A bioinformatics framework for modeling, visualization and simulation of gene regulatory networks
Evert-Jan Blom1, Dinne W.J. Bosman1,2, Patrick Ogao2, Oscar P. Kuipers1, Jos B.T.M. Roerdink2
1 Dept. of Molecular Genetics, University of Groningen
2 Dept. of Mathematics and Computing Science, University of Groningen

Transcriptome analysis using DNA-microarrays based on time-series of *B. subtilis* provides a wealth of information. To make optimal use of the large number of (transcriptome) data and results on regulatory pathways being generated, our key objective is to develop an automated gene regulatory identification system. For this purpose, a system is developed which combines transcriptome information with knowledge from literature in order to reconstruct gene regulatory networks. All the information concerning those networks is stored in an object oriented database which serves as a data repository. Our system will enable users to gain insight in the organization and behavior of large regulatory gene networks by dynamical systems modeling, simulation, interactive visualization, cluster analysis and pattern recognition.

Design and performance improvement of cellular factories for biotechnological production processes.
From black box to white box approaches and vice versa?
Roel Bovenberg, Marco van den Berg, Han de Winde en Herman Pel
DSM
P.O. Box 425, 2600 AK, Delft
The Netherlands
Postal point 624-0270; tel 31-152792998; fax 31 152793779; roel.bovenberg@dsm.com

At DSM a large number of production processes are based on microbial fermentations. Typical examples are processes for the production of Baker’s yeast (*S. cerevisiae*), penicillin and cephalosporin (*P. chrysogenum*), enzymes (*A. niger*, *K. lactis*, *Bacillus* sp, *E. coli*) and other primary or secondary metabolites (*E. coli*, *Streptomyces* sp.,). In addition, production of pharma proteins and monoclonal antibodies with mammalian cell systems has become an important business area. Further extensions lie ahead in the area of vitamins and nutritional pigments. Traditionally, DSM, The Netherlands and Europe are recognized strongholds in these areas of Biotechnology. The production processes of today are the result (and subject) of continuous improvement programs. Strains are exposed to a variety of genetic techniques (both classical and modern), analyzed in screening or selection regimes and tested on performance at different scales under different physiological and physical constraints. Despite major scientific advancements of the last couple of years, the whole process is still largely a black box approach.

Investments have been made by DSM in the last few years to enable us to analyze (some of) our strains and processes at various intra-cellular levels: genome, transcriptome, proteome, metabolome and cellular infrastructure. In addition, relevant metabolic modeling and flux analysis skills have been developed as well as a Bio-IT infrastructure. This will allow us to analyze our processes at various scales and subsequently, to use the various data sources to build comprehensive models to help
understand the complex systems we are dealing with. The ultimate dream is to come to a fully predictive model for strain and process design.

Typical examples of complex problems encountered nowadays are:

- Control of filamentous fungal morphology, compartmentation and spore formation
- Optimal design of product pathways, in tune with the rest of metabolism
- Improvement of secretion pathways
- Design of pre-set genetic or physiological switches
- Data handling requires convenient interface software tools (MES, SCADA like?) and skills. How do you build relevant models efficiently?

The presentation will selectively address one or two of these problems in more detail. Moreover, the resulting system will be applied for the determination and visualization of gene networks in other prokaryotes.

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A molecular jungle organizing signal transduction

Dennis Bray.
Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK.

The pathway controlling chemotaxis in Escherichia coli is the simplest and most well understood cell signalling system to date. However, quantitative models based on the available data still fail to reproduce important features of the pathway. Most notably, the observed sensitivity of cells to very small changes in stimulus concentrations cannot be reproduced by conventional models based on the measured concentrations, binding affinities and rate constants of the proteins involved. This discrepancy, together with recent experimental findings, drew our attention to the spatial organization of molecules within the cell and in particular to the clusters of receptors localised at the cell poles. A stochastic simulator for chemical reactions, STOCHSIM, was previously developed to model the chemotaxis pathway at the level of individual molecular interactions. This program has now been extended to incorporate a spatial representation that allows the interaction between molecules in a two-dimensional lattice to be simulated. In silico 'experiments' using this new version of STOCHSIM demonstrate that lateral interactions between clustered receptors can significantly enhance the excitation response. The adaptation reactions may also exploit the proximity of receptor molecules, and a hypothetical mechanism by which this may occur is currently being tested.

Cf: Novartis Found Symp 2002;247:162-77

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System Biology Illustrated I: modules, robustness, and silicon cells

Frank J. Bruggeman\textsuperscript{1,2}, Fred C. Boogerd\textsuperscript{1}, Boris N. Kholodenko\textsuperscript{1,3}, and Hans V. Westerhoff\textsuperscript{4}

\begin{enumerate}
\item Molecular Cell Physiology, FALW, CRBCS, Biocentrum aMSTERDAM, Vrije Universiteit, Amsterdam, The Netherlands
\item frankb@bio.vu.nl (www.angelfire.com/scifi/frankb)
\item Dept. of Pathology, Anatomy, and Cell Biology, Thomas Jefferson University, Philadelphia, USA
\end{enumerate}

