Complex microbial systems across different levels of description

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TOPICAL REVIEW

Complex microbial systems across different levels of description

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Abstract

Complex biological systems offer a variety of interesting phenomena at the different physical scales. With increasing abstraction, details of the microscopic scales can often be extrapolated to average or typical macroscopic properties. However, emergent properties and cross-scale interactions can impede naïve abstractions and necessitate comprehensive investigations of these complex systems.

In this review paper, we focus on microbial communities, and first, summarize a general hierarchy of relevant scales and description levels to understand these complex systems: (1) genetic networks, (2) single cells, (3) populations, and (4) emergent multi-cellular properties. Second, we employ two illustrating examples, microbial competition and biofilm formation, to elucidate how cross-scale interactions and emergent properties enrich the observed multi-cellular behavior in these systems.

Finally, we conclude with pointing out the necessity of multi-scale investigations to understand complex biological systems and discuss recent investigations.

1. Reductionism and emergence in complex biological and physical systems

In both physics and biology, finding appropriate descriptions for complex systems is a challenge. In part, this difficulty arises from a hierarchy of description levels that one uses to order the world around us [1]. For example, certain atoms build molecules which interact to build the intracellular machinery, which in turn drive the cells within organs, that are the building blocks of human bodies and so on.

In using multiple description levels, two (not necessarily contradictory) concepts become important, reductionism and emergence. From this multiple-decade old philosophical discussion, we want to extract only the most relevant definitions to aid in the challenge to appropriately describe complex systems.

From a reductionist perspective, all phenomena can be *reduced to* more fundamental constituents. In both physics and biology, this ultimately corresponds to explaining everything from fundamental particles or molecules and their interactions [2, 3]. In turn, this reductionist perspective includes the idea of 'upward causation' in which the lower level constituents generate higher level states [3]. In many cases, this works well and higher order behavior can be *extrapolated* from the microscopic level. For example, in physics, the pressure of a gas can be extrapolated from collision on the single particle scale (figure 1(A)); in biology, the population growth of large microbial assemblies can often be extrapolated from single cell replication and decay (figure 1(B)).

However, in some cases such naïve extrapolations break down and new phenomena *emerge*. A phenomenon is called *emergent,* if it cannot be reduced to, explained by, or predicted from its individual constituent parts [1], or in short: 'More is different' [4]. A canonical example from physics is the emergence of ferromagnetism (figure 1(C)). In ferromagnets the collective coupling of spins cannot be naively extrapolated from the individual spins and generates a spontaneous symmetry breaking. Such symmetry breaking often accompanies emergent phenomena such as super-conductivity or super-fluidity [4–6]. In biology, emergent phenomena are among others life itself as the transition from inanimate to living matter [2], or as a more concrete example the collective migration of independently non-migrating organisms (figure 1(D)) [7, 8]. Although not considered a source for emergence in physical systems [6], stochasticity due to small constituent numbers and the related fluctuations can play an important role for emergence of bistable states in deterministically monostable biological systems [9–12].

To conclude: while it is useful to abstract from lower to higher scales to make a system conceptually

tractable, phenomena can emerge that impede naïve extrapolation.

Here, we argue that the trade-off between useful simplification and necessary precision calls for multi-scale descriptions in biological systems, that passes the necessary information between scales and simplifies if possible.

In the following, we use microbial examples to elucidate this idea. Section 2 presents a general description hierarchy for microbial systems, reviews the most-relevant literature from both prokaryotic and eukaryotic systems and comments on the simplification-precision-trade-off. Subsequently, we illustrate two examples of emergent properties of multi-cellular microbial communities. First, in section 3, we focus on bacterial competition and describe a model system in which the competitive interaction of one of the populations arises through division of labor. In this system, the division of labor fundamentally originates from stochastic gene expression and a bistable gene regulatory network that has a multitude of interesting consequences on different levels of abstraction. We will argue how a purely reductionist approach, that averages all details from lower levels of abstraction, breaks down because stochastic single cell behavior can influence the long-term behavior of the interaction. This will be illustrated via reviewing recent findings [13] and new theoretical considerations (box 1). Second, in section 4, we review biofilm formation as another type of emergent multi-cellular properties and link biofilms to the study of micromechanics, which enables the description of complex materials at the continuum scale. Finally, we conclude by discussing recent theoretical and experimental approaches to understand multi-cellular properties of microbial systems.

