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The Physics behind Systems Biology

Nicole E. Radde[1*] and Marc-Thorsten Hütt[2]

*Correspondence:
nicole.radde@ist.uni-stuttgart.de
[1]Institute for Systems Theory and Automatic Control, University of Stuttgart, Pfaffenwaldring 9, 70569 Stuttgart, Germany
Full list of author information is available at the end of the article

Abstract

Systems Biology is a young and rapidly evolving research field, which combines experimental techniques and mathematical modeling in order to achieve a mechanistic understanding of processes underlying the regulation and evolution of living systems. Systems Biology is often associated with an Engineering approach: The purpose is to formulate a data-rich, detailed simulation model that allows to perform numerical ('*in silico*') experiments and then draw conclusions about the biological system. While methods from Engineering may be an appropriate approach to extending the scope of biological investigations to experimentally inaccessible realms and to supporting data-rich experimental work, it may not be the best strategy in a search for design principles of biological systems and the fundamental laws underlying Biology.
Physics has a long tradition of characterizing and understanding emergent collective behaviors in systems of interacting units and searching for universal laws. Therefore, it is natural that many concepts used in Systems Biology have their roots in Physics. With an emphasis on Theoretical Physics, we will here review the 'Physics core' of Systems Biology, show how some success stories in Systems Biology can be traced back to concepts developed in Physics, and discuss how Systems Biology can further benefit from its Theoretical Physics foundation.

Keywords: Complex systems, Statistical physics of networks, Nonlinear dynamics, Mathematical models, Robustness, Model inference

Introduction

It is a prominent current trend in the Life Sciences to proceed from the detailed study of individual molecular elements to the analysis of interactions between a large number of such elements. Systems Biology is often seen as the discipline of choice for this step from single components to systems. Systems Biology is an interdisciplinary research field, which combines experimental (in particular, 'omics') techniques with mathematical modeling and model analysis, with the ultimate goal of understanding the emergence of biological function on the basis of interdependencies among molecular components.

Systems Biology is often seen as the application of modeling and simulation strategies from Engineering to biological questions. Similar to the goal of representing technical systems in a simulation framework, Systems Biology sets out to represent biological systems *in silico*, in order to interpret experimental observations, contextualize experimental data and extend the scope of investigations by numerical experiments. Over the last decade, the large-scale numerical simulations of biological systems have been facilitated by high-throughput technologies.

In addition to the Engineering perspective, there is a second research direction in Systems Biology, which focuses on the search for universal principles behind biological observations. One of the main foundations of this direction is Physics. *EPJ Nonlinear Biomedical Physics* has devoted a topical issue to studies showing examples of research on biological systems either initiated by Physics or employing methods rooted in Physics. In this introductory article to the topical issue, we put these examples into a broader context. At the same time, we want to illustrate this fascinating research area at the interface of Systems Biology and Theoretical Physics and show the wide range of physical principles underlying approaches in Systems Biology.

Why is this second research direction so vital for Systems Biology? In recent years, we can observe a trend towards the creation of increasingly large and complex models of intracellular processes that are only accessible via numerical simulations. Since many of these models describe large networks of interacting components, whose dynamics are described in a nonlinear way, these models are usually very flexible and can show a huge variety of different behaviors. Combined with the fact that parameters of these models are characterized by a high degree of uncertainty, this renders model calibration a difficult task. Thus the predictive power of these models is also often questionable. Consequently, this development must be accompanied by approaches facilitating the detection of underlying general principles, which is crucial for an ultimate deeper mechanistic understanding (see, e.g., [1, 2]). Many theories and concepts developed in Physics are particularly suited for this purpose, since Physics, by its nature, searches for unifying theories and frameworks.

The article is structured as follows. In the next section, we describe achievements and challenges in Systems Biology, with an emphasis on the relationship of Systems Biology and Theoretical Physics. Then we focus on a few selected examples, which illustrate how concepts and ideas from Physics have shaped research in Systems Biology. And finally, we provide an outlook on possible future research directions at the interface of Physics and Systems Biology.

Review

Physics and systems biology – some rules of interaction

Any generalization about the historical development of research fields and the interplay of scientific disciplines will have to be simplistic and will leave out important threads in the complicated web of research trends and scientific endeavors. With this *caveat* in mind, a few 'stylized facts' about the relationships of the disciplines involved need to be emphasized. In Physics the search for universal laws was fueled by the deep, century-long interplay of Mathematics and Physics, with an innovation in one discipline often triggering progress in the other.

Although Engineering is a heavily mathematized discipline (as seen, among other aspects, in the relevance of numerical simulations), an important theoretical framework is derived from Systems Theory and Cybernetics, rather than from Mathematics. As a consequence, regulation and systemic control, rather than universal laws, tend to define the disciplinary agenda.

When starting from these differences in their theoretical frameworks, it becomes clear that the interactions of these two disciplines with Biology, each of which can be seen as an important foundation of Systems Biology, strive for markedly different forms of systemic

understanding: design principles and universal laws on the one hand, detailed numerical *in silico* simulations of systems and control tasks on the other hand. Figure 1 summarizes this highly simplified, but instructive, interdisciplinary 'landscape'.

