Portfolio of showcases illustrating the impact of modelling

Deliverable D8.1

Work package 8

Modelling infrastructure and expertise

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ISBE Infrastructure for Systems Biology Europe

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Background information

Objectives of WP8

ISBE Work Package 8, Modelling infrastructure and expertise, aims to provide the scientific and organisational foundation for a rapid and highly coordinated pan-European implementation of *modelling as a service*, for academia and industry across all major disciplinary fields of biology.

Mathematical modelling is central in tying together theory and experiment, and interpreting and integrating data and model resources in the context of other commensurable data and models. Mathematical modelling has a proven capability of (i) connecting a comprehensive amount of empirical data into a functional whole, (ii) enforcing more explicit formulations of various hypotheses, (iii) increasing the prediction space of hypotheses, (iv) initiating and canalising experimental or empirical work by pointing out key questions and the type of data needed, (v) ensuring that models and 'ways of thinking' are consistent with fundamental principles of physics, chemistry, and biology, and (vi) functioning as highly efficient intellectual meeting places for various disciplines. Modelling comes in many types, ranging from single-molecule stochastic modelling to organ level physiological modelling, and addressing some or all of the multiple dimensions of space, time, chemistry and the cellular control hierarchy.

This document

This document, Deliverable D8.1, marks the fulfilment of Task 8.1, set out as follows:

Identify showcases illustrating the impact of modelling on biological discovery and the added-value of integrated theoretical-experimental research.

The Description of Work for this task was as follows:
• Illustrate the impact of modelling through existing showcases. This will be done by providing, for each of 6-10 existing modelling methodologies, two examples where they have led to a quantum increase in understanding of biology or medicine.
• Show by 5 representative examples how experimental data have driven the development of new modelling approaches capable of delivering the required methodology.
• Illustrate by 5 outstanding, representative, examples of the added value of integrated theoretical-experimental research programmes.
• Document how modelling will have to guide the development of a mature phenomics discipline.

We loosely define the term "modelling methodology" as the intersection of a mathematical framework, a law of nature (perhaps approximate), and a set of key assumptions. For instance, flux balance analysis is defined by the mathematics of ordinary differential equations and matrix algebra, stoichiometry (which ties in much "hard science" from biochemistry), and the

assumption of equilibrium and optimization of some relevant biological objective such as biomass production or nutrient uptake.

An agreed-upon, structured classification of modelling methodologies does not yet exist, but will be needed to characterize and classify the competence in respective ISBE nodes. The showcases below exemplify many important modelling methodologies, and ISBE members are part of the recent MAMO (mathematical modelling ontology) initiative, http://sourceforge.net/p/mamo-ontology/wiki/Home. We will revisit the classification of modelling methodologies later in the planning of ISBE services.

Showcases

As we collected these showcases, a common theme was that success stories were formed by the complementary use of multiple mathematical models to study different aspects of a phenomenon. Therefore, rather than rigidly structuring showcases e.g. by mathematical formalism, we focus first on the biological story, then exemplify which modelling formalisms have proved useful, and in what way. We have aimed for a balanced selection of showcases with respect to modelling formalisms, as defined above.

Heart modelling: From diffusing molecules to the electrically controlled, mechanical pump

As familiar as the heart is, it takes a lot of different science to understand its function and failures. The heart is an electrically controlled, mechanical pump, built from the interaction of trillions of molecules. It performs its function of circulating the blood through the body by rhythmic muscle contractions, signalled by small electric impulses that originate in pacemaker cells, get propagated from cell to cell, and set off chains of events that trigger muscle proteins to shorten and eventually relax again. Your heart beats every second, yet people die from heart disease only once in their lifetime. Clearly, a thorough mathematical description of this wonder of nature must cover vastly disparate scales of size and time, and branches of physics from biochemistry to electricity and mechanics.

Biological question: The underlying mechanisms of many heart diseases have been studied and mathematically modelled in great detail, at levels from the subcellular (e.g. ion channels, muscle proteins) to cells (action potential, contraction) to the whole organ. An important goal for such mathematical models is to suggest therapies, possibly tailored to each patient via patient-specific parameters of heart geometry (Lamata *et al.*, 2011) or ion channel function (Silva *et al.*, 2009).

Mathematical methods: The heart is by nature a multiphysics system, being an electrically controlled mechanical pump (Plank *et al.*, 2013).