2. Hierarchical description of complex microbial systems

Complex microbial systems can be understood using a hierarchy of physical description levels (figure 2). The first level of description we want to focus on is the level of biochemical reaction networks and, in particular, gene regulatory networks. Fundamentally, the genetic program encoded on the DNA is converted into biochemically active proteins according to the famous central dogma [14]. Gene expression itself is subject to regulation not only on the transcriptional level [15], but on nearly all levels of the protein synthesis pathway [16]. Collectively, the interconnected genes build large gene regulatory networks that can be modeled by differential equations [17] and further extended with additional data, such as information on metabolic pathways [18], for example.

The large interconnectedness of signaling pathways can lead to emergent properties [19]. However, in many cases the relevant information is only contained in sub-networks, justifying a modular analysis [20]. On an even smaller scale, network motifs, recurring interaction circuits from which the networks are built, are useful abstractions and can be handled easily by theoretical analysis and computational approaches [20–23].

Experimentally, a wealth of molecular biology techniques gives a handle to alter the genetic sequences and thereby the network structure *in vitro* and *in vivo* [24, 25] to understand regulatory pathways. In particular, the usage of the green fluorescent protein (GFP) and its derivatives [26–28] allows the investigation of protein expression dynamics *in vivo*. Furthermore, combined theoretical and experimental approaches enable creation of synthetic regulatory motifs for which the repressilator unit [29], the genetic toggle switch [30], or the linearizing fine-tuner circuit [31] are beautiful examples. Even such small model systems of few interacting genes can exhibit rich dynamic behavior (oscillations [29], multi-stability [30, 32], excitability [33], etc) due to nonlinearities and or feedback mechanisms. In addition, gene expression noise [34, 35] adds to the inherent complexity of networks and can—in combination with multi-stable gene circuits—lead to qualitatively different behavior [32] that cannot be neglected in accurate models [36]. This necessitates the incorporation of stochasticity into the models. Chemical master equations are the framework to describe the temporal evolution of state probability distributions analytically [37, 38]. In many cases, solving these equations is only possible numerically using a stochastic simulation algorithm (SSA) also known as Gillespie algorithm [39].

Abstracting from the details of the underlying gene regulatory networks, single cells are the fundamental self-replicating units of living matter [24, 40] and can be considered the hardware on which genome encoded instructions run. This black box picture [41] suggests a deterministic mode of action with rigid input–output relations. However, due to multi-stable gene networks, gene expression noise, and other factors [42], there is considerable variability in cell state distributions of genetically identical cells [43].

To account for this phenotypic heterogeneity, single cell methods are needed. Basically, there are two types of methods. First, methods such as single cell 'omics' [44] approaches and flow cytometry [45] that generate cell state distribution snapshots (cross-sectional data) and second, time-lapse methods, mainly single cell fluorescence microscopy, that generate time traces (longitudinal data) [46–48] of cell states.

Understanding differentiation into distinct cell states using the 'epigenetic landscape' abstraction was initially developed for eukaryotes [49] but later extended to incorporate the role of noise and applied to various levels of biological scales [50]. A given cell state can be interpreted as a point in a high dimensional phase space and represents a certain phenotype [51]. The abstraction into distinct phenotypes instead of continuous cell state measures, such as protein con-

centrations, underlines emergent qualitative differences between phenotypes.

If gene expression is costly, phenotype and fitness are coupled giving rise to phenotype-specific cell growth rates that differ from the population growth rate. This phenotype-fitness coupling can alter the phenotype distribution pattern in changing environments due to a trade-off between functional benefit and gene expression cost [52]. Furthermore, knowledge on the optimal trade-off value can be used to study the evolutionary adaption of the underlying genetic network to non-optimal conditions [53].

On the population level, genetically identical cells can interact to generate qualitatively new behavior that is not possible on the single cell level. They can do so either directly, e.g. via communication, or indirectly by phenotypic heterogeneity. Some bacteria use quorum sensing, the most common form of bacterial communication, to monitor their environment and coordinate their behavior [54]. Such direct forms of interaction also depend on the topology of the interaction network between individual cells. In spatially extended systems, as opposed to well mixed liquid systems, the spatial organization of the cells can clearly change the way how an individual cell senses its environment and can alter the interaction strength to other individuals [55]. Only recently, an automated single cell setup synthetically coupled individual cells to imitate population behavior [56]. The mechanisms for phenotypic heterogeneity originate on the single cell level and have been discussed recently [42]. However, only on the population level bacteria can profit from the diversity, for example by division of labor or bet hedging [57, 58]. The population composed of different phenotypes can exhibit multiple behaviors to achieve a population response that neither of the phenotypes could achieve alone.

Many microbial populations exhibit emergent multi-cellular properties that cannot be extrapolated from individual cells, but are still fundamentally encoded in the underlying gene regulatory network. Among others, examples are physical properties of biofilms [59], ecological competition [60], collective migration [8], or communication via protein secretion and sensing [61].