Modeling of phenomena observed in biological systems and the search for underlying mechanisms and principles have a long history, with remarkable contributions from Mathematical Biology. The development of the Hodgkin-Huxley model as an explanation of the temporal behavior of excited neurons or the class of predator-prey models for describing the dynamics of competing populations are prominent examples. Their principles can still be found in many areas of research (see e.g. Chapter 2 in [3] or Chapter 3 in [4]). Moreover, although classical Cell Biology was in the past a predominantly reductionist approach, in which cells were broken down into small subsystems, this was always accompanied by models on the system level. Regulation processes that drive cellular decisions, such as the lytic and lysogenic pathway in bacteriophage lambda [5, 6], the diauxic switch between glucose and lactose uptake in bacterial cells [7] or apoptotic decision processes [8], have been in the focus of interest for a long time. Norbert Wiener, known as the founder of Biological Cybernetics, raised the idea of pursuing research towards a system-level understanding and control of biological and technical systems [9]. Several years later, Ludwig van Bertalanffy made first attempts at developing a consistent framework for open systems that are not in thermodynamic equilibrium. Although his concepts were described at an abstract level, important system-level properties of such open systems can be found in his work. Examples are the terms *dynamic steady state* and *equifinality*, which refers to the ability of reaching the same final state via different paths and independently of the exact initial condition. The latter characterizes open systems [10]. A later example of an already established system-level approach is Metabolic Control Analysis (MCA), an approach to analyzing the sensitivity of metabolic networks with in- and outflow with respect to small perturbations (see e.g. [11, 12]).

Even in these early developments, long before the term 'Systems Biology' was coined, we can discern two principal trends: the strive for biological realism (see, e.g., [13]) and the search for fundamental design principles or 'natural laws' underlying the biological systems (see, e.g., [14]). These two trends are still visible today, e.g., comparing realistic simulations of the yeast cell cycle [15] with minimal model approaches [16] for the same

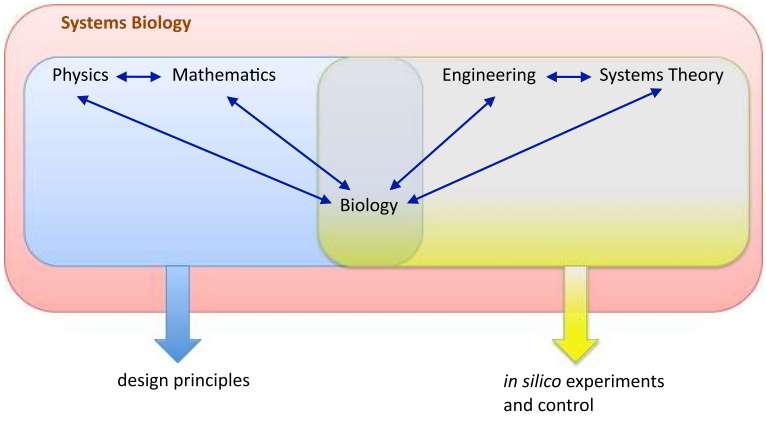

Fig. 1 Relationship between the disciplines. This schematic representation illustrates how the two foundations of Systems Biology, one associated with Engineering, the other with Physics, can be related to two different sets of goals

system. In the case of the MAPK cascade (one of the systems with the richest modeling history in Systems Biology [17]), there seems to be a clear trend towards ever more detailed and realistic simulations. This research is of high relevance for the mathematical description and numerical exploration of a specific cellular function. One example is the work of Hatakeyama et al. [18], who studied the different regulatory events triggered by a single kinase, PI3K, via a detailed mathematical model (see also [19]). However, when searching for the disruptive research result that provided insight into the design principles of the cascade and its evolutionary constraints, the seminal work by Huang and Ferrell [20] stands out, where the cascade was related to ultrasensitivity. A recent progress in understanding further generic principles is reported in [21], where the broad range of dynamical behaviors a MAPK cascade can potentially display was studied with a technique developed in Physics – generalized modeling [22].

The history of interactions between Physics and Biology is rich and diverse. Here our emphasis is on interactions on the theoretical side. Therefore, the progress in instrumentation and experimental methods as well as the whole discipline of Biophysics are not discussed. Also, it is clear that there is a regular and important flow of concepts, inspirations and methods in the opposite direction, from (Systems) Biology to Physics, all of which is not addressed here.

In addition to the many clear and direct influences of theoretical concepts from Physics on biological questions, there are fascinating examples of scientific developments in some field serving as an inspiration for a large research endeavor in Physics, which in turn facilitated (or even enabled) the application to Biology.

A prominent example is the development of Random Boolean Networks (RBNs) in the late 1960s by S. Kauffman, a physician who became a prominent figure in the study of complex systems. Kauffman's model [23], which was intended as a minimal model of gene regulatory networks, served as a starting point for an avalanche of research in Theoretical Physics (see [24] for a review).

