Abstract
We discuss and review different ways to map cellular components and their temporal interaction with other such components to different non-spatially explicit mathematical models. The essential choices made in the literature are between discrete and continuous state spaces, between rule and event-based state updates and between deterministic and stochastic series of such updates. The temporal modelling of cellular regulatory networks (dynamic network theory) is compared with static network approaches in two first introductory sections on general network modelling. We concentrate next on deterministic rate-based dynamic regulatory networks and their derivation. In the derivation, we include methods from multiscale analysis and also look at structured large particles, here called macromolecular machines. It is clear that mass-action systems and their derivatives, i.e. networks based on enzyme kinetics, play the most dominant role in the literature. The tools to analyse cellular reaction networks are without doubt most complete for mass-action systems. We devote a long section at the end of the review to make a comprehensive review of related tools and mathematical methods. The emphasis is to show how cellular reaction networks can be analysed with the help of different associated graphs and the dissection into modules, i.e. sub-networks.

Keywords: cellular reaction systems; modelling; graph theory; master equations; continuum limit; qualitative behaviour

SYSTEM IDENTIFICATION:
MAPPING CELLULAR REACTION SYSTEMS TO GRAPHS
We start this review with two small sections (this section and ‘static and dynamic network concepts’ section) on general network modelling and system identification. This is necessary because network theory is rapidly expanding, and we have to fix some ideas and concepts that are sometimes called differently or stay undefined in some areas. We then start the discussion of dynamic cellular regulatory networks in ‘stochastic versus deterministic reaction networks’ section.

As most ‘camps’ inside the philosophy of science would agree, experimental data can only be understood in the presence of concepts that allow their interpretation in a context. These concepts themselves are often called ‘a priori knowledge’ since Kant introduced this notion into the philosophical discussion. But they are surely in praxis just something that might have been established on the basis of experimental evidence before the actual data were taken, or these concepts are so basic that they simply establish a rational choice between several logical possibilities. Such a concept in the context of cellular reaction systems is surely the graph. The graph is a fundamental mathematical object consisting of two sets, \( G = (V, E) \), the vertex and the edge sets. In our context, the vertices will represent the system components and the edges the interaction between them. In the following we describe how to systematically introduce graphs as tools to translate a time-dependent cell biology situation where space can be neglected into an either stochastic or deterministic dynamical system. At the end of the process, there appears a mathematical model that describes how the system’s states (attached in an abstraction process to several components like molecules, membranes, etc. and linked together inside a network) change in time. Later we will also go the reverse
way, and attach graphs to already established types of reaction networks in order to analyse their qualitative behaviour.

**Interacting components**

The natural situation to analyse the state of a cell, still the most central entity in biology, is the first to identify components that, due to their presence, establish the basis of cellular function, and secondly to study the components’ interaction and transformations that will cause the cell to change and possibly adapt to signals stemming from outside. Even the first step in this blueprint concept is presently still cumbersome, in some cases even impossible. Why should this be the case? Typical system components for cellular systems, frequently used in bioinformatics and systems biology, are molecular species, even different variants or conformations of the same molecule, molecular complexes or slightly more abstract entities such as genes. This list already shows that cellular system components can be chosen as being embedded into and acting on different spatial and temporal scales. This will make it often necessary to introduce a mathematical multiscale analysis in order to understand how system components influence or interact with each other. We will come back to such multiscale ideas later in this review.

If, for example, a specific signalling pathway is under investigation, most often many of the different molecules involved are unknown, or there is a high probability that some yet unknown molecules will have a role in this pathway, at least under slightly changed experimental conditions. It is therefore no wonder that different ways of experimental component detection, typically high-throughput methods and respective tools from bioinformatics, are presently core techniques to establish biological knowledge. These ‘system component detection’ methods are essential for biological research, and there is much overlap with the methods discussed in this review. Possibilities include the detection of missing components by measured dynamical anomalies, very much analogous to the detection of new planets disturbing the orbits of known ones. But typically biological system component detection by large-scale screening is for good reasons a static (non-temporal) procedure. The yet unclassified newly detected objects are clustered into groups according to some detected function and its disruption or disturbance, for example realized in mutagenesis experiments [1, 2]. Sometimes detection and (non-temporal) functional characterization of components can be automatized as described in King et al. [3]. In the following we will not discuss such methods, as our goal is to focus on dynamical models predicting the temporally varying states of previously identified cellular components. In other words we will assume that the system components are already well characterized and proceed to analyse their temporal state changes.

Bioinformatics has traditionally its biggest successes in the component detection area. One just needs to think about the detection of genes inside DNA strings. All such algorithms are based on discrete data, but as we will explore reaction systems there appear additional challenges in the sense that the use of a combination of discrete and continuous data objects is needed.

**Interaction**

After the choice of the system components such as (a certain family of) proteins, the type of interaction between them needs to be established. It is very important to note that both the choice of system components as well as the type of interaction will depend on the experimental set up. In many, if not most cases, the experimental design and technology will be available before any modelling and analysis, and therefore before the system identification step. In general, theory and experiment have to adapt to each other, and this will be done in a recursive way while stepping through a guiding diagram like Figure 1. In a first step inside the identification of interaction a fundamental test has to performed: Is the interaction symmetric or non-symmetric? What we describe here is much the same as to translate a typical cell biology diagram into a mathematical model. We first design a graph by representing the system components as vertices and find their relations (interactions), which is already describing the system mathematically. The interaction is symmetric if there exists a binary relation between the components. It is non-symmetric if a component is influencing another component, but not necessarily the other way round. The most obvious symmetric relationship in this context is ‘component A binds to component B’, which is a common property of (bio-)molecules. This leads to a description of the system as an undirected graph. An example for a non-symmetric relation is ‘component A influences B’, or ‘component A modifies component B’. An example is post-translational modification or when component A produces the substrate of component B in a metabolic context. On a more
abstract level, this directed relation holds when a gene A is ‘upstream’ of gene B. The system now is represented as a directed graph with arrows as edges. Once a static graph topology has been identified, we can investigate its properties in terms of network connectivity, looking at scale-free or exponential network architectures (see the classical papers [7, 8]). In this review, however, we will go a step further and as stated before will be primarily interested in temporal dynamics defined on networks. It will turn out that the (static) network architecture or topology is important, but that it cannot for good reasons determine all the possible qualitative temporal behaviour of the network. This review focuses on some situations where such a close link between architecture (meaning the set of possible interactions between system components) and the system’s temporal behaviour can indeed be made.

**STATIC AND DYNAMIC NETWORK CONCEPTS**

The next step is, therefore, to introduce a (temporal) process, i.e. modelling a changing link structure over time (varying network topology) and/or the states attached to the nodes representing a quantity or quality measured in some experimental set up. This step, i.e. introducing a (stochastic) process acting on a newly introduced state space structure, will be called process identification in the following, see the next layer in Figure 1. If—based on experimental evidence—the network structure needs to vary temporarily, and/or the number of components (this means either the set E or in addition the set V of the graph \( G = G(V,E) \) is changing over time) is not fixed, there need to be rules included into the model how this process is uniquely defined. We will discuss this case briefly with the help of protein–protein networks to explain this model possibility inside a cell biology context. But even if we go back to a fixed network topology (edge distribution) with a given fixed number of components (nodes or vertices), in most experimental situations a state space has to be attached to the components for conceptual closure of the problem. This is illustrated in Figure 2.

Introducing such state spaces to the nodes will then also make it necessary to define a temporal process on the network, which updates or changes the state of the nodes in time. This abstract description is best understood in the context of molecular reaction systems. In this case, the network components are most often associated with the molecular species themselves, and there are experimental data available showing the changing number of these molecules in a given volume of observation. This will be indeed our generic case. Each node representing a molecular species will have a non-negative number (either an integer or a real number) attached, depending on whether we describe molecular number or concentration. Of course also other, for example, finite discrete state spaces are possible. In the most extreme case, only binary states occur that might describe whether a gene is switched on or off. Such Boolean networks have been introduced to describe genetic interaction by Kauffman [16].

**Fluxes**

Both graph theory and articles on biological regulatory networks often speak of fluxes or flows running through the network (the flow defined on the graph is realized as weights attached to the edges). A most famous related concept is the Kirchhoff laws of electric circuit theory, making a conservation statement for fluxes at nodes that are branching points. We will look at fluxes again in ‘Enzyme Kinetics’ Section. Here we define the flux along a given edge of the network as the weight attached to it. The change of

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**Figure 1:** The different layers in the process of identifying cellular reaction systems, mapping to a mathematical model and comparison with experimental data.
this weight will be described by the dynamical system defined on the network.

**Static network concepts as special cases**

We have already introduced static graph descriptions like trees produced by a clustering algorithm in the context of component identification. Evolutionary trees of descendence would also fall into this category. In this new context of networks equipped with state spaces and processes, the static network becomes a special case of the dynamic network. The processes and so-called initial conditions (initial conditions are the states of the network at the beginning of the experimental observation) can be such that the states will be in a dynamic equilibrium. Often this is also related to the concept of homoeostasis. To analyse equilibria is often the best way of entry to understand dynamic networks. It is therefore no wonder equilibrium concepts play a dominant role in the bioinformatics literature. Once equilibria are introduced for dynamic networks, a natural mathematical question arising is about their stability, i.e. whether any initial conditions ‘close’ to the equilibrium will converge in time back towards this equilibrium. In other words, the static networks are now embedded inside a dynamical context, and such analysis of the system will be done with the help of mathematical methods we associate with a *temporal layer* of the problem (Figure 1).

**Static and evolving network topologies, protein–protein networks**

Very often in cell biology and bioinformatics, the analysis is restricted to one type of molecule, for example, the important class of proteins. As there are binding assays for proteins, a binary relation can be defined as ‘Protein A is binding to protein B’. A complete map of proteins identified in a cell can be mapped to an undirected graph \( G = (V,E) \), with \( V \) being the set of proteins, and \( E \) being the set of links between proteins. Clearly, such a link always exist whenever two proteins can bind to each other. Again the situation is static, i.e. the proteins bind to each other whenever they are present in the system, which here is assumed to be at ‘all times’. Knowing the protein binding property implies that we know the experimental nature of the interaction in the graph, something which was not necessary for the previously introduced combinatorial studies (component analysis) we mentioned. A classic paper on protein–protein networks is Jcong *et al.* [4]. Due to refined mass spectroscopy, the whole set and even the number of proteins in a cell can be measured [5]. Such numbers can be mapped to weights attached to the vertices of the protein–protein network, at any given moment in time (Figure 2). If protein evolution is modelled, the network topology must be modelled to change on the respective evolutionary time scale (see, for example, [6]).

