamental motif that has been found in all signaling networks is the protein phosphorylation/dephosphorylation cycle—the cascade cycle. At this time, the computational function of many of the signaling networks is poorly understood. However, it is clear that it is possible to construct a huge variety of control and computational circuits, both analog and digital from combinations of the cascade cycle. In this review, we will summarize the great versatility of the simple cascade cycle as a computational unit and towards the end give two examples, one prokaryotic chemotaxis circuit and the other, the eukaryotic MAPK cascade.

- *Design principles and control mechanisms of signal transduction networks*, B. Binder, Dissertation HU Berlin, 133 pages, 2005

Abstract This work is based on the hypothesis that signal transduction networks in living cells are the result of an evolutionary development which is governed by mutation mechanisms and natural selection principles. This concerns their structural design as well as kinetic parameters. Therefore, it can be assumed that these properties have adopted values which imply certain optimal features with respect to the biological function of signal transduction. Based on this working hypothesis, two approaches are presented to investigate the structural design and control mechanisms of signal transduction networks. Both strategies have as a common research objective the explanation of these properties of signalling networks using certain efficiency criteria. In the first approach, a model is developed to analyse the structural design of signalling networks. A simplified model is used to describe signalling systems consisting of receptors, kinases and phosphatases. This

description includes important systems like MAP kinase pathways, the PI3K pathway, but also larger networks which exhibit complex crosstalk activations. Following the hypothesis mentioned above, optimisation principles have been applied to determine optimal network structures regarding certain biological functions. Based on the effect of small structural perturbations on the dynamic functions, a quantitative definition of the robustness of signalling networks is provided. Ratios of phosphatase and kinase activities maximising this robustness are identified. The general validity of these design principles is supported by the analysis of a large kinase network containing 86 kinases which has been retrieved from the TRANSPATH database. This network is indeed characterised by a very low connectivity and a lack of positive feedback cycles. This large signalling network is further investigated regarding several design properties. It is shown that the kinases group into two functional classes. The presented results show that the structural properties of signalling networks drastically differ from those of random networks. Moreover, import characteristic features, such as the length of signalling cascades can be explained by optimisation principles.

- *Interrelations between Dynamical Properties and Structural Characteristics of Signal Transduction Networks*, B. Binder and R. Heinrich, *Genome Informatics* 15(1):13–23, 2004

Abstract We present a theoretical approach for understanding the interrelations between dynamics and structure of signal transduction pathways. We consider large sets of networks with a specific number of kinases and phosphatases. Our methods are based on nonlinear differential equations and pathway dynamics is characterised in terms of signal amplification and signal duration. We show that networks with a high number of kinases, high connectivities and low phosphatase activities tend to be unstable and run, therefore, the risk to display autoactivation. Analysis of signal transduction pathways retrieved from databases reveals that several structural characteristics required for pathway stability are fulfilled for networks of very large size.

- *Mathematical Models of Protein Kinase Signal Transduction*, R. Heinrich et al., *Molecular Cell*, 9:957–970, 2002

Abstract We have developed a mathematical theory that describes the regulation of signaling pathways as a function of a limited number of key parameters. Our analysis includes linear kinase-phosphatase cascades, as well as systems containing feedback interactions, crosstalk with other signaling pathways, and/or scaffolding and G proteins. We find that phosphatases have a more pronounced effect than kinases on the rate and duration of signaling, whereas signal amplitude is controlled primarily by kinases. The simplest model pathways allow amplified signaling only at the expense of slow signal propagation. More complex and realistic pathways can combine high amplification and signaling rates with maintenance of a stable off-state. Our models also explain how different agonists can evoke transient or sustained signaling of the same pathway and provide a rationale for signaling pathway design.

- *Network Motifs: Simple Building Blocks of Complex Networks*, R. Milo et al., *Science* 298(5594):824–827, 2002

Abstract Complex networks are studied across many fields of science. To uncover their structural design principles, we defined "network motifs," patterns of interconnections occurring in complex networks at numbers that are significantly higher than those in randomized networks. We found such motifs in networks from biochemistry, neurobiology,

ecology, and engineering. The motifs shared by ecological food webs were distinct from the motifs shared by the genetic networks of *Escherichia coli* and *Saccharomyces cerevisiae* or from those found in the World Wide Web. Similar motifs were found in networks that perform information processing, even though they describe elements as different as biomolecules within a cell and synaptic connections between neurons in *Caenorhabditis elegans*. Motifs may thus define universal classes of networks. This approach may uncover the basic building blocks of most networks.

- *Bayesian analysis of signaling networks governing embryonic stem cell fate decisions*, P.J. Woolf et al., *Bioinformatics* 21(6), 2005

Abstract Bayesian networks are statistical tools to extract the causal connections between different parts of a system. In this paper, data is available on the phosphorylation states of several components of a signalling pathway, and the BN technique is used to reconstruct the causal connections. Although a BN gives only a rough idea of the structure of a signalling network, it nevertheless is a good way to make sense of large amounts of data. The approach to find the "right" network used here is basic: guess a lot of starting points and optimise them locally, then take the best. There is certainly room for improvement here.

2.3 Modelling CSNs: Concept and Examples

- *Toward rigorous comprehension of biological complexity: modeling, execution, and visualization of thymic t-cell maturation*. S. Efroni, D. Harel, and I. R. Cohen., *Genome Res*, 13(11):24852497, November 2003.

Abstract One of the problems biologists face is a data set too large to comprehend in full. Experimenters generate data at an ever-growing pace, each from their own niche of interest. Current theories are each able, at best, to capture and model only a small part of the data. We aim to develop a general approach to modeling that will help broaden biological understanding. T-cell maturation in the thymus is a telling example of the accumulation of experimental data into a large disconnected data set. The thymus is responsible for the maturation of stem cells into mature T cells, and its complexity divides research into different fields, for example, cell migration, cell differentiation, histology, electron microscopy, biochemistry, molecular biology, and more. Each field forms its own viewpoint and its own set of data. In this study we present the results of a comprehensive integration of large parts of this data set. The integration is performed in a two-tiered visual manner. First, we use the visual language of Statecharts, which makes specification precise, legible, and executable on computers. We then set up a moving graphical interface that dynamically animates the cells, their receptors, the different gradients, and the interactions that constitute thymic maturation. This interface also provides a means for interacting with the simulation.

- *Computational Systems Biology*, L. You. *Toward Cell Biochemistry and Biophysics*. (2004) 40:1-19.

Abstract The development and successful application of high-throughput technologies are transforming biological research. The large quantities of data being generated by these technologies have led to the emergence of systems biology, which emphasizes large-scale, parallel characterization of biological systems and integration of fragmentary information into a coherent whole. Complementing the reductionist approach that has dominated biology for the last century, mathematical modeling is becoming a powerful tool to achieve an

integrated understanding of complex biological systems and to guide experimental efforts of engineering biological systems for practical applications. Here an overview is given of current mainstream approaches in modeling biological systems, highlight specific applications of modeling in various settings, and point out future research opportunities and challenges.

- *Signaling Complexes: Biophysical Constraints on Intracellular Communication.*, D. Bray, Annu. Rev. Biophysics. Biomol. Struct. (1998) 27:59-75.

Abstract This review surveys the kinds of protein complex that participate in cell communication and identifies, where possible, general principles by which they form and act. It also advances the notion that biophysical constraints imposed by macromolecular crowding and diffusion have had a controlling influence on the evolution of cell signaling pathways. Complexes associated with the bacterial aspartate receptor, with eucaryotic tyrosine kinase receptors, with T-cell receptors, and with focal contacts are examined together with proteins that serve as adaptors, anchors, and scaffolds for signaling complexes. The importance of diffusion in controlling the numbers and locations of signaling complexes is discussed, as is the special role played by membranes in signaling pathways.

- *The Spatial Organization of Cell Signalling Pathways.*, T.S. Shimizu., PhD Thesis. Darwin College, Cambridge. (2002)
- *Signal Transduction in bacteria: phospho-neural network(s) in E.Coli.*, K.J. Hellingwerf and P.W. Postma and J. Tommassen and H.V. Westerhoff. FEMS Microbiology Reviews (1995) 309-321

Abstract The molecular basis of many forms of signal transfer in living organisms is provided via the transient phosphorylation of regulatory proteins by transfer of phosphoryl groups between these proteins. The dominant form of signal transduction in prokaryotic microorganisms proceeds via so-called two-component regulatory systems. These systems constitute phosphoryl transfer pathways, consisting of two or more components. Most of these pathways are linear, but some converge and some are divergent. The molecular properties of some of the well-characterised representatives of two-component systems comply with the requirements to be put upon the elements of a neural network: they function as logical operators and show the phenomenon of autoamplification. Because there are many phosphoryl transfer pathways in parallel and because there also appears to be cross-talk between these pathways, the total of all two-component regulatory systems in a single prokaryotic cell may show the typical characteristics of a 'phospho-neural network'. This may well lead to signal amplification, associative responses and memory effects, characteristics which are typical for neural networks. One of the main challenges in molecular microbial physiology is to determine the extent of the connectivity of the constituting elements of this presumed 'phospho-neural network', and to outline the extent of intelligence-like behaviour this network can generate. *Escherichia coli* is the organism of choice for this characterization.

- *Computational insights into Caenorhabditis elegans vulval development.*, J. Fisher, N. Piterman, E. J. A. Hubbard, M. J. Stern, and D. Harel. Proceedings of the National Academy of Science, 102:1951-1956, February 2005.

Abstract Studies of *Caenorhabditis elegans* vulval development provide a paradigm for pattern formation during animal development. The fates of the six vulval precursor cells

are specified by the combined action of an inductive signal that activates the EGF receptor mitogen-activated PK signaling pathway (specifying a primary fate) and a lateral signal mediated by LIN-12/Notch (specifying a secondary fate). Here we use methods devised for the engineering of complex reactive systems to model a biological system. We have chosen the visual formalism of statecharts and use it to formalize Sternberg and Horvitz's 1989 model [Sternberg, P. W. & Horvitz, H. R. (1989) *Cell* 58, 679-693], which forms the basis for our current understanding of the interaction between these two signaling pathways. The construction and execution of our model suggest that different levels of the inductive signal induce a temporally graded response of the EGF receptor mitogen-activated PK pathway and make explicit the importance of this temporal response. Our model also suggests the existence of an additional mechanism operating during lateral specification that prohibits neighboring vulval precursor cells from assuming the primary fate.

- *Combining state-based and scenario-based approaches in modeling biological systems.* Jasmin Fisher, David Harel, E. Jane Albert Hubbard, Nir Piterman, Michael J. Stern, and Naamah Swerdlin. In CMSB, pages 236-241, 2004.

Abstract Biological systems have recently been shown to share many of the properties of reactive systems. This observation has led to the idea of using methods devised for the construction (engineering) of complex reactive systems to the modeling (reverse-engineering) of biological systems, in order to enhance biological comprehension. Here we suggest to combine the two formal approaches used in our group the state-based formalism of statecharts and the scenario-based formalism of live sequence charts (LSCs). We propose that biological observations are better formalized in the form of LSCs, while biological mechanistic models would be more natural to specify using statecharts. Combining the two approaches would enable one to verify the proposed mechanistic models against the real data. The biological observations can be compared to the requirements in an engineered system, and the mechanistic model would be analogous to the implementation. While requirements are used to design an implementation, here the observations are used to motivate the invention of the mechanistic model. In both cases consistency of one with the other must be established, by testing or by formal verification.

- *Computational models of immunological pathways.* Mike Holcombe and Alex Bell. In IPCAT97: Proceedings of the second international workshop on Information processing in cell and tissues, pages 213-226, New York, NY, USA, 1998. Plenum Press.

Abstract A computational model of part of the immune system is introduced. The model is novel in respect of it representing the populations of the T cells and other related components of the system as a state-based parallel model. This is then analysed with respect to possible spatial and temporal interactions with the result of a number of simulations are presented and some conclusions drawn. One of the interesting aspects of the work is the attempt to construct an integrative model of a complex dynamic system such as the immune system.

- *The immune system as a reactive system: modeling t cell activation with statecharts,* N. Kam, I. Cohen, and D. Harel., 2001.

Abstract The construction of reliable reactive systems is considered to be one of the most challenging goals in the fields of software and system engineering. The definition of a reactive system suits biological systems at different levels, ranging from gene networks,

developing embryos and the immune system. We report here the application of a tool developed for constructing computerized systems to the modeling and analysis of a biological system, the immune system. We use the language of statecharts within the framework of object-oriented modeling. The results described here indicate that this modeling strategy can contribute to the transition of biology from the phase of analysis to the phase of synthesis.

- *Modeling biological reactivity: Statecharts vs. boolean logic*, N. Kam, D. Harel, and I. Cohen. 2001.

Abstract Remarkable progress in various fields of biology is leading in the direction of a complete map of the building blocks of biological systems. There is broad agreement among researchers that 21 century biology will focus on attempting to understand how component parts collaborate to create a whole. It is also well agreed that this transition of biology from identifying the building blocks (analysis) to integrating the parts into a whole (synthesis) should rely on the language of mathematics. In a recent publication, we described the results of a first attempt at confronting the above challenge using the visual formalism of statecharts. We presented a detailed model for T cell activation using statecharts within the general framework of object-oriented modeling. In this work, we compare the statechart-based modeling approach to a Boolean formalism presented by Thomas & D'Ari. This comparison was done by taking a model for T cell activation and anergy, which was constructed by Kaufman et al. using such a Boolean formalism, and translating it into the language of statecharts. Comparing these two representations of the same phenomena allows us to assess the advantages and disadvantages of each modeling approach. We believe that the results of this work, together with the results of our previous modeling work on T cell activation, should encourage the use of visual formalisms such as statecharts for modeling complex biological systems.

- *Application of a stochastic name-passing calculus to representation and simulation of molecular processes*. Corrado Priami, Aviv Regev, Ehud Shapiro, and William Silverman., *Inf. Process. Lett.*, 80(1):2531, 2001.

Abstract We describe a novel application of a stochastic name passing calculus for the study of biomolecular systems. We specify the structure and dynamics of biochemical networks in a variant of the stochastic P-calculus, yielding a model which is mathematically welldefined and biologically faithful. We adapt the operational semantics of the calculus to account for both the time and probability of biochemical reactions, and present a computer implementation of the calculus for biochemical simulations.

- *Representation and simulation of biochemical processes using the pi-calculus process algebra*. Aviv Regev, William Silverman, and Ehud Y. Shapiro. In *Pacific Symposium on Biocomputing*, pages 459-470, 2001.

Abstract Despite the rapidly accumulating body of knowledge about protein networks, there is currently no convenient way of sharing and manipulation of such information. We suggest that a formal computer language for describing the biomolecular processes underlying protein networks is essential for rapid advancement in this field. We propose to model biomolecular processes by using the pi-Calculus, a process algebra, originally developed for describing computer processes. Our model for biochemical processes is mathematically well-defined, while remaining biologically faithful and transparent. It is

amenable to computer simulation, analysis and formal verification. We have developed a computer simulation system, the PiFCP, for execution and analysis of pi-calculus programs. The system allows us to trace, debug and monitor the behavior of biochemical networks under various manipulations. We present a pi-calculus model for the RTK-MAPK signal transduction pathway, formally represent detailed molecular and biochemical information, and study it by various PiFCP simulations.

- *Cell cycle control in eukaryotes: a biospi model.* Paola Lecca and Corrado Priami, In Bioconcur 03, 2003.

Abstract This paper presents a stochastic model of the cell cycle control in eukaryotes. The framework used is based on stochastic process algebras for mobile systems. The automatic tool used in the simulation is the BioSpi. We compare our approach with classical ODE specifications.

- *Intracellular signalling as a parallel distributed process.*, D. Bray. J. Theor. Biol., 143(2):21531, 1990.

Abstract Living cells respond to their environment by means of an interconnected network of receptors, second messengers, protein kinases and other signalling molecules. This article suggests that the performance of cell signalling pathways taken as a whole has similarities to that of the parallel distributed process networks (PDP networks) used in computer-based pattern recognition. Using the response of hepatocytes to glucagon as an example, a procedure is described by which a PDP network could simulate a cell signalling pathway. This procedure involves the following steps: (a) a bounded set of molecules is defined that carry the signals of interest; (b) each of these molecules is represented by a PDP-type of unit, with input and output functions and connection weights corresponding to specific biochemical parameters; (c) a "learning algorithm" is applied in which small random changes are made in the parameters of the cell signalling units and the new network is then tested by a selection procedure in favour of a specific input-output relationship. The analogy with PDP networks shows how living cells can recognize combinations of environmental influences, how cell responses can be stabilized and made resistant to damage, and how novel cell signalling pathways might appear during evolution.

- *Bioengineering models of cell signaling*, Asthagiri, A.R. and Lauffenburger, D. A., Annual review of biomedical engineering, vol 2, 2000, p31-53

Abstract Strategies for rationally manipulating cell behavior in cell-based technologies and molecular therapeutics and understanding effects of environmental agents on physiological systems may be derived from a mechanistic understanding of underlying signaling mechanisms that regulate cell functions. Three crucial attributes of signal transduction necessitate modeling approaches for analyzing these systems: an ever-expanding plethora of signaling molecules and interactions, a highly interconnected biochemical scheme, and concurrent biophysical regulation. Because signal flow is tightly regulated with positive and negative feedbacks and is bidirectional with commands traveling both from outside-in and inside-out, dynamic models that couple biophysical and biochemical elements are required to consider information processing both during transient and steady-state conditions. Unique mathematical frameworks will be needed to obtain an integrated perspective on these complex systems, which operate over wide length and time scales. These may involve a two-level hierarchical approach wherein the overall signaling network is

modeled in terms of effective "circuit" or "algorithm" modules, and then each module is correspondingly modeled with more detailed incorporation of its actual underlying biochemical/biophysical molecular interactions.