- The electrical activity of heart cells was modelled using ordinary differential equations by (Hutter & Noble, 1960)(Noble, 1960), building on (Hodgkin & Huxley, 1952)'s Nobel-Prize-winning work with squid nerve cells. This mathematical framework has been

extended to include many ion channels (Fink *et al.*, 2011) and multiple cellular compartments.
- On the tissue and organ level, Peter Hunter introduced the application of continuum dynamics to heart function modelling in the 1970's to enable multi-scale physiological modelling and vizualisation of whole organ function (Hunter, 1975; Hunter *et al.*, 1975), and numerous methods such as operator splitting have been developed for the numerical solution of the resulting equations (Sundnes *et al.*, 2006).
- The coupling between heart electrophysiology and mechanics and deformation requires additional mathematical methods (Nordsletten *et al.*, 2011). The same goes for integration across scales of space and time (chapter in (Coveney *et al.*, in press)).

Key insights:

- The Hodgkin-Huxley model explained how cells can be excitable, that is, stable to a weak stimulus but responding to a stronger stimulus with a disproportionate positive feedback phenomenon, after which the cell is unsusceptible to further stimuli for a certain "refractory" period (well illustrated in Figure 1 of (Fitzhugh, 1961), which describes a two-dimensional simplification of the Hodgkin-Huxley model).
- Similar analyses continue to be useful; for instance, (Tran *et al.*, 2009) explained premature heartbeats (early afterdepolarizations) as a change in the stability properties of the dynamical system of the heart cell during the course of an action potential.
- Tissue electromechanics models show how infarctions can cause arrhythmia: the normal propagation of an electrical wave can be slowed down in places, so that cells that have passed their refractory period get prematurely re-activated. Thus, the activation front can double over backwards and generate spiral waves and arrhythmia that prevents effective pumping.
- Multiscale models of electrophysiology show how cellular action potentials give rise to the electrocardiogram (ECG) measured macroscopically on the surface of the body (Sundnes *et al.*, 2006).
- Markov models of ion channel function can explain how some genetic mutations cause abnormal action potentials and ECGs, e.g. prolonged action potentials (Clancy & Rudy, 1999), changes in the duration and levels of ECG phases (called P, Q, R, S, T) such as long QT or ST elevation, or early afterdepolarizations (Zhu & Clancy, 2007).
- Multiscale, multiphysics models can account for the effects of genetic mutations at levels from ion-channel structure, function, and macroscopic current; cell, tissue and organ function (Silva *et al.*, 2009).

Clancy CE & Rudy Y (1999). Linking a genetic defect to its cellular phenotype in a cardiac arrhythmia. *Nature* **400,** 566–569.
Coveney PV, Hunter PJ, Viceconti M, Noble D & Díaz V eds. (in press). *Computational Biomedicine*. Oxford University Press, Oxford.

Fink M, Niederer SA, Cherry EM, Fenton FH, Koivumäki JT, Seemann G, Thul R, Zhang H, Sachse FB, Beard D, Crampin EJ & Smith NP (2011). Cardiac cell modelling: Observations from the heart of the cardiac physiome project. *Prog Biophys Mol Biol* **104,** 2–21.
Fitzhugh R (1961). Impulses and physiological states in theoretical models of nerve membrane. *Biophys J* **1,** 445–466.
Hodgkin AL & Huxley AF (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. *J Physiol* **117,** 500.
Hunter P (1975). *Finite element analysis of cardiac muscle mechanics*. PhD thesis, University of Oxford, UK.
Hunter PJ, McNaughton PA & Noble D (1975). Analytical models of propagation in excitable cells. *Prog Biophys Mol Biol* **30,** 99.
Hutter OF & Noble D (1960). Rectifying properties of heart muscle.
Lamata P, Niederer S, Nordsletten D, Barber DC, Roy I, Hose DR & Smith N (2011). An accurate, fast and robust method to generate patient-specific cubic Hermite meshes. *Med Image Anal* **15,** 801–813.
Noble D (1960). Cardiac action and pacemaker potentials based on the Hodgkin-Huxley equations.
Nordsletten DA, Niederer SA, Nash MP, Hunter PJ & Smith NP (2011). Coupling multi-physics models to cardiac mechanics. *Prog Biophys Mol Biol* **104,** 77–88.
Plank G, Prassl AJ & Augustin C (2013). Computational Challenges in Building Multi-Scale and Multi-Physics Models of Cardiac Electro-Mechanics. *Biomed Eng Biomed Tech*; DOI: 10.1515/bmt-2013-4318.
Silva JR, Pan H, Wu D, Nekouzadeh A, Decker KF, Cui J, Baker NA, Sept D & Rudy Y (2009). A multiscale model linking ion-channel molecular dynamics and electrostatics to the cardiac action potential. *Proc Natl Acad Sci* **106,** 11102 –11106.
Sundnes J, Lines GT, Cai X, Nielsen BF, Mardal K-A & Tveito A (2006). *Computing the Electrical Activity in the Heart*, 1st edn. Springer.
Tran D, Sato D, Yochelis A, Weiss J, Garfinkel A & Qu Z (2009). Bifurcation and Chaos in a Model of Cardiac Early Afterdepolarizations. *Phys Rev Lett*; DOI: 10.1103/PhysRevLett.102.258103.
Zhu ZI & Clancy CE (2007). Genetic mutations and arrhythmia: simulation from DNA to electrocardiogram. *J Electrocardiol* **40,** S47–50.