The experimental methods to study these properties are as diverse as the phenomena themselves. A particularly interesting approach is to use multi-omics investigations to study complex microbiome ecosystems [62]. Here, features of the very abstract ecosystem level are investigated on the fundamental molecule level (genes, mRNA, and proteins). However, analyzing and interpreting the data is still challenging due to emergent properties at intermediate levels, such as cellular individuality [42] or complex relationships of multiple sub-populations [63].

Likewise, recent theoretical and computational approaches acknowledge the necessity to incorporate the whole scale of interactions from biochemical reaction networks to cellular reproduction [64]. Such an approach predicted the emergence of previously unknown metabolic cooperation in *E. coli* colonies [65].

3. Microbial ecology

3.1. Ecological interactions

From a top-down perspective, the composition of a microbial community emerges from the effective fitness differences between different populations due to complex interactions between individual microbes [66, 67] (figure 3). Broadly, these ecological interactions comprise competition and cooperation [66, 68–71]. An interaction between individuals or groups of microbes is said to be cooperative or competitive if it increases or decreases the fitness of the interaction partner [66, 72, 73]. Depending on the interaction partner, interactions can be classified as inter- or intra-species interactions [66]. In many cases, secreted molecules, such as digestive enzymes or toxins [74–76], mediate interactions on the microscopic level. Independent of the mode of action of these molecules on the recipient of another microbial population, the secretion itself is often achieved cooperatively by division of labor [77], and the secreted molecules are considered public goods. In some cases, production of public goods is coordinated by microbial communication via quorum sensing [78].

It is proposed that competition and not cooperation dominates the interactions among cultivable microbial species [79] partially because even in the absence of direct competition mechanisms, utilization of resources is often to one's competitor's disadvantage.

Due to their complexity, interactions present in microbial communities are either studied in well-defined experimental settings using well-studied model organisms [68, 80–82] or with the help of theoretical and computational modeling [72, 83–85].

In choosing an appropriate description, it is useful to start with the Lotka-Volterra equations (equation (1)). This model is commonly used to study ecological interactions of N different populations in which the temporal evolution of an abundance x for a (sub-) species i is described using a growth term with growth rate μ, and an interaction term with a parameter α_{ij} for the interaction of (sub-) species i and j [86].

$$\frac{\mathrm{d}x_i(t)}{\mathrm{d}t} = \mu_i x_i(t) + \sum_{j=1}^{N} \alpha_{ij}\, x_i(t)\; x_j(t) \qquad (1)$$

Depending on the sign of α_{ij} the interaction is said to be cooperative ($\alpha_{ij} > 0$) or competitive ($\alpha_{ij} < 0$) irrespective of the exact underlying interaction mechanisms [87]. A great benefit of the model is its generality and its conceptual simplicity. Growth and interactions between billions of individual cells are abstracted in two types of parameters.

However, this generality comes with the price of neglecting potentially important details.

For later use, we extend the model such that it explicitly takes into account reproduction and death reactions with rates ρ and δ, respectively and conversion between (sub-) species with a transition rate σ_{ij} from (sub-) species i to j. This yields equation (2):

$$\frac{\mathrm{d}x_i(t)}{\mathrm{d}t} = (\rho_i - \delta_i)\, x_i(t) + \sum_{j=1}^{N} \alpha_{ij}\, x_i(t)\; x_j(t) + \sum_{j=1}^{N} \left(\sigma_{ji}\, x_j(t) - \sigma_{ij}\, x_i(t)\right). \quad (2)$$

Other neglected details include but are not restricted to nonlinearities and details of the interaction, environmental parameters, spatial dimension, stochasticity and individuality of the microbes and have been taken into account in more advanced models [42, 72, 88–90].

3.2. Bacterial ecology—illustrated with the ColicinE2 system

Despite the detailed knowledge on the interaction mechanisms on the microscopic level, it is hard to assess their mechanistic role in enabling coexistence or affecting the composition of microbial communities. We argue that this is due to a complex hierarchy of interactions through which the community composition emerges.

The system of ColicinE2, a bacterial toxin, is an ideal model system to illustrate this hierarchy of interactions on different levels of abstraction (figure 4). In short, ColicinE2 is a bacterial toxin and has a fixed gene regulatory network (gene network level) that together with noise leads to a difference in the response of individual genetically identical cells to the same stimuli (single cell level). With changing environments, the population response (population level) effectively sets the interaction parameters for interactions with foreign populations resulting in the ecology of mixed bacterial communities (emergent properties) (figure 4). In order to gain a deeper insight, we discuss the different aspects in more detail in the following.

