OXFORD

Briefings in Functional Genomics, 16(2), 2017, 57–69

doi: 10.1093/bfgp/elv064
Advance Access Publication Date: 8 February 2016
Review paper

Facing the challenges of multiscale modelling of bacterial and fungal pathogen–host interactions

Jana Schleicher*, Theresia Conrad*, Mika Gustafsson, Gunnar Cedersund, Reinhard Guthke, and Jörg Linde

Corresponding author: Jörg Linde, Leibniz Institute for Natural Product Research and Infection Biology—Hans Knöll Institute, Jena, Germany. Tel.: +49-3641-532-1290; E-mail: Joerg.Linde@leibniz-hki.de
*These two authors contributed to this work equally.

Abstract

Recent and rapidly evolving progress on high-throughput measurement techniques and computational performance has led to the emergence of new disciplines, such as systems medicine and translational systems biology. At the core of these disciplines lies the desire to produce multiscale models: mathematical models that integrate multiple scales of biological organization, ranging from molecular, cellular and tissue models to organ, whole-organism and population scale models. Using such models, hypotheses can systematically be tested. In this review, we present state-of-the-art multiscale modelling of bacterial and fungal infections, considering both the pathogen and host as well as their interaction. Multiscale modelling of the interactions of bacteria, especially *Mycobacterium tuberculosis*, with the human host is quite advanced. In contrast, models for fungal infections are still in their infancy, in particular regarding infections with the most important human pathogenic fungi, *Candida albicans* and *Aspergillus fumigatus*. We reflect on the current availability of computational approaches for multiscale modelling of host–pathogen interactions and point out current challenges. Finally, we provide an outlook for future requirements of multiscale modelling.

Key words: infection; host–pathogen interaction; mathematical modelling; multiscale modelling

Introduction

A computational model is a simplified representation of a more complex, real system. Using models and data from the real system, one can deduct and infer properties about that system. Modelling has successfully been applied in various areas ranging from physics and economics to biology. There are numerous examples where

Jana Schleicher is a postdoctoral scientist in the Research Group Systems Biology and Bioinformatics (SBI) of the Leibniz Institute for Natural Product Research and Infection Biology—Hans Knöll Institute (HKI) in Jena, Germany. Her expertise is in both biology and mathematics. Her research focuses on mathematical modelling of molecular, cellular and organ scale processes to identify pathogenicity mechanisms.
Theresia Conrad is a PhD student at the SBI of the HKI within the Jena School for Microbial Communications. She is working on multiscale modelling of fungus–host interactions.
Mika Gustafsson is a senior lecturer at the Linköping University, Sweden, and a member of the Department of Physics, Chemistry, and Biology/Bioinformatics with expertise in bioinformatics and systems biology, especially constructing and analysing large-scale gene regulatory networks.
Gunnar Cedersund is a senior lecturer at the Linköping University, Sweden, a member of the Department of Clinical and Experimental Medicine and the Department of Biomedical Engineering. He is head of the Integrative Systems Biology Group, developed a new modelling approach—core-box modelling—and also developed a multiscale model for insulin resistance in type 2 diabetes.
Reinhard Guthke is Professor for Systems Biology at Jena University. He leads the Research Group SBI of the HKI. He is experienced in knowledge- and data-based modelling in medicine and biotechnology.
Jörg Linde is a postdoctoral scientist at the Research Group SBI of the HKI. He is experienced in genome-scale data analysis and network model inference with a focus on fungal infections and using dual RNA-Seq data.

even simple models are sufficient to draw conclusions that could not have been drawn without the models, or where previous conclusions drawn without the models have led to erroneous results [1, 2]. However, sometimes the structure of the underlying problem requires the use of more complex models: multiscale models.

Multiscale modelling is applied to systems that have important features across many orders of magnitude in time and space. For instance, computational weather forecasts became more realistic in the early 1980s by including the interactions of soil and vegetation with the atmosphere. The development of multiscale modelling started in the 1970s in various disciplines such as physics, meteorology and chemistry. This was driven by the advent of powerful computing platforms and the availability of a huge amount of measured data. In 2013, the Nobel Prize in Chemistry was awarded for the development of multiscale models of large complex chemical systems and biochemical reactions such as protein folding [3]. After 2000, with the development of more holistic approaches in biology and medicine (so-called 'systems biology' [4] and 'systems medicine'), the practice of multiscale modelling became more common in the life sciences. Its aim is to describe and support the understanding of human (patho)physiological functions. In the past few years, multiscale modelling has been applied successfully to the dynamics of the heart [5], liver [6–8], human metabolism [9–11] and immune system [12–15], which all are systems regulated at multiple scales of time and space and involve multiple compartments (e.g. cells, tissues and organs). This progress in the life sciences has been driven by the availability of a vast quantity of high-throughput measurements, so-called *omics* data, at the genome, transcriptome (i.e. microarray and RNA-Seq data), proteome and metabolome scales as well as progress in imaging technologies [16]. Walpole *et al.* [17] and Castiglione *et al.* [14] reviewed best practices in multiscale modelling of complex biological systems, coupling continuous and discrete modelling techniques. The 'Coordinating Action for the Implementation of Systems Medicine' across Europe published recommendations for multiscale modelling in systems medicine, including the establishment of ontologies, suitable information technology infrastructure and the development of standard operating procedures for data management and modelling [18].

Recently, we reviewed computational methods for modelling host–pathogen interactions (HPIs) [19]. It was highlighted that the systems biology of immune defence and pathogen activities needs to model HPI by including multiple scales. For example, models of the interplay between pathogens and immune cells have to include cellular interactions elucidated by the emerging image-based systems biology of infection [20, 21].

Current research in infection biology focuses on the involvement of multiple spatial and temporal scales in HPI as well as in the diagnosis and treatment of infections. Multiscale modelling in biology is the computational requisite for functional genomics studies with clinical applications; it is based on genome-wide approaches involving high-throughput methods rather than the more traditional 'gene-by-gene' approach. Here, systems medicine aims to develop multiscale computational models that integrate data and knowledge from the clinical and basic sciences. In other words, knowledge and data derived from *in vitro* experiments and animal models will be translated to the situation of individual patient's [18]. To cope with this task, modelling of HPI has to be carried out at different scales (Figure 1):

i. Molecular scale, including the genome, transcriptome, proteome and metabolome. This scale encompasses the interactome and complex molecular processes such as gene expression, gene regulatory networks, signalling and metabolic pathways involved in immunity and inflammation.
ii. Cellular scale, including the activities and behaviour of the different immune cells (e.g. T-cells and neutrophils) and different pathogen processes (e.g. bacteria or fungal conidia and hyphae formation).
iii. Inter-cellular and tissue scale, including inflammation processes and biofilm formation (e.g. quorum sensing mechanisms).
iv. Organ scale, including specific environmental conditions in each organ relevant for the infection process and the connection between organs (e.g. transfer of signals, toxins).
v. Body system scale, including multi-organ failure in sepsis and the population dynamics of the pathogen.

Our review provides a summary of state-of-the-art multiscale modelling of the interactions of microbial pathogens with the human host. While previous reviews mainly focus on bacterial infection, we additionally include results from the evolving modelling approach for fungal infections. Epidemiological studies and multiscale modelling of viral infections [22–24] are out of the scope of the present review. Vodovotz *et al.* ([25] and references therein) mainly focus on inflammation in the body, including multiscale models of sepsis. However, there is a lack of models considering both sides of HPI, i.e. both the pathogen and the host side. In future, research has to be focused more on these interaction, but bearing in mind that the interaction between pathogens themselves (see e.g. [26, 27]) is also important. Since the 2000s, papers have been published on multiscale modelling of bacterial HPIs (e.g. [28]), in particular for tuberculosis [29–38], whereas for fungal infections, the integration of multiple scales is currently in its early stages [39]. The low number of multiscale models simulating the interaction between a fungal pathogen and its host can be attributed to the more complex fungal genome and cell structure in comparison with bacteria, putting challenges on the development of suitable technical as well as computational approaches. But also important, research on fungal pathogens has attracted attention just in the past few decades, whereas bacterial pathogens had a longer research history. The increased attention may be attributed to the increasing infection rate of fungal pathogens [40]. We present—to our knowledge—the first overview of these early fungal HPI models. Our aim is to discover core areas for further research efforts and to identify the main challenges in the field of multiscale modelling.