In Biology, this model initially didn't have the same significance. In the early 2000s, however, building on Kauffman's original work and the subsequent research in Physics, several case studies demonstrated the usefulness of Boolean network models for the understanding of specific regulatory systems in Biology. Examples include the segment polarity network in *Drosophila* [25] and the cell cycles of the budding yeast *Saccharomyces cerevisiae* [16] and the budding yeast *Schizosaccharomyces pombe* [26].

Over the last years, Boolean network models have become an integral part of Systems Biology (see, e.g., [27–29]). One article in this Topical Issue [30] compares Boolean network models with models based on ODEs, as well as Boolean-ODE hybrid models, by using several regulatory motifs and biological examples.

In Fig. 2, a few historical publications and concepts, either originating in Theoretical Physics or with a strong impact on Physics, are listed, which can serve as examples of how such milestones have – directly or indirectly – shaped research strategies in Systems Biology.

Such a list is necessarily incomplete and also biased by one's own perception of scientific developments. It should therefore be emphasized that the purpose of Fig. 2 is to illustrate a few examples of such developments rather than serve as an 'encyclopedia' of physical concepts behind Systems Biology. An excellent account of some of the broader developments leading to Systems Biology is given in [31].

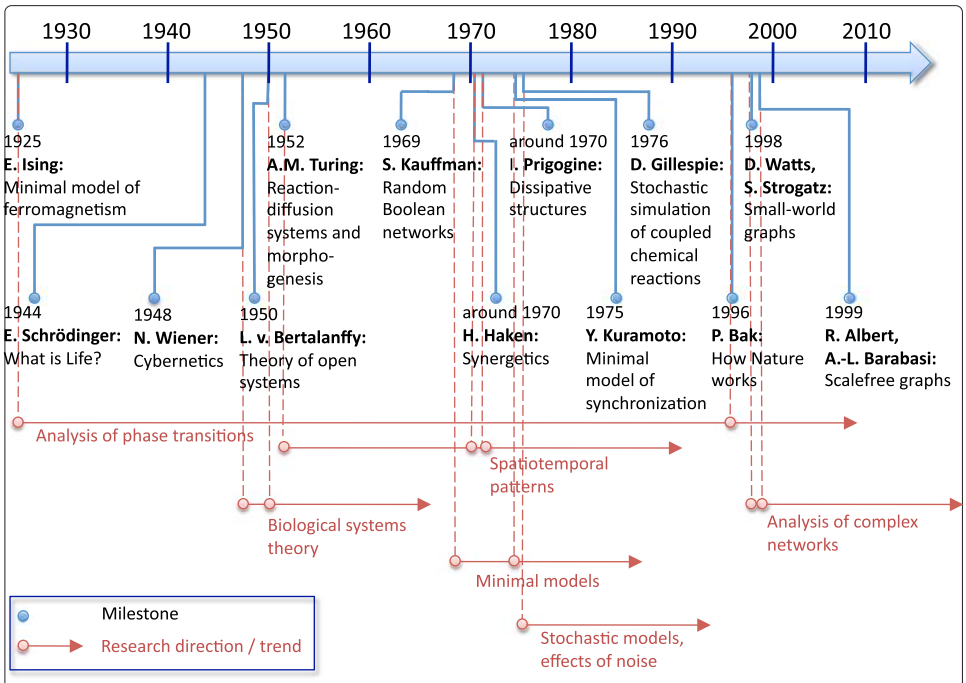

Fig. 2 Examples of historical milestones associated with the impact of Physics on Systems Biology. Upper half: This timeline provides a few examples of historical publications or concepts, some from Physics, others from neighboring disciplines, all of which had a strong impact on the relationship of Physics and Biology. Lower half: Research directions and trends in Systems Biology related to these milestone publications and concepts. This set of examples provides an illustration of the 'Physics core' of Systems Biology

While such a timeline will always suffer from severe omissions, the figure shows quite strikingly the deep historical roots of some of the concepts, which in Systems Biology are occasionally perceived as novelties.

Let us highlight one example in a little more detail. The Ising model of ferromagnetism [32, 33] is a simple thermodynamically motivated model, where the interaction of neighboring spins facilitates the alignment of spin orientations ('up' $= 1$, 'down' $= -1$), while temperature (which can be seen as the control parameter of the system) provides the energy for randomly occurring spin flips. The 'magnetization', i.e. the average of all spins (which serves as the order parameter of the system), can asymptotically be in three states, $+1$, 0 or -1. In the simplest form of the model (on a 2-dimensional lattice without an external magnetic field) one observes a second-order phase transition of magnetization as a function of temperature.[1]

In addition to its prominence in Theoretical Physics, this model – due to its simplicity and the fundamental relevance in systems of interacting two-state elements – has been applied to a multitude of real-world phenomena across a wide range of disciplines, ranging from opinion formation [34] to biological membranes [35]. The model itself and its diverse extensions (e.g., spin glasses, [36], or the Hopfield model, [37]) have also had a strong impact on Systems Biology. In [38] the signaling pathways underlying *E. coli* chemotaxis have been modeled using an Ising-type model. The emergence and regulation of long-range chromatin structures has also been studied with the help of Ising models in [39]. The Hopfield model, in which neurons are described as binary (active or inactive) variables that are connected via couplings and

switch their states in an asynchronous manner, was first introduced by Hopfield [37]. It has been used to understand brain structures and functions and was received, used and developed in quite different ways by theoretical physicists and neurobiologists, as described in [40].

