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Computational methodologies for modelling, analysis and simulation of signalling networks

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Abstract

This article is a critical review of computational techniques used to model, analyse and simulate signalling networks. We propose a conceptual framework, and discuss the role of signalling networks in three major areas: signal transduction, cellular rhythms and cell-to-cell communication. In order to avoid an overly abstract and general discussion, we focus on three case studies in the areas of receptor signalling and kinase cascades, cell-cycle regulation and wound healing. We report on a variety of modelling techniques and associated tools, in addition to the traditional approach based on ordinary differential equations (ODEs), which provide a range of descriptive and analytical powers. As the field matures, we expect a wider uptake of these alternative approaches for several reasons, including the need to take into account low protein copy numbers and noise and the great complexity of cellular organisation. An advantage offered by many of these alternative techniques, which have their origins in computing science, is the ability to perform sophisticated model analysis which can better relate predicted behaviour and observations.

Keywords: *biochemical signalling networks; modelling; analysis; simulation*

INTRODUCTION

The discipline of dynamic systems modelling originated in engineering and physics, but is increasingly being applied to biochemical problems. A dynamic model defines a fixed set of rules by which the temporal behaviour of the system and its variables can be traced.

Figure 1 depicts some biological systems to which dynamic modelling approaches have been applied. Applications range from the sub-molecular to

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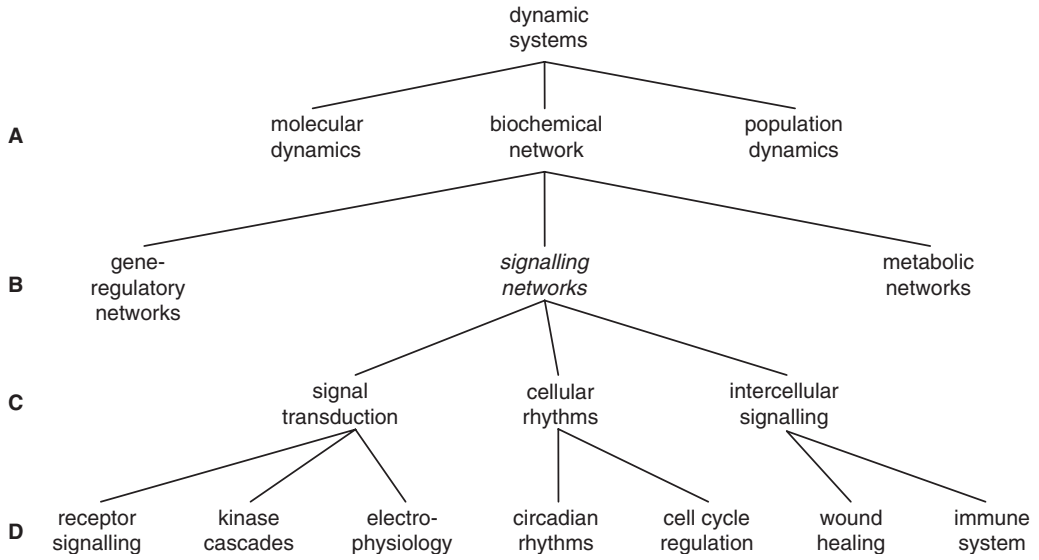

Figure 1: An ontological view of biological dynamic systems to which modelling approaches have been applied. While not meant to be exhaustive, this diagram gives an overview of the context, in which signalling networks are situated (A, B). Each of the three cellular functions in this diagram (C) is discussed in one of the three central sections of this review with concrete biological applications (D).

population level (Figure 1A). The fundamental elements of biochemical networks are biomolecules such as proteins (signalling networks), nucleic acids (gene regulatory networks) or small organic compounds (metabolic networks). In contrast, molecular dynamics simulations, in which conformational changes or interactions of a molecule result from the modelling of forces between its individual atoms, operate on a smaller scale. On a larger scale the entities of population dynamics models can be cells, individual organisms or people. The variables in a model of any such system generally describe a state (e.g. concentration or coordinates) of their basic entities. Although this article focuses on the modelling of signalling networks, we will address the problem of multiscaleness in intercellular communication.

Signalling networks, which are largely based on interactions between proteins, implement a variety of cellular functions: Signal transduction is the process by which a cell converts an external signal or stimulus into an appropriate cellular response. Cellular rhythms are periodic biological processes, such as the cell cycle or day–night cycles (circadian rhythms) of animals and plants. In multicellular organisms complex behaviour emerges from these basic cellular processes. Cells are organised to build an organism, defend it against pathogens (immune system) or repair wounded tissue.

In any of these areas computer-based modelling approaches are motivated by the inherent complexity of the system. Of all biochemical networks, signalling networks exhibit the highest degree of complexity. This is not only due to their non-linear network topology, but also because of the different types of interactions that these proteins can undergo: protein associations, enzymatic catalysis and reversible or irreversible protein modification, to name only the most common types.

Kinase networks, for example, are an important subclass of signalling networks. Kinases are enzymes that catalyse the covalent attachment of phosphate groups to various amino acid residues of proteins. These modifications can have a dramatic impact on the protein's enzymatic activity or its ability to participate in other interactions (allosteric effects). Cellular control systems exploit these effects to achieve complex signal processing tasks required in signal transduction or cellular rhythms. Diseases that arise from defects in signalling systems are usually not attributed to a single gene and are difficult to diagnose or treat in the absence of an accurate theoretical understanding of the underlying control system.