- *Elementary Arithmetic Operations by Enzymes: A Model for Metabolic Pathway Based Computing*, Baron, R. and Lioubashevski, O. and Katz, E. and Niazov, T. and Willner, I., *Angew. Chem.*, vol 118, 2006, p1602-1606
- *Computer simulation of the phosphorylation cascade controlling bacterial chemotaxis*, Bray, D. and Bourret, R. B. and Simon M. I., *Mol. Biol. of the Cell*, vol 4, p469-482, 1993

Abstract We have developed a computer program that simulates the intracellular reactions mediating the rapid (nonadaptive) chemotactic response of *Escherichia coli* bacteria to the attractant aspartate and the repellent Ni^{2+} ions. The model is built from modular units representing the molecular components involved, which are each assigned a known value of intracellular concentration and enzymatic rate constant wherever possible. The components are linked into a network of coupled biochemical reactions based on a compilation of widely accepted mechanisms but incorporating several novel features. The computer motor shows the same pattern of runs, tumbles and pauses seen in actual bacteria and responds in the same way as living bacteria to sudden changes in concentration of aspartate or Ni^{2+} . The simulated network accurately reproduces the phenotype of more than 30 mutants in which components of the chemotactic pathway are deleted and/or expressed in excess amounts and shows a rapidity of response to a step change in aspartate concentration similar to living bacteria. Discrepancies between the simulation and real bacteria in the phenotype of certain mutants and in the gain of the chemotactic response to aspartate suggest the existence of additional as yet unidentified interactions in the in vivo signal processing pathway.

- *Protein Molecules as Computational Elements in Living Cells*, Bray, D., *Nature*, vol 376, 2005, p307-312

Abstract Many proteins in living cells appear to have as their primary function the transfer and processing of information, rather than the chemical transformation of metabolic intermediates or the building of cellular structures. Such proteins are functionally linked through allosteric or other mechanisms into biochemical 'circuits' that perform a variety of simple computational tasks including amplification, integration and information storage.

- *Discovering Genomics, Proteomics, and Bioinformatics*, Campbell, A.M. and Heyer, L.J., chapter 8, Benjamin Cummings, 2002
- *Exact Stochastic Simulation of Coupled Chemical Reactions*, Gillespie, D. T., *Phys. Chem.*, vol 81:25, 1977
- *The stability of a stochastic CaMKII switch: dependence on the number of enzyme molecules and protein turnover.*, Miller, P and Zhabotinsky, A. M. and Lisman, J. E. and Wang, X. J., *PLoS biology*, vol 3:4, 2005

Abstract Molecular switches have been implicated in the storage of information in biological systems. For small structures such as synapses, these switches are composed of

only a few molecules and stochastic fluctuations are therefore of importance. Such fluctuations could potentially lead to spontaneous switch reset that would limit the lifetime of information storage. We have analyzed a model of the calcium/calmodulin-dependent protein kinase II (CaMKII) switch implicated in long-term memory in the nervous system. The bistability of this switch arises from autocatalytic autophosphorylation of CaMKII, a reaction that is countered by a saturable phosphatase-1-mediated dephosphorylation. We sought to understand the factors that control switch stability and to determine the functional relationship between stability and the number of molecules involved. Using Monte Carlo simulations, we found that the lifetime of states of the switch increase exponentially with the number of CaMKII holoenzymes. Switch stability requires a balance between the kinase and phosphatase rates, and the kinase rate must remain high relative to the rate of protein turnover. Thus, a critical limit on switch stability is set by the observed turnover rate (one per 30 h on average). Our computational results show that, depending on the timescale of fluctuations in enzyme numbers, for a switch composed of about 15 CaMKII holoenzymes, the stable persistent activation can span from a few years to a human lifetime.

- *A new method for assembling metabolic networks, with application to the Krebs citric acid cycle*, Mittenthal J. E. and Clarke, B. and Waddell, T. G. and Fawcett, G., J. theor. biol., volume 208, p361-382, 2001

Abstract To understand why a molecular network has a particular connectivity one can generate an ensemble of alternative networks, all of which meet the same performance criteria as the real network. We have generated alternatives to the Krebs cycle, allowing group transfers and B(12)-mediated shifts that were excluded in previous work. Our algorithm does not use a reaction list, but determines the reactants and products in generic reactions. It generates networks in order of increasing number of reaction steps. We find that alternatives to the Krebs cycle are very likely to be cycles. Many of the alternatives produce toxic or unstable compounds and use group transfer reactions, which have unfavorable consequences. Although alternatives are better than the Krebs cycle in some respects, the Krebs cycle has the most favorable combination of traits.

- *Modeling Cell Signaling Networks*, N.J. Eungdamrong and R. Iyengar, *Biology of the Cell* 96:355–362, 2004

Abstract Cell signaling pathways interact with one another to form networks in mammalian systems. Such networks are complex in their organization and exhibit emergent properties such as bistability and ultrasensitivity. Analysis of signaling networks requires a combination of experimental and theoretical approaches including the development and analysis of models. This review focuses on theoretical approaches to understanding cell signaling networks. Using heterotrimeric G protein pathways an example, we demonstrate how interactions between two pathways can result in a network that contains a positive feedback loop and function as a switch. Different mathematical approaches that are currently used to model signaling networks are described, and future challenges including the need for databases as well as enhanced computing environments are discussed.

- *Molecular Networks: The Top-Down View*, D. Bray, *Science* 301:1864–65, 2003

Abstract Network theory can give a useful overview of how a biological system works. But to make testable predictions, we need the details.

- *Protein molecules as computational elements in living cells*, D. Bray, Nature 376:307–312, 1995

Abstract Many proteins in living cells appear to have as their primary function the transfer and processing of information, rather than the chemical transformation of metabolic intermediates or the building of cellular structures. Such proteins are functionally linked through allosteric or other mechanisms into biochemical 'circuits' that perform a variety of simple computational tasks including amplification, integration and information storage.

- *Mechanistic systems models of cell signaling networks: a case study of myocyte adrenergic regulation*, J.J. Saucerman and A.D. McCulloch, Progress in Biophysics and Molecular Biology 85:261–278, 2004

Abstract Signal transduction networks coordinate a wide variety of cellular functions, including gene expression, metabolism, and cell fate processes. Understanding biological networks quantitatively is a major challenge to post-genomic biology, and mechanistic systems models will be crucial for this task. Here, we review approaches towards developing mechanistic systems models of established cell signaling networks. The ability of mechanistic system models to generate testable biological hypotheses and experimental strategies is discussed. As a case study of model development and analysis, we examined the functional roles of phospholamban, the L-type calcium channel, the ryanodine receptor, and troponin I phosphorylation upon beta-adrenergic stimulation in the rat ventricular myocyte. Model analysis revealed that while protein kinase A-mediated phosphorylation of the ryanodine receptor greatly increases its calcium sensitivity, calcium autoregulation may adapt quickly by negating potential increases in contractility. Systematic combinations of in silico perturbations supported the conclusion that phospholamban phosphoregulation is the primary mechanism for increased sarcoplasmic reticulum load and calcium relaxation rate during beta-adrenergic stimulation, while both phospholamban and the L-type calcium channel contribute to increased systolic calcium. Combined with detailed experimental studies, mechanistic systems models will be valuable for developing a quantitative understanding of cell signaling networks.

- *Emergent Properties of Networks of Biological Signaling Pathways*, U.S. Bhalla and R. Iyengar, Science 283:381–387, 1999

Abstract Many distinct signaling pathways allow the cell to receive, process, and respond to information. Often, components of different pathways interact, resulting in signaling networks. Biochemical signaling networks were constructed with experimentally obtained constants and analyzed by computational methods to understand their role in complex biological processes. These networks exhibit emergent properties such as integration of signals across multiple time scales, generation of distinct outputs depending on input strength and duration, and self-sustaining feedback loops. Feedback can result in bistable behavior with discrete steady-state activities, well-defined input thresholds for transition between states and prolonged signal output, and signal modulation in response to transient stimuli. These properties of signaling networks raise the possibility that information for "learned behavior" of biological systems may be stored within intracellular biochemical reactions that comprise signaling pathways.

- *Sniffers, buzzers, toggles and blinkers: dynamics of regulatory and signaling pathways in the cell*, J.J. Tyson, K.C. Chen, B. Novak, Current Opinion in Cell Biology 15:221–231, 2003

Abstract The physiological responses of cells to external and internal stimuli are governed by genes and proteins interacting in complex networks whose dynamical properties are impossible to understand by intuitive reasoning alone. Recent advances by theoretical biologists have demonstrated that molecular regulatory networks can be accurately modeled in mathematical terms. These models shed light on the design principles of biological control systems and make predictions that have been verified experimentally.

- *Chemical Computing*, P. Dittrich, In J.P. Banatre, P. Fradet, J.L. Giavitto, O. Michel, Proceedings Unconventional Programming Paradigms (UPP2004), LNCS Vol. 3566, Springer, 2005

Abstract All information processing systems found in living organisms are based on chemical processes. Harnessing the power of chemistry for computing might lead to a new unifying paradigm coping with the rapidly increasing complexity and autonomy of computational systems. Chemical computing refers to computing with real molecules as well as to programming electronic devices using principles taken from chemistry. The paper focuses on the latter, called artificial chemical computing, and discusses several aspects of how the metaphor of chemistry can be employed to build technical information processing systems. In these systems, computation emerges out of an interplay of many decentralized relatively simple components analogized to molecules. Chemical programming encompassed then the definition of molecules, reaction rules, and the topology and dynamics of the reaction space. Due to the self-organizing nature of chemical dynamics, new programming methods are required. Potential approaches for chemical programming are discussed and a road map for developing chemical computing into a unifying and well grounded approach is sketched.

- *Signaling Networks: The Origins of Cellular Multitasking*, J.D. Jordan, E.M. Landau, R. Iyengar, Cell 103:193–200, 2000

Abstract One characteristic common to all organisms is the dynamic ability to coordinate constantly one's activities with environmental changes. The function of communicating with the environment is achieved through a number of pathways that receive and process signals, not only from the external environment but also from different regions within the cell. Individual pathways transmit signals along linear tracts resulting in regulation of discrete cell functions. This type of information transfer is an important part of the cellular repertoire of regulatory mechanisms. However, as increasingly larger numbers of cell signaling components and pathways are being identified and studied, it has become apparent that these linear pathways are not free-standing entities but parts of larger networks. Several articles in this review series describe in exquisite detail how individual classes of signaling pathways are organized and function. As we understand the details of such functional organization and move to the next level of analyzing integrated cellular functions, it will become increasingly important to identify and study the properties and capabilities of signaling networks as a whole.

- *Signaling Complexes: Biophysical Constraints on Intracellular Communication*, D. Bray, Ann. Rev. Biophys. Biomol. Struct. 27:59–75, 1998

Abstract The review surveys the kinds of protein complex that participate in cell communication and identifies, where possible, general principles by which they form and act. It also advances the notion that biophysical constraints imposed by macromolecular crowding

and diffusion have had a controlling influence on the evolution of cell signaling pathways. Complexes associated with the bacterial aspartate receptor, with eucaryotic tyrosine kinase receptors, with T-cell receptors, and with focal contacts are examined together with proteins that serve as adaptors, anchors, and scaffolds for signaling complexes. The importance of diffusion in controlling the numbers and locations of signaling complexes is discussed, as is the special role played by membranes in signaling pathways.

- *Reconstruction of cellular signalling networks and analysis of their properties*, J.A. Papin, T. Hunter, B.O. Palsson, S. Subramaniam, *Nat Rev Mol Cell Biol.* 6(2):99–111, 2005

Abstract The study of cellular signalling over the past 20 years and the advent of high-throughput technologies are enabling the reconstruction of large-scale signalling networks. After careful reconstruction of signalling networks, their properties must be described within an integrative framework that accounts for the complexity of the cellular signalling network and that is amenable to quantitative modelling.

- *Motifs, modules and games in bacteria*, *Curr Opin Microbiol.* 6(2):125–34, 2003

Abstract Global explorations of regulatory network dynamics, organization and evolution have become tractable thanks to high-throughput sequencing and molecular measurement of bacterial physiology. From these, a nascent conceptual framework is developing, that views the principles of regulation in term of motifs, modules and games. Motifs are small, repeated, and conserved biological units ranging from molecular domains to small reaction networks. They are arranged into functional modules, genetically dissectible cellular functions such as the cell cycle, or different stress responses. The dynamical functioning of modules defines the organism's strategy to survive in a game, pitting cell against cell, and cell against environment. Placing pathway structure and dynamics into an evolutionary context begins to allow discrimination between those physical and molecular features that particularize a species to its surroundings, and those that provide core physiological function. This approach promises to generate a higher level understanding of cellular design, pathway evolution and cellular bioengineering.

- *Signal transduction networks: topology, response and biochemical processes*, O.S. Soyer, M. Salathe, S. Bonhoeffer, *J Theor Biol.* 238(2):416–25, 2006

Abstract Conventionally, biological signal transduction networks are analysed using experimental and theoretical methods to describe specific protein components, interactions, and biochemical processes and to model network behavior under various conditions. While these studies provide crucial information on specific networks, this information is not easily converted to a broader understanding of signal transduction systems. Here, using a specific model of protein interaction we analyse small network topologies to understand their response and general properties. In particular, we catalogue the response for all possible topologies of a given network size to generate a response distribution, analyse the effects of specific biochemical processes on this distribution, and analyse the robustness and diversity of responses with respect to internal fluctuations or mutations in the network. The results show that even three- and four-protein networks are capable of creating diverse and biologically relevant responses, that the distribution of response types changes drastically as a function of biochemical processes at protein level, and that certain topologies strongly pre-dispose a specific response type while others allow for diverse types of responses. This study sheds light on the response types and properties that could be expected from signal

transduction networks, provides possible explanations for the role of certain biochemical processes in signal transduction and suggests novel approaches to interfere with signaling pathways at the molecular level. Furthermore it shows that network topology plays a key role on determining response type and properties and that proper representation of network topology is crucial to discover and understand so-called building blocks of large networks.

2.4 Reverse Engineering of Biochemical Networks

- *Reverse Engineering of Biological Complexity.*, M.E. Csete and J.C. Doyle. Science (2002) 295:1664

Abstract Advanced technologies and biology have extremely different physical implementations, but they are far more alike in systems-level organization than is widely appreciated. Convergent evolution in both domains produces modular architectures that are composed of elaborate hierarchies of protocols and layers of feedback regulation, are driven by demand for robustness to uncertain environments, and use often imprecise components. This complexity may be largely hidden in idealized laboratory settings and in normal operation, becoming conspicuous only when contributing to rare cascading failures. These puzzling and paradoxical features are neither accidental nor artificial, but derive from a deep and necessary interplay between complexity and robustness, modularity, feedback, and fragility. This review describes insights from engineering theory and practice that can shed some light on biological complexity.

- *Bayesian network analysis of signaling networks: a primer.*, D. Peer. Sci STKE, 2005(281), April 2005.

Abstract High-throughput proteomic data can be used to reveal the connectivity of signaling networks and the influences between signaling molecules. We present a primer on the use of Bayesian networks for this task. Bayesian networks have been successfully used to derive causal influences among biological signaling molecules (for example, in the analysis of intracellular multicolor flow cytometry). We discuss ways to automatically derive a Bayesian network model from proteomic data and to interpret the resulting model.

- *Bayesian network approach to cell signaling pathway modeling.*, Tommi Jaakkola Peter Sorger Karen Sachs, David Gifford and Douglas A. Lauffenburger. Sci STKE, 148(EG10), 2002.

Abstract The modeling of cellular signaling pathways is an emerging field. Sachs et al. illustrate the application of Bayesian networks to an example cellular pathway involving the activation of focal adhesion kinase (FAK) and extracellular signal-regulated kinase (ERK) in response to fibronectin binding to an integrin. They describe how to use the analysis to select from among proposed models, formulate hypotheses regarding component interactions, and uncover potential dynamic changes in the interactions between these components. Although the data sets currently available for this example problem are too small to definitively point to a particular model, the approach and results provide a glimpse into the power that these methods will achieve once the technology for obtaining the necessary data becomes readily available.

- *Causal protein-signaling networks derived from multiparameter single-cell data.* Karen Sachs, Omar Perez, Dana Peer, Douglas A. Lauffenburger, and Garry P. Nolan. Science, 308(5721):523529, April 2005.

Abstract Machine learning was applied for the automated derivation of causal influences in cellular signaling networks. This derivation relied on the simultaneous measurement of multiple phosphorylated protein and phospholipid components in thousands of individual primary human immune system cells. Perturbing these cells with molecular interventions drove the ordering of connections between pathway components, wherein Bayesian network computational methods automatically elucidated most of the traditionally reported signaling relationships and predicted novel interpathway network causalities, which we verified experimentally. Reconstruction of network models from physiologically relevant primary single cells might be applied to understanding native-state tissue signaling biology, complex drug actions, and dysfunctional signaling in diseased cells.

- *Parameter Estimation in Biochemical Pathways: A Comparison of Global Optimization Methods*, C.G. Moles, P. Mendes and J.R. Banga, Genome Research

Abstract Here we address the problem of parameter estimation (inverse problem) of nonlinear dynamic biochemical pathways. This problem is stated as a nonlinear programming (NLP) problem subject to nonlinear differential-algebraic constraints. These problems are known to be frequently ill-conditioned and multimodal. Thus, traditional (gradient-based) local optimization methods fail to arrive at satisfactory solutions. To surmount this limitation, the use of several state-of-the-art deterministic and stochastic global optimization methods is explored. A case study considering the estimation of 36 parameters of a nonlinear biochemical dynamic model is taken as a benchmark. Only a certain type of stochastic algorithm, evolution strategies (ES), is able to solve this problem successfully. Although these stochastic methods cannot guarantee global optimality with certainty, their robustness, plus the fact that in inverse problems they have a known lower bound for the cost function, make them the best available candidates.

- *Stochastic Ranking for Constrained Evolutionary Optimization*, T.P. Runarson and X. Yao, IEEE Transactions on Evolutionary Computation, 4(3), 2000

Abstract Penalty functions are often used in constrained optimization. However, it is very difficult to strike the right balance between objective and penalty functions. This paper introduces a novel approach to balance objective and penalty functions stochastically, i.e., stochastic ranking, and presents a new view on penalty function methods in terms of the dominance of penalty and objective functions. Some of the pitfalls of naive penalty methods are discussed in these terms. The new ranking method is tested using a (μ, λ) evolution strategy on 13 benchmark problems. Our results show that suitable ranking alone (i.e., selection), without the introduction of complicated and specialized variation operators, is capable of improving the search performance significantly.