**STOCHASTIC VERSUS DETERMINISTIC REACTION NETWORKS**

In the rest of this article we restrict ourselves to systems with a fixed number of components and non-varying interactions (links) between them. Most scientists would agree that nature is inherently stochastic at ‘microscopic’ scales, for example (but not only) due to quantum mechanistic effects. This is indeed the philosophy we will adopt. The deterministic types of reaction systems are much easier to analyse, and in many cases they are more intuitive. Perhaps this is the reason why the most dynamic network models in cell biology use such deterministic reaction systems. But on the other side, there
are many situations where the understanding of stochastic effects is essential. The determinisitic systems should best be introduced as averages of the stochastic systems and/or after particle numbers can be assumed to be sufficiently large, such that stochastic fluctuations due to small numbers of particles/interactions can be neglected. Let us first assume that the entities/components of the systems we like to describe can be characterized by a set of molecular species $S_i$, say $s$ such species. The molecular species themselves are assumed to have no structure. Two different conformations of the same molecule would therefore have to be modelled as two different species (this can be problematic, as in principle both numbers of the species would need to be guaranteed to be the same inside the evolution for all times). For most metabolic systems such an assumption is very reasonable. We write $S = \{S_1, \ldots, S_s\}$. At this stage we can introduce the directed species graph $G_s$, discussed as a modelling tool in Figure 2, where the species are the vertex set $V$, and the edges/arrows contained in $E$ have to be determined from an investigation how the number or concentration of a species $S_i$ will influence the number/concentration of species $S_j$. The species graph $G_S$ will later reappear as the so-called interaction graph $G_I$, but then will be introduced as a graph that is assigned to an already specified reaction network. This difference is very important to note; in the first case we use the graph as a first model sketch, in the second case we have a working model and derive the graphs as associated mathematical objects needed for model analysis.

The different molecular species can now be combined in different ways. Each single reaction can change the number and composition of the different molecules in the system. In this case, a single reaction will be the basic event, and we need an event-driven stochastic process describing the evolution of the system. Next we assume there are $r$ possible transformations of molecules into each other, also including the possibility that molecules leave or enter the system. We also assume that only integer combinations of molecular species can be formed. In this case we can write each event/reaction $r_j$ in the form

$$a_{ij}S_1 + \cdots + a_{is}S_s \rightarrow \beta_{1j}S_1 + \cdots + \beta_{sj}S_s,$$

(1)

with $j = 1, \ldots, r$. The integer coefficients $a_{ij}$ and $\beta_{ij}$ represent the number of $S_i$ molecules participating in $j$-th reaction at reactant and product stages, respectively. The net amount of species $S_i$ produced or consumed by the reaction is named as the stoichiometric coefficient and is defined by $n_{ij} = \beta_{ij} - a_{ij}$. The integer coefficients $n_{ij}$ can be assembled inside the so-called stoichiometric $(s \times r)$ matrix $N$. In the chemical literature [10], the linear combinations of species to the left and right of the reaction vector are called complexes. They can be interpreted as a higher level of system components building a new graph with the complexes as nodes. We can define a directed link between these nodes whenever a reaction between them is defined. This directed graph $G$ will be called the reaction graph $G_R$. Clearly the number $c$ of nodes of $G_R$ is smaller or equal to $2r$. With respect to a complex $C_i$, let $c_{ij}$, $1 \leq i \leq s$ (index is running through all species), $1 \leq j \leq c$ (index is running through all complexes), be their integer coefficients. This means we do not differentiate between the reactant and product aspect (the $\alpha$ and $\beta$ notation for reactions above, respectively), but only look inside which complexes these species dependent integer coefficients appear. The coefficients are then assembled inside a $(s \times c)$ matrix $V$.

The master equation

A natural way to describe the changing number of molecules of each species (i.e. to assign a law of evolution to the system’s state and which components are attached to each node and representing a species’ number occurring in the reaction volume) $S_i$ is by introducing a continuous-time Markov jump process for each reaction. In the following, we use the notation and reasoning given in Gadgil et al. [9]. Indeed we should consider Equation (2) below as the most basic way to introduce a dynamical behaviour for molecules, in case their spatial position can be neglected. As always when space can be neglected, we should consider a given homogeneous reaction volume where we just need to count the appearance or disappearance of certain molecules by recombination, or be leaving or entering the system. Let therefore $N_i(t)$ be a random variable that represents the number of molecules of species $S_i$ at time $t$, and let $N$ denote the vector of $N_S$. Further, let $P(n,t)$ be the joint probability that $N_i(t) = n_i$ i.e. $N_1 = n_1$, $N_2 = n_2$, $\ldots$, $N_s = n_s$. Clearly, the state of the system at any time is now a point in $\mathbb{Z}_+^r$, where $\mathbb{Z}_+$ is the set of non-negative integers.
Formally the master equation that governs the evolution of \( P \) is then
\[
\frac{d}{dt} P(n, t) = \sum_{m \in S(n)} \mathcal{R}(m, n) \cdot P(m, t) - \sum_{m \in T(n)} \mathcal{R}(n, m) \cdot P(n, t),
\]
(2)
where \( \mathcal{R}(m, n) \) is the probability per unit time of a transition from state \( m \) to state \( n \), \( \mathcal{R}(n, m) \) is the probability per unit time of a transition from state \( n \) to state \( m \), \( S(n) \) is the set of all states that can terminate at \( n \) after one reaction step and \( T(n) \) is the set of all states reachable from \( n \) in one step of the feasible reactions. As pointed out in Gadgil et al. [9], the notation is meant to suggest the source and target states at \( n \); one could also call \( S(n) \) the predecessors of state \( n \) and \( T(n) \) the successors of state \( n \). The predecessor states must be non-negative for production reactions and positive for conversion, degradation and catalytic reactions. Similar bounds on the target states are naturally enforced by zero rates of reaction when the reactants are absent. The sets \( S(n) \) and \( T(n) \) are easily determined using the reaction graph \( G_R \). Let \( C = \{ C_1, \ldots, C_r \} \) be the set of complexes, i.e. the nodes of \( G_R \). Let \( r_l \in E(G_R) \) (the edge set of \( G_R \)), \( 1 \leq l \leq r \), whenever there exist two complexes with a reaction defined between them. Define the incidence matrix \( \mathcal{I} \) by [this is a matrix with \( c \) rows (number of complexes) and \( r \) columns (number of reactions)]
\[
\mathcal{I}_{il} = \begin{cases} +1 & \text{if } r_l \text{ is incident at } C_i \text{ and is directed toward it}, \\ -1 & \text{if } r_l \text{ is incident at } C_i \text{ and is directed away from it}, \\ 0 & \text{otherwise}. \end{cases}
\]
The sets \( S(n) \) and \( T(n) \) can now be determined using the \( G_R \) graph structure. It follows from the definition of \( \mathcal{V} \) and \( \mathcal{I} \) that the \( l \)-th reaction \( r_l \) between say \( C_i \rightarrow C_j \) induces a change \( \Delta n^{(l)} = \mathcal{V} \mathcal{I}_{(l)} \) in the number of molecules of all species after one reaction event, where the subscript denotes the \( l \)-th column of \( \mathcal{I} \). Therefore the state \( m = n - \mathcal{V} \mathcal{I}_{(l)} \) is a source or predecessor to \( n \) under one step of the \( l \)-th reaction. Similarly, states of the form \( m = n + \mathcal{V} \mathcal{I}_{(l)} \) are reachable from \( n \) in one step of the \( l \)-th reaction. With this insight we can now sum over reactions instead of sources and targets to get
\[
\frac{d}{dt} P(n, t) = \sum_{l=1}^{r} \mathcal{R}(n - \mathcal{V} \mathcal{I}_{(l)}) \cdot P(n - \mathcal{V} \mathcal{I}_{(l)}, t) - \sum_{l=1}^{r} \mathcal{R}(n) \cdot P(n, t),
\]
(3)
with \( \mathcal{R}(n) \) being the probability per unit of time that a reaction event \( n \) is taking place. The number of different species is \( \mathcal{S} \) before the event is given by the vector \( n \). The master equation is now conforming to the chemical complex structure and describes the reaction network on the microscopic level. It can be used to investigate how noise is occurring in the system when particle numbers are small, and how this noise depends on the network structure. We can use the master equation for direct simulation of the reaction network.

**Rule-based networks**

It is instructive to introduce (Boolean networks are explained in 'Boolean networks' Section. They can be interpreted as a rule-based framework with a simple binary state space. We give no literature review here, which would be impossible due to the variety of approaches. The remarks of this section apply, however, to all rule-based systems that can be interpreted to have a network structure) rule-based frameworks in this context, especially those which have either been inspired by or themselves been applied to cellular reaction problems. Indeed the single reaction step (1) can be interpreted as a rule. A reaction step is nothing else than a rule or description specifying which species of molecules can be transformed into each other during a single reaction event. Generally rules are defined on an alphabet \( A \) mapping each network node’s state \( a \in A \) (before the event) to another element \( a' \) of the alphabet (the alphabet in this context of rule-based systems is replacing or equivalent to the state space attached to each node. The alphabet is the better concept here, as rules are often expressed in terms of different conditional commands using other state concepts than numbers) (after the event). The alphabet can be either finite or infinite. An example of a framework using a finite alphabet is Boolean network, where the nodes can be either at state 0 or 1, i.e. the alphabet is \( \{0, 1\} \). For the reaction system, the nodes represent (for example) the different molecular species, and the alphabet is the integers counting how many molecules of a given species are in the system. In this interpretation, the total system state is clearly a vector of length \( |E| \), the number of vertices or nodes of the graph \( G = (V, E) \) associated with rule-based model. Each event will change or update the states of a subset of the node set, generally not all the nodes simultaneously. Typically the network architecture is exactly defined such that if an
event effects a number of nodes, these are the nodes or vertices that are connected with each other, i.e. there is an edge in the graph between any two vertices in this vertex subset whose states are updated. This gives a useful new insight to the nature of the network topology in this dynamic setting: the network architecture defines or tells us how local events—if they happen to take place—can effect which other states of neighbouring or linked nodes.

Rule-based systems have not necessarily a notion of time-scale, i.e. they are not necessarily based on a rate defining the numbers of events per some unit of time. Note that in Equation (3) a rate is defined, in terms of transition probabilities per unit of time. This should be compared with the simplest rule-based system, the Turing machine acting on an infinite band, i.e. the ‘linear infinite graph with the nodes’ alphabet being {0, 1} again. The Turing machine will run, i.e. evaluate its simple rules until the stopping criteria is met. It is important that this criteria is hit in a finite number of steps, it is, however, not important at which rate these steps (events) occur.