Building on the general ideas put forward by Schrödinger (on the thermodynamic interpretation of biological life, [14]) and von Bertalanffy (on open systems, [10]), the work by Turing (on patterns arising in reaction-diffusion systems [41]), Nicolis and Prigogine (on dissipative structures [42]) and Haken (on self-organization and synergetics [43, 44]) have shown how thermodynamically open systems can give rise to complex structures and spatial as well as spatio-temporal patterns.

Turing's fundamental paper [41] was able to trigger an avalanche of research in Physics on spatio-temporal patterns, with a strong emphasis on applications in Biology. In particular, via the concept of activator-inhibitor models [45], Turing patterns have shaped a long debate in Developmental Biology on the origin of gene expression patterns during development [46].

Over many years, Physics has consistently put forward minimal models for a wide range of complex phenomena. The disciplinary and interdisciplinary successes of the Ising model illustrate the amount of guidance such a model can provide. The Kuramoto model of coupled phase oscillators [47] has enabled a deep theoretical understanding of synchronization phenomena [48] and continues to serve as a template for identifying, classifying and understanding synchronized behavior in diverse application fields [49]. The idea of self-organized criticality [50] is intimately linked to the minimal model of avalanches on a sandpile [51].

Dissipative structures [42] and the theory of self-organization [43] provide a formal framework for the relationships between local interactions and collective behaviors that many of these models describe.

Given the long history of interactions of the disciplines involved, it is an interesting question how the necessity of a systemic description of biological systems has emerged from these early concepts and how Systems Biology could become this fascinating 'melting pot' of diverse scientific ideas. The two seminal articles by Hiroaki Kitano, both published in 2002, helped define the research agenda of Systems Biology [52, 53]. The following factors stand out as key factors in this development (see also e.g. [54]):

1. *Advances in experimental techniques and the 'omics' era.*
   In the last decades, there was an enormous development of new experimental techniques in Molecular Biology and Cell Biology. Starting with new sequencing methods for whole genomes and transcriptomes, these high-throughput techniques have enabled the study of cells and cellular systems with unprecedented levels of detail and lead to the onset of the 'omics' era, which provides us with huge amounts of data on the genome, the transcriptome, the proteome or the metabolome levels. For the first time, it seemed in principle possible to get a mechanistic and quantitative understanding at a molecular level. Importantly, parallel to these achievements, methods of perturbing networks of interacting biomolecules, such as loss-of function knockouts of genes or RNA interference,

have also been established. These new possibilities also raised the question of data storage, appropriate data preprocessing, visualization and analysis, which are required to make use of these new kinds of information.

2. *New developments in Bioinformatics.*
   Driven by the necessity of organizing and contextualizing these increasing volumes of biological data, the similarly young discipline of Bioinformatics expanded rapidly. Bioinformatics provides diverse statistical methods and algorithms, together with approaches from pattern recognition and machine learning, for these purposes. In line with the development of 'omics' technologies and Bioinformatics methods, the question of public data availability and transparency of data analysis and modeling became relevant. One consequence of this discussion was an explosion of the number of databases for data and knowledge sharing. This development is accompanied by consistent demands for the formulation of standards for formats and preprocessing steps. The requirement to provide raw data or to make source codes available, allowing reviewers to reproduce each step in the analysis, for publication in renowned journals is also a result of this debate.
3. *Quantitative models for molecular networks become feasible.*
   In contrast to pure data analysis, Systems Biology aims at a mechanistic understanding of biological processes at a molecular level. Thus quantitative or qualitative dynamic models of interacting molecular components play a key role here. Networks (or, more precisely, graphs consisting of nodes and links) have become a very popular data structure in Biology, as a wide range of biological information can be represented as interaction patterns between molecular components. As a consequence, the analysis of biological networks has become an integral part of Systems Biology.
   Besides high-throughput techniques enabling simultaneous monitoring of numerous components, experimental approaches that resolve processes in time and space and on a quantitative level also play a role for model development in Systems Biology. The availability of experimental data for the modeling process is a major advance compared to many models described in Mathematical Biology textbooks. On the one hand, intracellular dynamics can now, in principle, be captured quantitatively and not only phenomenologically. On the other hand, this gives rise to completely new challenges for modeling and model analysis. Some aspects will further be detailed later on. More globally speaking, the connection of 'omics' opportunities and new modeling challenges is at the core of the emergence of Systems Biology.
4. *Complex models can be handled with increasing computer power.*
   Increasing computer power principally enables the study of large and complex (nonlinear) models via numerical simulations, and we can indeed observe a trend in Systems Biology towards larger and more complex models (see for example [55, 56]). While computer simulations have largely influenced various fields of nonlinear dynamics (prominent examples are the understanding of phase transitions and chaotic behavior [57]), they cannot replace concepts for the search of simpler underlying principles. Moreover, in the presence of a high degree of uncertainty in model parameters and structure, which is typical for these models, simulation-based studies are associated with certain risks when these uncertainties