The benefit of state-of-the-art multiscale models

Systems biology of microbial infections intents to describe and analyse the confrontation of a host with bacterial and fungal pathogens [41]. Therefore, the interactions of the host s immune system with components of the pathogen should be elucidated by iteratively using computational approaches and experimental studies that provide spatiotemporal data. The ultimate aim of systems biology is to unravel the key mechanisms of pathogenicity and then apply this knowledge to identify diagnostic biomarkers and potential drug targets, thereby improving the treatment of infectious diseases. For instance, multiscale and multicompartment models of tuberculosis were used for integration of data from multiple model systems over multiple length and time scales of the *in vivo* immune response to *Mycobacterium tuberculosis* [29–38]. Modelling development of decades has reached a state that allows the application of

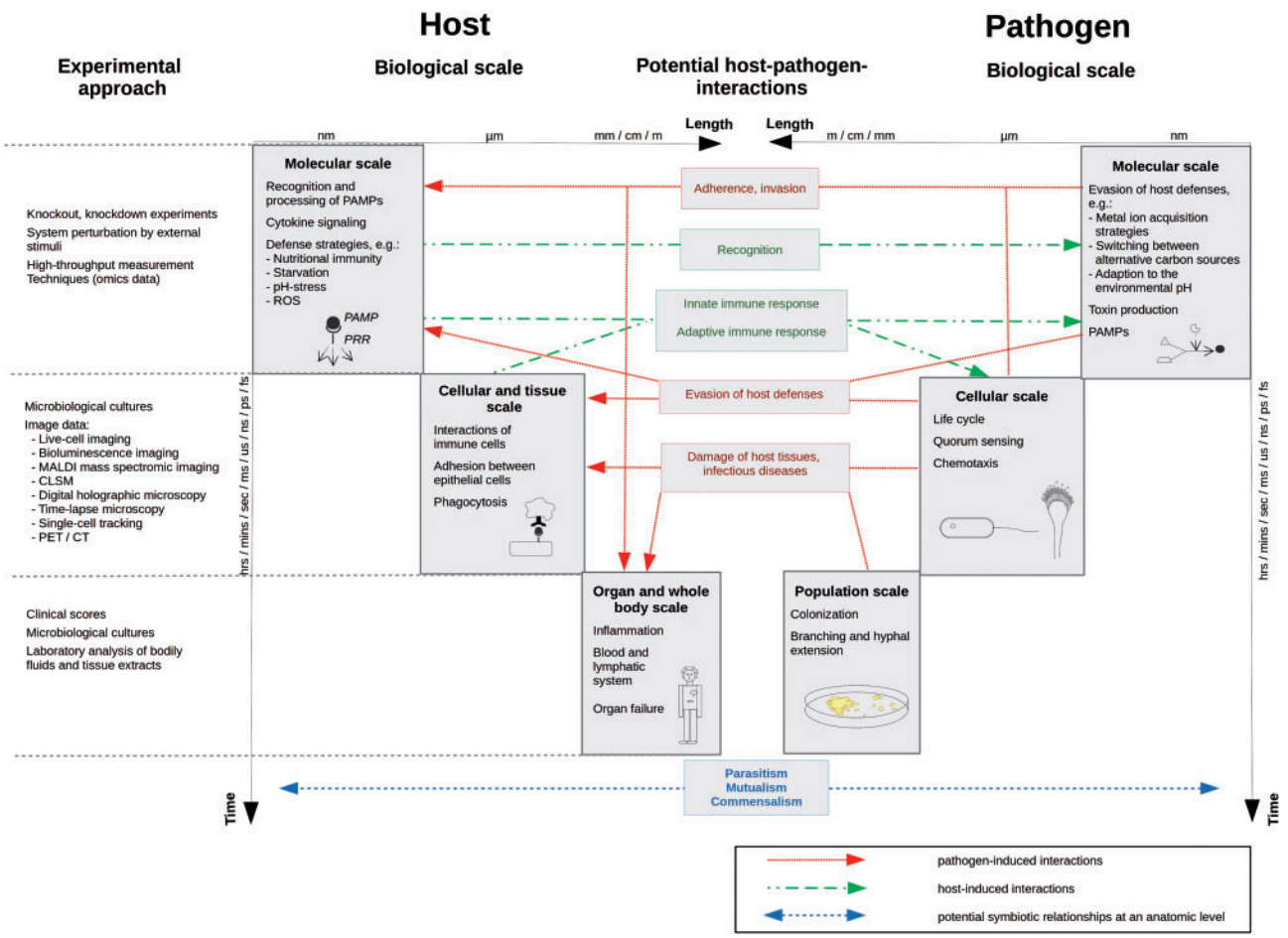

Figure 1. Schematic diagram of the complex spatiotemporal nature of HPIs, including a summary of experimental methods, which can be used at each scale. PAMP = pathogen-associated molecular pattern; PRR = pattern recognition receptor; PET = positron emission tomography; CT = computer tomography; CLSM = confocal laser scanning microscopy; MALDI = matrix-assisted laser desorption/ionization.

model predicted hypothesis in clinical settings. With help of model-based simulations, Linderman *et al.* [37] designed therapeutic interventions by immunomodulation with tumor necrosis factor alpha and interleukin 10, i.e. pro-inflammatory and anti-inflammatory cytokines, by antibiotic administration and, finally, the effect of vaccination. Cilfone *et al.* [36] applied such models to compare different therapeutic regimes for the treatment of tuberculosis. They found that inhaled formulation of the antibiotic isoniazid given at a significantly reduced dose frequency has better sterilizing efficacy and reduced toxicity than the conventional oral regimen. For modelling, they combined dynamics of lung granuloma, carrier release kinetics, pharmacokinetics and pharmacodynamics.

In general, multiscale models of HPI share the same benefits as other models in systems biology. They provide a deeper insight into the complex interplay of hosts and pathogens by providing a mechanistic understanding of the interaction network. In particular, agent-based models (ABMs) are used to model the interaction between hosts and pathogens and to improve our understanding of the underlying mechanisms (see below 'Multiscale modelling approaches of HPI'). This is because the investigated system can be modelled in a natural way by interacting individuals, and the model output allows us to capture emergent phenomena (i.e. complex patterns emerge on a higher scale through the interaction of individuals on a lower scale; [42]). HPI models can also be used to guide the setting up of experiments by *in silico* generation of hypotheses, which can be experimentally validated, frequently in an iterative cycle [43, 44]. In the field of translational systems biology, multiscale models are used to improve diagnosis of infectious diseases by biomarker discovery or to predict the clinical outcome of infections. Furthermore, such models can be used to make predictions about how a patient reacts under defined conditions or how a therapy can be optimized (i.e. therapy decision support and therapy optimization [25]).

For decades, multiscale models have been applied in pharmaceutical research and industry for drug development to predict the absorption, distribution, metabolism and excretion of synthetic or natural substances in the host [45]. Historically, such models have only been multiscale in the sense that they include both descriptions of the internal drug dynamics within an individual and the variation of key parameters across the population. In other words, in such models, which often are formulated using so-called non-linear mixed-effects models, the pharmacokinetics and pharmacodynamics are captured using simple quasi-phenomenological descriptions (PKPD models). In the past one to two decades, there has been an increasing push to also develop more realistic models, based on the physiological understanding of the involved processes. Such so-called physiologically based pharmacokinetic (PBPK) models are

compartmental and regression models, which include human or animal anatomy, physicochemical and biochemical mechanisms or toxicological effects. This push has gained further momentum through the rise of the field Systems Pharmacology, which attempts to combine intracellular systems biology models with whole-body scale PBPK models. In general, these kinds of pharmacometric models, independently of the degree of detail, have been used to successfully optimize the drug administration regimes and to extrapolate from animal models to the human host.

A typical application of multiscale models in infection biology is antibiotic administration. Frequently, different drugs with different molecular features are compared. Predictive chemistry models, namely the so-called quantitative structure–activity relationship (QSAR) models, may be integrated in multiscale models. QSAR models have been used for risk management. They are recommended by regulatory authorities for registration, evaluation, authorization and restriction of chemicals [46].

An important potential and benefit of multiscale modelling of HPI is the replacement, refinement and reduction of animal trials in research, the so-called '3Rs', by *in silico* experiments during the transition from *in vitro* experiments to clinical trials. Regarding this, a major breakthrough was recently achieved in type 1 diabetes: now the Food and Drug Administration allows for the usage of a multi-PBPK model for glucose homeostasis instead of test animals when certifying certain insulin treatments [47].

Experimental methods relevant for computational modelling

A central requirement for multiscale modelling in HPIs is the availability of suitable measurement data (Figure 1). These data are necessary to estimate model parameter values and to refine model structure, as well as to validate the models by testing the model-derived predictions.