Regardless of whether organisms are of eukaryotic/prokaryotic or unicellular/multicellular nature, they all have in common that they are extraordinarily complex. Living systems
Emerging System Biology NL; March 6-7, Egmond aan Zee, NL, EU; ABSTRACTS, page: 3

are composed of large numbers of nonlinearly interacting (macro)molecular species: biochemical networks of metabolic pathways and genetic circuitry. Somehow, the properties of living systems are to be explained mechanistically in terms of the properties of the (macro)molecules and the strength of their interactions. This holds in particular for a special class of interesting systemic properties, i.e. emergent properties. Emergent properties are properties manifested at the systemic level and not displayed by any of the components in isolation, e.g. homeostasis, robustness, oscillations, apoptosis, etc. This implies that emergent properties are brought about by (macro)molecular interactions and may critically depend on their strengths! The strengths of these interactions depend nonlinearly on the concentrations of the interacting (macro)molecules and therefore on the state of the living system. This necessitates system reconstruction: the state of the system at time $t$ should be calculated from the properties of the (macro)molecules, boundary conditions, and initial conditions, yielding; catalytic rates, phosphorylation states, strengths of interactions, etc. In some cases, these calculations necessitate appreciation of the spatial organization of the living system or the inherent stochastic of processes among small numbers of (macro)molecules. Performing such calculations with silicon cells and determining what level of description is sufficient (homogenously stirred volume, stochastic description?) is one of the challenges for system biology. Considering our knowledge of molecular-cell biology and physiology we can start building silicon cell kinetic models for some characterized systems (e.g. *E. coli*, baker’s yeast), starting from the experimentally determined kinetic parameters that characterize the (macro)molecules. Another challenge for system biology is to provide tools and concepts to analyze complex living systems. For instance, techniques that allow (i) decomposition of living systems into modules, (ii) quantitative analysis of control, regulation, homeostasis, robustness, adaptation, etc., and (iii) analyze relationships between function and design of living (sub)systems.

We illustrate; (i) how silicon cells can be constructed from experimental and literature data, (ii) how steady-state biochemical networks can be described in terms of modules, and (iii) robustness and adaptation of a metabolic model of ammonium assimilation in *Escherichia coli*.

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**A genetical genomics approach to identify transcriptional pathways in hematopoietic stem cells**

*Leonid V. Bystrykh, Ellen Weersing, Edo Vellenga, Kenneth F. Manley, Robert W. Williams, Michael Cooke and Gerald de Haan*

Dep. of Stem Cell Biology, University of Groningen, the Netherlands; Roswell Park Cancer Institute, Buffalo, NY; University of Tennessee, Memphis, TN; Genomics Inst. Novartis Research Foundation, San Diego, CA.

We have previously documented that hematopoietic stem cells (HSC) isolated from genetically distinct inbred strains of mice show highly variable functional properties. We have mapped a locus responsible for the cycling activity to chromosome 11, and an additional locus, associated with stem cell frequency to chromosome 18. To elucidate molecular pathways associated with such variation, we have now subjected highly purified HSC from C57BL/6, DBA/2 and 30 fully genotyped BXD recombinant inbred strains of mice to a genome-wide Affymetrix microarray analysis. Such a genetic genomics analysis is far more powerful than a regular profiling approach. The data were deposited in a Web-database (The Web-QTL Project,
which allows a custom genetic linkage analysis of all differentially expressed transcripts. Comparing the physical position of each transcript with the mapped controlling locus resulted in two types of expression pattern. First, we identified approximately 300 cis-regulated transcripts, variation in which should be a direct cause of much inheritable variation between B6 and DBA stem cells. Selected analysis of these loci indeed showed a high frequency of polymorphism in these transcripts. Second, we identified large clusters of genes, located throughout the genome, that are controlled by a single, transacting locus. These "transbands" are caused by a single, or a collection of closely associated, gene variants, and they form the basis for the phenotypical manifestation of Quantitative Trait Loci (QTL). Our data immediately identify genetic pathways operating in stem cells, and provide a first analysis of complex networks controlling hematopoietic stem cell functioning.

The geometrical model for cell wall texture: a systems biology approach
Anne Mie C. Emons 1 and Bela M. Mulder 2

1Laboratory of Experimental Plant Morphology and Cell Biology, Department of Plant Sciences Wageningen University, Arboretumlaan 4, 6703 BD Wageningen, The Netherlands
2FOM Institute for Atomic and Molecular Physics (AMOLF), Kruislaan 407, 1098 SJ Amsterdam, The Netherlands

Plant cell walls are made by apposition of new material from the side of the plasma membrane. This material is contained in Golgi vesicles that deliver their content into the existing cell wall after fusing with the plasma membrane. During exocytosis the cellulose synthases present in the membrane of the Golgi vesicles are inserted into the plasma membrane where they are active and produce the building blocks of cell wall texture, the cellulose microfibrils (CMF). CMFs form lamellae and the orientation of the CMFs within a lamella is constant, but may vary from lamella to lamella. The most striking texture is the helicoidal wall, which is formed by subsequent lamellae in which the orientation of the CMFs changes by a constant angle. Other wall textures are the axial, helical, crossed-polylamellate and the random wall textures, and combinations of these. The question of the origin of these wall architectures remains unanswered. The synthases channel UDP-glucose through the plasma membrane while polymerizing the glucoses into cellulose, a 1,4-α-glucan polymer that in turn crystallizes into CMFs (36 cellulose polymers).