- *Reverse engineering of biochemical equations from time-course data by means of genetic programming*, M. Sugimoto et al, BioSystems 80:155–164, 2005

Abstract Increased research aimed at simulating biological systems requires sophisticated parameter estimation methods. All current approaches, including genetic algorithms, need pre-existing equations to be functional. A generalised approach to predict not only parameters but also biochemical equations from only observable time-course information must be developed and a computational method to generate arbitrary equations without knowledge of biochemical reaction mechanisms must be developed. We present a technique to predict an equation using genetic programming. Our technique can search topology and

numerical parameters of mathematical expression simultaneously. To improve the search ability of numeric constants, we added numeric mutation to the conventional procedure. As case studies, we predicted two equations of enzyme-catalyzed reactions regarding adenylylate kinase and phosphofructokinase. Our numerical experimental results showed that our approach could obtain correct topology and parameters that were close to the originals. The mean errors between given and simulation-predicted time-courses were 1.6×10^{-5} to identify metabolic reactions from observable time-courses.

- *Automated Reverse Engineering of Metabolic Pathways from Observed Data Using Genetic Programming*, J.R. Koza, W. Mydlowec, G. Lanza, J. Yu, M.A. Keane, Proceedings Pacific Symposium on Biocomputing 6, 2001

Abstract Recent work has demonstrated that genetic programming is capable of automatically creating complex networks (e.g., analog electrical circuits, controllers) whose behavior is modeled by linear and non-linear continuous-time differential equations and whose behavior matches prespecified output values. The concentrations of substances participating in networks of chemical reactions are modeled by non-linear continuous-time differential equations. This chapter demonstrates that it is possible to automatically create (reverse engineer) a network of chemical reactions from observed time-domain data. Genetic programming starts with observed time-domain concentrations of substances and automatically creates both the topology of the network of chemical reactions and the rates of each reaction of a network such that the behavior of the automatically created network matches the observed time-domain data. Specifically, genetic programming automatically created a metabolic pathway involving four chemical reactions that consume glycerol and fatty acid as input, use ATP as a cofactor, and produce diacyl-glycerol as the final product. The metabolic pathway was created from 270 data points. The automatically created metabolic pathway contains three key topological features, including an internal feedback loop, a bifurcation point where one substance is distributed to two different reactions, and an accumulation point where one substance is accumulated from two sources. The topology and sizing of the entire metabolic pathway was automatically created using only the time-domain concentration values of diacyl-glycerol (the final product).

- *Chemical Kinetics is Turing Universal*, M.O. Magnasco, Physical Review Letters 78(6):1190–1193, 1997

Abstract We show that digital logic can be implemented in the chemical kinetics of homogeneous solutions: We explicitly construct logic gates and show that arbitrarily large circuits can be made from them. This proves that a subset of the constructions available to life has universal (Turing) computational power.

- *Evolutionary optimization with data collocation for reverse engineering of biological networks*, K-Y Tsai and F-S Wang, Bioinformatics, 21(7):1180–1188, 2005

Abstract Modern experimental biology is moving away from analyses of single elements to whole-organism measurements. Such measured time-course data contain a wealth of information about the structure and dynamic of the pathway or network. The dynamic modeling of the whole systems is formulated as a reverse problem that requires a well-suited mathematical model and a very efficient computational method to identify the model structure and parameters. Numerical integration for differential equations and finding global parameter values are still two major challenges in this field of the parameter estimation

of nonlinear dynamic biological systems. We compare three techniques of parameter estimation for nonlinear dynamic biological systems. In the proposed scheme, the modified collocation method is applied to convert the differential equations to the system of algebraic equations. The observed time-course data are then substituted into the algebraic system equations to decouple system interactions in order to obtain the approximate model profiles. Hybrid differential evolution (HDE) with population size of five is able to find a global solution. The method is not only suited for parameter estimation but also can be applied for structure identification. The solution obtained by HDE is then used as the starting point for a local search method to yield the refined estimates.

- *Extracting Biochemical Reaction Kinetics from Time Series Data*, E.J. Crampin et al., 2004

Abstract We consider the problem of inferring kinetic mechanisms for biochemical reactions from time series data. Using a priori knowledge about the structure of chemical reaction kinetics we develop global nonlinear models which use elementary reactions as a basis set, and discuss model construction using top-down and bottom-up approaches.

- *Decoupling Dynamical Systems for Pathway Identification from Metabolic Profiles*, E.O. Voit and J. Almeida, *Bioinformatics*, 2004

Abstract Without preprocessing, the inverse problem of determining structure from metabolic or proteomic profile data is challenging and computationally expensive. The combination of system decoupling and data fitting with universal functions simplifies this inverse problem very significantly. Examples show successful estimations and current limitations of the method.

- *Modelling the Nonlinear Dynamics of Cellular Signal Transduction*, J. Timmer, T.G. Muller, *International Journal of Bifurcation and Chaos*, 14(6):2069–2079, 2004

Abstract During the past decades the components involved in cellular signal transduction from membrane receptors to gene activation in the nucleus have been studied in detail. Based on the qualitative biochemical knowledge, signalling pathways are drawn as static graphical schemes. However, the dynamics and control of information processing through signalling cascades is not understood. Here we show that based on time resolved measurements it is possible to quantitatively model the nonlinear dynamics of signal transduction. To select an appropriate model we performed parameter estimation by maximum likelihood and statistical testing. We apply this approach to the JAK-STAT signalling pathway that was believed to represent a feed-forward cascade. We show by comparison of different models that this hypothesis is insufficient to explain the experimental data and suggest a new model including delayed feedback.

- *Iterative approach to model identification of biological networks*, K.G. Gadkar, R. Gnanawan, F.J. Doyle, *BMC Bioinformatics*. 6:155, 2005

Abstract Recent advances in molecular biology techniques provide an opportunity for developing detailed mathematical models of biological processes. An iterative scheme is introduced for model identification using available system knowledge and experimental measurements. The scheme includes a state regulator algorithm that provides estimates of all system unknowns (concentrations of the system components and the reaction rates of their inter-conversion). The full system information is used for estimation of the model parameters. An optimal experiment design using the parameter identifiability and D-optimality

criteria is formulated to provide "rich" experimental data for maximizing the accuracy of the parameter estimates in subsequent iterations. The importance of model identifiability tests for optimal measurement selection is also considered. The iterative scheme is tested on a model for the caspase function in apoptosis where it is demonstrated that model accuracy improves with each iteration. Optimal experiment design was determined to be critical for model identification. The proposed algorithm has general application to modeling a wide range of cellular processes, which include gene regulation networks, signal transduction and metabolic networks.

- *Discovery of regulatory interactions through perturbation: inference and experimental design*, T.E. Ideker, V. Thorsson, R.M. Karp, Pac Symp Biocomput. 305–16, 2000

Abstract We present two methods to be used interactively to infer a genetic network from gene expression measurements. The predictor method determines the set of Boolean networks consistent with an observed set of steady-state gene expression profiles, each generated from a different perturbation to the genetic network. The chooser method uses an entropy-based approach to propose an additional perturbation experiment to discriminate among the set of hypothetical networks determined by the predictor. These methods may be used iteratively and interactively to successively refine the genetic network: at each iteration, the perturbation selected by the chooser is experimentally performed to generate a new gene expression profile, and the predictor is used to derive a refined set of hypothetical gene networks using the cumulative expression data. Performance of the predictor and chooser is evaluated on simulated networks with varying number of genes and number of interactions per gene.

2.5 Real CSNs

1. Cardiac Calcium Oscillations

- *Understanding Calcium Dynamics Experiments and Theory*, M. Falcke, D Malchow, Springer- Verlag Berlin Heidelberg, 2003

Abstract Calcium is essential for eukaryotic life: from yeast to plants, animals and humans; it is required for the heartbeat, muscle contraction, stabilising of the bones and brain functionality. Calcium activates eggs at fertilisation, effects differentiation and development and is essential in central function like mitosis, ATP synthesis, oxidative phosphorylation, motility and cellular signalling. Another important aspect is secretion of hormones and neurotransmitter release. On the other hand, if Ca^{2+} concentrations raise too high, cell death occurs. This may be a desired outcome as in apoptosis; neuronal cell death impairs brain function. Calcium dynamics is the exchange of Ca^{2+} ions between intracellular stores and the cytosol, the interior and exterior of a cell or between cells, as well as the binding of Ca^{2+} to Ca^{2+} binding proteins. The active elements of the exchange process are channels and pumps in the membranes bounding the intracellular stores and the cell as a whole. Channels have an open and a closed state. They allow for flux of Ca^{2+} down its electro-chemical gradient, when they are open. Pumps transport Ca^{2+} against its electro-chemical gradient incurring consumption of energy. The most important Ca^{2+} binding proteins for the dynamics of Ca^{2+} itself are buffers that are located in Ca^{2+} stores. They bind most of the Ca^{2+} in a concentration dependent manner and thus affect time scales of Ca^{2+} transients. The cytosol contains many different Ca-binding proteins.

They are activated by increasing Ca^{2+} concentrations. A central mediator is calmodulin that regulates many enzymes and channels. The concentration dependent binding of Ca^{2+} to buffers serves as an indicator of the concentration of free Ca^{2+} concentration in intracellular measurements. Certain buffers change their fluorescence wavelength upon binding of a calcium ion. The intensity of fluorescence on that wavelength provides information on the concentration of buffer bound Ca^{2+} and hence on the concentration of free Ca^{2+} as well.

The interplay between the activation of an InsP_3 -metabolizing enzyme (InsP_3 3-kinase) by Ca^{2+} and Ca^{2+} -induced Ca^{2+} release is a plausible explanation for at least some complex Ca^{2+} oscillations. This regulation indeed provides a mechanism for the self-modulation of the InsP_3 signal, thus leading to a phenomenon analogous to the periodic forcing of an autonomous oscillator (G.Dupont, G.Houart and A.Goldbeter)

Calcium signalling often employs calcium oscillations. It is by now a known fact that information, e.g. about the amount of stimulus that evoked a calcium response is encoded in the oscillations. Information can be stored in the frequency, amplitude and shape of the oscillations. How this information is decoded by the many enzymes which are regulated by calcium is not thoroughly understood and so far only few experiments and theoretical studies have been made in order to elucidate the responsible mechanisms. A.Z. Larsen and U. Kummer presented both experimental and theoretical studies of calcium signal encoding and decoding in non-excitabile cells (e.g. hepatocytes), and tried to illuminate the mechanisms by which effective and versatile information processing is possible.

- *Analysing cardiac excitation-contraction coupling with mathematical models of local control*, Christian Soeller*, Mark B. Cannell, Progress in Biophysics & Molecular Biology 85 (2004) 141162

Abstract Cardiac excitation-contraction (EC) coupling describes the process that links sarcolemmal Ca^{2+} influx via L-type Ca^{2+} channels to Ca^{2+} release from the sarcoplasmic reticulum via ryanodine receptors (RyRs). This process has proven difficult to study experimentally, and complete descriptions of how the cell couples surface membrane and intracellular signal transduction proteins to achieve both stable and sensitive intracellular calcium release are still lacking. Mathematical models provide a framework to test our understanding of how this is achieved. While no single model is yet capable of describing all features of cardiac EC coupling, models of increasing complexity are revealing unexpected subtlety in the process. In particular, modelling has established a general failure of common-pool models and has emphasized the requirement for local control so that microscopic sub-cellular domains can separate local behaviour from the whole-cell average (common-pool) behaviour. The micro architecture of the narrow diadic cleft in which the local control takes place is a key factor in determining local Ca^{2+} dynamics. There is still considerable uncertainty about the number of Ca^{2+} ions required to open RyRs within the cleft and various gating models have been proposed, many of which are in reasonable agreement with available experimental data. However, not all models exhibit a realistic voltage dependence of EC coupling gain. Furthermore, it is unclear which model features are essential to producing reasonable gain properties. Thus, despite the success of local-control models in explaining many features of cardiac EC coupling, more work will be needed to provide a sound theoretical basis of cardiac EC coupling.

- *Computational approaches to cellular rhythms*, Albert Goldbeter, NATURE, VOL 420, 14,

NOVEMBER 2002, www.nature.com/nature

Abstract Oscillations arise in genetic and metabolic networks as a result of various modes of cellular regulation. In view of the large number of variables involved and of the complexity of feedback processes that generate oscillations, mathematical models and numerical simulations are needed to fully grasp the molecular mechanisms and functions of biological rhythms. Models are also necessary to comprehend the transition from simple to complex oscillatory behaviour and to delineate the conditions under which they arise. Examples ranging from calcium oscillations to pulsatile intercellular communication and circadian rhythms illustrate how computational biology contributes to clarify the molecular and dynamical bases of cellular rhythms.

- *Modeling the Heart from Genes to Cells to the Whole Organ*, D. Noble, www.sciencemag.org SCIENCE, VOL 295, 1 MARCH, 2002

Abstract Successful physiological analysis requires an understanding of the functional interactions between the key components of cells, organs, and systems, as well as how these interactions change in disease states. This information resides neither in the genome nor even in the individual proteins that genes code for. It lies at the level of protein interactions within the context of subcellular, cellular, tissue, organ, and system structures. There is therefore no alternative to copying nature and computing these interactions to determine the logic of healthy and diseased states. The rapid growth in biological databases; models of cells, tissues, and organs; and the development of powerful computing hardware and algorithms have made it possible to explore functionality in a quantitative manner all the way from the level of genes to the physiological function of whole organs and regulatory systems. This review illustrates this development in the case of the heart. Systems physiology of the 21st century is set to become highly quantitative and, therefore, one of the most computer-intensive disciplines.

- *Bcl-2 functionally interacts with inositol 1,4,5-trisphosphate receptors to regulate calcium release from the ER in response to inositol 1,4,5-trisphosphate*, Rui Chen, Ignacio Valencia, Fei Zhong, Karen S. McColl, H. Llewelyn Roderick, Martin D. Bootman, Michael J. Berridge, Stuart J. Conway, Andrew B. Holmes, Gregory A. Mignery, Patricio Velez, and Clark W. Distelhorst, *The Journal of Cell Biology* Volume 166, Number 2, 2004

Abstract Inositol 1,4,5-trisphosphate (InsP3) receptors (InsP3Rs) are channels responsible for calcium release from the endoplasmic reticulum (ER). We show that the anti-apoptotic protein Bcl-2 (either wild type or selectively localized to the ER) significantly inhibited InsP3-mediated calcium release and elevation of cytosolic calcium in WEHI7.2 T cells. This inhibition was due to an effect of Bcl-2 at the level of InsP3 Rs because responses to both anti-CD3 antibody and a cell-permeant InsP3 ester were decreased. Bcl-2 inhibited the extent of calcium release from the ER of permeabilized WEHI7.2 cells, even at saturating concentrations of InsP3, without decreasing luminal calcium concentration. Furthermore, Bcl-2 reduced the open probability of purified InsP3 Rs reconstituted into lipid bilayers. Bcl-2 and InsP3 Rs were detected together in macromolecular complexes by coimmunoprecipitation and blue native gel electrophoresis. We suggest that this functional interaction of Bcl-2 with InsP3 Rs inhibits InsP3 R activation and thereby regulates InsP3-induced calcium release from the ER.

- *Modelling of simple and complex calcium oscillations from single-cell responses to intercellular signalling*, Stefan Schuster, Marko Marhl and Thomas Hofer *Eur. J. Biochem.*

269, 13331355 (2002) FEBS 2002

Abstract This review provides a comparative overview of recent developments in the modelling of cellular calcium oscillations. A large variety of mathematical models have been developed for this wide-spread phenomenon in intra- and intercellular signalling. From these, a general model is extracted that involves six types of concentration variables: inositol 1,4,5-trisphosphate (IP3), cytoplasmic, endoplasmic reticulum and mitochondrial calcium, the occupied binding sites of calcium buffers, and the fraction of active IP3 receptor calcium release channels. Using this framework, the models of calcium oscillations can be classified into minimal models containing two variables and extended models of three and more variables. Three types of minimal models are identified that are all based on calcium-induced calcium release (CICR), but differ with respect to the mechanisms limiting CICR. Extended models include IP3calcium cross-coupling, calcium sequestration by mitochondria, the detailed gating kinetics of the IP3 receptor, and the dynamics of G-protein activation. In addition to generating regular oscillations, such models can describe bursting and chaotic calcium dynamics. The earlier hypothesis that information in calcium oscillations is encoded mainly by their frequency is nowadays modified in that some effect is attributed to amplitude encoding or temporal encoding. This point is discussed with reference to the analysis of the local and global bifurcations by which calcium oscillations can arise. Moreover, the question of how calcium binding proteins can sense and transform oscillatory signals is addressed. Recently, potential mechanisms leading to the coordination of oscillations in coupled cells have been investigated by mathematical modelling. For this, the general modelling framework is extended to include cytoplasmic and gap-junctional diffusion of IP3 and calcium, and specific models are compared. Various suggestions concerning the physiological significance of oscillatory behaviour in intra- and intercellular signalling are discussed. The article is concluded with a discussion of obstacles and prospects.

- *CaM kinase II as frequency decoder of Ca21 oscillations*, G. Dupont* and A. Goldbeter BioEssays 20:607610, 1998.

Abstract In many cell types, Ca21 signals are organized in the form of repetitive spikes. The frequency of these intracellular Ca21 oscillations increases with the level of stimulation, suggesting the existence of a frequency encoding phenomenon. The question arises as to how the frequency of Ca21 oscillations can be decoded inside the cell. Ca21/calmodulin kinase II has long been proposed as an attractive candidate, as it is a key target of Ca21 signals. By immobilizing the Ca21/calmodulin kinase II and subjecting it to pulses of Ca21 of variable amplitude, duration, and frequency, De Koninck and Schulman(1) have shown for the first time that the autonomous activity of Ca21/calmodulin kinase II is highly sensitive to the temporal pattern of Ca21 oscillations.

- *Cardiac excitationcontraction coupling*, Donald M. Bers, NATURE, VOL 415, 10 JANUARY 2002, www.nature.com

Abstract Of the ions involved in the intricate workings of the heart, calcium is considered perhaps the most important. It is crucial to the very process that enables the chambers of the heart to contract and relax, a process called excitationcontraction coupling. It is important to understand in quantitative detail exactly how calcium is moved around the various organelles of the myocyte in order to bring about excitationcontraction coupling if we are to understand the basic physiology of heart function. Furthermore, spatial microdomains

within the cell are important in localizing the molecular players that orchestrate cardiac function.