Taking the reaction arrow as an example of a rule, it is clear that every rule-based system can be transformed into a rate-based system by specifying how often the rule is applied to which subset of nodes (and therefore states) per unit of time. In this review, we only consider rate-based dynamic networks, because the underlying time series stemming from experimental data (and used to test the predictive power of the models) will necessarily introduce time scales.

The Gillespie formulation of stochastic mass-action kinetics

The condition on the state of the reaction system, described by the vector \(n\) with integer entries, is extremely important. As Gillespie and others [11, 12] have pointed out combinatorial effects causing ‘noise’ can be significant when the number of molecules is small. In this case the transition probability \(R_i\) in Equation (3) cannot be separated into a constant rate times a function of particle numbers or concentrations only. The latter is exactly what we do later when introducing the deterministic mass-action kinetics on a ‘macroscopic’ temporal scale in the next section. In mass-action kinetics, the basic assumption is that the probability to react per unit of time is proportional to the number of different molecules in the substrate complex (raised to the power of the substrate stoichiometric coefficient for non-mono molecular reactions) located in the reaction volume. If we include combinatorial effects inside mass-action kinetics defined on the microscopic scale, assume that \(r_i\) is the reaction \(C_i \rightarrow C_j\), and use Gillespie’s original notation, \(R_i\) can be further specified and becomes

\[ R_i = c_i h_{i|j}(n). \]

Here \(c_i\) is the probability per unit time that the molecular species in the \(i\)-th complex react, \(i|j\) denotes the reactant (or substrate) complex for the \(l\)-th reaction, and \(h_{i|j}(n)\) is the number of independent combinations of the molecular components in this complex. The term \(c_i\) can be written as

\[ c_i = \frac{k_i}{(AV)\sum_v v_{m;v}n^{-1}}. \]

Here \(v_{m;v}\) is the \((m, v)\)-th entry of the matrix \(V\), and \(A\) is Avogadro’s constant, \(V\) the reaction volume. The positive constant \(k_i\) is the, ‘mean’ reaction rate that will reappear in the definition of deterministic mass-action kinetics in ‘Mass-action Kinetics’ Section. Now \(h_{i|j}(n)\) is defined as

\[ h_{i|j}(n) = \prod_{m=1}^i \left( \frac{n_m}{v_{m;i}} \right), \]

with the usual convention that \(\binom{n}{0} = 1\). By looking at the mathematical structure of \(h_{i|j}(n)\), we see now how small numbers of some species can significantly reduce the reaction probability of a given reaction. The Gillespie method has been later implemented numerically and optimized in a series of papers [13–15] and others.

DETERMINISTIC REACTION SYSTEMS

Boolean networks

We discuss Boolean networks again as both a first simple example of a deterministic rule-based system and, at the same time, to investigate how assumptions made during the modelling process might reduce the dimension and nature of the different weights we have conceptually introduced in Figure 2. The use of such logical networks in biology to mimic regulatory networks goes back to Kauffman [16]. The reasoning was given for genetic regulatory networks, but could also be extended to other types of biological regulatory networks. In these networks, the state space of the nodes, representing genes, is just binary, representing ‘on’
and ‘off’. In modeling terms this means we are not interested in the precise rate of transcription events, but assume that the gene is regularly transcribed from time to time, or never. As outlined in ‘Rule-based networks’ Section, physical time becomes unimportant, because we are only interested in whether (after a series of events describing the mutual regulation among the binary genes) the dynamical system operating on these discrete state spaces locks into one of the attractors of the system. It is still an open question when and how such extremely simplifying assumptions can be justified in biology. One typical mathematically related problem is: How can we define limits that in case the rate-based dynamical system is ‘extremely discretized’, then (some of) its properties after the discretization remain invariant? Such reasoning could deliver strong justifications for the use of Boolean networks in biology. Besides such questions, there are indeed many situations not related to ‘regulation’ when physical time is indeed unimportant. Boolean networks should, for example, prove useful in experimental designs when different combinations of (discretely distinct) treatments (like different genetic knock-outs) and their discrete responses (discrete classification of the phenotype of the knock-out variants) need a logical analysis.

Mass-action kinetics
As we have seen in ‘The Gillespie formulation of stochastic mass-action kinetics’ Section, we need to make assumptions as to how reaction rates, i.e. molecular collision events per time unit, are determined from the number of molecules occurring in a reaction volume. In order to be able to write down the equations in a time-invariant (autonomous) framework many physical conditions need to be constant, like temperature and pressure. Furthermore, the particles need to be able to make a random walk without drift, also implying they are not getting stuck somewhere in the reaction volume. We will not look at ‘crowding effects’ that could cause severe alterations of these assumptions, although they are very relevant in the area of cell biology. In the following we introduce heuristically deterministic kinetics. The reaction velocity will be taken to be proportional to species concentration (this is of course only true for the monomolecular reactions. If several molecules are needed to form a new molecule, then we assume that this term becomes the concentration raised to the power of reactant molecules needed to form one new product molecule). If we now assume that molecule numbers are large for all species, and the expected concentration of each molecular species has no fluctuations, then the reaction system is indeed governed by a deterministic law of motion called the mass-action kinetics. Note that we have not presented any rigorous mathematical derivation of this type of kinetics from the master equation introduced earlier. What can be found in Gadgil et al. [9] is the computation of the expectation and variance for monomolecular reactions on a microscopic level. In addition, we would need to show that the variance of the Markov jump process does indeed decline to 0 for arbitrary reactions if particle numbers of each species become infinite. A transition from discrete to continuous state spaces needs to be introduced, a so-called continuum limit. We discuss this approach in ‘The macroscopic equation and the combined adiabatic and continuum limits. The average dynamics’ section. Let \( x_i \) be the concentration of molecules of species \( S_i \) in the reaction volume, and \( x = (x_1, \ldots, x_n)^T \) be the vector of all species’ concentrations. With all these assumptions the reaction velocity will be given by

\[
\nu_j(x, k_j) = k_j \prod_{i=1}^n x_i^{\kappa_{ij}},
\]

where \( \kappa_{ij} \) is the molecularity of the species \( S_i \) in the \( j \)-th reaction, the \( k_j > 0 \) are the kinetic constants describing reaction events per time. In strict mass-action kinetics, the kinetic exponent \( \kappa_{ij} \) reduces to being simply \( \alpha_{ij} \). Kinetic exponents are arranged in a kinetic matrix, denoted by \( \kappa \). Using the vector notation \( \nu(x, k) = (\nu_1(x, k_1), \ldots, \nu_n(x, k_n))^T \) the time evolution of the species concentrations is then described by the following initial value problem,

\[
\dot{x} = N\nu(x, k), \quad x(0) \geq 0.
\]
attached to all chemical species in the substrate complex of a given reaction. The reaction diagram information can be incorporated into two graphs, an observation made by Karin Gatermann [18], see also the thesis [19]. The first one is a weighted (the weights are the kinetic constants $k_j$) directed graph representing the reactions (this is the reaction graph $G_R$), and a weighted (the weights are the stoichiometric constants) bipartite undirected graph to describe the complexes. We draw these two graphs for a small reaction system in Figure 3.

For the directed reaction graph $G_R$ two incidence matrices are of importance. The first one has been explained in ‘The master equation’ section, the matrix $I$ contains the information whether the complex is the initial (entry $-1$) or the end vertex of an edge (entry 1). This entry distinguishes reactant complexes from product complexes. The second one, $I_k$, contains non-zero entries only for initial vertices, i.e. for reactant complexes. The entries are the weights of the corresponding edge, which is the rate constant $k_j$. For the bipartite undirected graph, called the complex graph $G_C$, we consider the incidence matrix defined by

$$
\begin{pmatrix}
0 & Y \\
Y^t & 0
\end{pmatrix}.
$$

The entry $y_{ij}$ is the weight of the edge containing the stoichiometric coefficients (integers). The reaction scheme (4) can now be rewritten in the form

$$
\dot{x} = YI_k\Psi(x),
$$

with $\mathcal{N} = Y\mathcal{I}$ and $v = I_k\Psi(x)$. The $\Psi$ is a vector of monomials in terms of the species concentrations. The final result is that the non-linearities of the dynamical system defining the mass-action reaction scheme can be defined and investigated in terms of incidence and adjacencies matrices, and a vector of monomials. Obviously something similar could be done on the level of the master Equation (2), i.e. on the microscopic scale.

The mass-action systems (including the stochastic Gillespie formulation) need to be solved numerically. This also holds for derived equations, like enzyme kinetics which we discuss in ‘Enzyme Kinetics’ Section below. There have been different conventions to facilitate the implementation and simulation process, like SBML (Systems Biology Markup Language) with which a machine-readable description of the reaction network can be given. Packages like COPASI [20] can read SBML and produce time-series by integrating the system of ODEs. Additional functionality for users include parameter estimation techniques, and more.

**S-systems**

Savageau [21, 22] proposed the power law, or ‘synergistic-system’ (S-system) approximation as an alternative approach for modelling reactions following non-ideal kinetics, such as those occurring under molecular crowding within cells [23]. In contrast to Equation (4), the power law approximation assumes that the rate of change of a state variable is equal to the difference of two products of variables raised to non-integer powers:

$$
\dot{x}_i = \alpha_i \prod_{j=1}^s x_j^{p_{ij}} - \beta_i \prod_{j=1}^s x_j^{q_{ij}}, \text{ for } i = 1, \ldots, s.
$$

The first term of the RHS represents the net production, and the second term the net removal rate for the $i$-th species. The S-system is our first encounter and an early idea of approximating the dynamics of a reaction network, an idea frequently encountered.
in the systems biology literature. The S-system has, for example, been motivated by linearizing enzyme kinetics (see ‘The enzyme kinetics’ section) rate expressions in terms of the concentrations [26, 27]. It is important to note the difference of philosophy adopted in this review — the derivation of the dynamics from microscopic rules — and the approximation concept. Along the lines of remarks using Boolean networks in biology (‘Boolean network’ Section), such kind of approximations are inherently dangerous when used outside a context where their approximation properties have been properly established. As the citations [26, 27] show a successful application of such an approximation might succeed in some contexts, but will fail in others. Problems arise when the approximating equations like S-systems cannot cover some qualitative behaviour that can be observed in experiments. Similar remarks must necessarily hold for a variety of parametric approximations of enzyme kinetics and genetic feedbacks. We give this cautious remark without citations.