We discuss an explicit mathematical model, which becomes dynamic by assuming that new active cellulose synthases are inserted into the plasma membrane through exocytosis of Golgi vesicles within moving, localized regions along the cell which we call insertion domains. The rate at which new rosettes become active is assumed to be under cellular control and regulated. Once inserted into the plasma membrane the cellulose synthases move forward by the forces generated in the deposition process. In the course of time, their angle of motion with respect to the cell axis is continuously adapted to the changing number of other cellulose synthases in their neighborhood in order to satisfy the geometrical close packing constraint. The CMFs that are being deposited follow the tracks of the rosettes and as such constitute a "recording" of the motion of the rosettes. The final ingredient of the model to make the typical helicoidal wall texture, from which the experimental data were derived, is that cellulose synthases have a finite active lifetime. The elements outlined above can be cast into the form of a
differential equation describing the evolution, both in space and in time, of the density of active cellulose synthases present in the plasma membrane. The solutions of these equations can be reinterpreted in terms of the tracks of the cellulose synthases, and hence the orientations of the deposited CMFs, thus leading directly to the cell wall texture. Because of its geometrical origin, the model only has a small number (4) of relevant parameters. We show that by varying these parameters, basically all known cell wall textures can be reproduced by this fully predictive mathematical model.

The presented research has the essential feature of SYSTEMS BIOLOGY, the iterative interaction between quantitative modeling from real theory and experiments that verify/falsify the theory. This research is the product of collaboration between a cell biologist and a theoretical physicist. It would not have been possible without mutual understanding of the cell process and techniques of both disciplines. We think that its power further was in 1. The vast amount of data that were available, 2. The well-delineated distinct cell process of cellulose microfibril formation, although this encompasses a number of complicated cell processes involving ER, Golgi system, cytoskeleton, exo- and endocytosis and wall matrix production. Based on the simulations performed, a next round of experiments has been defined and is being carried out by two PhD students and two technicians.

We plan to work further on cell wall formation and also on the cytoskeleton in similar ways in the future in more elaborate systems biology approaches and in collaboration with more cell wall and cytoskeleton groups.

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Perspectives for prebiotic life
Hans Fraaije, Agur Sevink, Alexander Kros, Kees van Sluis, Pascale Ehrenfreund
Soft Condensed Matter/Astrobiology group, Leiden Institute of Chemistry, PO BOX 9502, 2300 RA Leiden
j.fraaije@chem.leidenuniv.nl

The reconstruction of critical events in the origin of life is challenging, since there is no known geological record of prebiotic chemistry. We are then faced with the following problem: while we have available suggestive models for the organic compounds present on primitive Earth for the origin of life (from laboratory simulation, atmospheric modeling and the compounds present in carbonaceous chondrites), and we have detailed knowledge of modern biochemistry, we have only speculation to guide our understanding of the steps which connect these two. Indeed it is possible that a number of intermediate stages existed between these two extremes, on the nature of which we know little. Can computer models serve as rational design tools for a sustaining chemical system \textit{ab initio} in the laboratory, without reference to, or using chemicals from, known biological origin? If yes, this could contribute to our understanding of complex networks and the origin of life on Earth or other planets.

The paper discusses the efforts from the Leiden group addressing these issues from various angles: (1) reconstruction of chemical inventory on primitive Earth and Mars, (2) abiotic chemical synthesis of molecular self-replicating and self-propelling systems and (3) developing functional computer models for dissipative self-assembling patterns.


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**System Biology Illustrated II: Spatio-temporal metabolic modelling coupled to experiment**

Christof Francke⁴, Rechien Bader⁴, Joke Blom¹, Mark Peletier¹,² and Hans Westerhoff³,⁴  
¹) CWI, Amsterdam and 2) TU Eindhoven; ³) dept. Molecular Cell Physiology, VU; and ⁴) research group of Pieter J. Postma⁴, dept. Microbiology, UvA;

In order to understand the working of the cell, we rely on kinetic models. These models should be open to experimentation but above all they should be realistic. One of the attempts to create a kinetic model of the complete cell is the ‘silicon cell’ initiative. The basic features of the ‘silicon cell’ are that it will ultimately contain the complete cellular biochemistry characterised in space and time, and that it is firmly footed on experimental data. Because of the enormous complexity of metabolism it will be necessary to reduce. In our opinion this can only be cleverly done when we know the effects of the environment on kinetics and of the separation in space and time on connected processes (e.g. transport, signal transduction, gene regulation). In every instance of the construction of the model, one might need experimentation to confirm or guide the assumptions made. The above approach can be nicely illustrated by the work that was done in our groups on the phosphotransferase system of *E. coli*. Traditional biochemistry provided the data to construct a 0 D model of the system and the predictions were compared with results obtained in living cells. Using measured data on protein diffusion coefficients in living cells the model was expanded to three spatial dimensions and it was shown that although transport function was hardly affected by diffusion, protein gradients arose. Additionally, the presence of high concentrations of other macromolecules, also referred to as macromolecular crowding, was shown to affect kinetics. Iteration between ‘wet’ biochemistry and in silicon experimentation revealed that the underlying reaction mechanism of the PTS was more complicated than was thought previously, partly explaining the effects of crowding.