The terms 'pathway' and 'network' tend to be used interchangeably in the literature, with pathway being implicitly taken to be a part of a more general network. In this article, we follow the generally

accepted use of the term pathway to refer to the core of a biochemical network, comprising a sequence of activities, for example a kinase cascade. Thus, we will describe the extracellular signal regulated kinase (ERK) pathway as being embedded in a more general signal transduction network, and that the ERK pathway is a member of a large family of MAP kinase pathways.

In biology and biotechnology, dynamic systems modelling refers to attempts that aim to model, analyse and simulate biological systems and processes using techniques from mathematics, computing and information technology. In contrast to modelling techniques that provide a more or less static view of the studied system, dynamic systems modelling facilitates the computational capture and rendition of the dynamic behaviour of the system or process in question. Dynamic models provide a powerful framework for hypothesis generation and testing, and the identification of inconsistencies in a model. They are often used by life scientists as a means to explore their ideas about the organisation of a system.

In most cases, however, simulation is not the final stage. Dynamic models permit a range of analytical techniques that give insight about system-level features that emerge from the model's elementary interactions. Emergent properties such as bifurcations, robustness or oscillations are not obvious from the network topology and their discovery requires computational methodologies. Finally, analytical results can suggest novel pathways and interactions and thus inspire new research.

The 'correctness' of a model can be established in several ways. *Model checking* establishes whether a set of formal properties hold for a model, and is often automated using computer programs. *Biological model validation* establishes whether a model does not contradict our knowledge of a biological system, and hence requires some kind of data about the system. A biologically valid model can be *incomplete* and hence not describe all the observations we can make of a system, but should not incorrectly describe behaviours of the system.

In this review we will describe a range of methodologies for modelling and analysing signal transduction networks, ranging from discrete approaches such as discrete Petri nets and logic based descriptions, through continuous approaches such as ordinary differential equations (ODEs), to stochastic descriptions based on modelling at the molecular level.

Figure 2 illustrates the modelling methods presented throughout this article using a simple example. It shows various representations of a basic enzyme catalysed biochemical reaction, where a substrate is transformed into a product. Two alternative formulations of this reaction are presented (Figure 2A and B). Dynamic representations of such reactions (Figure 2C–F) are the building blocks of any signalling network model.

RECEPTOR SIGNALLING AND SIGNAL TRANSDUCTION CASCADES

Cells can receive external signals in a variety of forms, such as growth factors and hormones, which stimulate a plethora of cell surface receptors, such as G protein coupled receptors (GPCRs) and receptor tyrosine kinases (RTKs). In most cell types, signalling through RTKs and GPCRs activates mitogen activated protein kinase (MAPK) cascades, which appear to function as central integration modules in signal processing. MAPK modules are evolutionarily conserved in cells from yeast to mammals. They typically consist of three kinases, forming a three tiered cascade, which are activated by sequentially phosphorylating each other in response to stimuli [1]. The kinase in the first tier of the cascade is typically activated at the plasma membrane, whereas the third kinase is typically translocated from the cytoplasm to the nucleus upon activation, where it can regulate gene transcription through affecting chromatin structure and modifying the activity of transcription factors. Although the structure of the core MAPK cascade appears to be relatively simple, it can be used by the cell to generate a wide range of different cellular responses. The classical example of this is in PC12 cells where the transient activation of ERK by epidermal growth factor (EGF) triggers cellular proliferation while the sustained activation of ERK by nerve growth factor (NGF) triggers neuronal differentiation [2]. ERK is itself a MAPK and forms a three-tier MAPK cascade consisting of Raf, MEK and ERK.

Signal transduction pathways have traditionally been drawn as separate linear entities, reflecting the history of how they were discovered rather than their functional context. However, signalling pathways are extensively interconnected and embedded in networks with common protein