- *Modelling of simple and complex calcium oscillations from single-cell responses to inter-cellular signalling*, S.Schuster, M.Marhl, T.Hofer, Eur.J.Biochem. (2002) 269, 1333-1355.

Abstract This review provides a comparative overview of recent developments in the modelling of cellular calcium oscillations. A large variety of mathematical models have been developed for this wide-spread phenomenon in intra- and intercellular signalling. From these, a general model is extracted that involves six types of concentration variables: inositol 1,4,5-trisphosphate (IP₃), cytoplasmic, endoplasmic reticulum and mitochondrial calcium, the occupied binding sites of calcium buffers, and the fraction of active IP₃ receptor calcium release channels. Using this framework, the models of calcium oscillations can be classified into minimal models containing two variables and extended models of three and more variables. Three types of minimal models are identified that are all based on calcium-induced calcium release (CICR), but differ with respect to the mechanisms limiting CICR. Extended models include IP₃ calcium cross-coupling, calcium sequestration by mitochondria, the detailed gating kinetics of the IP₃ receptor, and the dynamics of G-protein activation. In addition to generating regular oscillations, such models can describe bursting and chaotic calcium dynamics. The earlier hypothesis that information in calcium oscillations is encoded mainly by their frequency is nowadays modified in that some effect is attributed to amplitude encoding or temporal encoding. This point is discussed with reference to the analysis of the local and global bifurcations by which calcium oscillations can arise. Moreover, the question of how calcium binding proteins can sense and transform oscillatory signals is addressed. Recently, potential mechanisms leading to the coordination of oscillations in coupled cells have been investigated by mathematical modelling. For this, the general modelling framework is extended to include cytoplasmic and gap-junctional diffusion of IP₃ and calcium, and specific models are compared. Various suggestions concerning the physiological significance of oscillatory behaviour in intra- and intercellular signalling are discussed. The article is concluded with a discussion of obstacles and prospects.

- *Analysing cardiac excitation-contraction coupling with mathematical models of local control*, C.Soeller, M.B.Cannel, Progress Biophys.and Molec.Biol. (2004) 85, 141-162.

Abstract Cardiac excitation-contraction (E-C) coupling describes the process that links sarcolemmal Ca²⁺ influx via L-type Ca²⁺ channels to Ca²⁺ release from the sarcoplasmic reticulum via ryanodine receptors (RyRs). This process has proven difficult to study experimentally, and complete descriptions of how the cell couples surface membrane and intracellular signal transduction proteins to achieve both stable and sensitive intracellular calcium release are still lacking. Mathematical models provide a framework to test our understanding of how this is achieved. While no single model is yet capable of describing all features of cardiac E-C coupling, models of increasing complexity are revealing unexpected subtlety in the process. In particular, modelling has established a general failure of 'common-pool' models and has emphasized the requirement for 'local control' so that microscopic sub-cellular domains can separate local behaviour from the whole-cell average (common-pool) behaviour. The micro-architecture of the narrow diadic cleft in which the local control takes place is a key factor in determining local Ca²⁺ dynamics. There is still considerable uncertainty about the number of Ca²⁺ ions required to open RyRs within

the cleft and various gating models have been proposed, many of which are in reasonable agreement with available experimental data. However, not all models exhibit a realistic voltage dependence of E-C coupling gain. Furthermore, it is unclear which model features are essential to producing reasonable gain properties. Thus, despite the success of local-control models in explaining many features of cardiac E-C coupling, more work will be needed to provide a sound theoretical basis of cardiac E-C coupling.

- *Cardiac Calcium Signalling*, M.J.Berridge, *Biochem. Soc. Trans.* (2003) 31(5), 930-933.
Abstract Calcium regulates three different aspects of cardiac contraction. It drives pacemaker activity, excitation-contraction coupling and the transcriptional events that remodel the Ca(2+) signalling system in both health and disease.
- *Computational approaches to cellular rhythms*, A.Goldbeter, *Nature* (2002) 420, 238-245.
Abstract Oscillations arise in genetic and metabolic networks as a result of various modes of cellular regulation. In view of the large number of variables involved and of the complexity of feedback processes that generate oscillations, mathematical models and numerical simulations are needed to fully grasp the molecular mechanisms and functions of biological rhythms. Models are also necessary to comprehend the transition from simple to complex oscillatory behaviour and to delineate the conditions under which they arise. Examples ranging from calcium oscillations to pulsatile intercellular communication and circadian rhythms illustrate how computational biology contributes to clarify the molecular and dynamical bases of cellular rhythms.
- *Cardiac excitation-contraction coupling*, D.M.Bers, *Nature* (2002) 415, 198-205.
Abstract Of the ions involved in the intricate workings of the heart, calcium is considered perhaps the most important. It is crucial to the very process that enables the chambers of the heart to contract and relax, a process called excitation-contraction coupling. It is important to understand in quantitative detail exactly how calcium is moved around the various organelles of the myocyte in order to bring about excitation-contraction coupling if we are to understand the basic physiology of heart function. Furthermore, spatial microdomains within the cell are important in localizing the molecular players that orchestrate cardiac function.
- *Modelling the heart - from genes to cells to the whole organ*, D.Noble, *Science* (2002) 295, 1678-1682.
Abstract Successful physiological analysis requires an understanding of the functional interactions between the key components of cells, organs, and systems, as well as how these interactions change in disease states. This information resides neither in the genome nor even in the individual proteins that genes code for. It lies at the level of protein interactions within the context of subcellular, cellular, tissue, organ, and system structures. There is therefore no alternative to copying nature and computing these interactions to determine the logic of healthy and diseased states. The rapid growth in biological databases; models of cells, tissues, and organs; and the development of powerful computing hardware and algorithms have made it possible to explore functionality in a quantitative manner all the way from the level of genes to the physiological function of whole organs and regulatory systems. This review illustrates this development in the case of the heart. Systems physiology of the 21st century is set to become highly quantitative and, therefore, one of the most computer-intensive disciplines.

- *Oscillation and waves of intracellular calcium*, A.Goldbeter, In: Biochemical oscillations and cellular rhythms. The molecular bases of periodic and chaotic behaviour, Cambridge University Press (1996) pp.351-406.

Book summary This book addresses the molecular bases of some of the most important biochemical rhythms known at the cellular level. The approach rests on the analysis of theoretical models closely related to experimental observations. Among the main rhythms considered are glycolytic oscillations observed in yeast and muscle, oscillations of cyclic AMP in *Dictyostelium amoebae*, intracellular calcium oscillation observed in a variety of cell types, the mitotic oscillator that drives the cell division cycle in eukaryotes, pulsatile hormone signaling, and circadian rhythms in *Drosophila*. This book will be of interest to life scientists such as biochemists, cell biologists, chronobiologists, medical scientists and pharmacologists. In addition, it will appeal to scientists studying nonlinear phenomena, including oscillations and chaos, in chemistry, physics, mathematics and theoretical biology.

- *Mechanisms of cardiac fibrillation*, R.A. Gray, J. Jalife, A.V. Panfilov, W.T. Baxter, C. Cabo, J.M. Davidenko, A.M. Pertsov, P. Hogeweg, A.T. Winfree (1995) Science, New Series, vol. 270, No.5239, 1222-1225.

2. Bacterial (Prokaryotic) Chemotaxis

- *Design and Diversity in Bacterial Chemotaxis: A Comparative Study in E.Coli and B. Subtilis.*, C.V. Rao and J.R. Kirby and A.P. Arkin., PloS Biology (2004) 2(2):0239-

Abstract Comparable processes in different species often involve homologous genes. One question is whether the network structure, in particular the feedback control structure, is also conserved. The bacterial chemotaxis pathways in *E. coli* and *B. subtilis* both regulate the same task, namely, excitation and adaptation to environmental signals. Both pathways employ many orthologous genes. Yet how these orthologs contribute to network function in each organism is different. To investigate this problem, we propose what is to our knowledge the first computational model for *B. subtilis* chemotaxis and compare it to previously published models for chemotaxis in *E. coli*. The models reveal that the core control strategy for signal processing is the same in both organisms, though in *B. subtilis* there are two additional feedback loops that provide an additional layer of regulation and robustness. Furthermore, the network structures are different despite the similarity of the proteins in each organism. These results demonstrate the limitations of pathway inferences based solely on homology and suggest that the control strategy is an evolutionarily conserved property.

- *Perfect and near-perfect adaptation in a model of bacterial chemotaxis.*, B.A. Melloi And Y Tu., Biophysical Journal (2003) 84:2943-2956.

Abstract The signaling apparatus mediating bacterial chemotaxis can adapt to a wide range of persistent external stimuli. In many cases, the bacterial activity returns to its prestimulus level exactly, and this perfect adaptability is robust against variations in various chemotaxis protein concentrations. We model the bacterial chemotaxis signaling pathway, from ligand binding to CheY phosphorylation. By solving the steady-state equations of the model analytically, we derive a full set of conditions for the system to achieve perfect adaptation. The conditions related to the phosphorylation part of the pathway are discovered for the first time, while other conditions are generalizations of the ones found in previous works.

Sensitivity of the perfect adaptation is evaluated by perturbing these conditions. We find that, even in the absence of some of the perfect adaptation conditions, adaptation can be achieved with near-perfect precision as a result of the separation of scales in both chemotaxis protein concentrations and reaction rates, or specific properties of the receptor distribution in different methylation states. Since near-perfect adaptation can be found in much larger regions of the parameter space than that defined by the perfect adaptation conditions, their existence is essential to understand robustness in bacterial chemotaxis.

- *Bacterial chemotaxis and the question of gain.*, D. Bray. PNAS (2002) 99:7-9.
- *Chemotactic-like response of E.Coli cells lacking the known chemotaxis machinery but containing overexpressed CheY.*, R. Barak and M. Eisenbach. Molecular Microbiology (1999) 31(4): 1125-1137. Download pdf. [Even without the proteins of the chemotaxis pathway, poor quality chemotaxis is still observed by an alternative mechanism. Could this be due to cross-talk?]

Abstract We describe a chemotactic-like response of Escherichia coli strains lacking most of the known chemotaxis machinery but containing high levels of the response regulator CheY. The bacteria accumulated in aspartate-containing capillaries, they formed rings on tryptone-containing semisolid agar, and the probability of counterclockwise flagellar rotation transiently increased in response to stimulation with aspartate (1010105 M; the response was inverted at ≈ 104 M). The temporal response was partial and delayed, as was the response of a control wild-type strain having a high CheY level. α -Methyl-DL-aspartate, a non-metabolizable analogue of aspartate as well as other known attractants of E. Coli, glucose and, to a lesser extent, galactose, maltose and serine caused a similar response. So did low concentrations of acetate and benzoate (which, at higher concentrations, act as repellents for wild-type E. coli). Other tested repellents such as indole, Ni²⁺ and Co²⁺ increased the clockwise bias. These observations raise the possibility that, at least when the conventional signal transduction components are missing, a non-conventional chemotactic signal transduction pathway might be functional in E. coli. Potential molecular mechanisms are discussed.

- *A Model of excitation and adaptation in bacterial chemotaxis.*, P.A. Spiro and J.S. Parkinson and H.G. Othmer., PNAS (1997) 94:7263-7268.

Abstract Bacterial chemotaxis is widely studied because of its accessibility and because it incorporates processes that are important in a number of sensory systems: signal transduction, excitation, adaptation, and a change in behavior, all in response to stimuli. Quantitative data on the change in behavior are available for this system, and the major biochemical steps in the signal transduction/processing pathway have been identified. We have incorporated recent biochemical data into a mathematical model that can reproduce many of the major features of the intracellular response, including the change in the level of chemotactic proteins to step and ramp stimuli such as those used in experimental protocols. The interaction of the chemotactic proteins with the motor is not modeled, but we can estimate the degree of cooperativity needed to produce the observed gain under the assumption that the chemotactic proteins interact directly with the motor proteins

- *Robustness in bacterial chemotaxis.* U. Alon and M.G. Surrrette and N. Barkai and S. Leibler., Nature (1999) 397:168

Abstract Networks of interacting proteins orchestrate the responses of living cells to a variety of external stimuli¹, but how sensitive is the functioning of these protein networks to variations in their biochemical parameters? One possibility is that to achieve appropriate function, the reaction rate constants and enzyme concentrations need to be adjusted in a precise manner, and any deviation from these ‘ne-tuned’ values ruins the network’s performance. An alternative possibility is that key properties of biochemical networks are robust²; that is, they are insensitive to the precise values of the biochemical parameters. Here we address this issue in experiments using chemotaxis of *Escherichia coli*, one of the best-characterized sensory systems^{3,4}. We focus on how response and adaptation to attractant signals vary with systematic changes in the intracellular concentration of the components of the chemotaxis network. We find that some properties, such as steady-state behaviour and adaptation time, show strong variations in response to varying protein concentrations. In contrast, the precision of adaptation is robust and does not vary with the protein concentrations. This is consistent with a recently proposed molecular mechanism for exact adaptation, where robustness is a direct consequence of the network’s architecture

- *Bacterial Patterns and Chemotaxis. Background and experimental results*, J.D.Murray, In: *Mathematical Biology*, 3d Ed. Springer (2003) vol.2, pp.254-310.
- *Robust perfect adaptation in bacterial chemotaxis through integral feedback control.*, T.M. Yi, Y. Huang, M.I. Simon, J. Doyle., *PNAS* (2000) 97(9): 4649-4653.

Abstract Integral feedback control is a basic engineering strategy for ensuring that the output of a system robustly tracks its desired value independent of noise or variations in system parameters. In biological systems, it is common for the response to an extracellular stimulus to return to its prestimulus value even in the continued presence of the signal a process termed adaptation or desensitization. Barkai, Alon, Surette, and Leibler have provided both theoretical and experimental evidence that the precision of adaptation in bacterial chemotaxis is robust to dramatic changes in the levels and kinetic rate constants of the constituent proteins in this signaling network [Alon, U., Surette, M. G., Barkai, N. & Leibler, S. (1998) *Nature* (London) 397, 168171]. Here we propose that the robustness of perfect adaptation is the result of this system possessing the property of integral feedback control. Using techniques from control and dynamical systems theory, we demonstrate that integral control is structurally inherent in the Barkai Leibler model and identify and characterize the key assumptions of the model. Most importantly, we argue that integral control in some form is necessary for a robust implementation of perfect adaptation. More generally, integral control may underlie the robustness of many homeostatic mechanisms.

- *Hydrodynamics of bacterial colonies: a model*, J.Lega and T.Passot, *Phys. Rev. E* (2003) 67 (1-18).

Abstract We propose a hydrodynamic model for the evolution of bacterial colonies growing on soft agar plates. This model consists of reaction-diffusion equations for the concentrations of nutrients, water, and bacteria, coupled to a single hydrodynamic equation for the velocity field of the bacteria-water mixture. It captures the dynamics inside the colony as well as on its boundary and allows us to identify a mechanism for collective motion towards fresh nutrients, which, in its modeling aspects, is similar to classical chemotaxis. As shown in numerical simulations, our model reproduces both usual colony shapes and typical hydrodynamic motions, such as the whirls and jets recently observed in wet colonies of

Bacillus subtilis. The approach presented here could be extended to different experimental situations and provides a general framework for the use of advection-reaction-diffusion equations in modeling bacterial colonies.

- *A computational model of the collective fluid dynamics of motile microorganisms*, M.Hopkins and L.J.Fauci, J. Fluid Mech. (2002) 455, 149-174.

Abstract A mathematical model and numerical method for studying the collective dynamics of geotactic, gyrotactic and chemotactic micro-organisms immersed in a viscous fluid is presented. The Navier Stokes equations of fluid dynamics are solved in the presence of a discrete collection of micro-organisms. These microbes act as point sources of gravitational force in the fluid equations, and thus affect the fluid flow. Physical factors, e.g. vorticity and gravity, as well as sensory factors affect swimming speed and direction. In the case of chemotactic microbes, the swimming orientation is a function of a molecular field. In the model considered here, the molecules are a nutrient whose consumption results in an upward gradient of concentration that drives its downward diffusion. The resultant upward chemotactically induced accumulation of cells results in (Rayleigh Taylor) instability and eventually in steady or chaotic convection that transports molecules and affects the translocation of organisms. Computational results that examine the long-time behaviour of the full nonlinear system are presented. The actual dynamical system consisting of fluid and suspended swimming organisms is obviously three-dimensional, as are the basic modelling equations. While the computations presented in this paper are two-dimensional, they provide results that match remarkably well the spatial patterns and long-time temporal dynamics of actual experiments; various physically applicable assumptions yield steady states, chaotic states, and bottom-standing plumes. The simplified representation of microbes as point particles allows the variation of input parameters and modelling details, while performing calculations with very large numbers of particles ($10^4 - 10^5$), enough so that realistic cell concentrations and macroscopic fluid effects can be modelled with one particle representing one microbe, rather than some collection of microbes. It is demonstrated that this modelling framework can be used to test hypotheses concerning the coupled effects of microbial behaviour, fluid dynamics and molecular mixing. Thus, not only are insights provided into the differing dynamics concerning purely geotactic and gyrotactic microbes, the dynamics of competing strategies for chemotaxis, but it is demonstrated that relatively economical explorations in two dimensions can deliver striking insights and distinguish among hypotheses.

- *Cooperative self-organization of microorganisms*, E.Ben-Jacob, I.Cohen, H.Levine (2000) 49 (4) 395-554.

Abstract In nature, microorganisms must often cope with hostile environmental conditions. To do so they have developed sophisticated cooperative behaviour and intricate communication capabilities, such as: direct cell-cell physical interactions via extra-membrane polymers, collective production of extracellular 'wetting' fluid for movement on hard surfaces, long range chemical signalling such as quorum sensing and chemotactic (bias of movement according to gradient of chemical agent) signalling, collective activation and deactivation of genes and even exchange of genetic material. Utilizing these capabilities, the colonies develop complex spatio-temporal patterns in response to adverse growth conditions. We present a wealth of beautiful patterns formed during colony development of various microorganisms and for different environmental conditions. Invoking ideas from

pattern formation in non-living systems and using 'generic' modelling we are able to reveal novel survival strategies which account for the salient features of the evolved patterns. Using the models, we demonstrate how communication leads to self-organization via cooperative behaviour of the cells. In this regard, pattern formation in microorganisms can be viewed as the result of the exchange of information between the micro-level (the individual cells) and the macro-level (the colony). As such, a full understanding of bacterial behaviour must focus simultaneously on individual cell responses and overall colony organization.