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**Principles in protein-kinase signal transduction**

Reinhart Heinrich  
Institute of Biology, Department of Biophysics, Humboldt-University, Berlin, Germany.

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.

Cf: Mol Cell 2002 May;9(5):957-70

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Adequacy versus redundancy in the evolved homeostatic control design of free energy transduction in skeletal muscle

Jeroen A.L. Jeneson
Dept of Physiology, School of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands

Skeletal muscle function can be modeled as a ‘chemo-mechano transducer’ that converts thermodynamic force of a highly non-equilibrium cytosolic concentration ratio of phosphorylated adenine nucleotides into mechanical force during contraction. Upon activation, muscle may increase its rate of ATP energy utilization up to 50-fold. Sustained high intensity excitation of a muscle cell does not, however, lead to a state of rigor contraction associated with ATP energy depletion, but instead leads to contractile failure (‘fatigue’). Using kinetic modeling and control analysis to integrate experimental data on muscle energetics, -mechanics and Ca$^{2+}$ cycling, we investigated the minimal set of components and interactions in the ATPase network in contracting muscle that is necessary and sufficient to explain this particular aspect of the physiology. The results of the analysis are put into the perspective of adequacy versus redundancy in the evolved homeostatic control design of free energy transduction in skeletal muscle.

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Modeling Developmental Regulatory Networks

Jaap Kaandorp

A model is discussed for simulating regulatory networks that is capable of quantitatively reproducing spatial and temporal expression patterns in developmental processes. The model is a generalization of the standard connectionist model used for modeling genetic interactions, where the terms for the regulation of gene products and the diffusion term have been separated. This model can be coupled with biomechanical models of cell aggregates and can be used to study the formation of spatial and temporal patterns of gene products during development in cellular systems.

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Unraveling the controversy of the mechanism of uptake of long-chain fatty acids in cells

Frits Kamp
Adolf Butenandt Institut, LMU, Schillerstr. 44, D-80336 München. Email: Fritz.Kamp@pbm.med.uni-muenchen.de

Uptake and metabolism of non-esterified long-chain fatty acids (FA) is a complex multi-step process. FA released in the blood are transported towards the plasma membrane, bind to the outer membrane leaflet and cross the membrane. A fraction of the FA at the inner leaflet disassociate into the cytosol to bind to membranes of cytosolic organelles and other binding sites. Transmembrane FA transport might be facilitated by specific transport proteins and cytosolic FA transport might be enhanced by Fatty Acid Binding Proteins, which might even direct the FA towards specific targets in the cell, but these issues remain very controversial. FA are trapped in the cytoplasm by acyl-CoA synthase. Acyl-CoA is not membrane permeable and a substrate for many enzymes: acyl-CoA can either be (partially) beta-oxidized, elongated and/or esterified into triglycerides or phospholipids. Little is known what mechanisms regulate the metabolic fate of FA.
Uptake of short-chain FA has traditionally been believed to occur by free diffusion, typical for weak hydrophobic acids, the protonated (uncharged) form being membrane permeable. Intuitively one would believe that diffusion of fatty acids would be slower when the acyl-chain gets longer, yet this is not true as will be illustrated by some model calculations. Diffusion of FA has to be seen as a 3-step process: association, flip-flop and dissociation. Membrane permeability of FA flows the Overton rule: the longer the chain length, the more hydrophobic the FA and hence the more permeable the FA. This rule remains valid so long as flip-flop is not hindered by the chain length.

In the past 10 years, experiments with model membranes (protein-free vesicles) with trapped fluorescent pH indicators have demonstrated that when FA is added at the outside, they bind immediately to the outer leaflet, get 50% protonated (the pKa of a FA in the membrane being about 7.6), flip-flop rapidly in the protonated form, get again partially ionized in the inner leaflet, releasing protons into the inner volume of the vesicles which cause a measurable and predictable change in internal pH. The half time of this process is less than 20 ms. To us this has been the key argument that free diffusion is the key mechanism for uptake of FA in cells. Nevertheless in the past 10 years evidence has also accumulated that FA uptake in cells is a saturable process, which can be inhibited by specific inhibitors. Several putative „FA transport proteins“ have been identified. A requirement for proteins for transporting FA across the plasma membrane would imply low partitioning of fatty acids into the membrane lipids, and/or a slower rate of flip-flop through the lipid domains than the rates of intracellular metabolism of fatty acids.

We used both vesicles of the plasma membrane of adipocytes (fat cells) and intact adipocytes to study transmembrane fluxes of externally added oleic acid. Binding of oleic acid to the plasma membrane was determined by measuring the fluorescent fatty acid-binding protein ADIFAB added to the external medium. Changes in internal pH caused by flip-flop and metabolism were measured by trapping a fluorescent pH indicator in the vesicles and cells. In cells, the metabolic end products of oleic acid were evaluated over the time interval required for the return of intracellular pH to its initial value. The primary findings were that: 1) oleic acid binds with high avidity in the lipid domains of the plasma membrane with a partition coefficient similar to that of protein-free phospholipid bilayers; 2) oleic acid rapidly binds to and crosses the plasma membrane by the flip-flop mechanism (both events occur within 5 s); and 3) the kinetics of esterification of oleic acid closely follow the time dependence of the recovery of intracellular pH, which takes about 1 min. We concluded that fatty acids enter the adipocytes primarily, if not exclusively, by free diffusion.