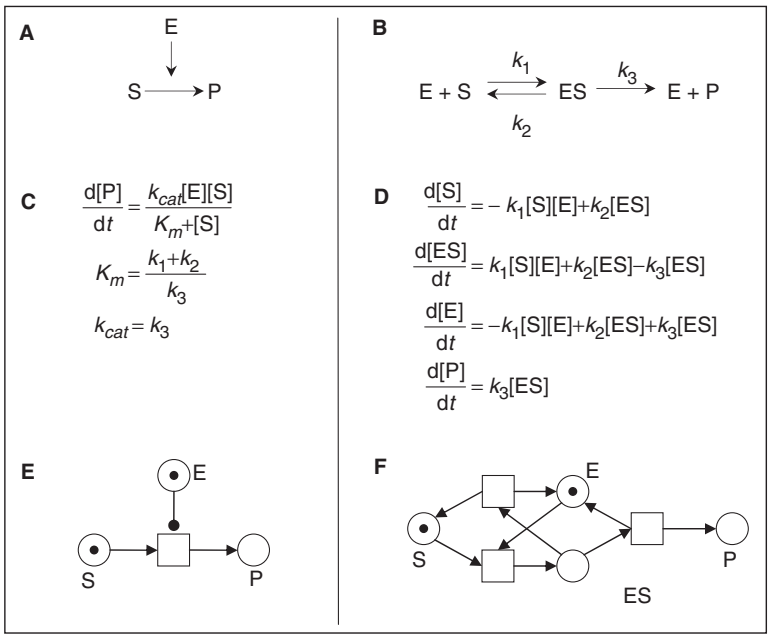

Figure 2: A single enzyme-catalysed reaction in various modelling representations. The left column shows Michaelis–Menten approximations, the right column mass-action kinetics, explicitly featuring an enzyme-substrate complex. (A, B) Conventional notation of the chemical reactions and kinetic constants. (C, D) A possible ODE (ordinary differential equations) representation. The differential equations mathematically describe the temporal change of each molecular species. (E, F) Discrete Petri net description. Circular nodes represent biochemical entities and boxes represent reactions. Enzymatic catalysis in E is represented using a special read arc (circled end). The marking of circular nodes with tokens indicates whether the biochemical entity is present in the state of the model. Reactions may occur if their preceding biochemical entities are marked.

components and a multitude of links and crosstalk between pathways. Owing to the complexity of these networks, computational modelling techniques are required in order to explain in detail how they function and predict possible behaviours.

ODE models of the ERK signalling pathway

The ERK pathway is implicated in various diseases, including cancer, making it an important drug target and has therefore been intensively studied in the laboratory and using computational models [1]. A large number of these models of this pathway are based on the ODE approach [3]. They have increased in both size and complexity through the years and have been used to investigate various aspects of the biological behaviour of this system, such as:

(i) Ultrasensitivity of the ERK cascade as a result of a two-step distributive activation mechanisms [4–6].
(ii) Oscillatory behaviour of the ERK cascade due to embedded negative feedback loops [7].
(iii) The effects of receptor location, trafficking and degradation on downstream ERK signalling [8, 9].
(iv) The dynamic differences between the transient and sustained activation of ERK by different growth factors. [10, 11].
(v) The influence of Raf kinase inhibitor protein (RKIP) on the ERK pathway [12].

While the ODE method is not the most visually intuitive, it interfaces well to higher- level modelling tools and graphical formalisms, which have been used to describe biochemical networks [13–16]. ODE modelling takes a population view of a system rather than modelling the stochastic behaviour of individual proteins, and requires exact knowledge of reaction rates and concentrations of the proteins. Partial differential equations (PDEs), the spatial counterpart to ODEs, have been used by Eungdamrong and Iyengar [17] to model spatially restricted reactions in signalling networks, taking into account spatial diffusion processes as well as chemical reactions.

Non-ODE modelling approaches applied to signal transduction pathways

Stochastic modelling approaches are based on representing the individual behaviour of molecules and hence variability in the overall behaviour of a system. For example, Phillips and Cardelli [18] have used the stochastic π-calculus to model the MAPK pathway by simulating the behaviour of individual molecules using the Gillespie algorithm. Their approach has shown that the overall behaviour of the system is highly robust to changes in reaction rates. A stochastic π-calculus description of the model enzymatic reaction is given in Figure 2B. A related approach, the stochastic process algebra PEPA (Figure 3A), was used by Calder *et al*. [19] to model the influence of RKIP on the ERK signalling pathway. This method can handle imprecise data, where concentrations are represented by levels rather than by exact values, and also permits different alternative formulations of a model to be formally compared. PEPA models can be simulated using the Gillespie algorithm or ODE solvers: Calder *et al*.[20] have shown how to automatically derive ODEs from process algebra models. A description in PEPA of the model enzymatic reaction is given in Figure 3A.

Calder *et al*. [21] have also used the PRISM model checker [85] to perform quantitative analysis of these PEPA models. Examples are: steady-state analysis of stability of a protein, for instance that a protein reaches and then remains within certain bounds, or that a protein is more likely to be stable for certain reaction rates, and transient analysis of protein activation sequences i.e. concentration peak ordering. Thus biochemists can directly pose queries of interest about a system, rather than attempt to manually interpret simulations, which may be very complex.

Petri nets (Figure 2E and F) are graphical notations for modelling concurrent systems, in which several processes can occur at the same time, and are widely used to analyse biochemical networks. These graphs give an immediate representation of the topology of a biochemical network; whilst the underlying mathematics may be complicated, their presentation is very intuitive for the life scientist. Yup-Lee *et al*. [22] have used Petri nets to model the molecular mechanisms of cell signalling and their pathological implications, applying their approach to modelling the IL-1β and TNF-α-induced signalling system. Several types of automated analyses can be performed on discrete Petri nets, for example structural properties which are independent of the marking, and behavioural properties. Gilbert and Heiner [23] have applied such analyses to a Petri net model of the influence of RKIP on the ERK signalling pathway, and have also shown how that analysis can be used to

A. PEPA

$E_H = (r_1,k_1).E_L$

$E_L = (r_2,k_2).E_H + (r_3,1).E_H$

$S_H = (r_1,k_1).S_L$

$S_L = (r_2,k_2).S_H$

$SE_H = (r_2,k_2).SE_L + (r_3,k_3).SE_L$

$SE_L = (r_1,k_1).SE_H$

$P_L = (r_3,k_3).P_H$

$P_H = (\text{stop},1).P_H$

$$S_H \underset{\{r_1,r_2\}}{\bowtie} (SE_L \underset{\{r_1,r_2,r_3\}}{\bowtie} E_H) \underset{\{r_3\}}{\bowtie} P_L$$