Biological timekeepers

Biological organisms have internal “clocks” that enable them to adapt their internal processes to the daily rhythm of night and day. Our understanding of these mechanisms is very much framed in terms of mathematical models and concepts, and has had applications e.g. in medicine (Goldbeter & Claude 2002; Altinok et al. 2009). Below, we describe a couple of other examples in some detail.

Altinok A, Lévi F & Goldbeter A (2009). Identifying mechanisms of chronotolerance and chronoefficacy for the anticancer drugs 5-fluorouracil and oxaliplatin by computational modeling. European Journal of Pharmaceutical Sciences 36, 20–38.
Goldbeter A & Claude D (2002). Time-patterned drug administration: insights from a modeling approach. Chronobiology international 19, 157–175.

The daily rhythms of plants

Circadian clocks enable biological organisms to adapt their internal processes to the daily rhythm of night and day. Properties like phase, period or amplitude are clearly systems properties and have no meaning on the level of individual molecular components. The investigation of circadian clocks speaks therefore for a combined experimental and theoretical approach. Circadian rhythms were first identified in plants, famous became the 'flower clock' of Carl von Linné. Andrew Millar et al. were the first who integrated disparate molecular results in mathematical models of the plant clock mechanism. The models revealed general operating principles, such as the flexible regulation of complex feedback circuits. Through an iterative experimental and theoretical work Millar et al. were able to show that the circadian clock of the model plant *Arabidopsis thaliana* consists of interlocked feedback loops, which includes a three-component repressilator circuit in its complex structure.

Locke, J. C. W., Millar, A. J. & Turner, M. S. Modelling genetic networks with noisy and varied experimental data: the circadian clock in Arabidopsis thaliana. *J Theor Biol* 234, 383–393 (2005).
Locke, J. C. W. *et al.* Extension of a genetic network model by iterative experimentation and mathematical analysis. *Mol Syst Biol* 1, 2005.0013 (2005).
Pokhilko, A. *et al.* The clock gene circuit in Arabidopsis includes a repressilator with additional feedback loops. *Mol Syst Biol* 8, (2012).
Saithong, T., Painter, K. J. & Millar, A. J. The contributions of interlocking loops and extensive nonlinearity to the properties of circadian clock models. *PLoS ONE* 5, e13867 (2010).

Understanding cell-cell synchronization of metabolic oscillations

Glycolysis is a process of breaking down sugars to provide energy for other bodily processes. The rate of glycolysis has been found to oscillate markedly in yeast cells under certain conditions, a phenomenon that could not be explained merely through experiments. Mathematical modelling has been a driver towards key insights from early on, through decades of hypothesis generation and testing, and close interaction between theory and experiment.

Initial discovery: Glycolytic oscillations were found in vivo and in cell extracts through intracellular NADH fluorescence (first demonstration Duysens & Amesz 1957; early developments reviewed in Hess & Boiteux 1971).
Mathematical methods:

Glycolytic oscillations have been modeled extensively with various network mechanisms (e.g. Sel'kov). Most involve kinetic modelling and numerical analysis, but also graphical tools to help identify oscillophores in networks (Goldstein et al.).
Key insights:
In several of these mechanisms control over the oscillation was distributed over multiple network components (Teusink et al.). Ccetaldehyde mediates the synchronization (Richard et al.). Where experiments with populations of intact cells had been transient only, Richard et al. found conditions where the NADH oscillations are sustained, and this finding was consolidated in a flow-through setting (Dano et al.). Modelling cell-to-cell synchronization, raised the issue whether the cells effectively form one single oscillator or a synchronized set of oscillators (one for each cell) (Bier et al.; Wolf et al.; Dano et al.), and a combined modeling and experimental approach inspecting individual cells showed that the latter is the case (Du Preez et al.).