are not properly taken into account. As a consequence, the predictive power of these models is often only moderate. This is particularly true, when they are used to predict yet untested scenarios. Statistical approaches for model inference and sensitivity analyses are powerful tools for dealing with these problems and are frequently used for this purpose (see e.g. [58, 59]). One paper in our topical issue addresses this point by discussing different variants of sensitivity analyses for ODE models of biological systems by using the example of the MAPK pathway [60].
Another example of such a transition from minimal models to high-dimensional detailed models is the decade-long theoretical work on circadian oscillations [see, e.g. [61]]. The more recent work is still covering both levels of modeling, the search for design principles [62, 63] and the construction of detailed, realistic models of circadian rhythms [64, 65].
At this point, the key duality underlying Systems Biology investigations becomes visible again: minimal models giving access to fundamental principles and universal laws potentially governing a particular class of biological systems vs. detailed high-dimensional models allowing a direct validation of experimental data for specific systems and thus complementing and supporting experimental investigations.

5. *New approaches also enter Medical Sciences.*
High-throughput 'omics' technologies have become an integral part of medical research, thus paving the way towards Personalized Medicine or 'Precision Medicine'. Accordingly, methods from Bioinformatics and Systems Biology need to be adapted (or even newly developed) for the context of clinical data [66]. The resulting new field, Systems Medicine [67], sets out to offer a multi-'omics' view on patient cohorts. Challenges to be addressed along this way include data integration (i.e., the cross-referencing of different types of patient-specific data and the development and use of standardized, universal data formats) and the capacity to analyze diverse data distributed over interdependent networks.

A detailed look at a few examples

In the following, we will show some of the contributions from Physics to Systems Biology by using a few selected examples. Figure 3 sketches four fields of research that have substantially been influenced and shaped by methodology originating in Physics. We have classified these fields into contributions to network analysis, functional robustness of biological systems, concepts for dynamic modeling and methodology for model inference and parameter estimation.

Network analysis

There is a broad interdisciplinary trend towards considering complex systems as networks of interacting dynamic components in different research areas, including Systems Biology (see e.g. [68]). This new view on complex systems has lead to a variety of new methods and concepts. In Systems Biology these methods have been applied to metabolic networks, gene regulatory networks and signaling networks in particular.

Together with Statistical Physics (e.g. [69]), Systems Biology has been one of the principal drivers behind the development of a theory of complex networks and a rich set of methods for network analysis [70]. In fact, metabolic networks were among the first examples of graphs with a power-law degree distribution [71], thus helping

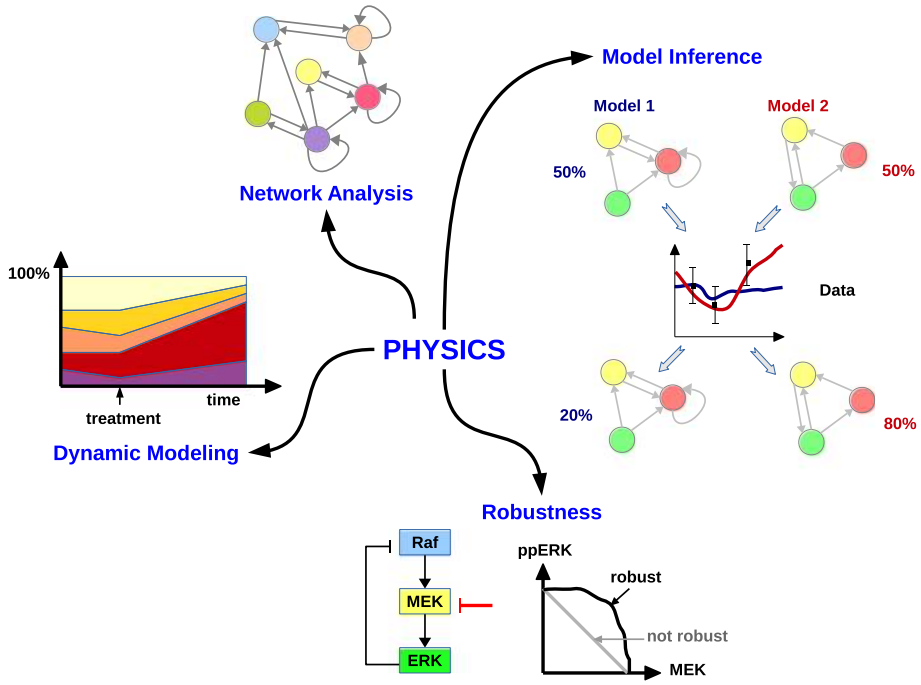

Fig. 3 Examples of research fields in Systems Biology that have profited from contributions from Physics. Methodology developed in Physics has been adapted and applied to biological systems, supporting a systemic understanding of these systems. In this survey we focus on physical contributions to progress in the development of dynamic modeling approaches, to concepts for the characterization and analysis of dynamic network models, to methodology for model inference and parameter estimation and to a unifying framework for functional robustness of biological systems

to define and analyze the corresponding category of *scale-free graphs* [72]. An analysis in terms of networks allows us to use terms and concepts from graph theory in order to understand organization principles of living systems. Examples of this viewpoint can be found in [1, 68, 73, 74]. More specifically, with their capacity to provide abstractions of biological systems, networks have been instrumental in the search for universal design principles governing biological systems. Results along these lines include the analysis of hierarchy [75] and modularity [76], the disassortativity of protein interaction networks [77] and the subgraph composition of gene regulatory networks [2].