ColicinE2 is a plasmid encoded bacteriocin of *Escherichia coli* and its operon comprises genes for the toxin protein, as well as an immunity and a lysis protein [91]. Coexpression of the toxin and lysis genes is necessary to ensure inactivity of the toxin within the producing cell before the toxin-immunity protein complex is released to the environment via cell lysis [92, 93]. Lysis entails the death of highly expressing cells [94]. Therefore, expression of the ColicinE2 operon cannot be active all the time. Indeed, expression of the three genes is transcriptionally regulated via the noisy SOS response which results in phenotypically heterogeneous expression [95, 96]. The SOS system can be triggered by DNA damaging agents such as the antibiotic mitomycin C [91]. In addition to the transcriptional regulation, the ColicinE2 system is post-transcriptionally regulated. Having two transcriptional terminators, two distinct mRNAs are produced, a long mRNA containing all three genes and a short one lacking the lysis gene [97]. Interestingly, the global regulator CsrA regulates translation of the long mRNA and times the dynamics of lysis protein production, creating a time delay between production and release [98, 99].

Neglecting the molecular details on the genetic level, recent single cell studies analyze the Colicin system via fluorescence time-lapse measurements to investigate the dynamics of Colicin expression [60, 95, 100]. In particular, these studies revealed stress-dependent tunable response dynamics, ranging from basal expression to synchronized responses [100] and stochastic state-switching between toxin production and non-production [60]. A common feature of these studies is the binary classification of cells into Colicin producers and non-producers, often by applying a fluorescence intensity threshold.

The phenotypic heterogeneity in the Colicin system comprised of self-sacrificing toxin-producers and non-producing proliferating cells constitutes a division of labor [13, 60]. This equips the population with a completely new functionality. Populations of neither pure toxin non-producers nor toxin producers could enjoy the benefits of both phenotypic variants. This population level response is an emergent phenomenon in which the resulting properties could not be extrapolated from the individual parts alone. In taking into account only the phenotype of cells, molecular details of the Colicin network reduce to effective phenotype-switching, growth and lysis rates that can be determined experimentally. Using these rates one can easily develop a phenomenological model to describe the population behavior (box 1).

Additional new features that arise on the multicellular level are interactions between cells. In particular, ColicinE2 cells were found to have an autoinducing effect of Colicin production [101] on the population level.

In the context of bacterial ecology, responses of uniclonal populations, such as Colicin production, can

Box 1. Phenomenological model of Colicin expression heterogeneity

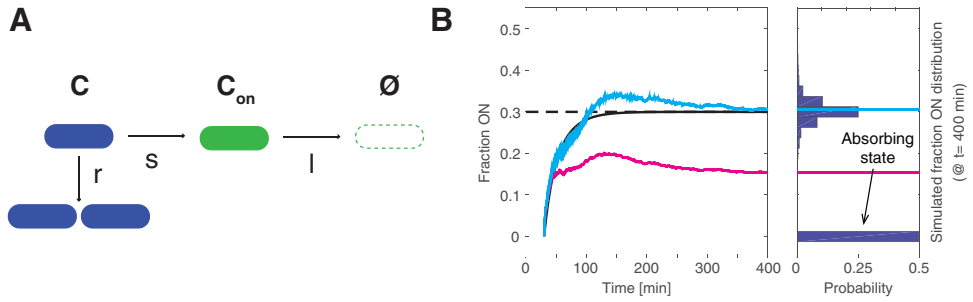

Figure B1. Colicin expression heterogeneity. (A) Schematic model of ColicinE2 heterogeneity; cells replicate with rate r, switch to the toxin producing ON state with rate s, and lyse subsequently with rate l. (B) Comparison of deterministic (solid black), steady state (broken black), complete average stochastic (magenta), and non-extinct stochastic (light blue) solution to model (left). Right hand distribution shows the corresponding final state distribution (blue) and averages (magenta and light blue) of the stochastic simulation with 1000 realizations (rates: $s = 0.015, r = 0.03, l = 0.02$).

Three microscopic processes: reproduction of non-producing cells (1), switching to the toxin producing 'ON' state (2), and cell lysis (3) of individual cells are hypothesized to determine the toxin producer fraction (figure B1(A)).