Bier et al. (2000) How yeast cells synchronize their glycolytic oscillations: a perturbation analytic treatment. Biophys J. 78: 1145-1153
Dano et al. (1999) Sustained oscillations in living cells. Nature 402: 320-322
Duysens LNM & Amesz J (1957). Fluorescence spectrophotometry of reduced phosphopyridine nucleotide in intact cells in the near-ultraviolet and visible region. Biochimica et biophysica acta 24, 19–26.
Hess B & Boiteux A (1971). Oscillatory phenomena in biochemistry. Annual review of biochemistry 40, 237–258.
Richard et al. (1994) Yeast cells with a specific cellular make-up and an environment that removes acetaldehyde are prone to sustained glycolytic oscillations. FEBS Lett. 341, 223-226
Richard et al. (1996) Acetaldehyde mediates the synchronization of sustained glycolytic oscillations in populations of yeast cells. Eur. J. Biochem. 235: 238-241
Sel'kov (1968) Self-oscillations in glycolysis. 1. A simple kinetic model. Eur. J. Biochem. 4: 79-86
Teusink et al. (1996) Control of frequency and amplitudes is shared by all enzymes in three models for yeast glycolytic oscillations. Biochim. Biophys. Acta 1275: 204-212
Wolf et al. (2000) Transduction of intracellular and intercellular dynamics in yeast glycolytic oscillations. Biophys J. 78: 1145-1153

Signal transduction

Signal transduction refers to cascades of biochemical reactions that enable a cell to respond appropriately to its state or environment. For example, conditions outside the cell can trigger a chain of events that eventually tell the nucleus to start expressing appropriate genes. The seminal mathematization by Goldbeter & Koshland (1981) spawned a large research program on mathematical modelling hand-in-hand with experiment.

Goldbeter A & Koshland DE (1981). An amplified sensitivity arising from covalent modification in biological systems. *PNAS* **78,** 6840–6844.

Pump priming of signal transduction as a general principle

Biological question:
The common way of looking at signal transduction was that of activation of a receptor, which then activates an enzyme that modifies a second enzyme, which again covalently modifies another enzyme, and somewhere down the chain a modified protein is a transcription factor. Although in some cases physical movement is involved, e.g. because the receptor resides in the plasma membrane and the DNA in the nucleus, the process has often been described, analysed and understood in terms of time dependence only. Key signalling systems properties like physical displacement, recruitment and active transport, were unaccounted for.
Mathematical methods:
Kinetic modelling; numerical analysis.
Key insights:
EGF induces signalling through the MAP kinase cascade essentially by causing the EGF receptor to accumulate SOS near the plasma membrane where RAS is located and thereby activated (Kholodenko and Westerhoff). Endocytosis of EGF-bound EGF receptor and movement towards the nuclear membrane may be the more important for signalling than diffusion of components of the MAP kinase cascade (Kholodenko and Brown). Circulation into and again out of the nucleus is crucial for JAK-STAT signalling (Klingmüller and Timmer). Signal transduction from hydrophobic signal molecules to transcription depends on a conveyor belt-like system in and out of the nucleus transporting the nuclear receptor plus or minus hormone (Kolodkin et al.). Such conveyor belt-like mechanisms also operates in lipid-mediated signalling (Bastiaens and coworkers). Trafficking of vesicles in cells contributes to signal transduction (Zerial and colleagues). Gibbs free energy hydrolysis is commonly driving the process, such that it can be described as a pumping of a relevant signalling molecule to a certain position in space, rather than covalent modification (like phosphorylation through ATP) that triggers the next step in the pathway.

Kholodenko et al. (2000) Why cytoplasmic signalling proteins should be recruited to cell membranes. Trends Cell Biol. 10: 173-178.
Kolodkin et al. (2010) Design principles of nuclear receptor signalling: How complex networking improves signal transduction. Mol. Syst. Biol. 6: 446
Rocks et al. (2010) The palmitoylation machinery is a spatially organizing system for peripheral membrane proteins. Cell 141: 458-471
Swameye et al. (2003) Identification of nucleocytoplasmic cycling as a remote sensor in cellular signaling by databased modeling. Proc. Natl Acad. Sci. USA 100: 1028-1033

Zerial & McBride (2001) Rab proteins as membrane organizers. Nat. Rev. Mol. Cell Biol. 2: 107-117

Reconciling in vivo and in vitro

Much of our understanding of biochemical pathways and their components derives from in vitro experimentation. It is an important issue whether the situation in vivo reflects the one in vitro. For one, concentrations of pathway components tend to be much higher in vivo than in most studies in vitro. Also concentrations of other components may be much higher. This may lead to enhancement of association reactions – particularly through macromolecular crowding – and to metabolite channeling.

Mathematical methods:

Kinetic modelling; stochastic modelling (Monte Carlo); numerical analysis.

Key insights:

There are substantial effects of molecular crowding for the glucose uptake system of E. coli (Rohwer and colleagues). With nonlinear kinetics channeling can enable chemical reactions that are otherwise impossible because of the second law of thermodynamics (Astumian et al.). The intracellular medium tends to differ from that in enzyme assays and this can significantly contribute to differences between in vitro data and in vivo observations (Van Eunen et al.).