3. Eukaryotic Chemotaxis

- *A modeling framework describing the enzyme regulation of membrane lipids underlying gradient perception in Dictyostelium cells*, J.Krishnan and P.A.Iglesias, J Theoretical Biology (2004) 229, 85-99.

Abstract Spatial sensing in Dictyostelium involves localization of the phosphoinositide lipids PI(3,4,5)P₃ and PI(3,4)P₂ at the leading edge of the cell in response to an external gradient. We have previously proposed a modelling framework describing the regulation of these lipids by the enzymes PI3K and PTEN. In this paper we analyse this regulation from an input-output perspective. When the inputs are homogeneous, we obtain explicit analytical expressions for the lipid concentrations as a function of enzyme concentrations and model parameters. We also show that the system can be cast as an open-loop bilinear control system, and employ control engineering tools to show that a local three-dimensional region in the four-dimensional phase space can be accessed by temporally varying either or both enzyme concentrations. For spatially graded enzyme profiles, we show that diffusion limits the extent to which lipid profiles can be manipulated by enzymes. However, we also demonstrate that for certain ranges of network parameters, increasing lipid diffusion can lead to an increase in steady-state leading-edge concentrations of PI(3,4,5)P₃ or PI(3,4)P₂, even though all lipid diffusion coefficients are equal. Finally, in order to determine the extent to which lipid profiles can be regulated by the enzymes, we formulate and solve inverse problems, where we determine the enzyme profiles required to realize particular lipid profiles at steady state.

- *Modeling the cell guidance system*, P.A.Iglesias and A.Levchenko, Sci. STKE (2002) 148, RE12.

Abstract Cell locomotion can be directed by external gradients of diffusible substances leading to chemotaxis. Recently, the mechanisms of gradient sensing, the cell guidance system, came under scrutiny both in experimental analysis and computational modeling. Here, we review several recent computational models of gradient sensing in eukaryotic cells, demonstrating why some of them predict little sensitivity to changes in the gradient and response locking, whereas others predict high gradient sensitivity at the expense of signal gain. We also propose a way to view chemotaxis regulation as a highly coupled combination of semi-independent control modules, leading to simplifying modeling of this complex cellular behavior.

- *Temporal and spatial regulation of chemotaxis*, M.Iijima, Y.E.Huang, P.N.Devreotes, Dev. Cell (2002) 3, 469-478.

Abstract The ability to sense and respond to shallow gradients of extracellular signals is remarkably similar in Dictyostelium discoideum amoebae and mammalian leukocytes.

Chemoattractant receptors and G proteins are fairly evenly distributed along the cell surface. Receptor occupancy generates local excitatory and global inhibitory processes that balance to control the chemotactic response. Uniform stimuli transiently recruit PI3Ks to, and release PTEN from, the plasma membrane, while gradients of chemoattractant cause the two enzymes to bind to the membrane at the front and back of the cell, respectively. Interference with PI3Ks alters chemotaxis, and disruption of PTEN broadens PI localization and actin polymerization in parallel. Thus, counteracting signals from the upstream elements of the pathway converge to regulate the key enzymes of PI metabolism, localize these lipids, and direct pseudopod formation.

- *Models of eukaryotic gradient sensing: application to chemotaxis of amoebae and neutrophils*, A.Levchenko and P.A.Iglesias, *Biophysic J.* (2002) 82, 50-63.

Abstract Eukaryotic cells can detect shallow gradients of chemoattractants with exquisite precision and respond quickly to changes in the gradient steepness and direction. Here, we describe a set of models explaining both adaptation to uniform increases in chemoattractant and persistent signaling in response to gradients. We demonstrate that one of these models can be mapped directly onto the biochemical signal-transduction pathways underlying gradient sensing in amoebae and neutrophils. According to this scheme, a locally acting activator (PI3-kinase) and a globally acting inactivator (PTEN or a similar phosphatase) are coordinately controlled by the G-protein activation. This signaling system adapts perfectly to spatially homogeneous changes in the chemoattractant. In chemoattractant gradients, an imbalance between the action of the activator and the inactivator results in a spatially oriented persistent signaling, amplified by a substrate supply-based positive feedback acting through small G-proteins. The amplification is activated only in a continuous presence of the external signal gradient, thus providing the mechanism for sensitivity to gradient alterations. Finally, based on this mapping, we make predictions concerning the dynamics of signaling. We propose that the underlying principles of perfect adaptation and substrate supply-based positive feedback will be found in the sensory systems of other chemotactic cell types.

- *A diffusion translocation model for gradient sensing by eukaryotic cells*, M.Postma, P.J.M. Van Haastert, *Biophys. J.* (2001) 81, 1314-1323.

Abstract Small chemotactic cells like Dictyostelium and neutrophils transduce shallow spatial chemoattractant gradients into strongly localized intracellular responses. We show that the capacity of a second messenger to establish and maintain localized signals, is mainly determined by its dispersion range, $\lambda = \sqrt{D_m/K_{-1}}$, which must be small compared to the cell's length. Therefore, short-living second messengers (K_{-1}) with diffusion coefficients D_m in the range of $0 - 5\mu m^2 s^{-1}$ are most suitable. Additional to short dispersion ranges, gradient sensing may include positive feedback mechanisms that lead to local activation and global inhibition of second-messenger production. To introduce the essential nonlinear amplification, we have investigated models in which one or more components of the signal transduction cascade translocate from the cytosol to the second messenger in the plasma membrane. A one-component model is able to amplify a 1.5-fold difference of receptor activity over the cell length into a 15-fold difference of second-messenger concentration. Amplification can be improved considerably by introducing an additional activating component that translocates to the membrane. In both models, communication between the front and the back of the cell is mediated by partial depletion of cytosolic

components, which leads to both local activation and global inhibition. The results suggest that a biochemically simple and general mechanism may explain various signal localization phenomena not only in chemotactic cells but also those occurring in morphogenesis and cell differentiation.

- *Interplay of cell-cell signalling and multicellular morphogenesis during Dictyostelium aggregation*, T.Hfer and P.K. Maini, In: *Computation in Cellular and Molecular Biological Systems (1995)* (Eds: R.Cuthbertson, M.Holcombe, R.Paton) World Scientific, IPCAT95, 3-28.
- *A model based on receptor Desensitization for cyclic-AMP signalling in Dictyostelium cells*, J.L.Martiel and A.Goldbeter, *Biophys J* (1987) 52, 807-828.

Abstract We analyze a model based on receptor modification for the cAMP signaling system that controls aggregation of the slime mold *Dictyostelium discoideum* after starvation. The model takes into account both the desensitization of the cAMP receptor by reversible phosphorylation and the activation of adenylate cyclase that follows binding of extracellular cAMP to the unmodified receptor. The dynamics of the signaling system is studied in terms of three variables, namely, intracellular and extracellular cAMP, and the fraction of receptor in active state. Using parameter values collected from experimental studies on cAMP signaling and receptor phosphorylation, we show that the model accounts qualitatively and, in a large measure, quantitatively for the various modes of dynamic behavior observed in the experiments: (a) autonomous oscillations of cAMP, (b) relay of suprathreshold cAMP pulses, i.e., excitability, characterized by both an absolute and a relative refractory period, and (c) adaptation to constant cAMP stimuli. A two-variable version of the model is used to demonstrate the link between excitability and oscillations by phase plane analysis. The response of the model to repetitive stimulation allows comprehension, in terms of receptor desensitization, of the role of periodic signaling in *Dictyostelium* and, more generally, the function of pulsatile patterns of hormone secretion.

4. The Cell Cycle

- *Modeling the fission yeast cell cycle: Quantized cycle times in wee12 cdc25D mutant cells*, Akos Sveiczler*, Attila Csikasz-Nagy*, Bela Gyorffy*, John J. Tyson, and Bela Novak* *PNAS* u July 5, 2000 u vol. 97 u no. 14 u 78657870

Abstract A detailed mathematical model for the fission yeast mitotic cycle is developed based on positive and negative feedback loops by which Cdc13yCdc2 kinase activates and inactivates itself. Positive feedbacks are created by Cdc13yCdc2-dependent phosphorylation of specific substrates: inactivating its negative regulators (Rum1, Ste9 and Wee1yMik1) and activating its positive regulator (Cdc25). A slow negative feedback loop is turned on during mitosis by activation of Slp1yanaphase-promoting complex (APC), which indirectly re-activates the negative regulators, leading to a drop in Cdc13yCdc2 activity and exit from mitosis. The model explains how fission yeast cells can exit mitosis in the absence of Ste9 (Cdc13 degradation) and Rum1 (an inhibitor of Cdc13yCdc2). We also show that, if the positive feedback loops accelerating the G2yM transition (through Wee1 and Cdc25) are weak, then cells can reset back to G2 from early stages of mitosis by premature activation of the negative feedback loop. This resetting can happen more than once, resulting in a quantized distribution of cycle times, as observed experimentally in

wee12 cdc25D mutant cells. Our quantitative description of these quantized cycles demonstrates the utility of mathematical modelling, because these cycles cannot be understood by intuitive arguments alone.

- *A minimal cascade model for the mitotic oscillator involving cyclin and cdc2 kinase*, ALBERT GOLDBETER, Proc. Natl. Acad. Sci. USA Vol. 88, pp. 9107-9111, October 1991 Cell Biology

Abstract A minimal model for the mitotic oscillator is presented. The model, built on recent experimental advances, is based on the cascade of post-translational modification that modulates the activity of cdc2 kinase during the cell cycle. The model pertains to the situation encountered in early amphibian embryos, where the accumulation of cyclin suffices to trigger the onset of mitosis. In the first cycle of the bicyclic cascade model, cyclin promotes the activation of cdc2 kinase through reversible dephosphorylation, and in the second cycle, cdc2 kinase activates a cyclin protease by reversible phosphorylation. That cyclin activates cdc2 kinase while the kinase triggers the degradation of cyclin has suggested that oscillations may originate from such a negative feedback loop [FMIix, M. A., LabbW, J. C., Doree, M., Hunt, T. & Karsenti, E. (1990) Nature (London) 346, 379-3821. This conjecture is corroborated by the model, which indicates that sustained oscillations of the limit cycle type can arise in the cascade, provided that a threshold exists in the activation of cdc2 kinase by cyclin and in the activation of cyclin proteolysis by cdc2 kinase. The analysis shows how mitotic oscillations may readily arise from time lags associated with these thresholds and from the delayed negative feedback provided by cdc2-induced cyclin degradation. A mechanism for the origin of the thresholds is proposed in terms of the phenomenon of zero-order ultrasensitivity previously described for biochemical systems regulated by covalent modification. Recent advances in the characterization of the biochemical

- *Selected biological processes*, E.Klipp, R.Herwig, A.Kowald, C.Wierling, H.Lehrach, In: Systems Biology in Practice. Concepts, Implementation and Application (2005) Wiley-VCH Verlag GmbH&Co.KGaA, Weinheim, pp. 234-240.
- *Modelling the fission yeast cell cycle: Quantized cycle times in "wee1- cdc25?" mutant cells*, A. Sveiczler, A. Csikasz-Nagy, B. Gyorffy, J.J. Tyson, B. Novak (2000) PNAS, vol.97, No.14, 7865-7870.

Abstract A detailed mathematical model for the fission yeast mitotic cycle is developed based on positive and negative feedback loops by which Cdc13yCdc2 kinase activates and inactivates itself. Positive feedbacks are created by Cdc13yCdc2-dependent phosphorylation of specific substrates: inactivating its negative regulators (Rum1, Ste9 and Wee1yMik1) and activating its positive regulator (Cdc25). A slow negative feedback loop is turned on during mitosis by activation of Slp1yanaphase-promoting complex (APC), which indirectly re-activates the negative regulators, leading to a drop in Cdc13yCdc2 activity and exit from mitosis. The model explains how fission yeast cells can exit mitosis in the absence of Ste9 (Cdc13 degradation) and Rum1 (an inhibitor of Cdc13yCdc2). We also show that, if the positive feedback loops accelerating the G2yM transition (through Wee1 and Cdc25) are weak, then cells can reset back to G2 from early stages of mitosis by premature activation of the negative feedback loop. This resetting can happen more than once, resulting in a quantized distribution of cycle times, as observed experimentally in wee12 cdc25D mutant cells. Our quantitative description of these quantized cycles demonstrates

the utility of mathematical modeling, because these cycles cannot be understood by intuitive arguments alone.

- *Modelling the mitotic oscillator driving the cell division cycle*, A.Goldbeter, In: *Biochemical oscillations and cellular rhythms*. The molecular bases of periodic and chaotic behaviour, Cambridge University Press (1996) pp.409-456.
- *The Cell Cycle: An Introduction*, A.W.Murray & T.Hunt (1993) Oxford Univ. Press, Oxford.
- *A minimal cascade model for the mitotic oscillator involving cyclin and cdc2 kinase*, A. Goldbeter (1991) Proc. Natl. Acad. Sci. USA, vol. 88, 9107-9111.

Abstract Better understanding of the molecular mechanisms underlying the cell cycle has given rise to the theory that there may be one universal, homologous mechanism controlling the onset of mitosis. Studies with yeast and embryonic cells suggest that mitosis is triggered by the periodic activation of cdc2 kinase. Using this experimental data, Albert Goldbeter developed a minimal mathematical model which describes the mitotic oscillator involving cyclin and cdc2 kinase (see Figure 1 below). This model is based on the situation in amphibian embryos. As cyclin, a protein signalling molecule, accumulates and exceeds a certain threshold concentration, mitosis is triggered. In the first cycle of the bicyclic cascade model, cyclin promotes the activation of cdc2 kinase through reversible phosphorylation. In the second cycle, cdc2 kinase activates a cyclin protease by reversible phosphorylation. Since cyclin activates cdc2 kinase, and in turn, active cdc2 kinase indirectly triggers the degradation of cyclin, cyclin oscillations may originate from a negative feedback loop. Model simulations support this theory. Oscillations can arise as long as thresholds exist in the activation of cdc2 kinase by cyclin, and in the activation of cyclin protease by cdc2 kinase. Time lags associated with these thresholds, together with the delayed negative feedback from the cdc2-induced cyclin degradation, can readily lead to sustained mitotic oscillations.

- *Discontinuities and singularities in the timing of nuclear division*, A.T.Winfree (1984) In: *Cell Cycle Clocks*. L.N.Edmunds Jr, Ed. Marcel Dekker, New York and Basel, pp.63-80.

5. Intracellular Calcium Oscillations

- *Decoding of intracellular calcium spike trains*, K. Prank, L. Laer, A. von zur Muhlen, G. Brabant, C. Schofl (1998) *Europhys. Lett.* 42 (2), 143-147.

Abstract Cells respond to external signals, such as hormonal stimuli, by generating repetitive spikes in the intracellular free-calcium concentration ($[Ca^{2+}]_i$). These $[Ca^{2+}]_i$ spikes, which can be modulated in their frequency and amplitude, regulate diverse cellular processes. Experimentally, $[Ca^{2+}]_i$ can be assessed continuously in contrast to cellular responses represented by the activation of proteins. We propose a mathematical model that allows for the on-line decoding of $[Ca^{2+}]_i$ spike trains into cellular responses represented by the activation of proteins.

- *CaM kinase II as frequency decoder of Ca^{2+} oscillations*, G. Dupont and A. Goldbeter (1998) *BioEssays* 20.8, 607-610.

Abstract In many cell types, Ca^{2+} signals are organized in the form of repetitive spikes. The frequency of these intracellular Ca^{2+} oscillations increases with the level of stimulation, suggesting the existence of a frequency encoding phenomenon. The question arises as to how the frequency of Ca^{2+} oscillations can be decoded inside the cell. Ca^{2+} /calmodulin kinase II has long been proposed as an attractive candidate, as it is a key target of Ca^{2+} signals. By immobilizing the Ca^{2+} /calmodulin kinase II and subjecting it to pulses of Ca^{2+} of variable amplitude, duration, and frequency, De Koninck and Schulman have shown for the first time that the autonomous activity of Ca^{2+} /calmodulin kinase II is highly sensitive to the temporal pattern of Ca^{2+} oscillations.

2.6 Verification of CSN behaviour

- *Model Building and Model Checking for Processes*, M. Antoniotti and A. Policriti and N. Ugel B. Mishra, Cell Biochemistry and Biophysics, vol 38:3, 2003, p271-286

Abstract A central claim of computational systems biology is that, by drawing on mathematical approaches developed in the context of dynamic systems, kinetic analysis, computational theory and logic, it is possible to create powerful simulation, analysis, and reasoning tools for working biologists to decipher existing data, devise new experiments, and ultimately to understand functional properties of genomes, proteomes, cells, organs, and organisms. In this article, a novel computational tool is described that achieves many of the goals of this new discipline. The novelty of this system involves an automaton-based semantics of the temporal evolution of complex biochemical reactions starting from the representation given as a set of differential equations. The related tools also provide ability to qualitatively reason about the systems using a propositional temporal logic that can express an ordered sequence of events succinctly and unambiguously. The implementation of mathematical and computational models in the Simpathica and XSSYS systems is described briefly. Several example applications of these systems to cellular and biochemical processes are presented: the two most prominent are Leibler et al.'s repressilator (an artificial synthesized oscillatory network), and Curto- Voit-Sorribas-Cascante's purine metabolism reaction model.

- *Symbolic Model Checking for Biochemical Networks*, N. Chabrier and F. Fages, Computational Methods in Systems Biology: First International Workshop, CMSB 2003, Rovereto, Italy, February 24-26, 2003. Proceedings, Lecture Notes in Computer Science 2602, Springer, 2003, p149-162

Abstract Model checking is an automatic method for deciding if a circuit or a program, expressed as a concurrent transition system, satisfies a set of properties expressed in a temporal logic such as CTL. In this paper we argue that symbolic model checking is feasible in systems biology and that it shows some advantages over simulation for querying and validating formal models of biological processes. We report our experiments on using the symbolic model checker NuSMV and the constraint-based model checker DMC, for the modeling and querying of two biological processes: a qualitative model of the mammalian cell cycle control after Kohn's diagrams, and a quantitative model of gene expression regulation.