B. Stochastic π-calculus

$E(k_1) \stackrel{\Delta}{=} \nu k_2 \nu k_3 !k_1(k_2,k_3).(?k_2.E(k_1) + ?k_3.E(_1))$

$S(k_1) \stackrel{\Delta}{=} ?k_1(k_2,k_3).(!k_2.K(k_1) + !k_3.P())$

run 100 of $E(a) \mid S(a)$

Figure 3: Stochastic representations of the single enzyme-catalysed reaction. (A)**:** Stochastic process algebra description in PEPA; the upper part defines the biochemical components, where the concentrations of each one can be either high or low (e.g. for the substrate either S_H or S_L). The reactions are referred to by the labels r_1,r_2,r_3 and k_1,k_2,k_3 represent the rates. The last line describes how the components are composed together to form the model. Simulations are via ODEs or the Gillespie algorithm and queries about the model can be made with the PRISM model checker. (B): Stochastic π-calculus description; the first two lines are rules describing the behaviours of the enzyme and substrate respectively. The product is also defined in the second rule. The third line is the instruction to simulate the model with 100 molecules each of the enzyme and substrate using the Gillespie algorithm.

derive the sets of initial concentrations required by the corresponding continuous ordinary differential equation model.

CELL-CYCLE REGULATION

Eukaryotes use a highly conserved process to divide and create new cells, called the *cell cycle*. This periodic process comprises the replication of chromosomal DNA (S phase), the division of the nucleus, called *mitosis* (M phase). Each of these stages is preceded by a gap phase (G1 and G2). *Cytokinesis*, the physical separation of the mitotic cell, ends with two daughter cells in G1, concluding the cycle.

The cell cycle is a biological example of a periodic chemical reaction, such as the famous Belousov-Zhabotinsky reaction. This chemical system is capable of performing self-sustained oscillations, which can be visualised using a chemical indicator. It was the first chemical oscillator to be mathematically described as a set of differential equations in the 1950s [24–26]. Since then, oscillatory cellular processes such as circadian rhythms in animals and plants, cardiac and respiratory rhythms or oscillations in metabolic systems have gained wide interest [27]. The elucidation of the core molecular mechanisms of the central cell cycle 'engine', comprising interactions of cyclins with cyclin-dependent kinases (CDKs), has triggered intensive modelling efforts in the last decades [28–30].

CDKs and their activators, the periodically expressed cyclin proteins, trigger important cell cycle specific events. The complex of cyclin and its specific CDK is catalytically active and phosphorylates serine and threonine residues in target proteins, some of which feed back to the cyclin-CDK system. Early cell-cycle models showed that this simple system can perform self-sustained oscillations [31, 32]. More recently it has been discovered that cell-cycle regulation consists of a number of self-contained, but interlinked modules [33–35]. This organisation facilitates *open-loop approaches*, in which subsystems are decoupled from the larger, complex closed-loop system, enabling a detailed study of the subsystem's behaviour.

ODEs represent the predominant approach in cell-cycle modelling. As with signalling cascades, dynamic models of cell-cycle kinase networks are constructed from basic kinetic building blocks of enzymatic reactions (Figure 2). This is known as a *bottom-up* approach. Its aim is to deduct physiological behaviour by simulating underlying molecular details. From the analysis of the basic equations, higher-level phenomena arise in the form of *emergent properties* [36]. In contrast, top–down modelling is concerned with inferring lower-level mechanisms and parameters from information at system level.

The differential equations in Figures 2C and D contain non-linear terms: the system variables are contained in products and fractions. An analytical approach to integrating or characterising even a simple system with only two or three reactions can be nearly impossible, and numerical methods are needed. Schmidt and Jacobsen [37] on the other hand, have developed a method for reducing non-linear ODE systems to a set of interacting, linearised subsystems. The application of their technique to a previously published cell-cycle model allowed them to attribute roles to particular sub-networks regarding the dynamic behaviour they implement: two sub-networks consisting of three components each create sustained oscillations and bistability, respectively.

As a continuous modelling method, the applicability of ODEs to modelling cellular signalling processes has been questioned. In cases where proteins are expressed in low copy numbers, the assumption of continuous variables may not hold [38]. As an extreme example, a system described by Vilar *et al.* [39] oscillates only in the presence of noise for a given set of parameters.

Emergent behaviour

Even simple non-linear ODEs can exhibit complex and sometimes chaotic behaviour. ODEs are amenable to symbolic or numerical analysis techniques, including bifurcation analysis. These techniques aim to characterise the emerging behaviour in either a qualitative or quantitative sense.