There is increasing evidence that dynamic properties of biological networks are tightly related to network architecture. This constitutes an important starting point for generalizing abstractions, in which complex intracellular networks are reduced to network structures and graphs, which are, to a large extent, independent of the choice of kinetics or the exact values of the kinetic rate constants. This abstraction has, for example, lead to the identification of *network motifs*, which are small subnetworks that are ubiquitous in nature and might fulfill certain biological tasks [78]. Feedback circuits are an example of such motifs, and single circuits are already well understood from a theoretical point of view. Pioneering work has been done by Thomas and colleagues [79–84]. Positive feedback, which amplifies an external perturbation, is related to bistability, switch-like behavior, memory and hysteresis effects, and cellular decision making. In contrast, negative feedback, which counteracts external perturbations, can cause oscillating behavior,

but also has a stabilizing effect and plays a major role in maintaining homeostasis (see for example [81, 85–89]). Similarly, feed-forward control might enable a system to show perfect adaptation to a signal, as it is typical of chemotactic systems [89]. In this case, the system shows a transient response to a persisting input signal. The design of feedback and feed-forward loops has already entered the world of Synthetic Biology. An impressive early example was the repressilator model [90], and in the meantime many more regulatory modules have been built up in Synthetic Biology (for examples see e.g. [89]).

One also observes an interesting interplay between network approaches and modeling: Statistical approaches are used to identify unexpected topological properties (compared to suitable random networks), and subsequent modeling supports the functional relevance of these topological properties. Network motifs and their dynamical functions are an example of this interplay [2, 89].

A class of mathematical models relating networks and dynamical modeling are Boolean network models (see also the previous Section), where few discrete states (e.g., $0 =$ inactive/off and $1 =$ active/on) of the dynamical variables are iteratively updated using Boolean rules, thus generating a time evolution of the system along discrete time. Due to their stylized representation of dynamics, Boolean networks are frequently used to reveal fundamental principles of large networks that are not yet accessible to a detailed quantitative description. Boolean networks have been successfully applied to various biological systems [1, 24, 91–94].

Facilitated by these studies, new methodology has also been developed for the analysis of these network models [95–97]. Ultimately, these will allow to reveal unifying regulation principles for reliable functioning that are found across different organisms, which is in the spirit of the search for basic principles.

This topical issue contains two examples of methodologies for network analysis. First, in [98], the match between transcriptome profiles and two levels of gene regulation, namely the transcriptional regulatory network and the arrangement of genes on the (circular) chromosome, are statistically analyzed, revealing a time-dependent interplay of 'digital' (network-based) and 'analog' (chromosomally regulated) control in bacterial gene expression. Second, a fascinating example of a new type of network analysis is the 3D reconstruction of a (eukaryotic) chromosome from a contact network of genomic sites (as obtained from a Hi-C data), which is enabled by an elegant, graph-theoretical treatment of constraints on the distance matrix for a given contact network [99].

Functional robustness

In his seminal papers [52, 53], which laid an important foundation for Systems Biology, Kitano emphasized the huge diversity of systemic properties often summarized in the term *robustness*. This robustness manifests itself, for example, in bacteria, which are extremely flexible and capable of maintaining functions essential for survival under a wide variety of conditions [100]. Signaling pathways take over a key role in these adaptation processes in all kinds of cells, and show amazingly reliable functioning [101–104]. Another example are pathogens such as viruses and bacteria, which rapidly adapt and are able to become resistant to various treatments, which today constitutes a major health care problem in hospitals [105, 106].

Robustness of dynamical processes is an important concept at the interface between Physics and Biology. A central question is whether conceptions of robustness originating

in Physics can be directly transfered in any way to Biology or whether extensions and new concepts are required (see for example [107, 108]). This topic is furthermore tightly related to questions concerning function and evolution of networks [109]. Systems Biology is creating a theory of *functional robustness*, a term or concept that is used to describe reliable and robust functioning despite perturbations, fluctuations and varying environmental conditions. Appropriate definitions and quantifications of such functional robustness measures have been developed at the interface of Physics and Systems Biology [110, 111]. This research direction also aims at finding unifying underlying principles and mechanisms for robust functioning.

Biological systems do not only face perturbations and variations in external conditions but also have to cope with intrinsic noise and stochasticity [112, 113]. An elegant stochastic approach enabling the separation of extrinsic and intrinsic noise from data of a heterogeneous cell population is presented in [114, 115]. These ideas were later used in a recent study for the design of robust synthetic signaling modules [116]. Several studies carried out in the last few years hint at the fact that network complexity is intimately linked to functional robustness, meaning that the network structure contributes to a reliable performance of tasks in the presence of perturbations and noise [101, 117–121].