At the molecular scale, various high-throughput measurement techniques have been developed over the past decades. Next-generation sequencing [48] allows us to assemble complete high-quality genomes of microbes, to structurally and functionally annotate genomes [49] and to identify genomic changes as risk factors on the host side [50]. Expression data can be used for diagnosis. For instance, in a genome-wide expression study, a supervised machine learning approach was applied for classification of bacterial and fungal whole-blood infections [51]. The latest advances in hybrid tandem mass spectrometry [e.g. triple quadrupole, quadrupole time-of-flight, Orbitrap hybrid mass spectrometer (tandem-in-space instruments) and ion-trapping mass spectrometers (tandem-in-time instruments)] make it possible to analyse complex proteoms with a high resolution, sensitivity and mass accuracy. In addition, various mass spectrometry imaging [e.g. matrix-assisted laser desorption/ionization (MALDI imaging)] and Raman spectroscopic imaging techniques can be used to measure the abundances and spatial distributions of proteins and metabolites in a tissue.

As eukaryotes, fungi have larger and more complex genomes than bacteria. Therefore, complete sequenced genomes of fungi were available at a later time point than bacterial genomes. Availability of the genome sequence allows identification of specific infection and interaction pathways, the discovery of drug targets, as well as species-specific microarrays. Moreover, genetic manipulations (knock-out, knock-down, overexpression) of fungi are more challenging.

A challenge in connecting the molecular scale to the cellular scale is the heterogeneous nature of biological samples, i.e. samples are composed of cell types with different gene expression profiles. In infection biology, this issue is most pronounced for organ samples (e.g. lung, liver and brain) and blood assays. To deal with mixed samples in gene expression analyses, in the past decade, several groups developed expression deconvolution algorithms, e.g. [52–57]. These algorithms allow the extraction of information on a cell-based scale from heterogeneous biological samples (for an introduction see [58, 59]). A variety of these algorithms were combined in the R package CellMix [60], which allows for an efficient estimation of cell type proportions and cell type-specific expression profiles in mixed samples. Similarly, the R package DeconRNASeq also enables deconvolution of mRNA-Seq data from mixed samples [61].

For storage and access of *omics* data, several data repositories are available (e.g. GenBank, Gene Expression Omnibus, ArrayExpress, PRIDE). Other repositories provide knowledge on functional genomics, i.e. genome annotation of both hosts and pathogens [19, 39, 62]. The database PHISTO, a web-based HPI search tool, stores known molecular relations between pathogens and the human host, extracted by text mining from scientific papers [63]. Such molecular biological databases have been used to infer interolog-based networks for the molecular interaction of the pathogen *Candida albicans* with its animal and human hosts [64, 65].

While advances in *omics* techniques drive the progress of multiscale modelling on the molecular scale, there has also been significant progress on the cellular scale based on imaging data from positron emission tomography/computer tomography, bioluminescence imaging, confocal laser scanning microscopy, live cell imaging, time-lapse microscopy, single-cell tracking, digital holographic microscopy and MALDI mass spectrometric imaging. Although the automated analysis of image and video data from HPI remains a challenging task [66–68], it holds great potential because it automatically extracts important parameters such as velocity or turning angles for individual cells. Moreover, automatic analysis identifies interactions between individual host and pathogen cells, such as touching events, adherence or phagocytosis. Such data drive the emerging image-based systems biology of infection [20, 21]. The integration of both *omics* and image-based sub-models in multiscale models is challenging owing to the requirement of combining different modelling techniques. Here, an outstanding task is to combine the non-spatial *omics* data with the image-based sub-models that generally have an inherent spatial scale.

For modelling at the cellular, tissue and organ scales, biomechanical, rheological and physicochemical parameters become important. For example, cytometric data and data quantifying the deformability of erythrocytes (e.g. *Plasmodium falciparum*-parasitized red blood cells) were analysed and modelled using a particle-based simulation technique (i.e. dissipative particle dynamics) for different stages of malaria [69].

In general, the analysis tools to investigate HPI at the cellular, tissue, organ and whole body scales stem from various medical disciplines such as radiology, clinical/medical microbiology, clinical immunology, cytopathology, clinical chemistry/medical biochemistry, haematology and clinical pathology. Diagnostics of infectious diseases affecting the whole body are based on the laboratory analysis of body fluids, such as blood, urine, sputum and tissue extracts by macroscopic or microscopic analysis. Clinical scores summarize the status of an infection by

Table 1: A selection of modelling approaches used to examine HPIs

Reference	Host	Pathogen	Scales*	Time-independent modelling approach		Continuous time modelling approach		Discrete time modelling approach				
				Constraint-based	Game theory	ODEs	PDEs	Agent-based	State-based	Cellular automata-based	Boolean	Pro-babilistic
Single application of the main modelling approach												
Thakar *et al.* [74]	Mammalian host	*Bordetella bronchiseptica*	1: Cytokines 2: Immune cells 3: Lung, lymph nodes, bacterial growth	x								
Hummert *et al.* [75]	Human host	*Candida albicans*	2: Macrophages, ingested yeast cells, fungal survival strategies 3: Fungal growth (fitness)		x							
Eswarappa [76]	Mammalian host	Pathogenic bacteria (persistent infections)	1, 2: Extra-, intra-cellular compartments and defence mechanisms 3: Bacterial growth		x							
Tierney *et al.* [77]	Murine host	*Candida albicans*	1: Gene expression, cytokines 2: Innate immune cells			x						
Boswell *et al.* [78]	Plant root system	*Rhizoctonia solani*	1: Fungal uptake of external substrate 2: Growth of fungal mycelia				x					
Tokarski *et al.* [79]	Human host	*Aspergillus fumigatus*	1: Chemical communication 2: Phagocytes, chemotaxis, clearing efficiency, conidia, lung 3: Fitness					x				
Hünniger *et al.* [80]	Human host	*Candida albicans*	1: Antifungal effector molecules, cytokines 2: Immune cells, whole-blood 3: Distribution of fungal cells						x			
Wcislo *et al.* [81]	Wheat	*Fusarium graminearum (invasion, colonization)*	1: Nutrient concentrations, secreted substances 2: Plant cells, fungal cells 3: Fungal growth							x		
Thakar and Albert [82]	Mammalian host	*Helicobacter pylori, Bordetella bronchiseptica, Bordetella pertussis*	1: Cytokines, antibodies 2: Immune cells, bacterial cells								x	
Grant *et al.* [83]	Murine host	*Salmonella enterica*	1: Bacterial genetic diversity 2: Infected cells 3: Liver, spleen, blood; bacterial growth and death									x

(continued)

Table 1. Continued

Reference	Host	Pathogen	Scales*	Time-independent modelling approach		Continuous time modelling approach		Discrete time modelling approach				
				Constraint-based	Game theory	ODEs	PDEs	Agent-based	State-based	Cellular automata-based	Boolean	Probabilistic
Combined application of different modelling approaches												
Cilfone *et al.* [36]	Non-human primates	*Mycobacterium tuberculosis*	1: Cytokines, granuloma function, antibiotics (carrier) 2: Immune cells, granulomas formation, receptor-ligand dynamics, lung			x	x	x				
Linderman *et al.* [37]	Non-human Primates	*Mycobacterium tuberculosis*	1: Cytokines, antibodies, granuloma function, antibiotics 2: Immune cells, granuloma formation, receptor-ligand dynamics, lung 3: Bacterial growth			x	x	x				
Tyc [84]	Human host	*Candida albicans*	1: Drug treatment, environmental conditions, virulence factors 2: Immune cells, virulence factors 3: Fungal growth and phenotypes		x	x		x				
Carbo *et al.* [85]	Murine host	*Helicobacter pylori*	1: Virulence factors, cytokines 2: Immune cells, gastric lumen, epithelium, lamina propria, lymph nodes 3: Gastric mucosa, bacterial colonization			x		x				
Pollmächer *et al.* [86]	Human host	*Aspergillus fumigatus*	1: Chemokines 2: Leucocytes, alveoli, conidia					x**				

Besides introducing models that only use one modelling approach to simulate various scales, we also provide references of models in which multiple approaches were combined. These models provide valuable ideas how the problem of combining different models of various scales can be sorted out.

ODE = ordinary differential equation; PDE = partial differential equation.

*1: Molecular scale; 2: Cellular and tissue scale; 3: Organ and whole body scale, population scale.