**Lotka–Volterra systems**

Lotka–Volterra (LV) systems have their origins in food web research, so describe primarily an ecological situation. We cite them here for two reasons: the first reason is that they form a subset of mass-action reaction networks because their right-hand side (RHS) is polynomial, so can be back-translated into reaction schemes. The so-called replicator equations (hypercycle), introduced by Manfred Eigen and Peter Schuster [24] and intimately related to research on the origins of life, can in turn be transformed into LV systems [25]. The second reason to introduce LV systems is that much theory on the stability and permanence of regulatory dynamical networks has its origin in ecology, and was based on LV systems [25].

Enzyme kinetics

We can only briefly review enzyme kinetics as it is a huge field with many applications. All textbooks covering theoretical biochemistry and cellular biology have chapters devoted to this topic [28–31]; chapter on molecular events. An excellent book is still the one of Siegel [32]. The major idea of enzyme kinetics is to derive typical so-called functional responses from reaction networks of mass-action type. These functional responses can then be compared with measured data within the cell. For enzyme kinetics, the functional response is giving the speed at which a constant amount of enzymes (as we are determining mass-action kinetics as a basis, this amount is a concentration, i.e. we assume there are infinitely many enzyme molecules in the reaction volume. Compare this with ‘Mixed reaction systems including structured particles’ section) can convert the enzyme’s substrate. The best known and oldest example in this context is the widely used Michaelis–Menten kinetics, modelling an increasingly ‘busy’ enzyme when the substrate concentration is increasing, eventually reaching a highest conversion rate. In other words any further increase of substrate concentration would not increase this conversion rate.

Enzyme kinetics is best understood by discussing the quasi steady-state assumption (QSSA).

QSSA

In order to understand the relationship between non-linearities, which are the functional responses of enzyme kinetics, and the QSSA, we introduce so-called slow and fast variables. In the following we use the reasoning and notation in Deuflhard and Heroth [33]. Let \( f \) denote the RHS of (4), i.e. \( f(x; k) = Nv(x, k) \). We split the vector of concentrations \( x \) according to \( (y, z) = (P_x, Q_x) \), where \( P \) and \( Q \) are the projections on the dynamically ‘slow’ and ‘fast’ parts, respectively. Let \( d < s \) denote the number of slow components (\( s \) was defined as the number of species, so the length of vector \( x \)), so \( d = \text{rank}(P) \). Both projection operators may depend on the solution itself, which is an important point we adress in a moment. Upon (formally) applying the projections to the reaction system (4) and (5), the following relations hold:

\[
P \dot{x} = P f(x), \quad Q \dot{x} = Q f(x), \quad x(0) = x_0
\]

Upon imposing the QSSA, which assumes that the ‘fast’ species are in steady-state (same as equilibrium),
i.e. $Q \dot{x} = 0$, the result is a differential algebraic equation (DAE):

$$P \dot{x} = Pf(x), \quad Q(x)f(x) = 0, \quad x(0) = x_0.$$  

A necessary condition for this DAE to have a solution is that the initial value $x_0$ lies within the slow manifold defined by $M = \{ x : Qf(x) = 0 \}$. Otherwise, a projection of $x_0$ onto this manifold will be necessary to make the problem consistent. But, as already shown in [34], not every DAE in above form has a unique solution, even if the initial values are consistent with the algebraic conditions. This has led to a classification of DAE systems with respect to the so-called indext. In our case to guarantee a unique solution the index should be 1. Often the problem of fast and slow variables is written in the notation of singular perturbation theory, the reason we repeat the formulation. After the splitting into $(Px, Qx)(y, z)$, a perturbation parameter $\epsilon$ is introduced. With the help of $\epsilon$ we can write

$$\dot{y} = g(y, z), \quad y(0) = Px_0, \quad (6)$$

$$\epsilon \dot{z} = h(y, z), \quad z(0) = Qx_0. \quad (7)$$

Letting formally $\epsilon \to 0$ we get back a DAE:

$$\dot{y} = g(y, z), \quad y_0(0) = \bar{y}_0, \quad (8)$$

$$0 = h(y, z), \quad z_0(0) = \bar{z}_0. \quad (9)$$

For simplicity we assume that the initial conditions are consistent, i.e. $\{(y_0, \bar{z}_0) \in M_G = \{ (y, z) : h(y, z) = 0 \}$. Leaving away mathematical detail we can try to use the implicit function theorem to make the slow $z$ a function of the fast $y$, therefore reducing it (at this stage, we still need to discuss whether we can do this dimensional reduction for all times, or only for a restricted period of time) the dimension of the state space. In this review, we interpret this variable reduction as a reduction of the number of nodes in the regulatory network. As the respective nodes representing species are deleted in the interaction (or species) graph $G_I$ (see ‘The interaction graph’ section), also the associated edges are necessarily deleted, and with them both the weights defined on nodes and edges of the graph associated with this regulatory network. Clearly this implies that these weights are incorporated into the weights defined on the remainder of the graph. This will lead to a re-definition of the fluxes described by these weights. If those fluxes were originally described by deterministic mass-action kinetics and assuming the dimensional reduction holds for all times, this elimination process will lead to the classic non-linearities of enzyme kinetics instead of the well-known mass-action polynomials describing the network fluxes before dimensional reduction.

It should be noted that dimensional reduction, as defined above, can sometimes only be valid for certain parameter ranges and only for certain periods of time. This observation has been used in [33] to define a dynamic, i.e. temporally varying reduction inside a numerical approximation.

**The role of enzymes**

We discuss the important role of enzymes in this context. In the simplest case, which we adopt in the following, the enzymes are much fewer in number than metabolites, and the enzyme copy number can be assumed constant over time. Moreover, the enzymes fall into the category of fast-acting machines, i.e. they are modelled as fast variables. Let, therefore, $\bar{z} = E$ for all observation time, where $E$ is the vector of enzyme concentrations (now a vector of additional system parameters). Moreover, let $J$ be the new vector of fluxes defined on the reduced network. Note that by these assumptions we have $s - e$ variables in the reduced system, with $s$ being the number of species in the original mass-action system, and $e < s$ being the number of enzymes. Moreover, we have reduced the number of reactions to $r - r_e$, with $r_e$ being the number of reactions deleted by the dimensional reduction process. It is therefore the preservation of enzymes and their catalytic properties that allows to significantly simplify the regulatory cellular network under investigation.

**Metabolic control analysis**

Metabolic control analysis (MCA) originally aimed at describing the control of fluxes in metabolic pathways controlled by the different sets of enzymes catalysing the conversion of substrates into products. In the meanwhile, it has been extended to all regulatory networks based on derivations from (deterministic) mass-action kinetics, therefore including all enzyme kinetics as discussed in the previous ‘Enzyme Kinetics’ section. This implies we are considering systems of ordinary differential equations describing the continuously varying fluxes (or weights) defined on a graph $G$ (Figure 2). In mathematical terms, the starting point for MCA was to define deviations of steady-state fluxes when the total concentration of an enzyme in the system is varied. The so-called
flux-control coefficients have been introduced by Kacser and Burns [35] as

\[ C^k_j = \frac{E_k}{J_j} \frac{\partial J_j}{\partial E_k} \]

Here \( J_j \) is the \( j \)-th component of the flux vector \( J \), which is created by the \( j \)-th reaction in the (reduced) reaction network, and \( E_k \) is the \( k \)-th enzyme concentration. The quantity \( C^k_j \) is a weighted sensitivity \( \partial J_j/\partial E_k \) incorporating a ratio of enzyme concentration to flux. We have followed here the notation given in Chapter 5 of [36]. As \( E_k \) has become a parameter (not a variable), this can be easily extended to any parameter defined on the (reduced) reaction network. The respective work in this direction started with references [37 and 26] and was extended in different articles by Fell, Westerhoff, Kholodenko, Hofmeyr and others, see reference [36], and the recent review by Westerhoff [38]. In our context, it should be noted that MCA can be established both for static and dynamic networks, i.e. for steady states and transients of the system. Moreover, it was later argued to be applicable to other attractors such as limit cycles. For limit cycles the parameters used in the definition of the flux–control coefficient can become the frequency or the amplitude, making it attractive for the application to biological clocks. As there is a huge combinatorial complexity, it is useful to plot sensitivities with the aid of heat maps [39].

Viewed from a mathematical perspective, sensitivities are a local property along trajectories defined in the (reduced) state space of the system when certain system parameters are varied. We will later also look at bifurcations that do much the same reasoning. Bifurcations are best understood in the Cartesian product of state and parameter space. Here the perspective is not to assume that a certain attractor like a steady state remains stable during the analysis, but one is actively looking whether the system can be in different regimes after such parameter changes. The challenge for regulatory networks in the future is to combine both approaches, as this would guarantee that there is no regime change when the sensitivity analysis is performed.

The quantities defined in MCA like flux–control coefficients possess some intuitive properties called (generalized) summation laws. They describe the fact that the strength of control (normalized to unity) inside entire pathway fluxes [here we mean fluxes defined on the whole paths of the graph \( G_r \); note that this includes both paths without and with branching (chains)] in the regulatory network, as enabled by parameter changes, can be distributed inhomogeneously along the different control strength of the parameter defined for single fluxes (associated with a single edge).

**MIXED REACTION SYSTEMS INCLUDING STRUCTURED PARTICLES**

**Motivation**

As we have seen earlier, enzyme kinetic theory has to deal with more complex macro-molecules, proteins, which potentially can have a number of properties that affect cellular reaction mechanisms. For example, a protein can have several binding sites modifying its action and allowing for a differentiated control of the metabolic pathways in which it is acting. A similar example is genetic regulation, where a variety of molecular machines orchestrates the biogenesis of proteins according to the current needs of the cell. These machines are tightly controlled, which means they can be set into different states.

In the previous sections we have considered unstructured molecules. The system state was given either by the number of molecules or the concentration of different molecular species. This turns out not to be sufficient from a modelling perspective. For example, we like to capture different conformational states of a protein. In addition, we also like to classify molecules that are either present in large numbers or, in contrast, present only in a very small copy number. This means mathematically we like to take continuum limits, but not necessarily for all species like we did implicitly for deterministic mass-action systems. The typical answer from a mathematical point of view is to enrich the state space of the ‘microscopic’ model in a first step. This will imply that also the types of different reactions that are possible will necessarily increase. The formulations in the next section follow references [40–42].