Dynamic modeling approaches

Intracellular networks are nowadays commonly described by ODEs based on chemical reaction kinetics and regulatory interactions. This results in systems of coupled nonlinear equations, which show phenomena beyond the superposition principle. Here, Physics has made valuable contributions to the general field of nonlinear dynamics by developing approaches to the analysis of such systems, and by equipping mathematicians with physical examples of systems that show highly nonlinear behaviors [57].

Almost all nonlinear models are only accessible via simulations, and the broad acceptance and wide application of numerical simulations in diverse new fields also possess an inherent danger. As Robert May pointed out several years ago [122], we are approaching a point in science where the vast majority of 'users' numerically studies systems of nonlinear ODEs without an education in (Applied) Mathematics. It is certainly difficult for them to recognize pitfalls such as numerical instabilities, to apply stability analyses, to reduce model complexity (e.g., via time scale separation) or to handle uncertain parameters.

Simulating a model usually requires detailed knowledge of the systems. For example, parameters and initial conditions have to be known a priori, information that is often lacking in Systems Biology. Especially if model parameters can only vaguely be defined and the model is sensitive to small changes of some of these parameters, an analysis of these models solely based on simulations can be misleading. In consequence, these models might show a large variation when used to predict new scenarios, and their predictive power is low. There are several ways to deal with this problem. One direction is the development of identification approaches that are able to track uncertainty in measurements and model parameters to respective uncertainties in model predictions, as further outlined below. Another approach is the search for minimal models that facilitate the qualitative understanding of generic system properties. Such minimal models can be found in many Mathematical Biology textbooks, but also in Theoretical Physics. Successful reductions to

minimal models in Systems Biology, which are driven by physical concepts, can be found for gene regulation networks [1] or for biological self-organization [123].

As already discussed above, Boolean models aim to reduce model complexity by operating on a reduced state space. They are particularly helpful in analysing large networks and relations between phenotypes and intracellular network states. As an example, a Boolean model of the apoptosis network can be found in [92]. Systems Biology has also influenced further development of this kind of qualitative models, e.g. network inference from data, or various model extensions (for an overview see e.g. [94]). One article in this topical issue [30] also deals with Boolean modeling by providing a detailed comparison of ODE models with Boolean models and an intermediate, continuous-time class of models, called hybrid models.

New technologies even enable the quantification of phenomena at the single cell level that cannot be captured with ODE models. Examples include stochasticity in gene expression or stochastic fluctuations due to low copy numbers, the spatio-temporal organization within mammalian cells, or heterogeneity in populations of genetically identical cells. This opens the field for the development of novel modeling and analysis approaches beyond nonlinear ODEs. An accurate processing and description of single cell data has become a rapidly developing field in recent years, and physicists are also actively contributing. Examples are described in [124]. A major contribution of a physicist to stochastic modeling certainly is the Gillespie algorithm [125, 126], which is a dynamic Monte Carlo method for the creation of sample paths of chemical reaction networks with a low number of molecules. This algorithm is extensively used in Systems Biology, and several variations have been developed in the past decades. Further important milestones in methodology for stochastic processes such as system size expansions stem from Statistical Physics (The book [127], written by the theoretical physicist van Kampen, certainly belongs to the most famous references in this field). Linear noise approximation techniques, in particular, are nowadays frequently used in Systems Biology, e.g. for the analysis of variance of molecular species in signaling networks [114].

Model inference

A key obstacle in the development of quantitative models that capture the dynamic behavior of intracellular processes is the calibration of these models with experimental data. Since quantitative measurements are complex and expensive, the data available for model calibration is often sparse. The data contain insufficient information for the unique identification of all model parameters, and the corresponding optimization problems are ill-posed. Standard point estimators such as least squares or maximum likelihood estimators are not suitable in this context. They might not be unique, or they do not take sloppy parameters into account. This issue can partly be dealt with by data-driven modeling approaches, which take the data available for model calibration into account when choosing the granularity of the model. Good examples for such approaches can be found in [128] and [59]. Physics has also contributed to method development for revealing and handling non-identifiable parameters, especially for nonlinear models [129]. Furthermore, driven by current problems and challenges in Systems Biology, statistical methods have been established, which are able to deal with sloppy or non-identifiable parameters. Examples consist of profile likelihoods [130], sampling-based Bayesian approaches, and, related to this, stochastic model approaches. A good introduction into sampling-based

model inference in Systems Biology can be found in [131], important applications are, for example, described in [115, 132]. Related to sampling-based approaches for the estimation of model parameters, statistical methods for model comparison, model selection and model validation have also been introduced and adapted to the specific needs in Systems Biology.

What is the contribution of Physics in this context? Sampling-based approaches are computationally expensive, which limits applicability to models of medium size at most. They are (with a few exceptions, when many parameters are well-identifiable) not yet feasible for large models comprising several hundred parameters and states. However, increasing computing power, combined with advances in algorithms for the production of samples from arbitrary distributions, has, in recent years, led to a rapid increase in the size of the problems that can be handled. Substantial progress has been made in this area via Markov Chain Monte Carlo (MCMC) sampling algorithms, which produce correlated samples, but often with much higher efficiency than direct samplers. MCMC methods have their origins in Statistical Physics [133], and the Statistical Physics community has, in the past, also contributed to making naïve algorithms more efficient. An impressive example are Hamiltonian Monte Carlo methods [134], in which proposals for the next move are not completely random, but take the target distribution into account by solving Hamiltonian equations of motion [135–137]. This allows for a more efficient exploration of the target distribution and therefore faster convergence.