- *Model Checking Genetic Regulatory Networks Using GNA and CADP*, G. Batt and D. Bergamini, H. de Jong and H. Garavel and R., Model Checking Software: 11th Interna-

tional SPIN Workshop, Barcelona, Spain, April 1-3, 2004. Proceedings, Lecture Notes in Computer Science 2989, Springer, 2004, p158-163

Abstract The study of genetic regulatory networks, which underlie the functioning of living organisms, has received a major impetus from the recent development of high-throughput genomic techniques. This experimental progress calls for the development of appropriate computer tools supporting the analysis of genetic regulatory processes. We have developed a modeling and simulation method [5,7], based on piecewise-linear differential equations, that is well-adapted to the qualitative nature of most available biological data. The method has been implemented in the tool Genetic Network Analyzer (GNA) [6], which produces a graph of qualitative states and transitions between qualitative states. The graph provides a discrete abstraction of the dynamics of the system.

- *Representation and Simulation of Biochemical Processes using the Pi-Calculus Process Algebra*, A. Regev and W Silverman and E. Shapiro, Pacific Symposium on Biocomputing, 2000, p459-470

Abstract Despite the rapidly accumulating body of knowledge about protein networks, there is currently no convenient way of sharing and manipulation of such information. We suggest that a formal computer language for describing the biomolecular processes underlying protein networks is essential for rapid advancement in this field. We propose to model biomolecular processes by using the pi-Calculus, a process algebra, originally developed for describing computer processes. Our model for biochemical processes is mathematically well-defined, while remaining biologically faithful and transparent. It is amenable to computer simulation, analysis and formal verification. We have developed a computer simulation system, the PiFCP, for execution and analysis of pi-calculus programs. The system allows us to trace, debug and monitor the behavior of biochemical networks under various manipulations. We present a pi-calculus model for the RTK-MAPK signal transduction pathway, formally represent detailed molecular and biochemical information, and study it by various PiFCP simulations.

- *Qualitative Modelling of Genetic Networks: From Logical Regulatory Graphs to Standard Petri*, C. Chaouiya and E. Remy and P. Ruet and D. Thieffry, Applications and Theory of Petri Nets 2004: 25th International Conference, ICATPN 2004, Bologna, Italy, June 21, 2004. Proceedings, Lecture Notes in Computer Science 3099, Springer, 2004, p137-156

Abstract In this paper, a systematic rewriting of logical genetic regulatory graphs in terms of standard Petri net models is proposed. We show that, in the Boolean case, the combination of the logical approach with the standard Petri net framework enables the analysis of isolated regulatory circuits, confirming their most fundamental dynamical properties. Furthermore, two more realistic applications are also presented, the first dealing with the control of the early cell cycles in the developing fly, the second dealing with flower morphogenesis. The combination of logical and Petri net formalisms open new prospects for the delineation of specific relationships between the feedback structure and the dynamical properties of complex regulatory systems. Moreover, this approach should ease the definition of integrated models of networks encompassing various kinds of interactions: genetic or metabolic regulations, signal transduction cascades.

2.7 Miscellaneous

- *Investigation of in vivo cross-talk between key two-component systems in E. Coli.*, D.T. Verhamme, J.C. Arents, P.W. Postma, W. Crielaard, K.J. Hellingwerf., Microbiology (2002) 148:69-78

Abstract Intracellular signal transfer in bacteria is dominated by phosphoryl transfer between conserved transmitter and receiver domains in regulatory proteins of so-called two-component systems. *Escherichia coli* contains 30 such systems, which allow it to modulate gene expression, enzyme activity and the direction of flagellar rotation. The authors have investigated whether, and to what extent, these separate systems form (an) interacting network(s) in vivo, focussing on interactions between four major systems, involved in the responses to the availability of phosphorylated sugars (Uhp), phosphate (Pho), nitrogen (Ntr) and oxygen (Arc). Significant cross-talk was not detectable in wild-type cells. Decreasing expression levels of succinate dehydrogenase (reporting Arc activation), upon activation of the Pho system, appeared to be independent of signalling through PhoR. Cross-talk towards NtrC did occur, however, in a *ntrB* deletion strain, upon joint activation of Pho, Ntr and Uhp. UhpT expression was demonstrated when cells were grown on pyruvate, through non-cognate phosphorylation of UhpA by acetyl phosphate.

- *Two-component signal transduction pathways regulating growth and cell cycle progression in a bacterium: A System-Level Analysis.*, J.M. Skerket and M.S. Prasol and B.S. Perchuk and E.G. Biondi and M.T. Laub., PLoS Biology. 3(10): 1770-

Abstract Two-component signal transduction systems, comprised of histidine kinases and their response regulator substrates, are the predominant means by which bacteria sense and respond to extracellular signals. These systems allow cells to adapt to prevailing conditions by modifying cellular physiology, including initiating programs of gene expression, catalyzing reactions, or modifying protein-protein interactions. These signaling pathways have also been demonstrated to play a role in coordinating bacterial cell cycle progression and development. Here we report a system-level investigation of two-component pathways in the model organism *Caulobacter crescentus*. First, by a comprehensive deletion analysis we show that at least 39 of the 106 two-component genes are required for cell cycle progression, growth, or morphogenesis. These include nine genes essential for growth or viability of the organism. We then use a systematic biochemical approach, called phosphotransfer profiling, to map the connectivity of histidine kinases and response regulators. Combining these genetic and biochemical approaches, we identify a new, highly conserved essential signaling pathway from the histidine kinase CenK to the response regulator CenR, which plays a critical role in controlling cell envelope biogenesis and structure. Depletion of either *cenK* or *cenR* leads to an unusual, severe blebbing of cell envelope material, whereas constitutive activation of the pathway compromises cell envelope integrity, resulting in cell lysis and death. We propose that the CenK-CenR pathway may be a suitable target for new antibiotic development, given previous successes in targeting the bacterial cell wall. Finally, the ability of our in vitro phosphotransfer profiling method to identify signaling pathways that operate in vivo takes advantage of an observation that histidine kinases are endowed with a global kinetic preference for their cognate response regulators. We propose that this system-wide selectivity insulates two-component pathways from one another, preventing unwanted cross-talk.

- *The Computational Versatility of Proetomic Signaling Networks.*, H.M. Sauro, Current Pro-

teomics (2004) 1:67-81

Abstract Almost all proteomic signaling networks in prokaryotes and eukaryotes are based on the simple phosphorylation/dephosphorylation cycle; from this simple unit it is possible to construct a huge variety of control and computational circuits, both analog and digital. With the characterization of many signaling networks, researchers are turning to address the question of how a particular physiological response can be understood in terms of the proteins that make up the network; this is one of the central questions in Systems Biology. In this article I wish to summarize the great versatility of the basic protein cycle as a means to construct complex functional behaviors including the central role that feedback plays in determining the properties of protein based networks.

- *Cell signaling pathways as control modules: Complexity for simplicity?*, D.A. Lauffenburger., PNAS (2000) 97(10) 5031-5033

- *Biological Robustness.*, H. Kitano., Nature Reviews. (2004). 5:826

Abstract Robustness is a ubiquitously observed property of biological systems. It is considered to be a fundamental feature of complex evolvable systems. It is attained by several underlying principles that are universal to both biological organisms and sophisticated engineering systems. Robustness facilitates evolvability and robust traits are often selected by evolution. Such a mutually beneficial process is made possible by specific architectural features observed in robust systems. But there are trade-offs between robustness, fragility, performance and resource demands, which explain system behaviour, including the patterns of failure. Insights into inherent properties of robust systems will provide us with a better understanding of complex diseases and a guiding principle for therapy design

- *The systems biology markup language (SBML): a medium for representation and exchange of biochemical network models*, Hucka, M. et al, Bioinformatics, volume 19:4, 2003, pages 524-531

Abstract MOTIVATION: Molecular biotechnology now makes it possible to build elaborate systems models, but the systems biology community needs information standards if models are to be shared, evaluated and developed cooperatively. RESULTS: We summarize the Systems Biology Markup Language (SBML) Level 1, a free, open, XML-based format for representing biochemical reaction networks. SBML is a software-independent language for describing models common to research in many areas of computational biology, including cell signaling pathways, metabolic pathways, gene regulation, and others. AVAILABILITY: The specification of SBML Level 1 is freely available from <http://www.sbml.org/>

- *Formation of regulatory patterns during signal propagation in a Mammalian cellular network.*, Ma'ayan, Avi and Jenkins, Sherry L and Neves, Susana and Hasseldine, Anthony and Grace, Elizabeth and Dubin-Thaler, Benjamin and Eungdamrong, Narat J and Weng, Gehzi and Ram, Prahlad T and Rice, J Jeremy and Kershenbaum, Aaron and Stolovitzky, Gustavo A and Blitzer, Robert D and yengar, Ravi, Science, vol. 309:5737, 2005, p1078-1083

Abstract We developed a model of 545 components (nodes) and 1259 interactions representing signaling pathways and cellular machines in the hippocampal CA1 neuron. Using

graph theory methods, we analyzed ligand-induced signal flow through the system. Specification of input and output nodes allowed us to identify functional modules. Networking resulted in the emergence of regulatory motifs, such as positive and negative feedback and feedforward loops, that process information. Key regulators of plasticity were highly connected nodes required for the formation of regulatory motifs, indicating the potential importance of such motifs in determining cellular choices between homeostasis and plasticity.

- *Reaction kinetics in intracellular environments with macromolecular crowding: simulations and rate laws*, Schnell, S and Turner, T.E., Progress in biophysics and molecular biology, volume 85:2-3, 2004, p235-260

Abstract We review recent evidence illustrating the fundamental difference between cytoplasmic and test tube biochemical kinetics and thermodynamics, and showing the breakdown of the law of mass action and power-law approximation in in vivo conditions. Simulations of biochemical reactions in non-homogeneous media show that as a result of anomalous diffusion and mixing of the biochemical species, reactions follow a fractal-like kinetics. Consequently, the conventional equations for biochemical pathways fail to describe the reactions in in vivo conditions. We present a modification to fractal-like kinetics following the Zipf-Mandelbrot distribution which will enable the modelling and analysis of biochemical reactions occurring in crowded intracellular environments.

- *Thermodynamic Theory of Structure, Stability and Fluctuations*, Glansdorff, P. and Prigogine, I., Wiley Interscience, 1971
- *Enzymes*, Dixon, Malcolm and Webb, Edwin C., Longman Group Limited, 1979
- *Nonlinear Differential Equations of Chemically Reacting Systems*, Gavalas, George R., Springer, 1968
- *Robustness of Cellular Functions*, J. Stelling et al., Cell, 118:675–685, 2004

Abstract Robustness, the ability to maintain performance in the face of perturbations and uncertainty, is a long-recognized key property of living systems. Owing to intimate links to cellular complexity, however, its molecular and cellular basis has only recently begun to be understood. Theoretical approaches to complex engineered systems can provide guidelines for investigating cellular robustness because biology and engineering employ a common set of basic mechanisms in different combinations. Robustness may be a key to understanding cellular complexity, elucidating design principles, and fostering closer interactions between experimentation and theory.

- *Evolving Inventions*, J.R.Koza, M.A. Keane, M.J. Streeter, Scientific American 288(2):52, 2003

Abstract Evolution is an immensely powerful creative process. From the intricate biochemistry of individual cells to the elaborate structure of the human brain, it has produced wonders of unimaginable complexity. Evolution achieves these feats with a few simple processes-mutation, sexual recombination and natural selection-which it iterates for many generations. Now computer programmers are harnessing software versions of these same processes to achieve machine intelligence. Called genetic programming, this technique has designed computer programs and electronic circuits that perform specified functions.

3 Online Databases and Tools

1. An extensive list of databases can be found at

http://www.mpiem.gwdg.de/Forschung/Biol/biol_index_en.html.

Modelling

- W.I.S. - Home Page <http://www.weizmann.ac.il/>
- Cambridge Structural Database at W.I.S.
- Computational Molecular Modelling at NIH
- Molecular Modelling Database (MMDB) at NCBI

Scientific literature

- Max-Planck-Society: Access to Literature Databases
- Baylor College of Medicine Human Genome Center - Libraries and Literature
- BioMedNet Literature
- Deutsches Institut für medizinische Dokumentation und Information
- Medline Search at NCBI
- National Institute of Health (NIH) Library and Literature Resources
- National Library of Medicine (NLM)
- Ovid Home-Page at GWDG (NLM)
- Ovid-Entrance at GWDG (NLM)
- Association for Computing Machinery (ACM, www.acm.org)

Server for bio- and medical technology

- Antibody Resource Page
- Baylor College of Medicine Human Genome Center - Link collection
- Biotech Europe
- Columbia University -The Human Genome Project - Link collection
- UTHSCSA Genome Center - Link collection

2. Databases and services of the EU Bioinformatics Institute

- <http://www.ebi.ac.uk/Databases>
- <http://www.ebi.ac.uk/services/>

3. Databases of the National Centre for Biotechnology

- <http://www.ncbi.nlm.nih.gov/>

4. Literature databases

- Through **PubMed**, anyone can access MEDLINE's 15,000,000 biomedical journal citations to research biomedical questions. Clicking the "Related articles" link for each abstract can expand your search.
- **PubMed Central** is a digital archive of life sciences journal literature. Integrated into the Entrez retrieval system, PMC provides free and unrestricted access to the full text of over 160 life sciences journals, with more to come.
- **Bookshelf** Look for background information or research new topics with freely accessible, online biomedical textbooks. The growing NCBI Bookshelf can be searched directly or accessed via PubMed abstracts by clicking the "Books" link. The database can also be searched by choosing "Books" from the Entrez pull-down menu.
- **Online Mendelian Inheritance in Man (OMIM)**, With over 15,000 entries, OMIM, maintained by Dr. Victor A. McKusick and his colleagues at Johns Hopkins University, represents a comprehensive and constantly updated catalog of inherited diseases.

4 Software

- **Genomic Object Net**

Genomic Object Net is an environment for simulating and representing biological systems. It is an integrated tool consisting of several kinds of software tools for describing biopathways, visualizing simulation results, evaluating a hypothesis, biopathways recreation from biopathway databases, and so on.

<http://www.genomicobject.net>

- **Gepasi**

Gepasi is a software package for modeling biochemical systems. It simulates the kinetics of systems of biochemical reactions and provides a number of tools to fit models to data, optimize any function of the model, perform metabolic control analysis and linear stability analysis. Gepasi simplifies the task of model building by assisting the user in translating the language of chemistry (reactions) to mathematics (matrices and differential equations) in a transparent way. This is combined with a set of sophisticated numerical algorithms that assure the results are obtained fast and accurate. Gepasi is intended primarily for research purposes but because of its user-friendly interface it is equally good for education. Latest version is 3.30, released on September 2002, added SBML support and other features

<http://www.gepasi.org/>

- **Cellware**

Cellware has not only been designed to conduct modeling and simulation of gene regulatory and metabolic pathways but also offer an integrated environment for diverse mathematical representations, parameter estimation and optimization. In addition, a user-friendly

graphical display and capability to run large and complex models would be provided by default. A very special feature of Cellware is that it would be the first grid based modeling and simulation tool in the field of Systems Biology, to our best knowledge.

<http://www.bii.a-star.edu.sg/achievements/applications/cellware/index.asp>

- **Virtual Cell**

The Virtual Cell has been specifically designed to be a tool for a wide range of scientists, from experimental cell biologists to theoretical biophysicists. Likewise the creation of models can range from the simple, to evaluate hypotheses or to interpret experimental data, to complex multi-layered models used to probe the predicted behavior of complex, highly non-linear systems. Such models can be based on both experimental data and purely theoretical assumptions.

<http://www.nrcam.uchc.edu/login/login.html>

- **CellML**

The CellML language is an open standard based on the XML markup language. CellML is being developed by the Bioengineering Institute at the University of Auckland and affiliated research groups. The purpose of CellML is to store and exchange computer-based mathematical models. CellML allows scientists to share models even if they are using different model-building software. It also enables them to reuse components from one model in another, thus accelerating model building.

<http://www.cellml.org/>

- **CellDesigner**

CellDesigner is a structured diagram editor for drawing gene-regulatory and biochemical networks. Networks are drawn based on the process diagram, with graphical notation system proposed by Kitano, and are stored using the Systems Biology Markup Language (SBML), a standard for representing models of biochemical and gene-regulatory networks. Networks are able to link with simulation and other analysis packages through Systems Biology Workbench (SBW).

<http://www.celldesigner.org>

- **StochSim, a Stochastic Simulation Modeling System**

The computer program StochSim was written by Carl Firth (formerly Carl Morton-Firth) as part of his PhD work at the University of Cambridge (Morton-Firth, 1998). It was developed as part of a study of bacterial chemotaxis as a more realistic way to represent the stochastic features of this signalling pathway and also as a means to handle the large numbers of individual reactions encountered (Morton-Firth and Bray, 1998; Morton-Firth et al., 1999). The program now provides a general purpose biochemical simulator in which individual molecules or molecular complexes are represented as individual software objects. Reactions between molecules occur stochastically, according to probabilities derived from known rate constants. An important feature of the program is its ability to represent multiple post-translational modifications and conformational states of protein molecules.

<http://www.anat.cam.ac.uk/pages/comp-cell/StochSim.html>

- **E-Cell: A Multi-Algorithm, Multi-Timescale Simulation Software Environment**

E-Cell System is an object-oriented software suite for modeling, simulation, and analysis of large scale complex systems such as biological cells, architected by Kouichi Takahashi and written by a team of developers. Core part of the system, E-Cell Simulation Environment version 3, allows many components driven by multiple algorithms with different timescales to coexist. E-Cell System consists of the following three major parts:

E-Cell Simulation Environment (or E-Cell SE) E-Cell Modeling Environment (or E-Cell ME) E-Cell Analysis Toolkit

<http://ecell.sourceforge.net/>

- **COPASI (Complex Pathway Simulator)**

Based on a precursor project, Gepasi, COPASI incorporates a model generator, different simulation techniques, optimization routines, methods from nonlinear dynamics and user-friendly visualization platforms. This new software will enable experimental biochemists around the world to simulate complex metabolic processes in cells without having to master complex mathematical and computer skills. COPASI software will greatly expand our knowledge of cellular processes, merging the future of biology with computational modeling and simulation.

http://www.vbi.vt.edu/research/projects/resproj_mendes_copasi.htm

- **Systems Biology Software Project**

Researchers in quantitative systems biology make use of a large number of different software packages for modeling, analysis, visualization, and general data manipulation. The Systems Biology Workbench (SBW), is a software framework that allows heterogeneous application components-written in diverse programming languages and running on different platforms-to communicate and use each others capabilities via a fast binary encoded-message system. Our goal was to create a simple, high performance, open-source software infrastructure which is easy to implement and understand. SBW enables applications (potentially running on separate, distributed computers) to communicate via a simple network protocol. The interfaces to the system are encapsulated in client-side libraries that we provide for different programming languages.