**A reaction network extension**

We introduce different types of particles. The first type of particle will be structured by attaching discrete states to it. This type of particle in the reaction system will also be assumed not to be very abundant. For simplicity we even assume that there is only one such molecule around in the reaction volume. This will be our discrete-state macro-molecular machine, which effects we like to study in the following.
The assumption of having one such particle can be easily extended to any finite number. In contrast, the second kind of particle is assumed to be small but very abundant, like the ones we have modelled in the mass-action reactions in ‘Mass action kinetics’ section. They will follow a birth–death process on the microscopic level as before, i.e. they can also appear and leave the system very frequently. This kind of particle need not be structured, i.e. we only take note of their numbers. Next we define two sets of scales, a vector of size scales \( \delta = (\delta_1, \ldots, \delta_i) \), \( \delta_i > 0 \) attached to each species of small molecules (a typical interpretation is the average number of such particles in the system in some time interval), and a time scale \( \tau > 0 \). Also let \( \mathbb{L}_\delta \) be the following lattice:

\[
\mathbb{L}_\delta = \{ n_\delta = (n_1, \delta_1, \ldots, n_i, \delta_i) : n = (n_1, \ldots, n_i) \in \mathbb{N}^i \}.
\]

(10)

We need this discrete structure for discussing the continuum limit (which was implicitly assumed for deterministic mass action systems), the essential method of any multiscale analysis. It allows to go from a particle-based system to a continuum description, i.e. a model incorporating molecular concentrations. In our case we assume only two scales. This could be generalized. We will assume there is a Markov process describing the possible jumps between the discrete states of the molecular machine. Typical macro-molecular examples of such a machine are ion channels, translocons, DNA (see Figure 4). The new ‘microscopic’ reaction system is then defined as follows:

**Definition 1** Let the tuple \((\zeta, R, \text{and } P)\) determine a stochastic process by specifying the state \(\zeta\), a set of reactions \(R\), and a vector of probabilities \(P\) such that

(i) the state \(\zeta\) of the system is fully specified by \(n_1, \ldots, n_i\), infinite states (i.s.) of populations of small particles, and a second variable, the finite states \(\sigma\) (f.s.) attached to a structured large particle. The state \(\zeta\) of the system has the composition

\[
\zeta = (n_1, \delta_1, \ldots, n_i, \delta_i, \sigma) = (n_\delta, \sigma) \in \mathbb{L}_\delta \times \Sigma,
\]

where \(\mathbb{L}_\delta = \mathbb{N}_\delta\), \(n\) is an \(n\)-tuple of natural numbers and \(\sigma\) runs in a finite set \(\Sigma\), with \(|\Sigma| = g\) being the number of discrete states [e.g. this could correspond to the number of possible (meta-stable) conformations of a protein].

(ii) the time evolution of the stochastic process is defined via the set of reactions \(R\) is defined as follows:

(a) Reactions involving only small molecules (i.s.) represented by reactions (possibly reversible) of the form

\[
(n, \sigma) \rightarrow (n', \sigma).
\]

The operator describing these reactions in the master Equation (12) below is the transpose \(\mathcal{K}^T\) of the Markov chain generator of the process governing the transitions among the discrete states \(\sigma = 1, \ldots, g\). Here \(\delta_{\sigma \sigma'} = 1\) for \(\sigma = \sigma'\), and zero otherwise.

(b) Reactions involving only the macromolecular machine (f.s.) represented by reactions (possibly reversible) of the form

\[
(n, \sigma) \rightarrow (n, \sigma').
\]

The operator describing these reactions in the master Equation (12) below is the transpose \(\mathcal{K}^T\) of the Markov chain generator of the process governing the transitions among the discrete states \(\sigma = 1, \ldots, g\). The Markov chain is finite dimensional with a space of stationary states \(M_\mathcal{K}\) of dimension strictly less than \(g\).

(c) Reactions involving both i.s. and f.s. represented by reactions (possibly reversible) of the form

\[
(n, \sigma) \rightarrow (n', \sigma').
\]

The operator describing these reactions in the master Equation (12) below is the transpose \(\mathcal{K}^T\) of the Markov chain generator of the process governing the transitions among the discrete states \(\sigma\) which affect processes involving i.s.

(iii) each realisation of the process is valued in \(\mathbb{L}_\delta^N \times \Sigma\). The state \(\zeta\) at time \(t\) is given by the vector of probabilities

\[
P(t, n) = (P_1(t, n), \ldots, P_g(t, n)),
\]

with

\[
\sum_{n \in \mathbb{N}^i} \sum_{\sigma = 1}^g P_\sigma(t, n) = 1.
\]

(11)

The time evolution of \(P\) is given by a master equation (ME) of the form

\[
\frac{\partial P(t, n)}{\partial t} = (\mathcal{L}_R^* + \mathcal{L}_E^*)P(t, n) + \mathcal{K}^T(t)P(t, n),
\]

(12)
with $P$, $\mathcal{L}_R^\pi$, $\mathcal{L}_E^\pi$, and $K^T$ being sufficiently regular such that (12) has a unique solution for all times $t > 0$. Then the tuple $(\zeta, R, P)$ is called a (microscopic) system with infinite and finite states, or short an IFSS (Infinite–Finite State System).

Clearly, reactions of type (a) are the ‘traditional’ reactions, which in case there is no molecular machine [and therefore no reactions of type (b) and (c)] would lead us back to events described by (1). But note in contrast to (1) we have not introduced any chemical complex structure that would need to be done in addition. Type (b) reactions describe spontaneous changes of the states of the molecular machine. They are not triggered by binding of small molecules to the machine. In contrast, type (c) reactions combine the two previous types, there is binding of the small molecule to the macromolecule which at the same time changes its state. This reaction structure explains the structure of the master Equation (12), which now in contrast to the master Equation (2) becomes a vector equation with as many components as there are discrete states of the molecular machine. Note that we have not introduced a complex structure for the master Equation (12), this could be done in a similar way as for (2).

The same holds, therefore, for the limit equations introduced in the next section.

### The macroscopic equation and the combined adiabatic and continuum limits. The average dynamics

As we could have done with master equation (2) to derive deterministic mass-action kinetics, we can also introduce deterministic limits for the vector Master Equation (12). Because of the additional Markov chain (MC) structure with which we describe the molecular machines, there will be a combination of two limits, a so-called adiabatic limit, and a continuum limit. The adiabatic limit essentially assumes that the MC consists of fast variables in comparison with its environment, i.e. is in partial equilibrium (this is the same as the QSSA in ‘QSSA’ section). The equilibrium state of a MC is given by its so-called invariant measure, which we will call $\mu$. The vector $\mu$ has as many components as the master Equation (12). The continuum limit is a large number limit on the microscopic time-continuous Markov jump process describing the small very abundant particles (molecules) of the system. At the same time, the former discrete state

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**Figure 4:** Shown are different examples of molecular machines that are best modelled as structured particles. (A) A typical enzyme that can be in different states, either ‘silent’ or ‘active’ (usually associated with phosphorylation), and which only in its active state processes some substrate. (B) A string of DNA is shown, with binding sites to a repressor molecule which itself can be ‘inactivated’, i.e. by binding to some other smaller molecules loses the ability to bind to the DNA string. Finally (C) Different membrane proteins, which act as protein translocation machines, or as ion channels, etc.
space becomes a continuous state space under this operation, i.e. when letting the number \( n \) of molecules of each such species tend to infinity. As it is well known for the diffusion operator, this limit can only be meaningful if at the same time the vector of spatial scales \( \delta \) and the time scale \( \tau \) both tend to 0 in a meaningful way. The continuum limit is defined in terms of an expansion, where the first-order term determines the deterministic vector field, the second-order term describes the noise terms, etc. \([43]\). As discussed, when introducing the Gillespie algorithm (‘The Gillespie formulation of stochastic mass-action kinetics’ section), the role of noise is in itself a most interesting topic when discussing biological regulatory networks, but we will omit it completely in the following. This means we will only consider first-order terms in the expansion defining the continuum limit. With these preparatory remarks, i.e. when using a combination of adiabatic and continuum limit in the way described, we will get the following system of equation from (12):

\[
\begin{align*}
\frac{dx_i(t)}{dt} &= \sum_{\sigma=1}^{s} \mu_\sigma(x(t)) f_i^\sigma(x(t)), \\
x_i(0) &= x_{i,0}.
\end{align*}
\]

(13)

Here \( x_i \) is the \( i \)-th concentration of a small unstructured molecular species, \( 1 \leq i \leq s \), \( \mu_\sigma \) is the \( \sigma \)-th entry of \( \mu \) and \( f_i^\sigma \) is the \( \sigma \)-th contribution of the MC to the \( i \)-th component \( f_i \) of the vector field \( f \) describing the dynamics of the different species after up-scaling. For obvious reason Equation (13) is also called average dynamics. Note that the average vector field can be written in a matrix compact form:

\[
\dot{x}(t) = \mu(x(t)) F(x(t)),
\]

(14)

with \( \mu(x) = (\mu_1(x), \ldots, \mu_s(x)) \) and \( F(x) = \{ f_i^\sigma(x) \}_{\sigma=1}^{s} \). We interpret Equation (14) as the description of a regulatory network of \( s \) molecular species, regulated by a single molecular machine structured by \( g \) discrete states of an MC. This is an alternative description when compared with deterministic mass-action kinetics. Here we used additional model ingredients and a new set of assumptions, i.e. the appearance of a regulatory machine (single, so finite in number) that always works at steady state, completely independent of the dynamics of the regulatory network.

**Application to synthetic biology**

Model (14) can be used to represent detailed descriptions of genetic regulatory networks. This is useful when certain properties of genetic circuits are to be designed synthetically \([44]\). Each genetic switch involving a single gene is described as a molecular machine in the sense described above. Transcription factors are modelled as small unstructured particles. The framework can be easily extended to a finite number of molecular machines interacting via the small unstructured molecules. This is very close to the situation encountered in genetic regulation. The framework can be used to systematically derive governing equations for detailed regulatory mechanisms, including DNA looping. An application was given to model a synthetic clock in *Escherichia Coli* (Figure 5), and the thesis \([45]\). The resulting equations are of the form (14).