The development of models for the regulation of uptake and metabolic fate of FA is highly desirable and this forms a challenge for system biology.

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Quantitative Modular and Mechanistic Analyses of Cellular Networks
Boris N. Kholodenko
Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, 1020 Locust \Street, Philadelphia, PA 19107, USA, Boris.Kholodenko@mail.tju.edu
Emerging technologies have enabled the acquisition of large genomics and proteomics data sets. However, current methodologies for analysis do not permit interpretation of the data in ways that unravel cellular networking. We propose a quantitative method for
determining functional interactions in cellular signaling and gene networks. It can be used to explore cell systems at a mechanistic level or applied within a "modular" framework, which dramatically decreases the number of variables to be assayed. This method is based on a mathematical derivation that demonstrates how the topology and strength of network connections can be retrieved from experimentally measured network responses to successive perturbations of all modules. Importantly, our analysis can reveal functional interactions even when the components of the system are not all known. Under these circumstances, some connections retrieved by the analysis will not be direct but correspond to the interaction routes through unidentified elements. The method is tested and illustrated by using computer-generated responses of a modeled mitogen-activated protein kinase cascade and gene network.


Non-linear dynamic properties of signaling networks in polarized cells

Werner J.H. Koopman, Stan C.A.M. Gielen, Peter H.G.M. Willems
KU Nijmegen

To ensure their survival and proper functioning, cells must respond adequately to changes in their environment. Such responses are generally mediated by receptor proteins on the cell surface that continuously feed information into a complex signal transduction network, which integrates this external information with intracellular signals to trigger an appropriate cellular response. With the completion of the human genome map it is to be expected that the number of identified players in the stimulus-response network will vastly increase. Knowing the biochemical activities and subcellular distributions of these players, one of the most important challenges in biology, will be to understand the dynamics of their mutual influences in terms of cellular functioning. Several members of the signaling network have already been identified and physico-chemically characterized in vitro during the past years. This has led to some insight into their mutual interactions. Because the signaling network features regulatory feedback/feed-forward loops, threshold behavior, non-linear diffusion of second messengers and time delays, it behaves intrinsically non-linear. This non-linearity poses severe restrictions on the usefulness of descriptive (and even mechanistic) biological models since an intuitive understanding of the total network (and its input-output behavior) cannot be achieved by abstract reasoning alone. Moreover, in contrast to the architecture of engineered control systems, the architecture and even some of the properties of network constituents become remodeled upon network stimulation. Fortunately, mathematical techniques can be used to condense experimental findings into a mathematical model of multiple interacting systems. By evaluating this model inside a computer using suitable simulation software, a quantitative understanding of the complexity involved becomes feasible. When paralleled by the appropriate experiments, this modeling approach can teach us how the dynamic behavior of the biochemical signaling network regulates cell physiological functioning. We will pursue this aim by answering the following key questions:

(I) What are the global dynamics of the stimulus-response network?
(II) What are its spatial dynamics?
(III) How do specific perturbations within the network affect its behavior?
We used pancreatic acinar cells to validate a one-dimensional model for IP$_3$-induced cytosolic calcium ([Ca$^{2+}$]$_c$) oscillations in polarized cells. The model includes various Ca$^{2+}$ in- and efflux pathways reported to exist in these cells and features the complex regulation of the IP$_3$-operated Ca$^{2+}$-release channel by IP$_3$ and [Ca$^{2+}$]$_c$. Bifurcation analysis revealed that the temporal dynamics of the model agreed well with experimental data. Next, we studied the collective dynamics of a diffusion-coupled two-dimensional network of these models. The network displayed hysteresis and bistability and was capable of generating different types of Ca$^{2+}$ waves. This indicates that the network can be used to simulate the spatio-temporal dynamics of intercellular Ca$^{2+}$ waves.

Some references:

Towards an integrative approach to cancer treatment: what to target and when?

*Jan Lankelma*
Tumor cell biology group FALW, and Dept. of medical oncology VUmc, VU Amsterdam, the Netherlands

Cancer can be seen as the result of competition between normal and malignant cells. The molecular cause of the success of such a competition can be diverse. Many mutations have been found in enzymes catalyzing biochemical processes favoring proliferation or suppressing apoptotic cell death.
Remarkably, many chemotherapeutic agents used to treat cancer increase rather than decrease the mutation rate. A useful working hypothesis on the working mechanism of these agents is the selective advantage of normal cells, which can better cope with the drug-induced damage than the cancer cells.
One of these agents is doxorubicin, used in the treatment of breast cancer. Due to its autofluorescence we have been able to follow its cellular and tissue transport.
An integrated model of transport of doxorubicin molecules to the target will be presented. The consequences at the cellular level will be discussed.