<http://sbw.kgi.edu/research/sbwIntro.htm>

- **MCell and DReAMM (A General Monte Carlo Simulator of Cellular Microphysiology)**

Cells are tightly packed with structures and molecules that carry out the day-to-day operations of living. Understanding how cellular design dictates function is essential to understanding life and disease, in the brain, heart, or elsewhere. MCell (Monte Carlo cell) is a program that uses spatially realistic 3-D cellular models and specialized Monte Carlo algorithms to simulate the movements and reactions of molecules within and between cells-cellular microphysiology.

<http://www.mcell.psc.edu/>

- **SigPath**

SigPath is a prototype of an information system for cell signaling pathways and networks. A primary emphasis of SigPath is that biochemical information can be stored both at the qualitative and quantitative levels. When information is stored quantitatively, SigPath can assist users in generating quantitative models that can be used to simulate how the concentrations of the molecules involved in a model change over time.

<http://icb.med.cornell.edu/services/sp-prod/sigpath/mainMenu.action>

- **BioSigNet-RRH**

BioSigNet-RRH is a knowledge-based system for Representing, Reasoning and Hypothesizing about signal networks. It consists of two components: BioSigNet-RR and BioSigNet-H. BioSigNet-H

BioSigNet-H takes input including a knowledge base (KB) and observations. The observations are assumed not to be explained by the KB. BioSigNet-H generates hypotheses which are modifications to the KB to account for the observations. It also provides the ranking of hypotheses. The KB and observations are encoded using the RR component. Both the reasoning engines of the H and the RR component are implemented using Smodels (AnsProlog).

<http://www.public.asu.edu/~nhtran/signet/>

- **Mathematica**

Mathematica and Matlab are two extensive general-purpose tools for computation and visualisation of any type of mathematical models. Mathematica is produced by Wolfram Research (<http://www.wolfram.com>) and exists currently as version 5 for the operating systems Microsoft Windows, Macintosh, Linux, and several Unix variants. A valuable source of help is the newsgroup

<news://comp.soft-sys.math.mathematica>.

- **Matlab**

Matlab is produced by MathWorks (<http://mathworks.com>). Matlab is available for the same platforms as Mathematica. A repository exists for user-contributed files (<http://www.mathworks.com/matlabcentral/fileexchange> and <http://www.mathtools.net/MATLAB/toolboxes.html>) as well as a newsgroup (<news://comp.soft-sys.matlab>) for getting help.

5 Publication

5.1 Journals

- **Bioinformatics**

The leading journal in its field, Bioinformatics publishes the highest quality scientific papers and review articles of interest to academic and industrial researchers. Its main focus is on new developments in genome bioinformatics and computational biology. Two distinct sections within the journal - Discovery Notes and Application Notes- focus on shorter

papers; the former reporting biologically interesting discoveries using computational methods, the latter exploring the applications used for experiments.

<http://bioinformatics.oxfordjournals.org/>

- **Molecular Biology and Evolution**

Molecular Biology and Evolution (MBE) publishes research at the interface between molecular and evolutionary biology. The journal publishes investigations of molecular evolutionary patterns and processes, tests of evolutionary hypotheses that use molecular data, and studies that use molecular evolutionary information to address questions about biological function at all levels of organization. Reports of work on comparative and evolutionary genomics and the evolution of molecular structure and function are particularly welcome.

<http://mbe.oxfordjournals.org/>

- **Journal of Computational Biology**

A peer-reviewed journal providing a forum for the communication of technical issues associated with the analysis, management, and visualization of cellular information at the molecular level. Includes papers on genomics, mathematical modeling and simulation, distributed and parallel biological computing, designing biological databases, pattern matching and pattern detection, linking disparate databases and data, new tools for computational biology, relational and object-oriented database technology for bioinformatics, biological expert system design and use, reasoning by analogy, hypothesis formation and testing by machine, and management of biological databases.

http://www.liebertpub.com/publication.aspx?pub_id=31

- **The Journal of Cell Biology**

The Journal of Cell Biology publishes papers on all aspects of cellular structure and function. Areas of interest include, but are not restricted to, nuclear organization and structure, protein and membrane trafficking, signal transduction, cytoskeleton and molecular motors, cell cycle and division, cell growth, survival and death, cellular adhesion and motility, and intercellular communication. The Editors also encourage the submission of manuscripts that define the interfaces between cell biology and other fields, especially those elucidating the cell biological basis of problems in immunology, neurobiology, microbial pathology, developmental biology, and disease. As the methodology of cell biology has come to encompass everything from structural biology to molecular biology, biochemistry to immunocytochemistry, genetics to live cell imaging, submissions are welcome regardless of experimental approach.

<http://www.jcb.org/>

- **ComPlexUs, Modelling and Understanding Functional Interactions in the Life Sciences**

ComPlexUs publishes original articles offering significant new insights into the functioning of complex systems of all kinds, that is systems in which important properties and functions arise from non-trivial collective organization, and which cannot be understood by analysis of the parts of the system alone. The formidable complexity of such systems ranging from genetic regulatory networks to marine ecosystems, human communities and organizations

to ecologies of interacting technologies demands new experimental and theoretical tools. Hence, ComPlexUs aims to facilitate communication between researchers in a variety of fields such as medicine, neuroscience, biology, sociology, ecology and bioinformatics, as well as physics, mathematics and economics, following the conviction that complexity science can only thrive on the basis of fruitful cross-disciplinary exchange.

<http://www.complexus.karger.com/>

- **In Silico Biology**

The major focus of this journal shall be on the data acquisition, development, and applications of theoretical / mathematical / computational tools onto biological systems rather than on the description of new algorithms. Thus, the journal will focus on publication of: results of applied computational biology algorithms provided they are presented together with an application program systematic compilations of biological as well as computational results from various sources evaluation of original experimental data with biocomputational tools WWW-online resources for experimental scientists

<http://www.bioinfo.de/isb/>

- **Genetic and Molecular Research**

GMR is a peer-reviewed, all-electronic journal available at no charge to readers via the Internet on the FUNPEC (Ribeiro Preto foundation for research) website. The overall aim of GMR is to publish original, outstanding research papers in the areas of Genetics, Molecular Biology and Evolution.

<http://www.funpecrp.com.br/>

- **Signal Transduction Knowledge Environment (STKE)**

The overarching goal of Sciences STKE is to identify and develop a mix of tools and approaches (algorithms, schemas, programs, and human organizational structures) that are stable, scalable, interoperable, and cost effective for providing access to information on cell signaling. All aspects of Sciences STKE are designed to facilitate the sites main purpose, which is to maximize the efficiency with which the reader gathers, assimilates, and understands information about cell regulatory processes. We strive to increase the likelihood of the scientist making new connections between facts from discrete sources, and to support educational, collaborative, and community building efforts. An additional goal of the site is to better understand and provide the tools and organizational structures scientific authorities need when they attempt to systematize knowledge of cell signaling into the Connections Map database. As we meet our goals of increasing the speed and effectiveness of information transfer, we expect all STKE users to benefit.

<http://stke.sciencemag.org/>

- **Biology of the cell**

Biology of the Cell publishes original research articles and reviews on all aspects of cellular and molecular biology and cell physiology, e.g. structure-function relationships with respect to basic cell and tissue functions, development, neurobiology, immunology, microbiology and protistology, and plant biology. The Journal also publishes a new specific discussion and general interest section called Scientiae Forum.

<http://www.biolcell.org/>

- **Cell Biology International**

Cell Biology International is the official publication of the International Federation for Cell Biology. Each month, the journal publishes easy-to-assimilate, up-to-the-minute reports of experimental findings by researchers using a wide range of the latest techniques. Promoting the aims of cell biologists world-wide, papers relating to structure and function - especially where the findings are seen in a whole cell (physiological) context - are very welcome. In covering all areas of the cell, the journal is both appealing and accessible to a broad audience.

http://www.elsevier.com/wps/find/journaldescription.cws_home/622803/description#description

- **Cell Calcium**

Cell Calcium covers all fields of calcium metabolism and signalling in living systems, publishing works from all branches of life science and medicine. This includes all aspects of calcium in biological systems from inorganic chemistry to physiology, molecular biology and pathology.

http://www.elsevier.com/wps/find/journaldescription.cws_home/623014/description#description

- **Cell Metabolism**

Cell Metabolism publishes reports of novel results in any area of metabolic biology, from molecular and cellular biology to translational studies. The unifying theme is homeostatic mechanisms in health and disease, from simpler model systems all the way to the clinic. Published work should not only be of exceptional significance within its field, but also of interest to researchers outside the immediate area. Cell Metabolism also provides expert analysis and commentary on key findings in the field. The journal's mission is to provide a forum for the exchange of ideas and concepts across the entire metabolic research community, cultivating new areas and fostering cross-disciplinary collaborations in basic research and clinical investigation.

http://www.elsevier.com/wps/find/journaldescription.cws_home/704292/description#description

- **Cellular Signalling**

This journal publishes full-length, original papers covering all aspects of mechanisms, actions and structural components of cellular signalling systems. The emphasis is on effector systems, such as protein kinases, lipid signalling pathways, cyclic nucleotide signalling processes, NO signalling and ion channels; the production, regulation, degradation and action of second messengers; the structure, regulation, degradation and action of receptors; guanine nucleotide regulatory proteins; bio-informatic studies related to cell signalling mechanisms; compartmentalisation/compartimentation of signalling systems; anchor/scaffold signalling proteins; the effect of cellular signalling events on the functioning, growth and differentiation of cells in normal and pathological states and also cellular oncogenes. Within the scope of the journal, manuscripts dealing with biochemistry, protein structure, cell biology, molecular biology, pharmacology, neurobiology, molecular endocrinology and molecular oncology are welcomed. The journal also publishes original reviews on topical subjects.

http://www.elsevier.com/wps/find/journaldescription.cws_home/525462/description#description

- **Current advances in cell & developmental biology**

Contents: Major subject areas covered include: Cell surface; Cell junctions and cell adhesion; Endocytosis, phagocytosis, exocytosis; Secretion; Signal transduction mechanisms; Cytoplasmic membranes; Intracellular transport and polarity; Cell growth and division; Growth factors and inhibitors; Cellular senescence and death; Cytoskeleton; Cell motility; Extracellular matrix; Tumor cell biology; Differentiation; Culture studies; Gamete biology and fertilization; Development of tissues and organs; Determination, pattern formation and morphogenesis; Regeneration; Developmental genetics; Molecular biology of development; Embryonic development; Post-embryonic development; Techniques.

http://www.elsevier.com/wps/find/journaldescription.cws_home/733/description#description

- **Trends in Cell Biology**

Trends in Cell Biology is among the leading review journals in molecular and cell biology (Impact Factor 19.6; ISI 2003). Review articles published each month monitor the breath and depth of current research in cell biology, reporting on new developments as they happen and integrating methods, disciplines and principles. All articles are commissioned from leading scientists and then subjected to stringent peer-review, ensuring balance and accuracy. In addition to Reviews, Trends in Cell Biology publishes Opinion and Research Focus articles. Opinion articles follow trends and innovative ideas whilst the Update section is designed to highlight recent advances in a particular research field providing insight on the implications of the new developments as well as future perspectives and directions.

http://www.elsevier.com/wps/find/journaldescription.cws_home/422552/description#description

5.2 Conferences

- **The Pacific Symposium on Biocomputing (PSB)**

The Pacific Symposium on Biocomputing (PSB) 2006 is an international, multidisciplinary conference for the presentation and discussion of current research in the theory and application of computational methods in problems of biological significance. Papers and presentations are rigorously peer reviewed and are published in an archival proceedings volume. PSB 2006 will be held January 3-7, 2006 at the Grand Wailea in Wailea, Maui. Tutorials will be offered prior to the start of the conference.

PSB 2006 will bring together top researchers from the US, the Asian Pacific nations, and around the world to exchange research results and address open issues in all aspects of computational biology. PSB is a forum for the presentation of work in databases, algorithms, interfaces, visualization, modeling, and other computational methods, as applied to biological problems, with emphasis on applications in data-rich areas of molecular biology.

The PSB has been designed to be responsive to the need for critical mass in sub-disciplines within biocomputing. For that reason, it is the only meeting whose sessions are defined dynamically each year in response to specific proposals. PSB sessions are organized by

leaders in the emerging areas and targeted to provide a forum for publication and discussion of research in biocomputing's "hot topics." In this way, PSB provides an early forum for serious examination of emerging methods and approaches in this rapidly changing field.

<http://psb.stanford.edu/>

- **Computational Methods in Systems Biology**

Molecular biology has traditionally considered biological molecules as isolated entities or as components of simple systems. However these molecules participate in very complex networks in living systems, including: regulatory networks for gene expression; intracellular metabolic networks; and both intra- and intercellular communication networks. Recent progress in biology in high-throughput data-production technologies has given the prospect of a new approach, focussing on how components work together as a system. The CMSB workshop is intended to catalyze the convergence between modellers (theoretical computer scientists from fields such as language design, concurrency theory or program verification, mathematicians and physicists) and biologists interested in such a systems-level understanding of cellular processes.

<http://homepages.inf.ed.ac.uk/v1bklin/cmsb05/>

<http://www.biopathways.org/CMSB04/>

<http://www.unitn.it/convegna/cmsb.htm>

- **2nd International Symposium on Computational Cell Biology (2003)**

The Symposium for Computational Cell Biology provides a unique opportunity for cell biologists who are interested in computational approaches to interact with theoreticians and computer scientists who are developing computational modeling applications for cell biological processes. The scientific focus of the meeting is on areas of cell biology for which modeling approaches are currently being developed, or that are ripe for computational modeling approaches. Topics at the meetings will encompass a range of cellular mechanisms including regulation of the cytoskeleton and molecular motors, membrane and protein trafficking, regulation of calcium dynamics, signal transduction pathways, and cell cycle control.

<http://www.nrcam.uchc.edu/2ndsymposium/main.html>

- **International Conference on Systems Biology (ICSB)**

The International Conference on Systems Biology (ICSB) aims at bringing together researchers working in the field of Systems Biology and related field to present the current status of their research and to discuss future directions of the research. The importance of the system-level understanding of biological systems is receiving increasing recognition recently. Nevertheless, the efforts are still sporadic and there is no conference specifically focused on this topic. While there are conferences on bioinformatics, the threads of the conferences are the use of computers in various aspects of biology, and not necessarily viewed from the systems-level perspectives. Thus, these conferences cover broadly from sequence database, protein structures to gene networks, and less emphasis on the system-level understanding. Therefore, the goal of the conference is to create a forum of discussion for those who are interested in system-level understanding from various approaches for various biological systems, so that such efforts can form a coherent landscape. Systems Biology that focuses on system-level understanding can be a major thrust in biology in the 21st century.

<http://www.symbio.jst.go.jp/systemsbiology/news/icsb/>

<http://www.icsb2001.org/>

<http://icsb2003.molecool.wustl.edu/>

<http://www.icsb2004.org/>

<http://csbi.mit.edu/icsb-2005>

- **SIAM Conference on the Life Sciences 2004**

The SIAM Activity Group on Life Sciences brings together researchers who seek to develop and apply mathematical and computational methods in all areas of the life sciences. This conference of the activity group will provide a cross-disciplinary forum for catalyzing mathematical research relevant to the life sciences. It will facilitate rapid diffusion of new mathematical and computational methods in the life sciences, and may stimulate more researchers to work in these important areas. Mathematicians, life scientists, computational biologists, bioengineers and others interested in mathematical and computational analysis of biological systems are encouraged to attend.

<http://www.siam.org/meetings/ls04/>

- **European Conference on Artificial Life (ECAL)**

<http://kal-el.ugr.es/ecal95.html>

<http://diwww.epfl.ch/lami/ecal99/>

<http://www.comdig2.de/Conf/ECAL2003/>

<http://www.ecal2005.org/>

- **International Conference on the Simulation and Synthesis of Living Systems (ALIFE)**

<http://alife6.alife.org/>

<http://alife7.alife.org/>

<http://www.alife.org/alife8/>

<http://www.alife9.org/>

<http://www.alifex.org/>

- **Foundations of Systems Biology in Engineering (FOSBE)**

FOSBE 2005 (Foundations of Systems Biology in Engineering) is the first in a series of conferences offered by the CACHE organization to address the emerging challenges in the field of Systems Biology. The conference is unique in that it addresses not only current research problems, but also the curricular developments and industrial needs and challenges in this important intersection of biology and engineering. FOSBE brings together researchers from biochemical engineering, systems engineering, complex systems research, computational biologists, computer science, and experimental biologists. Furthermore, the audience will include academic researchers, experts from industry (including pharmaceutical, biotech, and biomedical products), government laboratories (DOE, and Department of Defense), and federal funding agencies to discuss the advances, challenges, and emerging opportunities in systems biology.

<http://www.fosbe.org/>

- **Genetic and Evolutionary Computation Conference (GECCO)**

The Genetic and Evolutionary Computation Conference (GECCO) will present the latest high-quality results in the growing field of genetic and evolutionary computation. Topics include: genetic algorithms, genetic programming, evolution strategies, evolutionary programming, real-world applications, learning classifier systems and other genetics-based machine learning, evolvable hardware, artificial life, adaptive behavior, ant colony optimization, swarm intelligence, biological applications, evolutionary robotics, evolutionary combinatorial optimization, coevolution, artificial immune systems, and more.

<http://www.sigevo.org>

- **Workshops on Membrane Computing - At the Crossroads of Cell Biology and Computation (WMC)**

To bring together researchers working in membrane computing and related areas (such as DNA computing, artificial life, computational biology, theory of computation) in a friendly atmosphere enhancing communication and cooperation. As the subtitle suggests, the focus will be on bringing membrane systems back to biology.