**ABM combination of migration and interaction in continuous space with spatio-temporal modelling on a discrete grid.

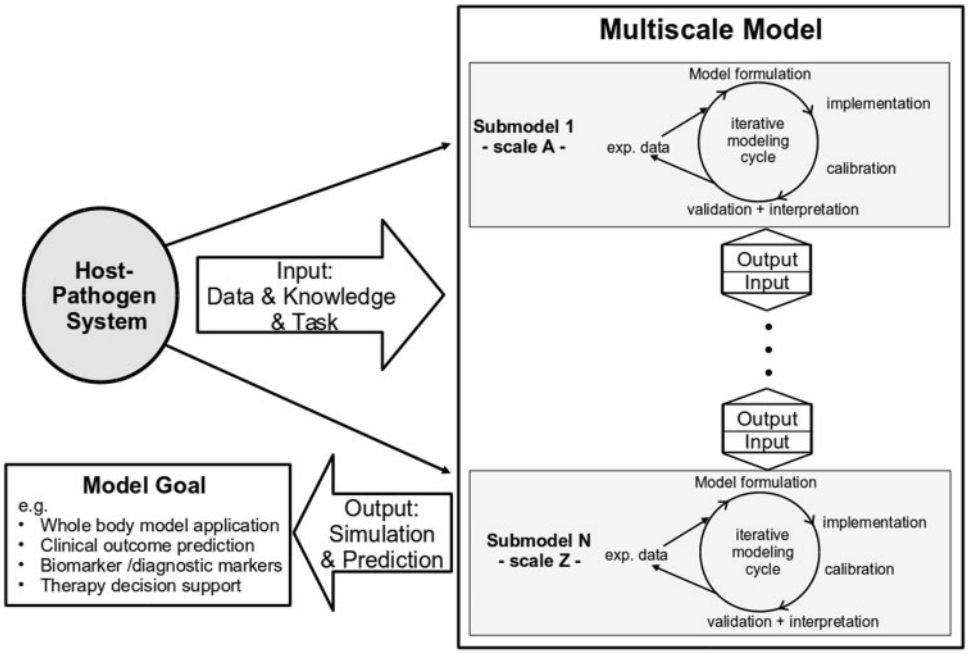

Figure 2. Schematic overview of a multiscale model structure. Sub-models on various scales are used to examine multiscale HPIs. In each sub-model the iterative cycle of modelling and experimental calibration and validation has to be passed through.

combining clinical parameters with observation of the infected individual. For example, the 'Clinical Pulmonary Infection Score' (CPIS) and the 'Sepsis-related Organ Failure Assessment Score' (SOFA) [70], used to classify patients with severe sepsis, are sometimes recorded as easily attainable data on the host side. Clinical scores are also used in animal trials of infection studies and usually include body temperature, weight, activity and feeding patterns. In contrast, pathogens are identified in the laboratory using microbiological cultures. The multiscale model-based personalized treatment of infectious diseases will be based on the stratification of patients by analysing both observed clinical phenomena of physiologic variability and molecular patterns that characterize the immunological state.

In general, for experimental validation of multiscale models, adequate experimental systems that focus on individual modules of interest are needed. To make model results reliable and useful, for example for clinics, verification of a multiscale model has to be conducted on each implemented scale. Therefore, it is necessary to obtain experimental data from the different spatio-temporal scales (Figure 1). In the future, so-called microphysiological systems, e.g. organs-on-chips or tissue-engineered 3D organ constructs that use human cells, provide an alternative to animal testing [71]. On the bioinformatics side of validation, Pârvu and Gilbert [72] developed a methodology for automatic validation of multiscale computational models.

Multiscale modelling approaches of HPI

Dada and Mendes [73] and Walpole *et al.* [17] have characterized the main modelling approaches; here, we provide an overview of their application in multiscale HPI modelling (Table 1). Simple modelling approaches, which can include multiple scales of HPI but neglect time, are (evolutionary) game theoretical concepts and constraint-based models. Often, HPI modelling requires the behaviour of the simulated system over time to be considered (e.g. with regard to infection time or time for immune response). In dynamic modelling, a system can be simulated in a continuous or discrete-time context, depending on the model aim and the chosen computational approach. In this section, we start by reviewing time-independent models of HPI, followed by models in continuous time and models using discrete time. Finally, combining the advantages of different modelling approaches on different scales offers an opportunity for multiscale modelling. Thus, we introduce mixed models linking different modelling approaches and exemplify their application to HPI.

In general, game theory concepts [87] are used to examine the possible outcomes of interactions, in which real world entities are represented as 'players' who take part in a 'game' with the aim of optimizing some sort of pay-off. Players can choose between different strategies. To find an optimal solution for the game, the approach takes into account the costs and benefits of each strategy in relation to the strategy chosen by the other player. The application of this concept to evolving organisms or populations is termed evolutionary game theory. With this approach the evolutionary dynamics of strategy changes of interacting species can be examined depending on the frequencies of strategies and the fitness gain for each strategy. As a recently published example, Li *et al.* [88] studied the *in vitro* population dynamics of two commensal bacteria that synergistically protect the metazoan host *Hydra vulgaris* from fungal infection. Another example is the modelling of interplay of drug-resistant and drug-sensitive pathogens under antibiotic treatment [89]. In HPI, evolutionary game theory may be used in future to elucidate the adaptation of evasion strategies of pathogens or defence strategies of the host over time. With a more phenotypic and generalized view, game theory can also be applied to model the interaction between pathogens and humans or the interaction between different pathogens. For example, this approach was used to understand what advantages the human fungal pathogen *C. albicans* experiences by changing its morphological form [75, 90] in the context of interacting with the

host's immune cells. Additionally, persistent bacterial infection was described by developing a game theoretical model; predictions regarding persistent bacterial infections were drawn by considering the ability of a pathogen to survive extracellularly and intracellularly, within an immune cell [76].

Another knowledge-based approach for large-scale modelling is so-called constraint-based modelling. The idea of constraint-based modelling is to describe a biological system by a set of knowledge-based constraints, which characterize its possible behaviours but in general do not allow a precise prediction to be made. This modelling approach has mainly been applied to the modelling of metabolic networks [91–93]. Jamshidi and Raghunathan [94] outlined a systematic procedure to produce constraint-based HPI models. Interestingly, a constraint-based network model of HPI was also presented to describe the dynamic outcome of the interplay between host immune components and *Bordetella bronchiseptica* virulence factors [74].

Typical modelling approaches using a continuous time context consist of ordinary differential equations (ODEs) or, if space is included in the model, partial differential equations. ODE-based modelling is widely used at the molecular scale, such as for gene regulatory network models [95]. Here, gene expression analysis by RNA-Seq offers the opportunity to monitor and model the transcriptome of both the pathogen and host, as shown for the interaction of *C. albicans* with murine dendritic cells using an ODE-based approach [77]. Generalizing this work, methods for exploiting dual RNA-Seq data for the inference of gene regulatory networks of HPI has been presented [96].

In addition to their utility at the molecular scale, ODE modelling is also applicable for whole cell simulations and at the body scale (e.g. pathogen population scale; PBPK models [45]). Palsson *et al.* [13] published a fully integrated immune response model (FIRM) consisting of multiple sub-models, a multi-organ structure, circulating blood, lymphoid tissue, different immune cell types and cytokines and immune cell recruitment. FIRM was tested by simulating the response to a blood-borne pathogen (i.e. tuberculosis infection). An ODE-based simulator has the flexibility to be expanded. It is suitable for step-by-step interactive integration of further sub-models, describing the processes within the pathogen and their interaction with the host. FIRM may be a starting point for multiscale modelling of HPI.

In addition, the Lotka-Volterra model, well known for simulations of predator–prey interactions, can be used for multiscale modelling of HPI. The system consists primarily of a pair of first-order, non-linear ODEs, but the equations can be generalized to include, for example, trophic interactions, spatial structures and more than two species (e.g. [97]). Stein *et al.* [98] studied the dynamic stability of intestinal microbiota by use of a generalized Lotka-Volterra model for focal species to account for external perturbations representing antibiotics or diet.

Some aspects of HPI require the application of discrete time intervals, therefore permitting the use of agent-based [99], state-based [80] and cellular automata-based [81] Boolean [82] or probabilistic models [83, 100]. These approaches were used to model the HPI taking into account individual genes or cells (e.g. immune and pathogen cells) in time and, partly, space [20, 21].

In an ABM, the behaviour and interaction of autonomous agents are simulated over time to examine the emergence of complex phenomena on a higher scale. Each agent gets a set of rules determining its method of interaction and behaviour, thus making ABMs a promising tool for studying HPI and, more generally, infectious diseases and inflammatory processes [101]. The advantage of the agent-based modelling approach is the possibility of relatively easily integrating space (e.g. as a discrete grid) and, additionally, accounting for variability (e.g. in behaviour or movement) among individual cells and/or cell types.