Bistability is now recognised as an important pattern in cellular signalling pathways, and specifically in the cell cycle. Bistable systems can assume either one of two discrete equilibrium states and allow for switching between them. Han *et al.* [40] employed an analytical study of a two-variable cell-cycle model to determine the importance of hysteresis and bistability. Using a random parameter search they showed that bistable systems were more likely to display dynamic instabilities, which are essential for sustained oscillations. In a series of computational and *in vitro* experiments, Pomerening

and colleagues [41] have established a relationship between bistability, hysteresis and oscillations in the biological model system Xenopus. Their findings add to the evidence that bistability is an essential phenomenon for robustness of biological signalling systems, in particular for oscillating systems. Their work also addresses the critical task of experimental model validation.

A phenomenon that is less frequently found in signalling systems is excitable behaviour. Excitable systems, such as the neural membrane potential, modelled in the classical study by Hodgkin and Huxley [42], are generally characterised by a transient response to a sufficiently large stimulus and a global resting state, which the system returns to after an excitation. A recently published model analysis of the Src tyrosine kinase subsystem, which is connected to CDK activity, has shown that Src activity can be excited in this way [43].

Applications of cell-cycle models

Cell-cycle models also have an impact on drug discovery. Chassagnole *et al*. [44] employed a cell-cycle model to quantitatively predict cytotoxicity of a set of kinase inhibitors based on IC_{50} values, which were measured *in vitro*. The results allowed them to assess the pharmaceutical value of these inhibitors as anti-cancer therapeutics.

Does molecular modelling help us to gain a better understanding of the cell cycle? While models of molecular interactions certainly provide a systematic way of determining gene functions, the way in which the organisation of macroscopic cellular events, such as cytokinesis, emerge from molecular interactions, is an unresolved issue [45]. Biron *et al*. [46] employed an ODE model to characterise the contractile ring, whose contraction divides the cell in two at cytokinesis. On a molecular level, this model takes into account the interactions between actin and myosin, two proteins from which cellular fibres are built. It also includes biophysical parameters, which describe the forces experienced by the contractile ring. Spatial characteristics of the ring are built into the model, rather than arising from dynamics on molecular level.

The gap between molecular and macroscopic is an important challenge in systems biology. Some approaches towards intercellular communication will be discussed in the next section.

INTERCELLULAR SIGNALLING

A fundamental aspect of cellular signalling is to respond appropriately to the cell's environment, which normally includes the presence of other cells. The importance of the interaction of cells in multicellular organisms is apparent when considering the organism itself as an emergent property of the interaction of the component individual cells. Without this ability tissue repair, immunity or homoeostasis would be impossible. Indeed, errors in cellular information processing are responsible for diseases such as cancer, autoimmunity, and diabetes.

The component processes of cell signalling operate over orders of magnitude in size and time. The ability to build models from the bottom up and integrate them with models of different paradigms and spatiotemporal dynamics is at the heart of what has become one of the grand challenges of systems biology. The bottom-up approach, despite being the most accurate, is limited as the computational cost increases rapidly with the number of interacting elements. Using hierarchical models that abstract the details of finer grain sub-models can mitigate this problem [47, 48]. The success in modelling the heart as a whole organ from numerous submodels exemplifies this point [49]. These models have been able to demonstrate a number of counterintuitive mechanisms, such as the mechano-electrical feedback in which the contractions of the heart influence its electrical properties [50].

Spatiotemporal models

Various modelling techniques deal with spatial dimensions, for example, partial differential equations. However, we will discuss here two discrete methodologies, which are suitable for representing intercellular communication [50].

The modelling paradigm based on cellular automata (CA) is versatile, simple and scalable. CAs map the space of interest as a regular lattice at a dimension that is optimal for the model requirements (Figure 4A). The representation of space within the model will generally include a state, for example empty or occupied, and will take into account the states of the adjacent spaces. The approach can be adapted for biophysical simulations [51] and to explicitly represent molecular crowding. CA is one of the computational frameworks that are most efficiently parallelisable, as exemplified by CyberCell [52].

In contrast to CA, agent-based models represent the discrete elements of the model as software agents (Figure 4B). These entities incorporate the attributes of elements [53] including time, spatial position and often using various artificial intelligence capabilities. The versatility of this paradigm, like CA, has lent itself for biophysical simulations [54].

Here, we consider two models that address the issue of wound healing in terms of cellular interactions, first from an agent-based paradigm and then as a CA. Both models accommodate multiscaleness in time and space by operating at an appropriate scale and abstracting a finer grained model to be represented as a set of rules.

Agent-based simulation

The epitheliome project [57] aims to develop a computational model that is able to predict the social behaviour of cells in epithelial tissues. These tissues form sheets of cells of approximately 10 cells thick that serve as protective barriers. The lining of the urinary tract, the urothelium, has attributes that make it of particular interest to this study. Urothelium cells *in vivo* multiply slowly but they have a fast proliferative response to injury. When tissue is injured, the ability of the urothelial cells to self-organize into cell tissue appears to be an emergent property that arises from the mutual interaction of the cells.

Walker *et al*. [55, 56] have investigated some of these mechanisms. The model tests the hypothesis that global behaviour of the urothelial tissue can be explained by cell-based rules of engagement between neighbours. The agents, representing the individual cells, adapt the rules which dictate their behaviour through interaction with their environment and with other agents. These rules relate to proliferation and attachment. The virtual cells are able to progress through the cell cycle and divide, form calcium-dependent bonds with one another, migrate, and die. In order to validate these models, wounding assays were developed *in vitro* for a parallel study. Urothelial monolayer cultures were subjected to scratch wounds and their healing monitored and compared with the model.