Apart from this, Physics has also substantially contributed to the huge field of model reduction, which deals with the question of reducing the size and complexity of a model without loosing important properties. Model reduction techniques are often required to overcome limiting storage and computational capacities. Moreover, they help to make models accessible to computationally expensive analysis methods such as for example sensitivity analysis or optimization methods for inverse model inference problems. In fact, many models in Physics, such as Newton's equations for multi-body dynamics, have suffered from expensive computations, and the Physics community has thus introduced several concepts for handling this problem, such as the well-known mean field theory for a simplified description of many interacting particles (see [138] for a review of such approaches with an emphasis on applications in Biology). One article in this topical issue introduces model reduction techniques based on optimized state aggregation for an efficient reduction of stochastic calcium release site models [139].

Conclusions

Systems Biology has two theoretical foundations: the Engineering perspective focusing on detailed, parameter-rich simulations of specific biological systems, and the Physics perspective employing minimal models and principles of coherence and collective states in order to search for design principles and universal laws. Our brief review focuses on this latter foundation that is less often discussed in other Systems Biology reviews. *EPJ Nonlinear Biomedical Physics* addresses this gap by devoting a topical issue to the Physics perspective on Systems Biology.

Core topics of contributions from (mainly Theoretical) Physics to Systems Biology include (1) statistical properties of networks, (2) the robustness of biological systems, and (3) minimal models and design principles. In this topical issue, the network view

is illustrated by [99], where a 3D chromosomal structure is derived from a contact network of genomic locations, and by [98], where time-resolved transcriptome profiles are mapped onto a transcriptional regulatory network. Robustness is discussed from the perspective of sensitivity analysis for ODE models of biological systems using the example of the MAPK pathway [60]. The notion of minimal models is nicely illustrated in the methodological comparison of modeling approaches of varying levels of detail in [30]. Another perspective on modeling is given in [139], where coarse-graining of the state space is used to simplify Markov-chain models of calcium dynamics.

The relationship between Physics and Biology has a rich, century-spanning history. An important segment of Systems Biology builds on, and continues, this history. In his defining paper on Computational Systems Biology [53], Hiroaki Kitano emphasized the danger of building up Systems Biology directly from the toolbox of complex systems (see also the more general remark by Fox Keller [140]). Physics will need to continue searching for the appropriate balance of simplicity and detail in modeling and analyzing biological situations.

It is worth noting in this context that, similar to Systems Biology, Physics, too, is a discipline which naturally deals with experimental data and their interpretation and integration into mathematical frameworks. Experimental and theoretical physicists often work in close collaboration and influence and enrich each other. Thus – in contrast to the majority of mathematicians and computer scientists – physicists are used to working with experimental data. They are, in principle, familiar with the cycle of modeling, experimentation and model refinement, which is often underestimated in other disciplines (see also [141] for an account of some of the social patterns underlying Systems Biology). This competence is certainly of great help for physicists entering into the field of Systems Biology.

At the same time, many of the methods from Physics have not yet reached their full potential in addressing biological questions. Examples include (1) the theoretical understanding of interdependent networks [142, 143], (2) a theory of dynamical processes on graphs, which may serve as an important foundation of the interpretation of 'omics' data in Biology and Medicine [144–146], (3) the effect (and potentially functional relevance) of noise on diverse levels of cellular organization – ranging from transcriptional noise to fluctuations in signaling [147] and variability in spatio-temporal pattern formation [148] and (4) a theory of biological information processing, in particular the distinction between the algorithmic level and the biochemical 'implementation' level [1].

In her remarkable essay, E. Fox Keller voices doubt as to whether the search for "all-encompassing laws" is the right approach for understanding biological systems. This is precisely why the two foundations of Systems Biology help to install the right balance between detail and generalization that any quantitative science requires. Similar to Astronomy[2], where the design of instrumentation (by Galileo) and accumulation of data (by Brahe) has lead to a theoretical understanding of patterns in the data (by Kepler) and subsequently to the formulation of natural laws (by Newton), a long path of data-driven modeling and attempts at abstraction may be required before a theoretical foundation of Biology will emerge. Contributions from Physics can be expected to play a decisive role along the way.

Endnotes

[1]More precisely, the common choice for the control parameter β is the coupling strength J divided by the temperature T, $\beta = J/k_B T$, with the Boltzmann constant k_B.

[2]This example goes back to a review by Orly Alter [149]; see also [150].

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Authors' contributions
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The authors declare that they have no competing interests.

Author details
[1] Institute for Systems Theory and Automatic Control, University of Stuttgart, Pfaffenwaldring 9, 70569 Stuttgart, Germany.
[2] School of Engineering and Science, Jacobs University Bremen, Campus Ring 1, 28759 Bremen, Germany.

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