HPI in anastomotic leaks was examined by using the agent-based modelling approach [102]. An ABM of epithelial restitution was augmented by individual *Pseudomonas aeruginosa* agents interacting with the epithelium. The simulation of different killing mechanisms leads to a mechanistic understanding of tissue destruction.

An agent-based approach was also used by Tokarski *et al.* [79] to investigate the clearance efficiency of *Aspergillus fumigatus* conidia by neutrophil granulocytes. A combination of live imaging and grid-based modelling of individual cells allows *in silico* testing of different hypotheses for hunting strategies of immune cells. This modelling approach demonstrated that chemokine sensing by immune cells is the most efficient strategy. The ABM was implemented in the free software tool NetLogo [103, 104]. This well-established tool facilitates a user-friendly and efficient programming of ABMs. SPARK (Simple Platform for Agent-based Representation of Knowledge) is an alternative tool for multiscale ABMs that runs faster [105].

Besides the ABM approach, theoretical modelling in discrete time can also be realized by the use of Boolean networks [106]. In the past, Boolean models were developed to describe and simulate within-host immune interactions (reviewed by [82]). This heuristic modelling approach allows prediction of new interaction pathways or drug targets within the host–pathogen infection system. For example, a Boolean modelling technique was applied to model the signal transduction of the hepatocyte growth factor pathway of the human host in response to infection by *Helicobacter pylori* [107]. This model predicts new molecular targets against *H. pylori* infection, which were experimentally verified.

The combined application of different modelling approaches on multiple scales may facilitate multiscale modelling of HPI. As a prominent example, for multiscale modelling of *M. tuberculosis* infection, a system of ODEs to capture intracellular signalling pathways was combined with a discrete probabilistic ABM that describes cellular behaviour at the tissue scale [29–38] and references therein). Also, for the interaction of *C. albicans* with the human host, ODE-based, agent-based and game theory-based modelling methods were compared and partially combined [84].

A combination of ODE-based modelling with ABM was used to model the mucosal responses during *H. pylori* infection [85]. This hybrid model considers immune effector cells (i.e. macrophages, T-helper cells and pro-inflammatory epithelial cells) that secrete cytokines and chemokines, which recruit immune cells and promote their activation and differentiation to inflammatory phenotypes, and, finally, secrete effector molecules that destroy bacteria and may cause tissue damage.

A multiscale model simulating the distribution of chemokine concentrations in *A. fumigatus*-infected human alveoli was developed by combining an ABM of migration and interaction in continuous space with spatiotemporal modelling on a discrete grid [86].

In general, multiscale modelling has to reuse and link different sub-models (Figure 2). This requires a multiscale computational infrastructure and (sub-)model repositories. The systems biology markup language is the most developed standard concept at the moment and is increasingly used to support the exchange of models in the modelling community. In future, this concept may be expanded to support also multiscale models.

Challenges and outlook

Systems biology of microbial infection encompasses all scales of the pathogen and the host's immune system, leading to a complex interaction network on multiple scales. A common challenge in systems biology is the successful combination of both experimental and theoretical approaches. In this context, an iterative cycle should be applied in which model development and refinement, parameter calibration and *in silico* experiments alternate with experimental data collection and hypothesis/prediction validation (Figure 2). The application of an iterative cycle of experiments and model refinement for fungal pathogens is at the moment connected to more effort than for bacterial pathogens. In bacteria, genes of the same pathway and also virulence genes are often clustered together in an operon allowing a shared regulation. This clustering facilitates the study of regulatory mechanisms and enables a relatively simple mathematical replication of the regulatory processes in bacteria. Such structured, regulatory units are not present in fungi which makes it more difficult to find virulence genes, to understand their regulation and finally to develop a representative mathematical model of the regulatory network. In addition, in fungi there are secondary metabolite gene clusters characterized by complex structures of co-regulation [108, 109].

Moreover, fungi have complex life cycles with multiple morphological forms. They may occur as unicellular yeast or in a filamentous form (dimorphism). This indicates the need of implementing a broader spatial scale in fungal models than in bacterial interaction models. Furthermore, fungi have developed multiple sophisticated, specific and unique pathogenicity mechanisms including immune evasion strategies. Only a few of them are modelled by game-theoretical methods and ABMs [110] and many of the pathogenicity mechanisms are not well understood, e.g. the production of hydrophobins on the spore surface as an immune evasion strategy of the environmental fungus *A. fumigatus*.

At the start of developing a multiscale model of a complex biological system, researchers have to bear in mind that the aim of a model is not to completely mirror the real system. Essentially, the most important aspect of modelling relates to the wise reduction of the complexity of the investigated system to identify key properties. The parts to be implemented in a model and the parts to be left out are dictated by the biological question(s) that will be addressed with the model. Kirschner *et al*. proposed a 'tunable resolution' for multiscale models [111], in which sub-models at different scales are defined and connected. In case a specific question requires additional parameters, these can be added to one of the sub-models. *Vice versa*, more coarse-grained sub-models can be applied if the details on lower scales are not needed for the question in focus.

A further challenge in multiscale modelling of HPI is the combination of different time scales. While regulatory interactions on the transcriptomic scale take place in minutes, it may cause effects on the cell-, tissue- or organ-scale hours or days later. Approaches allowing a transition from one time scale to another need to be developed. For example, Chaves *et al*. [112] presented three asynchronous algorithms to meet this challenge for genetic regulatory networks using the example of Boolean models, which could also be applicable to multiscale problems.

Moreover, the number of features per scale is variable. The human body consists of several organs; each organ itself consists of millions of cells, and each cell has several thousands of proteins and transcripts. Thus, to develop an efficient multiscale model, stringent feature selection with a strong focus on the simulated phenomenon and model aim is essential.

A large number of parameters is an integral part of multiscale models, but the many parameters are also associated with some problems that must be overcome. The allowance of many parameters has positive implications, because it allows for a more realistic description of the system. In contrast, few parameters and minimal models may often imply that overly simplified and lumped descriptions of states and processes have to be used, which may be hard to interpret physiologically. One of the reasons why a high degree of parameters is negative is that it is hard to ensure that all of them have realistic values. Granted, some of the parameters may have values that can be determined in independent experiments, but in biology, and especially for large multiscale models, there are often many parameters that have to be inferred simultaneously from systems-wide dynamic data. This determination is often not unique: a problem known as parameter unidentifiability [113]. If untreated, such unidentifiability implies that all model predictions come with an arbitrarily large uncertainty range, i.e. the predictions are suggestions and not unique consequences of the model and data. Fortunately, recent progress in model analysis has allowed for the identification of such uniquely inferred predictions. Such predictions, sometimes called core predictions, are predictions that are uniquely determined from the data, even though the parameter values are non-unique. In practice, a three-step approach has been proposed by Cedersund, which allows for the accurate identification of the outer boundaries of such predictions [113]. Nevertheless, these methods are still only applicable to small- and medium-sized models, ranging between 1 and 50 parameters. For truly large multiscale models, further method developments are needed.

For ethical reasons, experiments designed to calibrate parameter values in a human model might not be realizable in the human body or in animals. In these cases, the parameter values have to be identified using sub-models and exploiting data measured under *in vitro* conditions despite the fact that the value may be different *in vivo*. The same may be true for model validation, especially for HPI models. Suitable *in vitro* systems that mimic the pathophysiology of infection in humans have to be used.

In summary, HPIs should be described by a combination of spatiotemporal models with interacting molecular networks of both the host and the pathogen. Considerable advances in multiscale modelling of microbial HPI have been made in the study of tuberculosis. In this case, and for other human infections including fungal infection by *C. albicans* and *A. fumigatus*, ODE-based, state-based and ABMs are the main techniques for successful modelling at the molecular and cellular scales. In the future, high-throughput *omics* and image data should be simultaneously considered and modelled in an integrated manner.

A promising approach to multiscale modelling is hierarchical modelling (see also Figure 2), in which the sub-models at each scale appear in a well-defined place in a super-model, in a so-called tree-structure. Such models have a natural modular structure, where one version of sub-models can be replaced for one another, to better suit the particular data and question that is studied. This approach has been relatively well-developed in technical systems, and in biological systems an important application involves glucose homeostasis and diabetes [9]. By

combining input–output data with the data for a sub-module, one can consider the modelling of a sub-module as an isolated modelling problem. Any model for the sub-module can be expected to fit into the dynamics of the super-model as long as the sub-model reproduces the measured output when exposed to the measured input. Thus, these input–output data also allow for a meaningful way of combining both *in vitro* and *in vivo* data. This approach was presented in [9] and [10], and in [11] the approach was used to unravel both where insulin resistance appears inside individual fat cells, and how this resistance spreads to the rest of the body.