Astumian et al. (1987) Can free energy be transduced from electric noise? Proc. Natl Acad. Sci. USA 84: 434 438
Garcia-Contreras et al. (2012) Why in vivo may not equal in vitro-new effectors revealed by measurement of enzyme activities under in-vivo-like assay conditions. FEBS J. 279: 4145-4159
Kholodenko & Westerhoff (1995) The macroworld versus the microworld of biochemical regulation and control. Trends Biochem. Sci. 20: 52-54
Rohwer et al. (1998) Implications of molecular crowding for signal transduction and metabolite channeling. Proc. Natl Acad. Sci. USA 95: 10547-10552
Van Eunen et al. (2010) Measuring enzyme activities under standardized in vivo-like conditions for Systems Biology. FEBS J. 277: 749-760

Non-equilibrium thermodynamics of living systems

- trade-offs between efficiency, yield, rate, regulation; reversibility, irreversibility, active versus passive transport

Through evolution, life has addressed a great number of challenges, including life under extreme circumstances like at high temperatures or high acidity levels. Limitations that could not be fundamentally solved have often been worked around by evolution, like for instance limitations imposed by thermodynamics. Famous is the apparent violation of the second law of thermodynamics, apparently creating order out of chaos, hence reducing entropy. The workaround is being an open system that increases entropy, yet locally decreased by exporting more than is being produced. Metabolic systems are good at this (Westerhoff and Van Dam). It

remains an issue, however, how efficient living systems are thermodynamically, i.e. how much of the free energy that they absorb they actually fix on their biomass or other tasks. Even today many scientists assume that living systems are optimal vis-à-vis efficiency. This is in fact the basis of the third phase of flux balance analysis (see above). Stucki and Van Dam and coworkers have shown that, if anything, biology is not much after efficiency, but much more after growth or production rate, or a combination between rate and yield. Yet flux balance analysis predictions based on assumed maximal yield appeared to be validated experimentally (Edwards et al.), although a constant maintenance metabolism was assumed here. Baker's yeast does not choose the most efficient growth option unless there is glucose limitation (Simeonidis et al.). There appears to be a trade-off between efficiency or yield, rate and perhaps other functionalities. One such functionality is that of robust control. It may be useful to expend free energy (and thereby to reduce efficiency) by making certain transitions irreversible, such as is done in the cell cycle (Novak et al.). Or to expend free energy to activate a pathway by accumulating certain intermediates to high enough levels to drive the subsequent steps (Teusink et al.). The thermodynamic argument can be important in deciding on proposed structure-based model, for instance the proposal that ammonium transport into cells is passive, based on structure models of the transporters. Systems biology computation have shown that this would neither be feasible thermodynamically nor kinetically (Boogerd et al 2011).

Boogerd et al. (2011) Hypothesis. AmtB-mediated NH3 transport must be active and as a consequence regulation of transport by GlnK is mandatory to minimise futile cycling of NH4+/NH3. FEBS Lett. 585: 23-28
Edwards & Palsson (2000) The Escherichia coli MG1655 in silico metabolic genotype: its definition, characteristics, and capabilities. Proc. Natl. Acad. Sci. USA 97: 5528–5533
Edwards et al. (2001) In silico predictions of Escherichia coli metabolic capabilities are consistent with experimental data. Nat. Biotech. 19: 125-130 Simeonidis et al. (2010) Why does yeast ferment? A flux balance analysis study. Biochem. Soc. Trans. 38: 1225-1229
Novak et al. (2007) Irreversible cell-cycle transitions are due to systems-level feedback. Nat. Cell Biol. 9: 724-728 Teusink et al. (1998) Intracellular glucose concentration in derepressed yeast cells consuming glucose is high enough to reduce the glucose transport rate by 50%. J. Bacteriol. 180: 556-562
Stucki (1980) The optimal efficiency and the economic degrees of coupling of oxidative phosphorylation. Eur. J. Biochem: 109, 269-283
Westerhoff et al. (1983) Thermodynamic efficiency of microbial growth is low, but optimal for maximal growth rate. Proc. Natl. Acad. Sci. USA 80: 305-309

Subtlety in biology: distributed control and regulation and high robustness

Biological question:

A persistent misunderstanding in biochemistry is that pathway function is controlled by a single rate-limiting step, being the first committed and non-equilibrium step in the pathway. This notion abounds in textbooks and science communications, but has been proven wrong in multiple cases and for various phenomena in models, experiment-based models and experiments. How?
Mathematical methods:
Theory development: metabolic control analysis, sensitivity analysis, hierarchical regulation analysis; implementation in kinetic modelling.
Key insights:
The fundamental principle is not that a single step has all the control, but rather that total flux control can be distributed over all steps in the pathway (Kacser, Burns, Heinrich, Rapoport, and others). For flux control, the distribution is determined by the relative kinetic properties of the pathway enzymes (the so-called elasticity coefficients) (Kacser and Burns). Also for concentrations in metabolic, signal transduction and gene expression pathways, control tends to be distributed over pathway steps (Westerhoff, Chen and Kahn). In addition to flux and concentrations, this notion also holds for membrane potential; DNA supercoiling; frequency and amplitude of metabolic oscillations; amplitude, rate, efficacy and duration of signal transduction; and cell cycle progression. Metabolic control analysis mathematically combines models, experiments, discoveries and natural laws based on the established organization of biochemical networks. Applied to signal transduction, for instance, the approach revealed that phosphatases are equally important as the kinases for the amplitude of the transduced signal, but that phosphatases are more critical for the duration. Development of methodology to determine the extents to which an organism regulates a flux through a process metabolically, through signal transduction, through transcription or through translation (hierarchical regulation analysis) (Ter Kuile and Westerhoff) again showed regulation to be subtle, i.e. distributed over these four modes (Daran-Lapujade et al), varying between steps in the same pathway (Ter Kuile and Westerhoff; Rossell et al.) with challenges that cause the organism to regulate (Rossell et al) and with time after the challenge (Van Eunen et al.). These properties bestow biochemical networks with much higher robustness than single metabolic reactions would have (Quinton-Tulloch et al.).

Daran-Lapujade et al. (2007) The flux through glycolytic enzymes in Saccharomyces cerevisiae are predominantly regulated at posttranscriptional levels. Proc. Natl Acad. Sci. USA 104: 15753-15758
Groen et al. (1982) Quantification of the Contribution of Various Steps to the Control of Mitochondrial Respiration. J. Biol. Chem. 257: 2754 2757
Jensen et al. (2000) Extensive regulation compromises the extent to which DNA gyrase controls DNA supercoiling and growth rate of Escherichia coli. Eur. J. Biochem. 266: 865-877
Kacser & Burns (1973) The control of flux. Symp. Soc. Exp. Biol. 27: 65-104 Heinrich et al. (1977) Metabolic regulation and mathematical models. Progr. Biophys. Mol. Biol. 32: 1-82

Quinton-Tulloch et al. (2013) Trade-off of dynamic fragility but not of robustness in metabolic pathways in silico. FEBS J. 280: 160 -173
Rossell et al. (2006) Unraveling the complexity of flux regulation: a new method demonstrated for nutrient starvation in Saccharomyces cerevisiae. Proc. Natl Acad. Sci. USA 103: 2166-2171
Ter Kuile & Westerhoff (2001) Transcriptome meets metabolome: hierarchical and metabolic regulation of the glycolytic pathway. FEBS Lett. 500: 169-171
Van Eunen et al. (2009) Time-dependent regulation analysis dissects shifts between metabolic and gen-expression regulation during nitrogen starvation in baker's yeast. FEBS J. 276: 5521-5536
Westerhoff & Arents (1984) Two completely rate limiting steps in one metabolic pathway? The resolution of a paradox using control theory and Bacteriorhodopsin liposomes. Biosc. Rep. 4: 23-31
Westerhoff & Chen (1984) How do Enzyme Activities control Metabolite Concentrations? An additional theorem in the theory of metabolic control. Eur. J. Biochem. 142: 425 430
Westerhoff & Kahn (1993) Control involving metabolism and gene expression: the square-matrix method for modular decomposition. Acta Biotheor. 41: 75-83
Westerhoff et al. (2009) Systems Biology towards Life in silico: mathematics of the control of living cells. J. Math. Biol. 58: 7-34

The dangers of activated metabolism

- turbo explosions, their prevention, and new drug targets

Biological question:
Most catabolic pathways are activated by an investment of ATP-free energy in their beginning to yield a substantial return on investment later on in the pathway, a so-called turbo design. How are these core metabolic systems properly controlled?
Mathematical methods:
Kinetic modelling
Key insights:
Turbo design could lead to metabolic explosions in terms of continued accumulation of hexose phosphates. Yeast growing at high glucose concentrations can prevent such explosion from happening in through a regulatory feedback loop via trehalose phosphate with hitherto unidentified function (Teusink et al.). In trypanosomes the compartmentation of part of glycolysis into a special compartment would also protect the organism against turbo explosions (proposed and computed by Bakker et al.), which was subsequently confirmed experimentally (Haanstra et al.), providing a novel drug target in trypanosomes. The principle is under active investigation as to the involvement of other parts of the network and a possible anti-tumour network target.