The computational modelling of wound healing in parallel with *in vitro* assays has provided a deeper insight into the mechanisms of monolayer regeneration. In particular, modelling and experimental results show that cells in low calcium ion concentrations rapidly migrate into the wound, whereas cells in physiological calcium ion concentrations drift as confluent sheet, covering the wound at a much slower rate. The model also suggests that in low-calcium ion, the repair of scratch wounds may be associated with increase in the number of cells entering the mitotic cycle.

A cellular automata model of tissue growth

A complimentary model to the aforementioned utilises a three-dimensional scaffold to represent a spatial matrix in which cells proliferate, migrate, collide and adhere, and eventually reach confluence

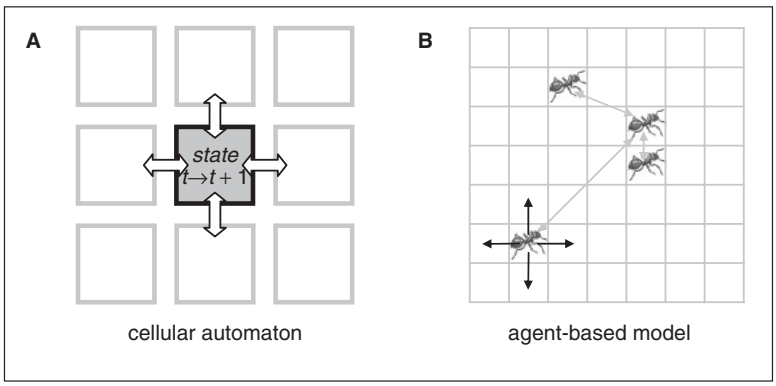

Figure 4: Comparison of two spatial modelling methodologies. (A): Cellular automata consist of a number of abstract elements ('cells') arranged in a grid. Each element is aware of its own state and the state of the adjacent elements (arrows), on which the dynamic rules are built. The elements may represent actual biological cells or any discrete volume of space. (B): In agent-based models any state information is encapsulated in the position and properties of agents, which move (black arrows) and interact with each other (grey arrows) on a spatial matrix. The agents may represent biological cells, with the state referring to intracellular signalling events.

(i.e. maximum density) [57]. Although this model is represented as a grid, functionally it represents space in the same explicit manner as a CA.

The model builds on that by Chang *et al*. [58] in that it takes into account the migration of the cells or the effect of collision between cells. It also extends Lee's model [59] that quantified the competing effects of migration and contact inhibition with a 2D random walk CA model. This model, however, includes an asynchronous cell population, whereby the proliferation period and speed of migration have a parametric distribution. This is regarded as being important because it has been shown that heterogeneity plays an important role in the complexity of behaviour and functionality of the model.

The spatiotemporal development takes place in a $100 \times 100 \times 100$ matrix to represent the 3D arena of cell population. The simulation starts with 0.1% of the confluent population density with various initial states of cell seeding distribution, random, uniform or a state representative of a wound-healing model. The different initial states also affect the outcome of the simulation.

Throughout the simulation the cells migrate in a random walk, dividing at intervals and occasionally colliding with each other. The effect of contact between the migratory cells is such that it will inhibit proliferation and tissue growth. Epithelial cells may adhere to each other irreversibly when they collide, forming small colonies that eventually grow into contiguous sheet of cells.

Fast cell migration mitigates the effects of adhesion as it increases the probability that a cell will move away from its neighbours and therefore have room to divide. The migration process is slowed down by cell collisions that either cause a pause in movement or adhesion and the formation of an aggregate.

Various parametric studies are conducted to test the sensitivity of the model's response to key parameters. Cell motility and average cell-division time both have a strong influence on time to confluence, although the mechanisms are non linear and complex. In general, after an initial lag phase the simulation progresses rapidly until about 45% confluence. At this point many cells are completely surrounded, are unable to divide and here cell speed has less of an effect on the simulation. Confluence occurs between 4 and 11 days depending on parameter values.

TOOLS AND RESOURCES

Several computational infrastructures, namely databases and model repositories, support model creation and exchange (Table 1). Databases like TRANSPATH [60] and aMAZE [61] are primary, searchable and annotated knowledge bases of molecular interactions. Comprehensive information concerning a variety of cellular processes can also be obtained from KEGG (Kyoto Encyclopedia of Genes and Genomes) [62]. BRENDA [63] and KDBI (Kinetic Data of Biomolecular Interactions) [64] are specialised databases collecting experimentally determined kinetic data, the knowledge of which is of major importance for biochemical modelling. CellML [67] and SBML [76] are markup languages which have been specifically designed to describe biochemical networks with the data required to perform ODE-based simulations.

Recently, model repositories have been constructed which allow the storage, curation and annotation of ready-to-use dynamic models. Examples include BioModels [65], DOQCS (Database of Quantitative Cellular Signalling) [66] and the repositories on the CellML [67] and SBML [76] websites. DOQCS, which is specific for signalling pathways, not only provides links to the GENESIS/Kinetikit simulator, but also allows the user to perform queries for kinetic parameters and reactions.