*In silico* and *in vivo* study of nucleotide excision repair

*P.O. Mari, B. Geverts, J.H.J Hoeijmakers, R. Kanaar, W. Vermeulen, A.B. Houtsmuller*
Depts of Genetics, Depts. of Pathology and Genetics, Erasmus MC Rotterdam, PO-BOX 1738 3000 DR Rotterdam, The Netherlands, a.houtsmuller@erasmusmc.nl

A computer modeling environment is presented for the simulation of (i) DNA-metabolizing processes in the cell nucleus, and (ii) quantitative fluorescence microscopy
methods like FRAP (fluorescence recovery after photobleaching), FRET (fluorescence resonance energy transfer) and FCS (fluorescence correlation spectroscopy). By using such a complete yet simple to use software, our aim is to focus on the concepts behind the models and create a more permeable interface between experiment-based computer simulations, the modeling of biological processes and live cell microscopy experiments. As a biological model system we use nucleotide excision repair (NER), which removes several types of single strand DNA-damage. Our approach is to study NER as a “complex system” (a large ensemble of strongly coupled entities) by using Monte Carlo simulations. At the heart of these simulations are the individual interacting proteins which are freely mobile on a very fine three-dimensional grid within an in silico cell. Conventional analysis of FRAP experiments relies on basic mathematical models of particle diffusion. Though very simple, this model has yet to be studied extensively. In addition to a model describing diffusion processes, protein-protein interactions (leading to complex formation) and protein-membrane interactions to create a model cell, we have introduced a refined model of the confocal microscope used for FRAP, FRET and FCS allowing the analysis of experimental measurements of living cells in a more straightforward manner.

Modeling the heart

Denis Noble
University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, UK.

The development of computer models of heart cells is used to illustrate the interaction between simulation and experimental work. At each stage, the reasons for new models are explained, as are their defects and how these were used to point the way to successor models. As much, if not more, was learnt from the way in which models failed as from their successes. The insights gained are evident in the most recent developments in this field, both experimental and theoretical. The prospects for the future are discussed.


On robustness of biological models

A.V. Panfilov
Theoretical Biology, UU

We would like to bring the problem of stability of biological models to the attention. During the last decades the complexity of biological models has enormously increased. Current models include more and more processes and involve more and more differential equations. For example in our area of modeling of action potential of cardiac cells, the number of equations was increased from 4 (Hodgkin Huxley 1952, or Noble 1962 model) to more than 50 equations (in the latest models), which does not yet include metabolic and gene regulation processes. Such huge numbers of equations result in the following familiar problems: many parameters in the models are not measured and are just filled in. In many cases the authors can easily change the parameters 3-4 fold, saying that this is 'within' the measured limits. Second, many models are very specialized and work only for proposed problem, but show huge
instabilities if one wants to use them for other problems. For example, in cardiac electrophysiology, models which describe good action potential in one cell, can easily fail to produce a stable propagation pulse of excitation, and in most of the cases do not reproduce the tissue properties in important pathological conditions, e.g. during cardiac arrhythmias.

Our suggestion is to think about developing some standard rules of verification of models, which increase their robustness. Here we propose several approaches:

1. Obvious. Step by step building of a model, but making sure that at each step the model is robust enough.
2. Mathematical. Study bifurcations which result in the onset of instability using the enter manifold theory and understand how we can tune the models to be more stable.
3. Statistical. Checking of robustness of the model by setting randomly generated coefficients in the equations, similar to Odell’s work.
4. Biological. Look for basic control mechanisms which make the biological systems stable. Study their development during the evolution.

Conclusion: to have a program for interaction of different modeling groups and mathematical groups and set up sort of ‘testing’ and ‘model improvement’ center. A possible good first problem here is improvement of existing models for cardiac cell. The last part highlights our approach for modeling cardiac tissue, and discusses importance of ‘tissue’ level in the system biology program.

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A large-scale mathematical model of Rheumatoid Arthritis in a joint - design principles and application in pharmaceutical research

J.A.C. Rullmann*, C.M. Meeuwisse*, C.L. Hofstra¶, H. Struemper‡, N. A. Defranoux‡, A. van Elsas§

* Dept. Molecular Design & Informatics, NV Organon, Oss
¶ Dept. Pharmacology, NV Organon, Oss
‡ Entelos Inc, Foster City CA, USA
§ Dept. Target Discovery, NV Organon, Oss

Rheumatoid arthritis (RA) is an autoimmune disorder characterized by chronic inflammation of the synovial tissue in joints and ultimately results in destruction of cartilage and bone within the joint. Based on proprietary technology and following a hypothesis-driven approach, Entelos — in collaboration with Organon — developed a mathematical model of a prototypical human RA joint using public literature data. The model integrates the behavior of chondrocytes, macrophages, fibroblast-like-synoviocytes and T cells as well as the result of their interaction. The model addresses population dynamics, cellular activation, mediator synthesis, cartilage matrix metabolism and regulation of these processes by the cytokine milieu and cell-cell contact. The model predicts clinically detectable parameters of disease and includes responses to drug-based therapies. Simulations provide in silico data and results on the human clinical effectiveness of various interventions that may form the basis of a new therapy. They can also provide insights to the factors that drive the underlying pathophysiology, providing greater scientific understanding into the pathways most relevant for RA. The presentation will describe the basic outline of the model, present the results of simulating therapy, and discuss possibilities and limitations of the hypothesis-driven modeling approach followed here.
Lactic acid bacteria: ideal model organisms for Dutch Systems Biology?