Bakker et al. (2000) Compartmentation prevents trypanosomes from the dangerous design of glycolysis. Proc. Natl Acad. Sci. USA 97: 2087-2092

Haanstra et al. (2008) Compartmentation prevents a lethal turbo explosion of glycolysis in trypanosomes. Proc. Natl Acad. Sci. USA 105: 17718-17723
Teusink et al. (1996) The danger of metabolic pathways with turbo design. Trends Biochem. Sci. 23: 162-169

Immunology

The "population dynamics" of white blood cells

den Braber et al. 2012. Maintenance of peripheral naive T cells is sustained by thymus output in mice but not humans.
Biological question: Mature T cells emerge from the thymus where they are selected for recognition of the body's MHC molecules and against recognition of self-peptides. The thymus progressively degenerates after puberty, and it has been a long-standing question of how long during life the pool of naïve T cells is replenished by thymic output. Among other implications, this question is relevant for understanding the aging of the immune system.
Mathematical methods: To answer this question, the lifetime of naïve T cells in the intact organism and influx from the thymus need to reliably estimated from multiple experimental data sets. **Population-dynamic models** based on ordinary differential equations have been developed and fitted to the data.
Key insights: Aging affected naive T cell maintenance fundamentally differently in mice and men. Whereas the naive T cell pool in mice was almost exclusively sustained by thymus output throughout their lifetime, the maintenance of the adult human naive T cell pool occurred almost exclusively through peripheral T cell division.

Buchholz et al. 2013. Disparate individual fates compose robust CD8+ T cell immunity.
Biological question: During the immune response, naïve T cells develop into short-lived effector cells that fight an invading pathogen and memory cells that protect against subsequent infections with this pathogen. The underlying developmental program has been controversial. This question has implications for vaccination as well as cell therapy of immunodeficiency and cancer.
Mathematical methods: **Stochastic modeling** of cell proliferation and differentiation, **statistically-based inference** of model topology ('developmental program') that accounts for the experimental data.
Key insight: Precursors of memory T cells arise early during the immune response and serve as stem cells for both primary and memory responses, giving rise to derived effector and effector memory cells.

Regulatory networks in immunology

T cells can discriminate between antigens of similar affinity, largely independent of ligand concentration. This exquisite specificity cannot be explained by mass-action receptor-ligand

binding. It is thought that the T cell receptor and its associated signal transduction machinery recognize the dwell time of the antigen at the receptor by a kinetic proofreading mechanism. Mechanistic understanding is relevant for rationalizing the pathogenesis of autoimmune diseases.

Key reference: Altan-Bonnet & Germain 2005. Modeling T cell antigen discrimination based on feedback control of digital ERK responses.
Mathematical methods: Detailed ordinary differential equation model of signal transduction downstream of the T cell receptor that accounts for the **combinatorial complexity** of protein complex formation and multi-site phosphorylation by rule-based modeling. Used to rationalize experimental data by semi-quantitative comparisons. This study made extensive use of automated model generation via BioNetGen.
Key insights: The results combining computation and experiment reveal that ligand discrimination by T cells is controlled by the dynamics of competing feedback loops that regulate a high-gain digital amplifier, which is itself modulated during differentiation by alterations in the intracellular concentrations of key enzymes.

Altan-Bonnet G & Germain RN (2005). Modeling T Cell Antigen Discrimination Based on Feedback Control of Digital ERK Responses. PLoS Biol 3, e356.
Buchholz VR, Flossdorf M, Hensel I, Kretschmer L, Weissbrich B, Gräf P, Verschoor A, Schiemann M, Höfer T & Busch DH (2013). Disparate Individual Fates Compose Robust CD8+ T Cell Immunity. Science 340, 630–635.
Den Braber I, Mugwagwa T, Vrisekoop N, Westera L, Mögling R, Bregje de Boer A, Willems N, Schrijver EHR, Spierenburg G, Gaiser K, Mul E, Otto SA, Ruiter AFC, Ackermans MT, Miedema F, Borghans JAM, de Boer RJ & Tesselaar K (2012). Maintenance of Peripheral Naive T Cells Is Sustained by Thymus Output in Mice but Not Humans. Immunity 36, 288–297.

Further examples of medical modelling

Regulatory networks and cancer: Analysis of the Wnt pathway

Lee et al. 2003. The roles of APC and axin derived from experimental and theoretical analysis of the Wnt pathway.
Biological question: Wnt signaling plays an important role in both cancer formation and normal development. Although the key molecules required for transducing a Wnt signal have been identified, a quantitative understanding of this pathway has been lacking.
Mathematical methods: **Ordinary-differential equation model** of the core Wnt pathway, sensitivity analysis, quantitative comparison with experimental data.
Key insights: Clarification of the role of the scaffold proteins APC and axin. The dependence of axin degradation on APC is shown to be an essential part of an unappreciated regulatory loop.