Regarding hierarchical modelling of HPI, the software tool SPARKS [106] might be useful, but the development of easy-to-use software applications for multiscale modelling will be an important task in the coming decades. Currently, there are further initiatives to establish a computational framework for multiscale modelling [114]. Andasari *et al*. [115] presented a multiscale, individual-based simulation environment that integrates a lattice-based Cellular Potts Model on the cellular scale (CompuCell3D) and an ODE-based Bionetsolver for intracellular modelling of reaction-kinetic network dynamics. This hybrid system has been applied to cancer research. The system may also be suitable for HPI modelling. Furthermore, the WholeCellKB is an open-source web-based software program for multiscale *omics* modelling and, in particular, WholeCellKB-MG enables whole-cell modelling of the human pathogen *Mycoplasma genitalium* by integrating diverse data sources into a single database [116, 117].

From a systems medicine perspective, the multi-layered HPI models should ideally also make use of all the available clinical information at hand, such as information regarding a patient's disease history and life-style factors, which probably will be extended to genotype information in the future. These factors may affect the system's response to stimuli via differences in the initial conditions of the state variables or by altering the effective regulatory interactions.

Finally, we want to highlight the fact that interactions also take place between different populations of bacteria and fungi (e.g. quorum sensing), which may positively or negatively influence the infection process in the host and determine the outcome of HPI (e.g. [118]). It is a future task to develop models that account for the interactions among three or more species (i.e. parasites, fungi, bacteria, viruses and host) by integrating the species and their unique characteristics at various temporal and spatial scales. Additionally, multiscale models need to combine models for intra-species communication (e.g. [26, 27]) with models for HPI.

Key Points

- Multiscale modelling of host–pathogen interactions has to consider processes at various temporal and spatial resolutions.
- The scales range from minutes to days and include the molecular, cellular, tissue and organism scales of the host and the molecular, cellular and population scales of the pathogen.
- Integrating across these scales requires multiple modelling approaches, such as ordinary and partial differential equations, state-based models and agent-based models.

Funding

This work was supported by the Jena School for Microbial Communication [to T.C.] and the Deutsche Forschungsgemeinschaft CRC/Transregio 124 'Pathogenic fungi and their human host: Networks of interaction' subproject INF [to J.L., R.G.].

References

1. Jullesson D, Johansson R, Rajan MR, *et al*. Dominant negative inhibition data should be analyzed using mathematical modeling–re-interpreting data from insulin signaling. *FEBS J* 2015;**282**(4):788–802.
2. Nyman E, Lindgren I, Lövfors W, *et al*. Mathematical modeling improves EC50 estimations from classical dose-response curves. *FEBS J* 2015;**282**(5):951–62.
3. Fersht AR. Profile of Martin Karplus, Michael Levitt, and Arieh Warshel, 2013 nobel laureates in chemistry. *Proc Natl Acad Sci USA* 2013;**110**(49):19656–7.
4. Kitano H. Systems biology: a brief overview. *Science* 2002;**295**(5560):1662–4.
5. Qu Z, Garfinkel A, Weiss JN, Nivala M. Multi-scale modeling in biology: how to bridge the gaps between scales? *Prog Biophys Mol Biol* 2011;**107**(1):21–31.
6. Schliess F, Hoehme S, Henkel SG, *et al*. Integrated metabolic spatial-temporal model for the prediction of ammonia detoxification during liver damage and regeneration. *Hepatology* 2014;**60**(6):2040–51.
7. Kuepfer L, Kerb R, Henney AM. Clinical translation in the virtual liver network. *CPT Pharmacometrics Syst Pharmacol* 2014;**3**:e127.
8. Schwen LO, Schenk A, Kreutz C, *et al*. Representative sinusoids for hepatic four-scale pharmacokinetics simulations. *PLoS One* 2015;**10**(7):e0133653.
9. Cedersund G, Strålfors P. Putting the pieces together in diabetes research: towards a hierarchical model of whole-body glucose homeostasis. *Eur J Pharm Sci* 2009;**36**(1):91–104.
10. Nyman E, Brännmark C, Palmér R, *et al*. A hierarchical whole-body modeling approach elucidates the link between *in vitro* insulin signaling and *in vivo* glucose homeostasis. *J Biol Chem* 2011;**286**(29):26028–41.
11. Brännmark C, Nyman E, Fagerholm S, *et al*. Insulin signaling in type 2 diabetes: experimental and modeling analyses reveal mechanisms of insulin resistance in human adipocytes. *J Biol Chem* 2013;**288**(14):9867–80.
12. Santoni D, Pedicini M, Castiglione F. Implementation of a regulatory gene network to simulate the TH1/2 differentiation in an agent-based model of hypersensitivity reactions. *Bioinformatics* 2008;**24**(11):1374–80.
13. Palsson S, Hickling TP, Bradshaw-Pierce EL, *et al*. The development of a fully-integrated immune response model (FIRM) simulator of the immune response through integration of multiple subset models. *BMC Syst Biol* 2013;**7**:95.
14. Castiglione F, Pappalardo F, Bianca C, *et al*. Modeling biology spanning different scales: an open challenge. *Biomed Res Int* 2014;**2014**:902545.
15. Azhar N, Vodovotz Y. Innate immunity in disease: insights from mathematical modeling and analysis. *Adv Exp Med Biol* 2014;**844**:227–43.
16. Watabe M, Arjunan SN V, Fukushima S, *et al*. A computational framework for bioimaging simulation. *PLoS One* 2015;**10**(7):e0130089.