<http://psystems.disco.unimib.it>

- **International Meetings on DNA Computing (DNA)**

Biomolecular computing has emerged as an interdisciplinary field that draws together chemistry, computer science, mathematics, molecular biology, and physics. Our knowledge of DNA nanotechnology and biomolecular computing increases dramatically with every passing year. The international meeting on DNA Computing has been a forum where scientists with different backgrounds, yet sharing a common interest in biomolecular computing, meet and present their latest results.

<http://hagi.is.s.u-tokyo.ac.jp/dna/>

- **Synthetic Biology**

<http://web.mit.edu/synbio/release/conference/speakers.html>

http://openwetware.org/wiki/BioBricks_abstraction_hierarchy

<http://syntheticbiology.org/>

6 Selected Research Groups

- **MIT, Doug Lauffenburger's Research Group**

Cell Signaling and Regulatory Networks: In this area we are attempting to understand, in terms of quantitative systems dynamics, regulation and dysregulation of cell phenotypic behavioral responses (e.g., death, proliferation, differentiation, migration) as governed by signaling networks activated by growth factors, cytokines, and extracellular matrix. We have a major focus on the EGF receptor family, which is strongly implicated in cancer progression, and on the TNF receptor family, which is involved in cell death/survival decisions in response to tissue. Our goal is to develop predictive computational models for cell phenotypic decisions in terms of underlying signaling network properties, with application to drug discovery and development.

<http://web.mit.edu/dallab/researcha.htm>

- **Stanford University, Nolan Lab**

The laboratory focuses on signaling in the immune system and study of host processes that HIV-1 exploits. Control of apoptosis, autoimmunity, angiogenesis, retrovirology, and blockade of HIV-1 infection are prominent in our studies. We use advanced Flow Cytometric analysis (FACS) of phosphoproteins in single cells and dominant effector genetics to achieve many of our goals. For this we have developed a range of FACS assays, cDNA and peptide expression systems using viruses, and single-cell genetic selections, to study pathways of interest to us.

<http://www.stanford.edu/group/nolan/>

- **University of New Mexico, Center for the Spatiotemporal Modeling of Cell Signaling Networks**

The STMC is a NIH/NIGMS Center of Excellence in Complex Biomedical Systems Research located in the Health Sciences Center (HSC) at the University of New Mexico, and with co-leaders and members in the College of Engineering and College of Arts and Sciences and at Sandia National Laboratory. Its goals are: to integrate mathematical, statistical and computational modeling into ongoing research on complex cell signaling networks; to provide students with the biological, mathematical, statistical and computational tools needed to conduct complex biomedical systems research; to disseminate modeling software and cell signaling knowledge to the biomedical and computational communities.

<http://cellpath.health.unm.edu/stmc/>

- **University of Connecticut Health Center, the National Resource for Cell Analysis and Modeling (NRCAM)**

NRCAM is developing a unique software modeling environment, the Virtual Cell, for quantitative cell biological research. NRCAM is currently funded through the NCCR, National Center for Research Resources, a component of the National Institutes of Health (NIH).

<http://www.nrcam.uchc.edu/>

- **Keck Graduate Institute, Computational Systems Biology**

This site is devoted to computational aspects of biochemical networks, their dynamics, function and evolution, be they metabolic, signal or gene networks. Various labels have been used to describe the study of such networks, including Systems Biology, 'Computational Biology or even perhaps Molecular Physiology. We will use the label Computational Systems Biology to cover an area of research concerned with how chemical networks within living systems behave.

<http://sbw.kgi.edu/>

- **Carnegie Mellon University, The Center for Quantitative Biological Simulation (CQBS)**

Research efforts encompass large scale, high performance programming development, predominantly MCell, PSC DX, and DReAMM, and their application to quantitative modeling and simulation in a variety of biological settings. Education efforts encompass workshops

on spatially realistic cell modeling conducted with the NRBSC, as well as extensive web-based technical and scientific tutorials and reference information on in-house software and computational microphysiology applications.

<http://www.mcell.psc.edu/index.html>

- **Cornell University, Institute for Computational Biomedicine (ICB)**

ICB realizes potential of mathematics and computation to enhance the study of medicine. Employing the tools of applied mathematics and computer-based technologies to enable physicianscientists to attack complex medical problems formerly beyond their reach.

<http://icb.med.cornell.edu>

- **University of Toronto, Attisano and Wrana Lab**

Study of molecular mechanism underlying TGF superfamily signaling. Use of biochemical and molecular genetic approaches.

<http://ana30.med.utoronto.ca/attisano.htm>

- **Columbia University, National Center for the Multiscale Analysis of Genomic and Cellular Networks**

Center activities will involve a significant, multidisciplinary effort that will forge new relationships between the biological and computational sciences at the interface between several disciplines including biochemistry and molecular biophysics, biomedical informatics, computer science, engineering, biology, and applied physics. As such, the Center will encompass and integrate diverse research areas at varying degrees of granularity.

<http://magnet.c2b2.columbia.edu>

- **University of Helsinki, Institute of Biotechnology and Institute of Biomedicine, DevSignal-Net**

The DevSignalNet has specialised in elucidating the molecular pathways that regulate organo- genesis in both vertebrate and invertebrate model systems. The focus is in signalling networks that mediate the communication between cells during morphogenesis and guide the differentiation of progenitor cells. Special interest are the following topics: Inductive interactions in organogenesis Regulation of stem cell behaviour Evolutionary developmental biology Regulation of gene expression at the level of RNA processing

<http://www.biocenter.helsinki.fi/devsignalnet/>

- **Facult de Mdecine Toulouse, Laboratoire dinteractions et Signalisation Cellulaire : relations hte-pathogne**

Thmes de recherche: Analyse de la rponse du pneumocoque la densit cellulaire, la concentration loxygne, la disponibilit en sources de carbone et la valeur du pH Pathognie, pidmiologie et taxonomie des bactries tropisme respiratoire : Pseudomonas aeruginosa et Burkholderia cepacia

http://www.ups-tlse.fr/RECHERCHE/le_laboratoire.php3?codeLabo=1936

7 Conclusion

This report surveys aspects of the state of the art with regard to workpackage 3. Considering a bibliography, online databases and tools, software, platforms for publication, and selected external research groups, it serves as a basic document for further studies.

8 Acknowledgements

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A Glossary

Allostery In biochemistry, an enzyme or other protein is allosteric if its activity or efficiency changes in response to the binding of an effector molecule at a so-called allosteric site. Changes that enhance activity are referred to as allosteric activation, while the opposite is called allosteric inhibition. "Allostery" is derived from the Greek "other site", referring to the typical scenario in which an enzyme's allosteric and active sites are distinct.

Anabolism the energy-requiring building phase of metabolism in which simpler substances are combined to form more complex substances.

Apoptosis A normal series of events in a cell that leads to its death.

ATP (Adenosine TriPhosphate) A molecule consisting of adenosine (adenine plus a ribose sugar) and three phosphate groups. The last two phosphates are joined by high energy bonds which provide energy used in chemical reactions such as respiration and glycolysis. In plants, ATP is formed in the chloroplasts during photosynthesis.

Autocatalysis A single chemical reaction is said to have undergone autocatalysis, or be autocatalytic, if the reaction product is itself the catalyst for that reaction.

Autopoiesis Autopoiesis literally means "self-production" (from the Greek: auto for self- and poiesis for creation or production) and expresses a fundamental complementarity between structure and function. The term was originally introduced by Chilean biologists Francisco Varela and Humberto Maturana in the early 1970s: The canonical example of an autopoietic system, and one of the entities that motivated Varela and Maturana to define autopoiesis, is the biological cell.

CAS/Crk signalling Molecular coupling of p130CAS and Crk occurs in response to integrin activation and plays a pivotal role in signalling pathways, culminating in cell proliferation, survival and migration [Chodniewicz and Klemke 2004].

Deregulated CAS/Crk signalling mechanisms are involved in the pathogenesis of malignant metastases [Chodniewicz and Klemke 2004]. The Abl protein is a negative regulator of CAS/Crk coupling, serving to suppress the CAS/Crk-mediated signalling cascade. CAS/Crk-mediated cell

invasion and survival requires activation of the small GTPase Rac, while uncoupling CAS from Crk prevents migration and induces apoptosis of the invasive tumour cells.

Catabolism The metabolic breakdown of large molecules in living organism, with accompanying release of energy.

Catalysis An increase in the velocity of a chemical reaction or process produced by the presence of a substance that is not consumed in the net chemical reaction or process; negative catalysis denotes the slowing down or inhibition of a reaction or process by the presence of such a substance.

Catalyst A chemical substance that increases the rate of a reaction without being consumed; after the reaction it can potentially be recovered from the reaction mixture chemically unchanged. The catalyst lowers the activation energy required, allowing the reaction to proceed more quickly or at a lower temperature. In a fuel cell, the catalyst facilitates the reaction of oxygen and hydrogen. It is usually made of platinum powder very thinly coated onto carbon paper or cloth.

Cell Smallest unit of life (single cell organism or bacteria) or unit of higher organisms, i.e., multicellular organisms. Cells are surrounded by a cell membrane (and cell wall in bacteria and plants = a membrane plus some chemically more stable structures, often mixtures of proteins and polysaccharides) and contain all necessary elements to sustain life; proteins, nucleic acids, lipids, minerals, and a diverse class of metabolites. Cells of higher organisms (known as eukaryotes) are subdivided into subcellular compartments called organelles such as the mitochondrion, the cell nucleus, the endoplasmic reticulum, the Golgi apparatus and many smaller organelles with highly specialized functions. While all these organelles are found in animal cells, plant cells in addition contain a central vacuole that controls pressure to stabilize the cell and chloroplasts, the site of photosynthesis or light depended biosynthesis of sugars (carbohydrates).

Cell-cell signaling Any process that mediates the transfer of information from one cell to another.

cell-cell signaling involved in cell fate commitment Signaling between cells that results in the commitment of a cell to a certain fate. This is often done by secretion of proteins by one cell which affects the neighboring cells and causes them to adopt a certain fate.

Chemotaxis Directional movement (migration) of biological cells or organisms in response to concentration gradients of chemicals, whereby the cells are attracted or repelled by substances exhibiting chemical properties.

Chemoton The chemoton model was introduced by Gnti in 1971 (see review Gnti 2002) as a fundamental unit model of living systems. It consists in three functionally dependent autocatalytic subsystems: the metabolic chemical network, the template polymerization and the membrane subsystem enclosing them all. The correct functioning of the chemoton lies in the precise stoichiometric coupling of the three subunits. It ensures that both the surface and the inner components evolve into doubling their initial value, leading to the subsequent division into

two identical chemotons. Besides the detailed introductory papers of Gnti, only a few studies of this model exist in the literature, presenting however contradictory conclusions. The present study aims toward a thorough survey of the chemoton's characteristics, such as replication period or optimal template length, in the parameters' space. Additionally, a comparative study between the deterministic approach and the stochastic one is performed.

CSNDB (Cell Signaling Networks Database) A database for cell signaling networks. <http://geo.nih.gov/jp/csndb.html>. Doesn't seem to be running anymore

Crosstalk The undesirable addition of one signal to another in a circuit usually caused by coupling through parasitic elements. An example would be inductive or capacitive coupling between adjacent conductors.

Cytokine Proteins manufactured by cells of various lineages that, when secreted, drive specific responses (eg, proliferation, growth, or maturation) in other susceptible cells.

Cytoplasm the protoplasm of a cell excluding the nucleus, The material between the nuclear and cell membranes; includes fluid (cytosol) organelles, and various membranes.

Cytosolic The cytosol (as opposed to cytoplasm, which also includes the organelles) is the internal fluid of the cell, and a large part of cell metabolism occurs here. Proteins within the cytosol play an important role in signal transduction pathways, glycolysis, and they act as intracellular receptors and form part of the ribosomes, enabling further protein synthesis. In prokaryotes, all chemical reactions take place in the cytosol.

Dimerization Dimerization is the formation of a polymer from two similar chemical structures.

Eukaryote Living organism composed of one or more cells with a distinct nucleus and cytoplasm. Includes all forms of life except viruses and bacteria (prokaryotes).

G protein

Synonym: *GTP binding protein*

A heterotrimeric guanine nucleotide binding protein that transduces a signal derived from a transmembrane receptor; also, small cytoplasmic proteins that regulate intracellular process.

Gap junction A specialized area of apposed plasma membranes containing connexons, proteins that bridge the extracellular space and that allow the cytoplasm of one cell to communicate with that of the other cell.

Genetic regulatory network A gene regulatory network (also called a GRN or genetic regulatory network) is a collection of DNA segments in a cell which interact with each other and with other substances in the cell, thereby governing the rates at which genes in the network are transcribed into mRNA.

Homeostasis In biology used to describe a condition where an organism maintains a stable structure where in fact a constant flux of molecules occurs. Although many organisms

can live for years, all cellular components like proteins, membranes, sugars, and nucleic acids are constantly recycled while never compromising the integrity of the organism as a whole. This turnover processes can be characterized by specific half-life values that for most proteins, membranes, and RNA (but not DNA structures) are measured in hours. In a more narrow sense homeostasis refers to the maintenance of water and salt concentration in cells

Kinase

Synonyms: *Phosphokinase, Phosphotransferases, Transphosphorylases*

An enzyme that catalyzes the transfer of phosphate groups from a high-energy phosphate-containing molecule (as ATP or ADP) to a substrate.

Ligand A small molecule that binds specifically to a larger one; for example, a hormone is the ligand for its specific protein receptor.

Lysis In biology, lysis refers to the breakdown of a cell caused by damage to its plasma (outer) membrane. Lysis can be caused by chemical or physical means (for example, strong detergents or high-energy sound waves) or by an infection.

Mesoscopic In physics and chemistry, the mesoscopic scale refers to the length scale at which one can reasonably discuss the properties of a material or phenomenon without having to discuss the behavior of individual atoms. For solids and liquids this is typically a few to ten nanometers, and involves averaging over a few thousand atoms or molecules. Hence, the mesoscopic scale is roughly identical to the nanoscopic or nanotechnology scale for most solids.

Metabolism Sum of the physical and chemical changes that take place in living organisms. These changes include both synthesis (anabolism) and breakdown (catabolism) of body constituents. In a narrower sense, the physical and chemical changes that take place in a given chemical substance within an organism. It includes the uptake and distribution within the body of chemical compounds, the changes (biotransformations) undergone by such substances, and the elimination of the compounds and their metabolites.

Metabolic control analysis (MCA)

Synonym: *Metabolic control theory*

Metabolic control analysis is a phenomenological quantitative sensitivity analysis of fluxes and metabolite concentrations. In MCA one studies the relative control exerted by each step (enzyme) on the system's variables (fluxes and metabolite concentrations). This control is measured by applying a perturbation to the step being studied and measuring the effect on the variable of interest after the system has settled to a new steady state.

Monomer (Chemistry) Any molecule that can be chemically bound as a unit of a polymer. Neutron Star: A collapsed star of extremely high density. Generally these objects have slightly more mass than the Sun, but are only about 10 km in radius. A neutron star has intense gravity, and may also have an intense magnetic field and fast rotational component. Oort Cloud: The spherical region around the Sun thought to contain a large number of cometary bodies.

Osmosis Osmosis is the diffusion of a solvent through a semipermeable membrane from a region of low solute concentration to a region of high solute concentration. The semipermeable membrane is permeable to the solvent, but not to the solute, resulting in a chemical potential difference across the membrane which drives the diffusion.

Organelle A subcellular structure having a specialized function for example the mitochondrion, the chloroplast, or the spindle apparatus.

Phosphorylation The addition of a phosphate group to a compound by an enzyme (eg, thymidine kinase, tyrosine kinase). Phosphorylation is an essential step in many cellular processes.

Polymer Large organic molecule formed by combining many smaller molecules (monomers) in a regular pattern.

Polymerization A chemical reaction in which two or more small molecules combine to form larger molecules that contain repeating structural units of the original molecules. A hazardous polymerization is the above reaction with an uncontrolled release of energy.

Prokaryote Organism made of simple cells that lack a well-defined, membrane-enclosed nucleus: a bacterium.

Protein A molecule made up of amino acids that are needed for the body to function properly. Proteins are the basis of body structures such as skin and hair and of substances such as enzymes, cytokines, and antibodies.

Regulatory sequences

Synonyms: *Genetic regulatory element, Regulator regions, nucleic acid, Regulatory regions*

Nucleic acid sequences involved in regulating the expression of genes.

Ribozyme A ribozyme, or RNA enzyme, is an RNA molecule that can catalyze a chemical reaction. Many natural ribozymes catalyze either their own cleavage or the cleavage of other RNAs, but they have also been found to catalyze the aminotransferase activity of the ribosome. Investigators studying the origin of life have produced ribozymes in the laboratory that are capable of catalyzing their own synthesis under very specific conditions.

Second messenger In biology, second messengers are low-weight diffusible molecules that are used in signal transduction to relay a signal within a cell. They are synthesized or released by specific enzymatic reactions, usually as a result of an external signal that was received by a transmembrane receptor and pre-processed by other membrane-associated proteins. There are three basic types of second messenger molecules:

- Hydrophobic molecules like diacylglycerol and phosphatidylinositols are membrane-associated and diffuse from the plasma membrane into the juxtamembrane space where they can reach and regulate membrane-associated effector proteins.
- Hydrophilic molecules are water-soluble molecules, like cAMP, cGMP, and Ca²⁺, that are

located within the cytosol.

- gases, nitric oxide (NO) and carbon monoxide (CO), that can diffuse both through cytosol and across cellular membranes.

These intracellular messengers have some properties in common:

- They can be synthesized/released and broken down again in specific reactions by enzymes.
- Some (like Ca²⁺) can be stored in special organelles and quickly released when needed.
- Their production/release and destruction can be localized, enabling the cell to limit space and time of signal activity.

<http://www.nationmaster.com/encyclopedia/Second-messenger>

SPAD (Signaling Pathway Database) The Signaling PATHway Database is an integrated database for genetic information and signal transduction systems.

<http://www.grt.kyushu-u.ac.jp/spad/>

Signal transduction The cascade of processes by which an extracellular signal (typically a hormone or neurotransmitter) interacts with a receptor at the cell surface, causing a change in the level of a second messenger (for example calcium or cyclic AMP) and ultimately effects a change in the cell's functioning (for example, triggering glucose uptake, or initiating cell division). Can also be applied to sensory signal transduction, eg. of light at photoreceptors.

Stoichiometric Calculation of the quantities of chemical elements or compounds involved in a chemical reaction.