A large number of software tools are available for simulation and analysis of cell- signalling models specified in ODEs, Petri nets and π-calculus. The software tools differ not only regarding their underlying techniques, but also the capabilities that they support. The majority of ODE-based tools provide users with a graphical interface to construct models. They allow users to define the network topology and kinetic laws, from which ODEs are generated and numerically solved. Most tools provide a variety of numerical algorithms for simulation and analysis.

MATLAB is a general-purpose mathematical environment that is widely used in the physical and engineering sciences, but recently program extensions for systems biology have become available [68], and a specialised toolbox is available for modelling, simulating and analysing biochemical networks. A major benefit of the MATLAB environment is the comprehensive library of mathematical and graphical functions, enabling

Table 1: A selection of databases and tools for modelling signalling networks

Databases

Name	Content	Website
TRANSPATH [62]	Signalling pathways	www.biobase.de/pages/index.php?id=39
aMAZE [63]	Annotated protein interactions	www.amaze.ulb.ac.be
KEGG [64]	Annotated metabolic and signalling pathways	www.genome.ad.jp/kegg
BRENDA [65]	Enzyme function and kinetic data	www.brenda.uni-koeln.de
KDBI [66]	Kinetic data	xin.cz3.nus.edu.sg/group/kdbi/kdbi.asp
BioModels [69]	Dynamic model repository	www.ebi.ac.uk/biomodels
DOQCS [70]	Dynamic model repository	doqcs.ncbs.res.in
CellML model repository [67]	Dynamic model repository	www.cellml.org/models

Tools

Name	Category	Model Representation	Function	URL
MATLAB, with SimBiology Toolbox [71]	Continuous and stochastic	Mathematical (e.g. ODE)	General-purpose mathematical environment, simulation and analysis	www.mathworks.com
XPPAut	Continuous and stochastic	ODE	General purpose; simulation, analysis	www.math.pitt.edu/∼bard/xpp/xpp.html
Copasi [73]	Continuous and stochastic	ODE	Simulation and analysis	www.copasi.org
Virtual Cell [75]	Continuous and stochastic	ODE-based, PDE	Simulation and parameter sensitivity analysis	www.nrcam.uchc.edu
Systems Biology Workbench [76], including Jarnac and JDesigner	Discrete, continuous and stochastic	ODE/SBML	Data-exchange framework for modelling, simulation and analysis	sbw.kgi.edu
Narrator [15]	Continuous and stochastic	Graphical, ODE-based	Modelling and simulation	www.narrator-tool.org
STOCHSIM [78]	Stochastic	Probabilistic	General-purpose biochemical simulator	www.pdn.cam.ac.uk/groups/comp-cell/StochSim.html
E-CELL [77]	Continuous	Object-oriented	Modelling and simulation	www.e-cell.org
SPiM [83]	Stochastic	Π-calculus	Simulation	http://www.doc.ic.ac.uk/∼anp/spim/
BioSigNet [85]	Discrete	Graphical	Reasoning, hypothesis testing	www.public.asu.edu/∼cbaral/biosignet
BIOCHAM [84]	Discrete and continuous	Logical + kinetic models	Simulation and analysis	contraintes.inria.fr/BIOCHAM
PRISM [24]	Discrete	Stochastic process algebra	General purpose; Analysis (model checking)	www.cs.bham.ac.uk/∼dxp/prism
PEPA Workbench [20]	Discrete	Stochastic process algebra	General purpose; Analysis	www.dcs.ed.ac.uk/pepa/tools

convenient visualisation, analysis and optimisation of biochemical models. MATLAB also allows for stochastic simulation, although some manual work is required. Another widely used tool for simulation and analysis of ODE models from all fields is XPPAut, particularly due to its interface to the bifurcation analysis tool AUTO [68].

On the other hand, tools specifically designed for biochemical modelling offer some advanced analysis techniques. Copasi [70], an updated and portable version of Gepasi [71], supports continuous and stochastic simulation, parameter estimation and optimisation, metabolic control analysis and linear stability analysis of biochemical models. Virtual Cell [72] uses PDEs to model and simulate the spatial aspects of cells and provides tools for sensitivity analysis and an interface to external kinetic and pathway databases. The Systems Biology Workbench [73] is a software framework permitting the combination of a wide variety of heterogeneous modelling tools. It incorporates Jarnac, a language for manipulating cellular system models and JDesigner, an open source visual design tool for building signalling, metabolic and gene networks.

E-CELL [74] is a software platform for modelling, simulation and analysis of complex, heterogeneous and multiscale systems like the cell. It provides a unified and object-oriented framework for integrative simulation of cellular process on several levels, and an advanced graphical interface allowing users to observe the cell's state and conduct virtual experiments *in silico*.

STOCHSIM [75] is dedicated to stochastic simulation, where molecules are treated as individual objects that react according probabilities computed using user-defined parameters.

All the above tools, including MATLAB, now support SBML [76], an XML-based model description language, designed with the aim of achieving compatibility among simulation packages. SBML accounts for dynamic features of pathways and allows quantitative data to be stored. Proposals for extensions to the current version of SBML incorporate the ability to include display and layout information in a model. SBML is also the basis of the Systems Biology Workbench (SBW), an effort to build a common infrastructure to develop, share and evaluate models effectively.