17. Walpole J, Papin JA, Peirce SM. Multiscale computational models of complex biological systems. *Annu Rev Biomed Eng* 2013;**15**:137–54.
18. Wolkenhauer O, Auffray C, Brass O, *et al*. Enabling multiscale modeling in systems medicine. *Genome Med* 2014;**6**(3):21.
19. Durmuş S, Çakir T, Özgür A, Guthke R. A review on computational systems biology of pathogen-host interactions. *Front Microbiol* 2015;**6**:235.
20. Figge MT, Murphy RF. Image-based systems biology. *Cytometry A* 2015;**87**(6):459–61.
21. Medyukhina A, Timme S, Mokhtari Z, Figge MT. Image-based systems biology of infection. *Cytometry A* 2015;**87**(6):462–70.
22. Heldt FS, Frensing T, Pflugmacher A, *et al*. Multiscale modeling of influenza A virus infection supports the development of direct-acting antivirals. *PLoS Comput Biol* 2013;**9**(11):e1003372.
23. Rong L, Perelson AS. Mathematical analysis of multiscale models for hepatitis C virus dynamics under therapy with direct-acting antiviral agents. *Math Biosci* 2013;**245**(1):22–30.
24. Murillo LN, Murillo MS, Perelson AS. Towards multiscale modeling of influenza infection. *J Theor Biol* 2013;**332**:267–90.
25. Vodovotz Y, An G. *Translational Systems Biology. Concepts and Practice for the Future of Biomedical Research*. Elsevier Science Publishing Co Inc, London, UK, 2014.
26. Ben-Jacob E, Becker I, Shapira Y, *et al*. Bacterial linguistic communication and social intelligence. *Trends Microbiol* 2004;**12**:366–72.
27. Ben-Jacob E. Social behavior of bacteria: from physics to complex organization. *Eur Phys J* 2008;**3**:315–22.
28. Foteinou PT, Calvano SE, Lowry SF, Androulakis IP. Multiscale model for the assessment of autonomic dysfunction in human endotoxemia. *Physiol Genomics* 2010;**42**(1):5–19.
29. Wigginton JE, Kirschner D. A model to predict cell-mediated immune regulatory mechanisms during human infection with *Mycobacterium tuberculosis*. *J Immunol* 2001;**166**(3):1951–67.
30. Fallahi-Sichani M, El-Kebir M, Marino S, *et al*. Multiscale computational modeling reveals a critical role for TNF-α receptor 1 dynamics in tuberculosis granuloma formation. *J Immunol* 2011;**186**(6):3472–83.
31. Marino S, Linderman JJ, Kirschner DE. A multifaceted approach to modeling the immune response in tuberculosis. *Wiley Interdiscip Rev Syst Biol Med* 2011;**3**(4):479–89.
32. Fallahi-Sichani M, Kirschner DE, Linderman JJ. NF-κB signaling dynamics play a key role in infection control in tuberculosis. *Front Physiol* 2012;**3**:170.
33. Fallahi-Sichani M, Flynn JL, Linderman JJ, Kirschner DE. Differential risk of tuberculosis reactivation among anti-TNF therapies is due to drug binding kinetics and permeability. *J Immunol* 2012;**188**(7):3169–78.
34. Ghosh S, Baloni P, Mukherjee S, *et al*. A multi-level multiscale approach to study essential genes in *Mycobacterium tuberculosis*. *BMC Syst Biol* 2013;**7**(1):132.
35. Cilfone NA, Perry CR, Kirschner DE, Linderman JJ. Multi-scale modeling predicts a balance of tumor necrosis factor-α and interleukin-10 controls the granuloma environment during *Mycobacterium tuberculosis* infection. *PLoS One* 2013;**8**(7):e68680.
36. Cilfone NA, Pienaar E, Thurber G, *et al*. Systems pharmacology approach toward the design of inhaled formulations of rifampicin and isoniazid for treatment of tuberculosis. *CPT Pharmacometrics Syst Pharmacol* 2015;**4**(3):193–203.
37. Linderman JJ, Cilfone NA, Pienaar E, *et al*. A multi-scale approach to designing therapeutics for tuberculosis. *Integr Biol* 2015;**7**(5):591–609.
38. Pienaar E, Cilfone NA, Lin PL, *et al*. A computational tool integrating host immunity with antibiotic dynamics to study tuberculosis treatment. *J Theor Biol* 2015;**367**:166–79.
39. Horn F, Heinekamp T, Kniemeyer O, *et al*. Systems biology of fungal infection. *Front Microbiol* 2012;**3**:1–20.
40. Rolston K. Overview of systemic fungal infections. *Oncology (Williston Park)* 2001;**15**(11 Suppl 9):11–4.
41. Guthke R, Linde J, Mech F, Figge MT. Systems biology of microbial infection. *Front Microbiol* 2012;**3**:328.
42. Bonabeau E. Agent-based modeling: methods and techniques for simulating human systems. *Proc Natl Acad Sci USA* 2002;**99**(Suppl 3):7280–7.
43. Somvanshi PR, Venkatesh KV. A conceptual review on systems biology in health and diseases: from biological networks to modern therapeutics. *Syst Synth Biol* 2014;**8**(1):99–116.
44. Cedersund G, Roll J. Systems biology: model based evaluation and comparison of potential explanations for given biological data. *FEBS J* 2009;**276**(4):903–22.
45. Jones HM, Chen Y, Gibson C, *et al*. Physiologically based pharmacokinetic modeling in drug discovery and development: a pharmaceutical industry perspective. *Clin Pharmacol Ther* 2015;**97**(3):247–62.
46. Dearden JC, Rowe PH. Use of artificial neural networks in the QSAR prediction of physicochemical properties and toxicities for REACH legislation. *Methods Mol Biol* 2015;**1260**:65–88.
47. Kovatchev B, Breton M, DallaMan C, Cobelli C. *In silico Model and Computer Simulation Environment Approximating the Human Glucose/Insulin Utilization*. Food Drug Administration Master File MAF-1521, Silver Spring, USA, 2008.
48. Mardis ER. Next-generation DNA sequencing methods. *Annu Rev Genomics Hum Genet* 2008;**9**:387–402.
49. Linde J, Duggan S, Weber M, *et al*. Defining the transcriptomic landscape of *Candida glabrata* by RNA-Seq. *Nucleic Acids Res* 2015;**43**(3):1392–406.
50. Liu JZ, Hov JR, Folseraas T, *et al*. Dense genotyping of immune-related disease regions identifies nine new risk loci for primary sclerosing cholangitis. *Nat Genet* 2013;**45**(6):670–5.
51. Dix A, Hünniger K, Weber M, *et al*. Biomarker-based classification of bacterial and fungal whole-blood infections in a genome-wide expression study. *Front Microbiol* 2015;**6**:171.
52. Bar-Joseph Z, Farkash S, Gifford DK, *et al*. Deconvolving cell cycle expression data with complementary information. *Bioinformatics* 2004;**20**(Suppl 1):i23–30.
53. Hoffmann M, Pohlers D, Koczan D, *et al*. Robust computational reconstitution - a new method for the comparative analysis of gene expression in tissues and isolated cell fractions. *BMC Bioinformatics* 2006;**7**:369.
54. Clarke J, Seo P, Clarke B. Statistical expression deconvolution from mixed tissue samples. *Bioinformatics* 2010;**26**(8):1043–9.
55. Shen-Orr SS, Tibshirani R, Khatri P, *et al*. Cell type-specific gene expression differences in complex tissues. *Nat Methods* 2010;**7**(4):287–9.
56. Gong T, Hartmann N, Kohane IS, *et al*. Optimal deconvolution of transcriptional profiling data using quadratic programming with application to complex clinical blood samples. *PLoS One* 2011;**6**(11):e27156.
57. Li Y, Xie X. A mixture model for expression deconvolution from RNA-seq in heterogeneous tissues. *BMC Bioinformatics* 2013;**14**(Suppl 5):S11.