**STABILITY, ROBUSTNESS AND MODULARIZATION OF REACTION SYSTEMS**

In the following discussion on qualitative behaviour of reaction networks, we restrict our attention again to deterministic reaction systems, although the same research could be carried out for stochastic systems in most cases. Nevertheless, the mathematical tools currently available are mainly based on the theory of time-continuous dynamical systems, i.e. systems of first-order differential equations. We will assume that such an ODE system has been derived inside a given modelling framework and with the help of multiscale methods as discussed in previous sections. Let therefore

\[
\dot{x} = f(x),
\]

(15)

\[
x(0) = x_0 \geq 0,
\]

(16)

be some autonomous (it is essential to understand first experimental situations that are time invariant, i.e. where, for example, the temperature is kept constant) system where without too much loss of generality we assume that the vector \( x \in \mathbb{R}_+^n \) has components \( x_1, x_2, \ldots, x_n \) that are describing the concentrations of some (unstructured) molecular species \( S_i \). The vector function \( f : \mathbb{R}_+^n \rightarrow \mathbb{R}^n \) in Equation (15) incorporates all the concentration-dependent non-linear feedbacks with which we will be mainly concerned with in the following.

This section will be a quite detailed discussion of new developments and tools that are available to analyse reaction systems. In this respect, reaction networks are playing a key role for the whole of systems
We will first address the complexity of solution behaviour for a given reaction system; for example, the number of steady states in which the system can be. Then we investigate whether the steady states are stable, and perhaps are even global attractors, making the system qualitatively very simple. Nevertheless, with respect to the latter one should realize that chemists in the 19th century would have believed that any reaction system goes asymptotically to a unique equilibrium state. The conditions to possess a single globally attracting steady state are therefore an important case of reference. Any such questions on qualitative behaviour is intimately related to the notion of robustness. We like to know whether, for example, evolution has favoured certain pathways or architectures, because the cell or organism is less vulnerable to perturbations of a certain kind. To answer such questions requires in turn that we can define modules of the reaction system in a meaningful way.

It will be important for our discussion that $f$ will be given at this stage (it will best have derived by one or a combination of the methods described earlier in this review. This would ensure its validity to describe a specific experiment situation. In addition, the model would have become better testable, for example in the sense of Popper), and act on the network of which the $S_i$ are the components (nodes). The vector $x_0$ determines the initial values of species concentrations, i.e. set the conditions at the start of experimental observation [Equation (16)]. Note that mass-action systems (4) and (5), the derived enzyme kinetics, the $S$-systems, the LV systems and the average dynamics (13) are such systems of non-linear ODEs. It is, however, clear that perturbations of $f$ itself should also be considered, for example to investigate structural stability questions.

The fundamental question in the following is how to assign a network structure or graph $G$ to the couple $(x, f)$ such that the qualitative behaviour of (15) and (16) can be determined from the graph structure (network topology) (it should be noted that the same question can be asked for the ‘microscopic’ stochastic reaction systems following the master Equation (3). This has been investigated for monomolecular reactions in [9]).

**Figure 5:** Diagram of regulatory interactions in a synthetic clock consisting of two gene modules. The activator modules consists of the structural gene glnG for the transcription factor NRI, driven by the NRI-responsive glnAp2 promoter. The repressor module consists of the gene of the lac repressor, driven by the glnKp promoter. The circuit realizes an auto-activating feedback of the activator module, as the phosphorylated form of NRI, NRI-P binds to enhancer sites in the glnAp2 promoter, that enhance its activity. Second, negative feedback is realized by NRI-P stimulating the expression of the lac repressor via enhancer sites in the glnKp promoter. The lac repressor then inhibits the expression of NRI by binding to lac operator sites inserted within the glnAp2 promoter of the activation module. Additional low affinity (governor) sites for NRI in the glnAp2 promoter inhibits its activity when NRI concentrations are high. Taken from Janus [45].
Other related ideas can be found in references [46–48]. The question has a long tradition in ecological research (see ‘Other qualitative behaviour of [bio-chemical] regulatory networks’ section), and was first asked by Robert May [49 and 50]. To get started with this idea, we go back to the beginning of this review. There we introduced the graph \( G \) in order to systematically approach and investigate a given cellular volume with respect to given molecular binding events. The nodes of the graph \( G \) are naturally associated with the species \( S_i \). After introducing averaging and up-scaling applied to the elementary set of reactions given in (1), the ‘reaction strength’ \( f \) could be derived on a ‘macroscopic’ scale (in the sense of average of large particle numbers inside any sufficiently small part of the reaction volume). The complex structure of the reactions (1) will determine which arrows are pointing to some node now identified with \( x_i \), which is the same in this view as listing the species concentrations \( x_1, \ldots, x_n \) on which the \( i \)-th component of \( f \) is depending on.

### The interaction graph \( G_I \)

It is possible to associate a weighted directed graph \( G_I \) to systems (15) and (16). The node set \( V \) incorporates the species concentrations (this is why in the reaction kinetics literature, the interaction graph \( G_I \) is also called the species graph \( G_S \)). Both graphs are identical and just refer to different circumstances, interaction is the more general concept acting between components \( x_i \), and the arrows in \( E \) are derived from \( f \). We do not incorporate the whole information, but look at the linearization of \( f \) at any fixed time \( t \geq 0 \). This implies the arrows can change in time, as we interpret the existence of a link to be associated with a non-zero entry in the Jacobian \( J \) of \( f \) evaluated at \( t \). The weights on the arrows are given by the sign pattern of \( J(f) \). As the signs can change the weights on the arrows may also change. Note that the network and weights are fixed for equilibria of systems (15) and (16). The same is true for a special class of non-linearities defining the quasi-monotone differential equations [52], or (less general, but with mathematically stronger results) monotone systems [53]. We give an example of such an interaction graph \( G_I \) in Figure 6 describing a Mitogen-activated protein kinase (MAPK) system, as shown in Domijan and Kirkilionis [54].

As introduced, the question is how the graph topology (evolving or fixed in time) determines the qualitative behaviour. First we have to fix which aspect of qualitative behaviour we like to or can address. It turns out that the number of equilibria a network might possess is an object that can be investigated with existing mathematical tools, and on basis of the interaction graph \( G_I \). As the most prominent example we state,

**Theorem 1 (Thomas-Soule)\)** If a system has no positive cycles in \( G_I(x) \) for any state \( x \in \mathbb{R}^n_+ \), then it cannot exhibit multi-stationarity.

This original conjecture by Thomas was proven by Soule in [51]. The positive cycles will be introduced below in the context of feedback loops, but they are easily intuitively explained when looking at the example of a \( G_I \) in Figure 6. We just need to follow arrows with only positive signs and have to be able to return to the same node.

### Non-uniqueness of associated graphs

It has to be noted that once we have assembled an interaction graph as a modelling device (as explained in the first two sections of this review) and arrive at systems (15) and (16), there are more different types of graphs that we can associate with the ODE (and perhaps other model frameworks, but that has not yet been investigated). Typically the non-linearities are further specified. Most results exist for mass-action kinetics, where \( f \) becomes a polynomial [see systems (4) and (5)]. Feinberg and co-workers [55–59] have developed a theory for this case, where during the process different types of graphs incorporating the complex set \( C = \{ C_1, \ldots, C_l \} \) of a mass-action system has been employed in different ways. Incorporating the complex structure is mathematically intuitive, as this gives additional information about the reaction system. The most often cited theorem in this context is (a variant of) the deficiency theorem. The deficiency of a reaction network (denoted by the symbol \( \delta \)) is defined by the formula

\[
\delta = \epsilon - l - \rho,
\]

where \( \epsilon \) is again the number of complexes, \( l \) is the number of linkage classes and \( \rho \) is the rank of the stoichiometric matrix \( N \). It holds that the deficiency index is always non-negative [55]. We need to define that complexes \( C \) and \( C' \) belong to the same linkage class if there exists an undirected path in the reaction diagram connecting the two complexes. This defines \( l \). The stoichiometric subspace for a reaction network is the span of the reaction...
vectors, namely $\text{Im}(\mathcal{N})$. Two complexes (represented as vectors) $C$ and $C'$ are stoichiometrically compatible if $C' - C \in \text{Im}(\mathcal{N})$. Stoichiometric compatibility is an equivalence relation that induces a partition of the space $\mathbb{R}^+_n$ into equivalence classes. Each positive stoichiometric compatibility class is a space of the form \(\{x_0 + \text{Im}(\mathcal{N})\} \cap \mathbb{R}^+_n\), where $x_0$ is some positive initial concentration. We state the following version of the deficiency theorems, as given in Feinberg [57]:

**Theorem 2 (Deficiency-Zero Theorem)** Consider a mass-action reaction network of deficiency zero. Assume that the network is weakly reversible [this assumption is a weaker form of the requirement that each of the reaction of the form (1) has a corresponding reaction in the reverse direction]. Then the following holds for any arbitrary parameter set:

1. The system admits neither a positive equilibrium, nor a positive periodic orbit.
2. The system has the following properties: each positive stoichiometric compatibility class contains precisely one equilibrium, this equilibrium is asymptotically stable, and there is no non-trivial periodic orbit.

The power of the deficiency-zero theorem is that by definition of the deficiency index, we can identify a class of networks that cannot have multiple positive steady states. These networks can be very complicated and contain hundreds of species. We also know about the asymptotic stability of the resulting equilibria. Most of the different graphs associated with deterministic reaction systems that can be found in the literature have been reviewed in the Domijan and Kirkilionis [54].

In conclusion, there are different types of graphs that can be associated with a reaction system, there is no uniqueness. The different graph topologies give rise to different types of theorems making conclusions about the possible dynamic behaviour of the system. The complex structure of reaction networks is usually exploited with much advantage. Existence or non-existence of multiple steady states (equilibria) for mass-action systems can be deduced from the graph topology alone. This is very valuable as we can decide, for example, whether a given system is able to have switching behaviour (bistability, etc.). Furthermore, we can rule out periodic solutions in some cases. To make such conclusions, we need first to fix the non-linearity $f$ or the class of non-linearities we like to investigate, so going the reverse way when compared with the modelling process.

**Other qualitative behaviour of [(bio-)chemical] regulatory networks**

As we have already mentioned for LV systems (see ‘Lotka–Volterra systems’ section) other types of qualitative behaviour are of relevance. A typical question in ecological food webs is whether the elimination of a species will cause other species to be eliminated from the system. Mathematically, this has been investigated with the help of definitions like permanence or persistence. Such questions are relevant for genetic regulatory networks, where extinction can be equivalent to knock-out of a certain gene. It is surprising how little we know on how the choice of certain non-linearities affect extinction behaviour. It is generally agreed in ecology that this behaviour is very sensitively reacting when non-linearities are changed [60, 61], so either heuristic arguments or mathematical derivation of the non-linearities used really matters. Early work on chemical network stability are Clarke [62], Tyson [63] and Sontag [64].