Bas Teusink\textsuperscript{1,2,3}, Oscar Kuipers\textsuperscript{4}, John van der Oost\textsuperscript{5}, Willem de Vos\textsuperscript{1,5}, Jeroen Hugenholtz\textsuperscript{1,2,5} and Roland Siezen\textsuperscript{1,2,3}

\textsuperscript{1}Wageningen Center for Food Sciences (www.wcfs.nl), \textsuperscript{2}NIZO food research, Ede (www.nizo.nl), \textsuperscript{3}Center for Molecular and Biomolecular Informatics (www.cmbi.nl), Nijmegen University, \textsuperscript{4}Molecular Genetics, Groningen University \textsuperscript{5}Microbiology Dept, Wageningen University

Lactic acid bacteria are important organisms in the food and fermentation industry. Many bacterial genomes, including those of many lactic acid bacteria, have been sequenced recently. Within the Wageningen Center for Food Sciences (WCFS) the complete genome of \textit{Lactobacillus plantarum} WCFS1 has been sequenced.

The complete sequence of an organism defines the genetic components; the next step is how these components interact and are organized in pathways. We are constructing databases of metabolic pathways, transport processes and regulatory pathways. For this we are collaborating within the NWO Biomolecular Informatics program between WCFS (Siezen), Groningen University (Kuipers, Roerdink), Wageningen University (Van Oost) and CMBI (Siezen) in Nijmegen. Within this program visualization of pathways and the projection of experimental data onto the pathways is an important aspect.

Experimental data (growth characteristics and mutant phenotypes, metabolomics, proteomics and transcriptomics) will be essential to be able to validate predictions based on \textit{in silico} reconstructions of metabolic pathways. Several projects in which LAB are involved will generate such data. Within the IOP Genomics program DNA microarray analysis of four gram-positive bacteria will be performed, a collaboration between WCFS (Molenaar), Groningen University (Kuipers, Roerdink), Wageningen University (Van Oost) and CMBI (Siezen). Within a WCFS project (Hugenholtz, Smid) data on transcriptomics and metabolomics will be generated on selected mutants in cofactor biosynthesis of \textit{Lactobacillus plantarum} and \textit{Lactococcus lactis}.

Once the pathways are reconstructed, understanding and ultimately prediction, simulation and manipulation of fluxes of important substrates and products will be the ultimate goal. Within the Kluyver Center for functional genomics of industrial fermentation, both theoretical and experimental tools will be implemented and partially developed for modeling large scale metabolic networks. The Kluyver Center comprises a collaboration between a.o. the Delft University (Pronk, Heijnen), Wageningen University (vd Berg), Utrecht University (Heck), TNO (vd Werff), WCFS (Hugenholtz), NIZO food research (Hylckama-Vlieg), CMBI (Siezen) and many industrial partners. The model organisms in this program are yeast, fungi and lactic acid bacteria.

Because of its relative simplicity and experimental accessibility, lactic acid bacteria are interesting organisms for a robust systems biology approach. This summary of national projects demonstrates that within the Netherlands there is sufficient theoretical, computational and experimental expertise to come to a leading network of systems biology of lactic acid bacteria. Many of the relevant partners participate already in one or more of the above-mentioned programs, but for this major scientific challenge we welcome all others interested to join.
Supercoiled DNA; plectonemic structure and liquid-crystal formation

J.R.C. van der Maarel (1), S.S. Zakharova (1), W. Jesse (1), C. Backendorf (1), S.U. Egelhaaf (2), and A. Lapp (3)
(1) Leiden University, The Netherlands
(2) University of Edinburgh, United Kingdom
(3) CE de Saclay, France

DNA often has the shape of closed rings. Before closing to form a ring the duplex is twisted and a superhelix is formed. Liquid-crystallinity provides an efficient packaging mechanism for supercoiled DNA. In addition to the usual parameters controlling liquid crystal formation (e.g., ionic strength, molecular weight), the topology of the supercoil is very important. The topology sets the dimensions (i.e., diameter, length, and pitch) of the superhelix and determines the volume excluded to another supercoil.

We have obtained the critical boundary concentrations of supercoiled pUC18 plasmid (2686 base pairs) from $^{31}$P NMR, polarized light microscopy, and phase equilibrium experiments. Furthermore, we have measured the small angle neutron scattering (SANS) to monitor the configuration of the superhelix through the phase transition. The transition is strongly first-order with a broad gap between the isotropic and anisotropic phases. The critical boundaries are strongly and reversibly dependent on temperature and weakly dependent on ionic strength. With polarized light microscopy on magnetically oriented samples, the anisotropic phase is assigned cholesteric with a pitch around 4 microns. The scattering experiments show that the dimensions of the supercoil, and, hence, the excluded volume, decrease significantly through the phase transition. The transition is interpreted with lyotropic liquid crystal theory, including the effects of charge, orientation entropy, and excluded volume effects. It was found that the molecular free energy associated with the change in topology is important in explaining the broadening of the phase gap, but the low concentration at which the anisotropic phase first appears is not reproduced.