58. Zhao Y, Simon R. Gene expression deconvolution in clinical samples. *Genome Med* 2010;**2**(12):93.
59. Shen-Orr SS, Gaujoux R. Computational deconvolution: extracting cell type-specific information from heterogeneous samples. *Curr Opin Immunol* 2013;**25**(5):571–8.
60. Gaujoux R, Seoighe C. CellMix: a comprehensive toolbox for gene expression deconvolution. *Bioinformatics* 2013;**29**(17):2211–2.
61. Gong T, Szustakowski JD. DeconRNASeq: a statistical framework for deconvolution of heterogeneous tissue samples based on mRNA-Seq data. *Bioinformatics* 2013;**29**(8):1083–5.
62. Galperin MY, Rigden DJ, Fernández-Suárez XM. The 2015 Nucleic Acids Research Database Issue and molecular biology database collection. *Nucleic Acids Res* 2015;**43**(Database issue):D1–5.
63. Durmuş Tekir SD, Ülgen KÖ. Systems biology of pathogen-host interaction: networks of protein-protein interaction within pathogens and pathogen-human interactions in the post-genomic era. *Biotechnol J* 2013;**8**(1):85–96.
64. Wang Y-C, Lin C, Chuang M-T, *et al*. Interspecies protein-protein interaction network construction for characterization of host-pathogen interactions: a *Candida albicans*-zebrafish interaction study. *BMC Syst Biol* 2013;**7**:79.
65. Remmele CW, Luther CH, Balkenhol J, *et al*. Integrated inference and evaluation of host-fungi interaction networks. *Front Microbiol* 2015;**6**:764.
66. Mech F, Thywissen A, Guthke R, *et al*. Automated image analysis of the host-pathogen interaction between phagocytes and *Aspergillus fumigatus*. *PLoS One* 2011;**6**(5):e19591.
67. Brandes S, Mokhtari Z, Essig F, *et al*. Automated segmentation and tracking of non-rigid objects in time-lapse microscopy videos of polymorphonuclear neutrophils. *Med Image Anal* 2015;**20**(1):34–51.
68. Kraibooj K, Schoeler H, Svensson C-M, *et al*. Automated quantification of the phagocytosis of *Aspergillus fumigatus* conidia by a novel image analysis algorithm. *Front Microbiol* 2015;**6**:549.
69. Fedosov DA, Lei H, Caswell B, *et al*. Multiscale modeling of red blood cell mechanics and blood flow in malaria. *PLoS Comput Biol* 2011;**7**(12):e1002270.
70. Macdonald SPJ, Arendts G, Fatovich DM, Brown SGA. Comparison of PIRO, SOFA, and MEDS scores for predicting mortality in emergency department patients with severe sepsis and septic shock. *Acad Emerg Med* 2014;**21**(11):1257–63.
71. Wikswo JP. The relevance and potential roles of microphysiological systems in biology and medicine. *Exp Biol Med (Maywood)* 2014;**239**(9):1061–72.
72. Pârvu O, Gilbert D. Automatic validation of computational models using pseudo-3D spatio-temporal model checking. *BMC Syst Biol* 2014;**8**:124.
73. Dada JO, Mendes P. Multi-scale modelling and simulation in systems biology. *Integr Biol (Camb)* 2011;**3**(2):86–96.
74. Thakar J, Saadatpour-Moghaddam A, Harvill ET, Albert R. Constraint-based network model of pathogen-immune system interactions. *J R Soc Interface* 2009;**6**(36):599–612.
75. Hummert S, Hummert C, Schröter A, *et al*. Game theoretical modelling of survival strategies of *Candida albicans* inside macrophages. *J Theor Biol* 2010;**264**(2):312–8.
76. Eswarappa SM. Location of pathogenic bacteria during persistent infections: insights from an analysis using game theory. *PLoS One* 2009;**4**(4):e5383.
77. Tierney L, Linde J, Müller S, *et al*. An interspecies regulatory network inferred from simultaneous RNA-seq of *Candida albicans* invading innate immune cells. *Front Microbiol* 2012;**3**:85.
78. Boswell GP, Jacobs H, Davidson FA, *et al*. A positive numerical scheme for a mixed-type partial differential equation model for fungal growth. *Appl Math Comput* 2003;**138**(2-3):321–40.
79. Tokarski C, Hummert S, Mech F, *et al*. Agent-based modeling approach of immune defense against spores of opportunistic human pathogenic fungi. *Front Microbiol* 2012;**3**:129.
80. Hünniger K, Lehnert T, Bieber K, *et al*. A virtual infection model quantifies innate effector mechanisms and *Candida albicans* immune escape in human blood. *PLoS Comput Biol* 2014;**10**(2):e1003479.
81. Wcislo R, Miller S, Dzwinel W. PAM: Particle automata model in simulation of *Fusarium graminearum* pathogen expansion. *J Theor Biol* 2016;**389**:110–22.
82. Thakar J, Albert R. Boolean models of within-host immune interactions. *Curr Opin Microbiol* 2010;**13**(3):377–81.
83. Grant AJ, Restif O, McKinley TJ, *et al*. Modelling within-host spatiotemporal dynamics of invasive bacterial disease. *PLoS Biol* 2008;**6**(4):757–70.
84. Tyc KM. A modeling perspective on *Candida albicans* interactions with its human host. Dissertation, Berlin, 2012.
85. Carbo A, Bassaganya-Riera J, Pedragosa M, *et al*. Predictive computational modeling of the mucosal immune responses during *Helicobacter pylori* infection. *PLoS One* 2013;**8**(9):e73365.
86. Pollmächer J, Figge MT. Deciphering chemokine properties by a hybrid agent-based model of *Aspergillus fumigatus* infection in human alveoli. *Front Microbiol* 2015;**6**:503.
87. Colman A. *Game Theory and its Application in the Social and Biological Sciences*. New York, NY: Routledge, 1999.
88. Li XY, Pietschke C, Fraune S, *et al*. Which games are growing bacterial populations playing? *J R Soc Interface* 2015;**12**(108):20150121.
89. Gao D, Lietman TM, Porco TC. Antibiotic resistance as collateral damage: the tragedy of the commons in a two-disease setting. *Math Biosci* 2015;**263**:121–32.
90. Tyc KM, Kühn C, Wilson D, Klipp E. Assessing the advantage of morphological changes in *Candida albicans*: a game theoretical study. *Front Microbiol* 2014;**5**:41.
91. Beste DJ, Hooper T, Stewart G, *et al*. GSMN-TB: a web-based genome-scale network model of *Mycobacterium tuberculosis* metabolism. *Genome Biol* 2007;**8**(5):R89.
92. Raghunathan A, Reed J, Shin S, *et al*. Constraint-based analysis of metabolic capacity of *Salmonella typhimurium* during host-pathogen interaction. *BMC Syst Biol* 2009;**3**:38.
93. Rienksma RA, Suarez-Diez M, Spina L, *et al*. Systems-level modeling of mycobacterial metabolism for the identification of new (multi-)drug targets. *Semin Immunol* 2014;**26**(6):610–22.
94. Jamshidi N, Raghunathan A. Cell scale host-pathogen modeling: another branch in the evolution of constraint-based methods. *Front Microbiol* 2015;**6**:1032.
95. Linde J, Schulze S, Henkel S, Guthke R. Data- and knowledge-based modeling of gene regulatory networks: an update. *EXCLI J - Exp Clin Sci* 2015;**14**:346–78.
96. Schulze S, Henkel SG, Driesch D, *et al*. Computational prediction of molecular pathogen-host interactions based on dual transcriptome data. *Front Microbiol* 2015;**6**:65.
97. Sprott JC, Wildenberg JC, Azizi Y. A simple spatiotemporal chaotic Lotka–Volterra model. *Chaos, Solitons and Fractals* 2005;**26**:1035.
98. Stein RR, Bucci V, Toussaint NC, *et al*. Ecological modeling from time-series inference: insight into dynamics and

stability of intestinal microbiota. *PLoS Comput Biol* 2013;**9**(12):e1003388.
99. Bauer AL, Beauchemin CA, Perelson AS. Agent-based modeling of host-pathogen systems: the successes and challenges. *Inf Sci (Ny)* 2009;**179**(10):1379–89.
100. Wylie DC, Hori Y, Dinner AR, Chakraborty AK. A hybrid deterministic-stochastic algorithm for modeling cell signaling dynamics in spatially inhomogeneous environments and under the influence of external fields. *J Phys Chem B* 2006;**110**(25):12749–65.
101. An G. Introduction of an agent-based multi-scale modular architecture for dynamic knowledge representation of acute inflammation. *Theor Biol Med Model* 2008;**5**(1):11.
102. Stern JR, Olivas AD, Valuckaite V, *et al*. Agent-based model of epithelial host-pathogen interactions in anastomotic leak. *J Surg Res* 2013;**184**(2):730–8.
103. Wilensky U. NetLogo. http://ccl.northwestern.edu/netlogo/ Center for Connected Learning and Computer-Based Modeling, Northwestern University, Evanston, IL, 1999.
104. Chiacchio F, Pennisi M, Russo G, *et al*. Agent-based modeling of the immune system: NetLogo, a promising framework. *Biomed Res Int* 2014;**2014**:907171.
105. Solovyev A, Mikheev M, Zhou L, *et al*. SPARK. *Int J Agent Technol Syst* 2010;**2**(3):18–30.
106. Albert I, Thakar J, Li S, *et al*. Boolean network simulations for life scientists. *Source Code Biol Med* 2008;**3**:16.
107. Franke R, Müller M, Wundrack N, *et al*. Host-pathogen systems biology: logical modelling of hepatocyte growth factor and *Helicobacter pylori* induced c-Met signal transduction. *BMC Syst Biol* 2008;**2**:4.
108. Brakhage AA, Schroeckh V. Fungal secondary metabolites - strategies to activate silent gene clusters. *Fungal Genet Biol* 2011;**48**:15–22.
109. Brakhage AA. Regulation of fungal secondary metabolism. *Nat Rev Microbiol* 2013;**11**:21–32.
110. Dühring S, Germerodt S, Skerka C, *et al*. Host-pathogen interactions between the human innate immune system and *Candida albicans* - understanding and modeling defense and evasion strategies. *Front Microbiol* 2015;**6**:625.
111. Kirschner DE, Hunt CA, Marino S, *et al*. Tuneable resolution as a systems biology approach for multi-scale, multi-compartment computational models. *Wiley Interdiscip Rev Syst Biol Med* 2014;**6**(4):225–45.
112. Chaves M, Albert R, Sontag ED. Robustness and fragility of Boolean models for genetic regulatory networks. *J Theor Biol* 2005;**235**(3):431–49.
113. Cedersund G. Conclusions via unique predictions obtained despite unidentifiability–new definitions and a general method. *FEBS J* 2012;**279**(18):3513–27.
114. McKeever S, Johnson D. The role of markup for enabling interoperability in health informatics. *Front Physiol* 2015;**6**:152.
115. Andasari V, Roper RT, Swat MH, Chaplain MAJ. Integrating intracellular dynamics using CompuCell3D and Bionetsolver: applications to multiscale modelling of cancer cell growth and invasion. *PLoS One* 2012;**7**(3):e33726.
116. Karr JR, Sanghvi JC, Macklin DN, *et al*. WholeCellKB: model organism databases for comprehensive whole-cell models. *Nucleic Acids Res* 2013;**41**(Database issue):D787–92.
117. Karr JR, Sanghvi JC, Macklin DN, *et al*. A whole-cell computational model predicts phenotype from genotype. *Cell* 2012;**150**(2):389–401.
118. Morales DK, Hogan DA. *Candida albicans* interactions with bacteria in the context of human health and disease. *PLoS Pathog* 2010;**6**(4):e1000886.