Other types of qualitative behaviour can be used to classify a certain type of non-linearity. For example, we can ask how dense the set of mass-action kinetic networks showing chaotic behaviour lies in the set of general mass-action networks. Or about the stability of topologically equivalent mass-action systems [65], or their ability to produce arbitrary behaviour [66]. Such questions or classifications [67] are intimately related to questions of feedback (see ‘Feedback loops’ section) and bifurcation behaviour (see ‘Bifurcation analysis based on external currents’ section) [68, 69], like the occurrence of oscillating behaviour [70–76].

The occurrence of bistability is also relevant, as this is necessary to construct biological switches [77, 78].

**Feedback loops**

Feedback loops are the essential objects to study any non-linear system. They are, therefore, important to understand any real-world problem that can be investigated by measuring time series. In the context of cell biology, it is the feedback loops of the various regulatory networks that allow the cell to react to new conditions, fine tune its own state, etc. The most natural way to define feedback loops in a given dynamical system (any dynamical system that can be linearized in a well-defined way) is via the interaction graph $G_r$. The feedback loops there can be defined by cycles of different length. A cycle in the
graph \( G_I(x) \) is a sequence of distinct vertices \( x_{i_1}, \ldots, x_{i_k} \) such that there is an edge connecting \( x_{i_1} \) to \( x_{i_2} \), \( x_{i_2} \) to \( x_{i_3} \) and so on, finishing with an edge between \( x_{i_k} \) and \( x_{i_1} \). The length of the cycle is the number of vertices that it contains. The special case of a cycle containing only one vertex is called a (self-)loop. A loop has obviously length one and is associated with the main diagonal entries of the Jacobian matrix. Each cycle is endowed with a sign, which is the product of the signs of its edges. In Figure 6, \( S_1, FS_1 \) and \( F \) form a negative (sign) cycle in the interaction graph of the MAPK cascade. Some definitions to feedback loops in mass-action systems can be found in Sensse and Eiswirth [79].

**Motifs**

The motif concept is frequently encountered and cited in the systems biology literature [80, 81]. It is best discussed in the context of feedback loops. But we will need to give several interpretations of a motif in order to compare it with other concepts that are previously introduced. In its simplest interpretation, a motif is a certain type of sub-graph occurring multiple times in a given network. For any given graph \( G \), we can indeed use methods from algebraic graph theory (see for example, the classical text [82]) to count the number of simple types of sub-graphs, for example triangles (the complete graph \( K_3 \)), etc. Such an investigation leads to a spectral analysis of the graph \( G \) and is an alternative characterization of the graph topology. This is very helpful for very large graphs as it allows to estimate whether the network topology is more scale-free or exponentially distributed, etc. In cell biology applications, it has been used for static or evolving graph analysis; for example, to characterize protein–protein networks (see ‘Static and evolving network topologies, protein–protein networks’ section). But can the concept of small sub-graph distribution be of any use for understanding dynamic regulatory networks, i.e. reaction networks? For both conceptual and mathematical reasons this is very unlikely. In this case, the network needs necessarily be equipped with some dynamical process, as explained in Figure 2. If we assume that the dynamical network is defined, say by systems (15) and (16) with a given non-linearity \( f \), we must observe that there is no theorem that would connect the number of (a certain type of) sub-graphs with the qualitative behaviour of the system. This is also not to be expected, as it is the combination and linkage of the feedback loops (as defined for the respective interaction graph \( G_I \)) inside the network that determines the dynamics, not the motif counting which in fact separates each such feedback loop represented by the motif from its context, i.e. the other feedbacks. It is therefore not feasible to take motifs as a kind of architectural principle for dynamic regulatory networks.

Of course, this does not exclude the necessity and importance to study simple (genetic) regulatory interactions in experimental isolation from other feedback loops. It is not surprising that this is exactly what can be found in the literature [83].

**Modules and stoichiometric network analysis: extremal currents (elementary flux modes)**

The so-called stoichiometric network analysis (SNA) was introduced in a seminal paper by Clarke [84], and is one of the most prominent methods of analysing chemical and biochemical networks of mass-action type, i.e. systems (4) and (5). Note that if \( \text{rank}(A) = \rho \leq s \) (\( s \) was the number of species), then the reaction system has \((s - \rho)\) conservation relations [36]. A conservation relation is a relation between a set of species with their total concentration being preserved. The set of conservation relations takes the form

\[
g_l^T x = c_l, \quad l = 1, \ldots, s - \rho,
\]
where each $g_i^T \in \text{ker}(\mathcal{N})$ and $c_l \in \mathbb{R}_+$ is a constant describing the total conserved concentration. The set of conservation relations forms an invariant space [85]. Non-trivial equilibria of the network (we characterize their constant state here by $x_0$) for a chosen set of parameters (reaction constants) $k_0$ are determined by the conditions

$$\mathcal{N} v(x_0, k_0) = 0, \quad (17)$$

$$x_0 > 0. \quad (18)$$

Any such equilibrium clearly depends on the kinetic parameters $k \in \mathbb{R}_+^r$ ($r$ was the number of reactions), but also on the constants $c_l$, $l = 1, \ldots, s$, stemming from the conservation relations. Due to (17) and (18), all stationary reaction rates belong to an intersection of the kernel of $\mathcal{N}$ and the positive orthant of the reaction space,

$$v(x_0, k_0) \in \left\{ z \in \mathbb{R}^r | \mathcal{N} z = 0, z \in \mathbb{R}_+^r \right\} = \text{Ker}(\mathcal{N}) \cap \mathbb{R}_+^r,$$

which form a convex polyhedral cone $K_v$ [86]. This cone $K_v$ is spanned by a set of minimal generating vectors $E_i$’s (called extremal currents, as introduced in Clarke [84]):

$$K_v = \left\{ \sum_{i=1}^t j_iE_i : j_i > 0 \forall i \right\}.$$

These generating vectors are unique up to scaling by a positive constant. They may be linearly dependent, as the number of extreme currents (better known as elementary flux modes in the systems biology literature) may be greater than $\dim(\text{ker}(\mathcal{N}))$. Extreme currents decompose the network into minimal steady state–generating sub-networks. We can also call these sub–networks modules, an important concept in cell biology [87, 88]. A current corresponds to a certain subset of the reaction set which when assembled into a dynamical system by the assumption of mass-action kinetics can produce positive equilibria (independent on the rest of other reactions not part of this specific extremal current).

In the biochemical literature, they are interpreted as switching on or off different parts of a metabolic pathway associated with the current. Mathematically, due to the polynomial RHS of mass-action networks, extremal currents are the best known example to know how to split a non-linear dynamical network into what could be called ‘modules’ or functional units. They clearly qualify as an architectural building block principle for complex networks.

But note that the splitting relies on the equilibrium assumption.

The influence of a sub–network on the full network dynamics (i.e. how much the given subnetwork plays a part in creating a certain steady state) depends on the constants $j_i$’s, which are called convex parameters. In fact the Jacobian of the network evaluated at any positive steady state, $x_0$, can be written as a convex combination of contributions from the extreme currents:

$$D_\nu \mathcal{N} v(x_0, k_0) = \left\{ \sum_{i=1}^M j_i\mathcal{N} \text{diag}(E_i)k^T \text{diag}(h) : j_i > 0, h \in \mathbb{R}_+^m \right\}. \quad (20)$$

Here $h$ is a vector defined as the inverses of any equilibrium concentrations $x_0$, $k$ the matrix of stoichiometric exponents (note, usually $k_{ij} = \alpha_{ij}$, i.e. the stoichiometry of the reactants in each reaction) (see ‘Mass-action Kinetics’ section) and diag$(v)$ is a diagonal matrix with the diagonal entries given by the vector $v$. The contribution of a single extreme current is given by the term

$$j_i\mathcal{N} \text{diag}(E_i)k^T \text{diag}(h),$$

which represents the Jacobian of the subnetwork generated by the extreme current $E_i$, evaluated at any of its positive steady states. The Jacobian description in the form of (20) comes from a simple observation that for positive steady states $x_0$,

$$D_\nu \mathcal{N} v(x_0, k_0) = N \text{diag}(v(x_0, k_0))k^T \text{diag}(x_0^{-1}). \quad (21)$$

Together with the newly defined parameters, $h_i = x_i^{-1}$, $s = 1, \ldots, n$, the convex parameters $j$ replace the reaction constants $k$ and the conserved mass constants $c$.

**Stability analysis and characterization of regulatory networks by extremal currents**

We can use the elementary current concept to investigate whether a combination of sub-networks giving rise to asymptotically stable (here we need a slightly stronger stability concept, i.e. mixing stability) positive equilibria produces such equilibria on the system level, i.e. whether the linear combination of stable sub-networks as given in (20) produces stable positive equilibria for the whole network [19]. It is important to characterize the dynamical stability of equilibria of extremal currents. It has been proposed in Stelling et al. [89] that extremal currents (without stability analysis) could serve as a structural characterization of cellular regulatory networks.
The advantage of the extremal currents is indeed that they can be computed without the knowledge of kinetic parameters, as they only depend on the stoichiometric matrix and the positivity condition (17,18). Unfortunately, the problem arises that most of the currents might be unstable, so their simple counting can be misleading as they do not correspond to feasible physiological states. Regulation and feedback are dynamic properties and cannot be captured by a measure neglecting both weights on vertices and edges of the associated interaction graph GC (Figure 2). On the other hand, the extremal currents are able to address stability questions of equilibria [Equation (21)], so there is no need to neglect this additional information.

**Bifurcation analysis based on extremal currents**

In a series of papers, Karin Gatermann has introduced methods from algebraic geometry, which in combination with the extremal current formalism allow to analyse bifurcations [18, 19, 90–92] away from equilibria. The results have been recently summarized and extended in Domijan and Kirkilionis [93]. In classical bifurcation theory, the behaviour of the reaction system would depend on the parameters ki and ci. It was noted by Karin Gatermann that when bifurcation theory is applied to SNA, this requires some additional restrictions on the convex polyhedral cone (19). In SNA, every ray in the convex cone, i.e. [z1, . . . , zpi] with $z \in \ker(N) \cap \mathbb{R}_+^p$, corresponds to some positive solution of (17) for some values of k. To find such a positive solution x it is sufficient to solve the system

$$z = v(x, k) = x_0 v(x, k),$$

(22)

where the constant x0 is introduced because of the ambiguous length of z. The construction is such that the interior of the convex cone corresponds to all positive solutions of (17) for any value of k. However, for fixed values of k and a given $z > 0$, a solution for the system (22) is not guaranteed. In fact, z needs to satisfy additional conditions that have been derived in Gatermann [18]. Using this, a positive solution exists if, and only if, $z \in V(I_{tor}^{def})$ where $V(I_{tor}^{def})$ is an affine deformed toric variety of the deformed toric ideal,

$$I_{tor}^{def} = \{ f \in \mathbb{Q}[z] | f(v(x, k)) = 0 \} \subseteq \mathbb{Q}(k)[z].$$

Since each ray of the cone is parametrized by a different choice of convex parameters j, there are also restrictions on j. Positive solutions will exist if $j \in V(f) \cap \mathbb{R}_+^M$, where V(f) is a variety of the new ideal created by substituting $z = \sum_{i=1}^M j_i E_i$ into $I_{tor}^{def}$.