INA (Integrated Net Analyser) [77] and PEP (Programming Environment based on Petri nets) [78] are general-purpose tool packages supporting the analysis of traditional, coloured and time Petri nets. Both incorporate features like transition invariants and structural analysis. The former also supports place invariants and performance analysis, such as simulation with time and Markov chains, while it fails to edit and represent nets graphically. TimeNET (Timed Net Evaluation Tool) [79] permits the user to perform modelling with continuous as well as discrete time. An overview of existing tools for Petri nets is available from the Petri nets Tools Database [80].

SPiM [81] is a modelling and simulation tool based on the stochastic π-calculus that can be used to simulate models of biochemical systems, and uses the Gillespie algorithm [82] as the basis of its computational engine. BIOCHAM (Biochemical Abstract Machine) [83] is a programming environment for modelling biochemical systems, making simulations and querying models in temporal logic.

Finally, a number of tools are specifically concerned with model checking and validation. BioSigNet [84] is a knowledge-based reasoning system for signalling networks. It allows the user to generate and test hypotheses about pathways based on experimental observations and biochemical knowledge. The PRISM probabilistic model checker [85] and the PEPA Workbench [86] are generic tools designed for the modelling and analysis of probabilistic systems and are used to simulate biochemical models specified using stochastic process algebra. These tools utilise temporal logic for the analysis of the models.

CONCLUSIONS

We have presented a variety of computational techniques for modelling, simulation and analysis of signalling networks exemplified by modelling approaches from three biological fields. Table 2 summarises the main properties of the presented methodologies.

The classical, differential equation approach in the form of ODEs and PDEs is currently the most prevalent. However, there are several reasons why alternative techniques may be of increased interest in the future.

First of all, there is a need for both discrete and stochastic approaches, because the validity of continuous representation of biochemical networks is disputed. Modellers will need to address this issue by transferring their continuous models to an

Table 2: Comparison of methods for description, simulation and analysis of biochemical systems

Method	Depiction/model	Simulation	Analysis
Pathway chart	Biochemical reactions/no formal model	None	None
Ordinary differential equations (ODEs)	Mathematical equations	Deterministic numerical solution: time-discretisation	Symbolic and numerical analysis (e.g. bifurcation analysis)
Partial differential equations (PDEs)	Mathematical equations	Deterministic numerical solution: space-time-discretisation	Symbolic and numerical analysis
Stochastic differential equations	Mathematical equations with random terms	Stochastic numerical simulation: time-discretisation	Symbolic and numeric analysis
Discrete Petri nets	Graph, labelled transition system	Animation via tokens	Qualitative: structural analysis and temporal logic
Continuous Petri nets	Graph, labelled transition system, rate information	Via ODEs	See ODEs
SBML-based graphical formalisms	Graph, rate information	Various (e.g. ODEs, Gillespie)	Various, tool-dependent
Stochastic π-calculus	Algebraic formulae	Stochastic numerical simulation via Gillespie algorithm	None
Process algebra (PEPA)	Algebraic terms, stochastic temporal logic	Stochastic numerical simulation via Gillespie algorithm; simulation from logical analysis	Quantitative, via temporal logic over models
Cellular automata	Spatiotemporal explicit model based on state and simple rules	Step-wise application of rules to discrete space state	Analysis of emergent properties
Agents	Spatiotemporal explicit model based on autonomous intelligent object behaviour	Representation of object(s) behaviour determined by history of encounters with environment	Analysis of emergent properties

environment that takes into account low protein copy numbers and noise.

Secondly, the characterisation of behaviour emerging from molecular interactions not only includes basic dynamic phenomena, such as limit cycle oscillations and multistability. It is commonly accepted that macroscopic cellular and intercellular events emerge from basic molecular processes, but due to the enormous complexity at the molecular level sophisticated computational methods, such as hierarchical models, are needed to bridge the gap. In fact, the most important challenges in bioinformatics and systems biology are concerned with bridging the gap between different levels of cellular organisation.

Model analysis is an important activity, which is often neglected by modelling tools. Frequently, theoretical models are only amenable to biological validation of an indirect nature, due to the lack of the experimental means to observe the behaviour of specific entities within the model. Sophisticated analysis steps are thus required to better relate predicted behaviour and observations.

The field of systems biology has attracted a great number of researchers from biological and computational backgrounds. The contribution of the latter is a new range of modelling techniques that has not been previously applied to biological signalling. New methodologies, such as intuitive, graphical modelling formalisms aim to bring the two scientific fields closer together.

Key Points

- Signalling networks can be modelled by a variety of continuous, stochastic and discrete techniques. The traditional modelling approach is based on differential equations, but several alternative modelling techniques have more recently been adapted from approaches in computing science and mathematics.
- The various modelling techniques have distinct advantages and disadvantages in terms of comprehensibility of the models, the power of their descriptions, and the kinds of simulation and analysis that can be performed.
- There are a large number of computational systems that are available to support biochemical system model construction, simulation and analysis.
- We describe several of these modelling approaches and show how they have been applied to signalling networks ranging from intracellular (receptor signalling and cell-cycle regulation) to intercellular (wound healing) signalling.

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