System identification of calcium handling in the heart

Natal A.W. van Riel and Ger J. van der Vusse

In this presentation, 2 different views on systems biology will be illustrated. As application, the calcium homeostasis in the heart will be discussed. Detailed biophysical descriptions exist with many variables and parameters. With current experimental techniques, these numerous quantities cannot be integrally addressed at a system level with experimental data. System and identification theory aim at compact descriptions of the system dynamics that are observable in the experimental data (the model output). A physiology-based model of calcium handling in the intact heart has been derived. This model incorporates the quantitatively most important processes involved in beat-to-beat calcium homeostasis. The (kinetic) parameters have been estimated using time-series data of the free calcium concentration in the sarcoplasm. The model enables a physiological system interpretation of disturbed calcium handling, such as when using beta-blockers.
Applications of Systems Theory in Molecular, Cell, and Neuro-Biology
Ivo H.M. van Stokkum
Physics Applied Computer Science Group, Department of Physics and Astronomy, Faculty of Sciences, Vrije Universiteit

Methods from systems theory can be successfully applied at all levels in biology. A parameterized model can be developed which describes the sufficiently rich experimental data and which contains meaningful parameters. On the molecular level, the system is described by an evolution of states, which are characterized by their spectral properties. At the neuronal level, the system is described by the dynamics of the neuronal potentials responsible for action potential generation. It is a challenge to find out how much molecular detail is needed for a system theoretical description of cells. An example from photosynthesis illustrates the surprising simplicity with which the steady state of a complex cellular system can be described.

A potential project is the system theoretical description of the dynamics of an in-vivo photosynthetic system, as probed by spectroscopic methods.

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Interactions and communication between cells and their environment in a landfill leachate-polluted Dutch aquifer
Henk W van Verseveld, Wilfred F.M. Röling and Hans V. Westerhoff
VU-FALW, Department of Molecular Cell Physiology, De Boelelaan 1087, 1081 HV Amsterdam.

The activity of microorganisms is indispensable for many biogeochemical processes. Modern molecular tools allow us now to determine the composition of microbial communities and the functions of individual species. Most of current research on biodegradation and intrinsic bioremediation is focusing on the microorganisms that are directly involved in the degradation of specific pollutants (like benzene, toluene, ethylbenzene and xylene degraders, dechlorinating bacteria etc.). However, the functioning of microorganisms degrading specific compounds are interlinked to the functioning of the ecosystem, and biodegradation can never be understood completely by studying the individual components alone, as has become clear from the Metabolic Control Analysis (MCA) of biochemical pathways. From the above it becomes clear that in order to understand and direct bioremediation it is of utmost importance to determine the contribution to biodegradation of (micro)organisms that are not directly involved in degradation, and the influence of microenvironment in which microbial interactions occur. Establishing what or who controls biodegradation rates requires a quantitative approach addressing the whole ecosystem. The determination of the factors controlling biodegradation starts with a network qualitatively describing the interactions between the most important functional groups of microorganisms (see Figure 1). Therefore, we experimentally assess also
functional groups that indirectly influence degradation, such as predators, mineral weathering microorganisms and anaerobically respiring microorganisms. The interactions between and within these functional groups are being determined with an ecological variant of MCA.

We developed a simple model in which fermenting microorganisms and Fe(III)-reducing organisms talk to each other via the hydrogen concentration.

**Figure 1.** Network showing the contribution of the proposedly most important functional groups of microorganisms (m.o.) to material fluxes in anaerobic subsurface groundwater ecosystems, and interactions between these groups. Green arrows indicate an activity by a certain physiological group that is beneficial for its growth. The red arrow indicates product inhibition. Please note that one species might be able to mediate two functions (e.g. iron reducing bacteria reduce iron(III) in minerals, this activity may also release phosphorus). Like competition within a functional group (e.g. sulphate reducers versus iron reducers) this is not shown, in order to keep the scheme simple.

The Silicon Cell

Hans V. Westerhoff, Barbara M. Bakker, Frank Bruggeman, Christof Francke, Fred C. Boogerd, Mark Peletier, Joke Blom, and *Jacky L. Snoep

Centre for Research on BioComplex Systems, BioCentrum Amsterdam, EU and *also Department of Biochemistry, University of Stellenbosch, South Africa

Living cells amaze because they appear to be well organized and highly adaptive to changes in conditions. In fact, were it not for the purely molecular nature of their composition, one might recognize intelligence in them. One of the tenets of System Biology ([www.systembiology.net](http://www.systembiology.net)) is that these fascinating properties of living cells do not solely derive from the properties of the individual macromolecules but also from the ways in which these macromolecules interact.

Since the genomes of simple autonomous living cells have been sequenced, we understand that those systems require more than 300 processes, and most probably many interactions. The number is large but not infinite. Moreover, aspects of living organisms might be understandable by examining less than the entire set of processes. For the examination of the effects of the interactions however, the human mind will need the help of computation. It becomes too complicated for the human mind to keep track of all information about all the processes in a living cell and then to integrate their behavior. Because the problem is of finite size, a computer should be able to help in this task.

The silicon cell project aims to do just this, i.e. make a computer replica of living cells, or, initially, of parts thereof. These replicas require precise and quantitative information concerning the participating processes. They can subsequently be used to help us understand how system behavior arises. The making of a silicon cell may not always be exciting, but the discoveries when using silicon cells are.

The Silicon Cell project ([see www.siliconcell.net](http://www.siliconcell.net)) is an international collaboration, which puts the silicon cells that have been made, on a web site ([http://www.jjj.bio.vu.nl/](http://www.jjj.bio.vu.nl/)) that is freely accessible for other scientists to make discoveries with. In the presentation we shall show how the silicon cells work and some of the discoveries that have been made by using them.