In conclusion, we have gained an algebraic method which when varying a j, later as a bifurcation parameter at the same time the existence of corresponding positive equilibrium solutions of the reaction system can be guaranteed under above conditions. In Domijan and Kirkilionis [93], this has been used to characterize (among others) saddle-node and Hopf-bifurcations, which by using the j parameters can be discussed in relationship to the contributions of different reaction sub-networks, i.e. the ones given by extremal currents.

**PRESENT SITUATION AND OUTLOOK**

The current situation in the field of cellular reaction networks is very heterogeneous, and there is probably a lot of confusion even among experts. The author agrees strongly with Hans Westerhoff in [38] who referring to the most interesting publication of Heinrich [94] also addresses this ‘Babylonian confusion’ in the area, making at the end of his paper a very illustrative example of how difficult it is for the human mind to understand what regulates what in a biochemical context. Heinrich (the author discussed with Heinrich shortly before his sudden death in a café in Yokohama, Japan, during an international systems biology conference) pointed out in his publication [94] that the major problem is the use of different scientific languages and something that could be called different ‘points of view’. The problem is indeed a general one and therefore effects heavily a newly emerging field like systems biology, where many different scientific disciplines are contributing. Perhaps everybody from mathematician, computer scientist, physicist, up to chemist and biologist uses a different language and looks very differently at the (same) situation. This makes the area very interesting for a philosopher of science, and more work on this should be encouraged. The situation is even more delicate because network theories are at the core of another newly emerging science, complex systems theory. In the end of course it is scientific success that counts, and this means predictive abilities. Not every concept can achieve this in every circumstance, there are indeed theories that need to be refused due to their lack of predictive power during the scientific progress. Of course, there is
plurality in the questions being asked, this is a particular strong point of systems biology at the moment.

**Derivation of reaction network models**

As a mathematician the author would like to support the idea that different approaches follow a hierarchical organization, and that, therefore, there should exist mathematical ways to derive observational quantities of a reaction network model from microscopic interactions. Here ‘microscopic’ is used in the sense that the basic events (or rules) of such an interaction can be well characterized and are self-consistent, i.e. there is no further deeper level that would significantly contribute to the behaviour of the chosen microscopic level. This thinking is indeed how this review is organized, starting with the basic event of reaction systems, the reaction event, (1). It is not so much that every theory would need to start on this level. But it has an enormous advantage to know that a theory can be traced back to basic events or rules in a given situation, at least in principle. A recipe for up-scaling and derivation of the dependent variables (observables) of the model is one way to achieve this goal. Such principles also guarantee that the quantities involved can be interpreted, and what it means measuring them. As an example, Westerhoff in [38] is right in his argument that a theory like MCA (see ‘Metabolic control analysis’ section) did contribute to clarify and therefore make control of enzymatic pathways ‘measurable’, simply because it became well-defined what quantities have what meaning in the context of regulation. It should be noted, however, that MCA itself is already a high level, i.e. macroscopic and derived theory as explained in this review (it is clear that, for example, membrane proteins have a different event statistics than freely diffusing enzymes, and this would lead to different MCA variants). The context of scales is most important when discussing regulation in cell biology. Scales are a natural object in physics and mathematics, but as discussed often neglected in the computer science area.

**Inference of network architecture and parameter estimation**

We have not discussed any methods of statistical inference or parameter fitting in this review. This has been done on purpose. A comprehensive review article for reaction mechanisms is Crampin *et al.* [96]. The review discussed what we could name ‘forward modelling’, i.e. a model is constructed from first principles as good as possible. In a next step, the model produces a time series (in ‘forward’ direction, i.e. with time following the time arrow), which can then be compared with data. The idea was to restrict ourselves to cases where spatial position of molecules can be neglected. This might not always be possible. Not only because of the importance of spatial gradients in cells, but also because reaction and transport are often intertwined, for example, in so-called ‘crowded environments’, and because different types of molecules will have different restrictions in their movements in the cell [97–99]. We are just at the beginning to understand the implications of such observations for the behaviour of reaction networks. With respect to system identification, we must find better ways to bring together methods from reaction networks with statistical approaches, like Bayesian networks. The latter are often used to interpret micro-array and other genetic data, most often not given as a time series. It nevertheless clear that even micro-array data must stem finally from regulatory interactions (typically measured from a number of cells in a tissue), which should be able to be described by models as presented in this review. A confirmed reaction mechanism should constitute additional information that can be incorporated into a Bayesian approach.

**Regulation and graph theory**

The review stressed the use of graphs to understand regulatory pathways. The graphs have been used for two different purposes. First as a tool to assemble a model of a regulatory network from scratch. Second to deduce the qualitative behaviour of the network from a graph attached to an existing reaction mechanism. The two aspects have to be strictly separated. The use of graphs to sketch relationships between certain components is very valuable, especially in biology. Different such diagrams and conventions exist; for example, in genetics. But it should be emphasized that the meaning of the relationships becomes only clear when, for example, the right time-scales of different processes have been set up. Which in other words means the weights drawn in Figure 2 have been properly set up. This coincides with the step-wise procedure that a dynamical system has been derived which then constitutes (in a further step) a measurable model or hypothesis that becomes refutable, i.e. scientific.
Relevance for bioinformatics

For bioinformatics this means that it enters new territory when dealing with cellular reaction systems and regulation. In reaction networks there is always a mixture of discrete and continuous objects, i.e. there are not exclusively discrete objects. As outlined, rule-based systems must become rate-based systems when a comparison with time-series is envisaged. It should be remembered that bioinformatics had its great breakthroughs in sequence analysis, a completely discrete method. Non-discreteness makes it harder to implement algorithms, as notions like approximation and averaging stemming from applied non-linear analysis will be definitely needed. On the other hand, discrete concepts from computer science, like methods from membrane computing [95], can offer new opportunities when combined with analysis techniques. It is the opinion of the author that purely discrete and static (i.e. non-temporal) network concepts, like the motifs (see ‘Motifs’ section), are not the correct way to capture the properties of regulatory pathways.

Open problems

There are many open and challenging problems in the field. We have already touched many situations where essential gaps in the theory did occur. The following list is made to encourage research in the area, but is of course not complete. We follow the sections of the review. Stated are mainly mathematical problems, which also should at the same time improve the applicability of the tools described in the review inside bioinformatics.

P1 (Mathematical Problem) There are many mathematical questions related to the ‘rate conversion’ of rule-based systems (see ‘Rule-based networks’ section). Can we, for example, define simple conditions such that the rate converted rule-based system asymptotically for \( t \to \infty \) converges to the same attractor as before, i.e. as the deterministic non-rate converted rule-based system did? Think about this problem in terms of Boolean networks or networks with a small number of discrete states. Can we allow some randomness of evaluation of the rules that are of Gillespie type, i.e. take combinatorial effects into account?

P2 (Mathematical Problem) A related problem to P1 is about the discretization of continuous state spaces or of general non-linearities \( f \), the weights on the edges of the interaction graph \( G_I \). There is already a rich literature on so-called piecewise linear \( f \) replacing the original non-linearity. It is a natural question to ask for consistency and error estimates, i.e. if the number \( n \) of intervals on which \( f \) is linear converges to infinity, the system has the same qualitative behaviour as the original one. Does this property hold already for finite but large \( n \)? Are there theorems specifying \( n \) and the length and positions of the respective intervals such that the consistency condition holds? With respect to discretization of continuous state spaces, there are similar questions that have already been stated. Define an equivalence between the type and number of attractors of the discrete and continuous state network. This definition should also describe the exact relationship between the rate-based rule or event structure of both the discrete and the continuous network, i.e. the ‘fluxes’. For which such definitions or assumptions do discrete and continuous dynamics on networks coincide? If this is not possible to decide, are there rational choices of discrete state spaces and their size given a time series stemming from a cellular regulatory network?

P3 (Mathematical Problem) This problem is about extensions of the Thomas-Soulé result in ‘The interaction graph’ section on the interaction...
graph $G_I$. There are obviously many possible ways of extensions. It should be noted that the Thomas–Soulé theorem makes no statement about the stability or instability of steady states. This is in contrast to the deficiency theorem presented in ‘Non-Uniqueness of associated graphs’ section, where the RHS $f$ is a polynomial (due to the fact that mass-action kinetics has been used). Can stability results be incorporated to theorems of Thomas–Soulé type if we specify a class of non-linearities? Does for example the following hold: Assume $f$ is (quasi-)monotone, and $G_I$ has no positive cycles (in this case we do not need to specify for which states $x \in G_I$ has this property). If a unique positive steady state exists, then it is globally asymptotically stable.

P4 (Mathematical Problem) Can elementary currents [see ‘Modules and stoichiometric network analysis: external currents (elementary flux models)’ section] as a constructive idea and design principle be generalized to non-polynomial reaction networks, i.e. is there a natural ‘modularisation’ in terms of sub-networks that are able to exhibit positive steady states? Can then the overall system behaviour be analysed as linear combinations of these sub-networks?

P5 (Computer Science and Mathematics) Can elementary currents [see ‘Modules and stoichiometric network analysis: external currents (elementary flux models)’ section] be implemented efficiently as a research tool where biological switches and oscillatory behaviour can be constructed by reverse engineering? Can this be combined with MCA (see ‘Metabolic control analysis’ section), i.e. can we generalize beyond stability results and incorporate sensitivity analysis?

Key Points

- Reaction networks are a key tool for understanding cellular processes. At the same time they are the best investigated examples of dynamic complex networks and therefore play again a key role for other areas of applications.
- Due to their dual basis in both discrete mathematics (graph theory) and time-continuous dynamical systems, they still offer many conceptual challenges.
- There are also experimental challenges due to the need of measuring detailed time-series inside the cell. To approach these challenges is a key scientific question in the coming years and will help to develop new drugs and therapies.
- The review discusses in detail different graphs that can be associated with reaction systems in order to retrieve knowledge on the qualitative behaviour of the system.

References


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