By applying control analysis to the mathematical model, tumor suppression and oncogenicity is quantified.

Lee E, Salic A, Krüger R, Heinrich R & Kirschner MW (2003). The Roles of APC and Axin Derived from Experimental and Theoretical Analysis of the Wnt Pathway. *PLoS Biol* **1,** e10.

Using mathematical models to characterise effects of blood cancer drugs

Chronic myeloid leukaemia is a cancer of the white blood cells, which are an important part of the body's immune system. To quantify the effects of drugs, it is useful to fit mathematical models to observe disease progression with and without drugs. The parameters in this mathematical model then form a very relevant and concise summary of the drug effects.

The clinical success of the ABL tyrosine kinase inhibitor imatinib in chronic myeloid leukaemia (CML) serves as a model for molecularly targeted therapy of cancer, but at least two critical questions remain. Can imatinib eradicate leukaemic stem cells? What are the dynamics of relapse due to imatinib resistance, which is caused by mutations in the ABL kinase domain?

Key reference: Michor et al. 2005. Dynamics of chronic myeloid leukaemia.
Mathematical methods: **Population-dynamic model** of hematopoiesis and development of leukemia ('compartment model').
Key insights: The model suggests that imatinib is a potent inhibitor of the production of differentiated leukaemic cells, but does not deplete leukaemic stem cells. It provides a quantitative framework for understanding the timescale at which therapy resistance emerges.

Michor F, Hughes TP, Iwasa Y, Branford S, Shah NP, Sawyers CL & Nowak MA (2005). Dynamics of chronic myeloid leukaemia. *Nature* **435,** 1267–1270.

Diseases with complex causes are systems-biology phenomena

Mankind has had appreciable success in treating unifactorial ailments, such as infectious disease and some inborn errors of metabolism (e.g.phenylketonuria). However, a one-gene one-disease type of paradigm has not worked well for understanding the major disorders that plague modern society, including diabetes, obesity, cancer, heart disease, and atherosclerosis.
Mathematical methods:
Kinetic modelling; topological modelling.
Key insights:
Cancer is a typical systems biology disease, with multiple systems biology studies showing that network functions depend on many factors and go awry upon changes in a number of alternative sets of simultaneous losses of molecular function (e.g. Hornberg and colleagues). Similar conclusions hold for other multifactorial diseases (e.g. Rehman et al.).

Hornberg et al. (2005) Control of MAPK signalling: from complexity to what really matters. Oncogene 24: 5533-5542
Hornberg et al. (2006) Cancer: A Systems Biology Disease. BioSystems 83: 81-90
Moreno-Sanchez et al. (2010) Metabolic control analysis indicates a change of strategy in the treatment of cancer. Mitochondrion 10: 626-639
Rehman et al. (2011) Dupuytren's: a systems biology disease. Arthritis Res. Ther. 13: 238.

Network-based drug design, and a new drug target

Most drug design has been either empirical (testing a battery of drugs in a high throughput experimental model of the disease) or through focusing on the binding of an inhibitor to a macromolecular target, such as an enzyme or receptor. Target selection is commonly based on, for example, its position at the beginning of a pathway, a suspicion that something would be the rate limiting step, or some correlation of its expression with disease occurrence. Since systems biology has shown that multifactorial diseases are network phenomena and that therefore one should target network function rather than single-component activity, the question was whether there is a more efficient and objective approach towards drug target selection in the parasite *Trypanosoma brucei* that causes sleeping sickness.

Mathematical methods:

Bakker and colleagues developed a systems biology methodology to identify the best target in a network comparing the action of that target between parasitic cells to be removed and healthy cells of the host. The studies involved a combination of computer simulations deploying ODE-based kinetic models and metabolic control analysis, and experimental studies of glycolysis in bloodstream-form *T. brucei*.

Key insights:

Applying the methodology to glycolysis in *T. brucei* it was found that (i) the traditionally used drug targets are unlikely to be effective when the metabolic network is taken into consideration, (ii) the glucose transporter is the much better target, and (iii) this is even more so when accounting for natural adaptation of the parasite to the low glucose conditions in the tsetse fly.

Bakker et al. (1999) What controls glycolysis in bloodstream form Trypanosoma brucei? J. Biol. Chem. 274: 24552-24559
Bakker et al (2002) Network-based selectivity of antiparasitic inhibitors. Mol. Biol. Rep. 29: 1-2
Haanstra et al. (2011) A domino effect in drug action: from metabolic assault to parasite differentiation. Mol. Microbiol. 79: 94-108
Bakker et al. (2010) The silicon trypanosome. Parasitology 137: 1333-1341