Neglecting spatial extension and further interactions, the dynamics of both ON and OFF populations were formulated in terms of the extended Lotka–Volterra equation (equation (2)) that incorporated growth of the OFF cells with rate r, switch to the toxin producing state with rate s, and lysis of the ON cells with rate l:

$$\begin{aligned} \partial_t x_{OFF} &= r\, x_{OFF} - s\, x_{OFF} \\ \partial_t x_{ON} &= s\, x_{OFF} - l\, x_{ON}. \end{aligned} \tag{B1}$$

This system of ordinary differential equations (ODEs) was solved analytically. In the limit $t \to \infty$ (large cell numbers) we could identify the steady state producer fraction (assuming $l, r, s \geqslant 0, r > s$):

$$Frac_{SS} = \left[\frac{x_{ON}}{x_{ON} + x_{OFF}}\right]_{t\to\infty} = \frac{s}{r+l}. \tag{B2}$$

In order to capture the stochasticity, the population dynamics were simulated using a stochastic simulation algorithm (figure B1(B), magenta). The resulting distributions (figure B1(B) right) underlined the importance of the absorbing boundary at $x_{ON} = x_{OFF} = 0$. Instead of always growing to a finite population that has its average producer fraction at 0.3, in some cases, the population dies out because all cells switch to the producing state and lyse subsequently. This is particularly probable if the switching rate is high compared to reproduction and the number of initial cells is low. Consequently, the average producer fraction obtained by stochastic simulations (figure B1(B), magenta curve) was lower than the analytically derived steady state value (figure B1(B), black curve). Stochastic simulations and steady state prediction (figure B1(B), black) coincide if the 'extinction' cases, in which $x_{ON} = x_{OFF} = 0$, were removed from the average stochastic trace (figure B1(B), light blue).

Taken together, this illustrative example shows how demographic noise can lead to strong deviations of the population dynamics from the deterministic prediction for small initial system sizes, even in the simplest two phenotype systems. This finding underlines the necessity to use individual-based models on the cell-scale instead of population-level approaches. Likewise, in physics, it would be hard to explain the phase transition from para- to ferromagnetism assuming an *a priori* average magnetization without going to the spin level.

average out to effective interaction parameters. These parameters often determine the outcome of competitions between, for example, the potentially Colicin producing (C), Colicin sensitive (S) and resistant (R) strains. Many studies do not differentiate the two phenotypes of the C strain and the details of phenotype switching and concomitant lysis reduce to a growth rate reduction or cost parameter. Likewise, the details of Colicin release and action on the S strain reduce to a toxin effectivity. Consequently, many studies try to predict the competition outcome in terms of these effective interaction parameters [82, 102–104]. Furthermore, a result of multi-cellularity is the necessity of taking into account the spatial arrangement of cells as well as habitat structure [102, 103]. In case of colicinogenic interaction, the spatial distribution, i.e.

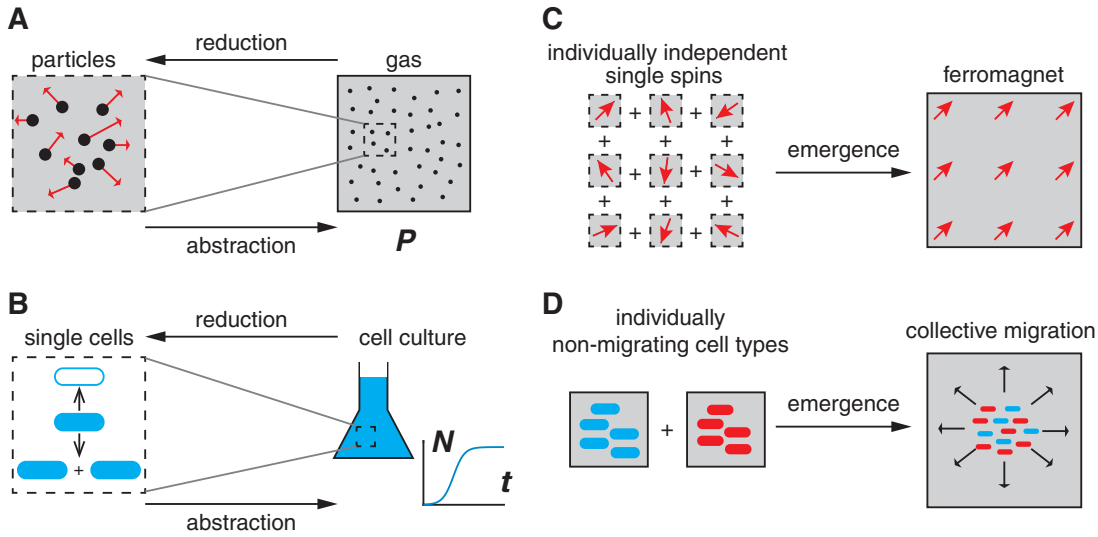

Figure 1. Examples of reducible and emergent phenomena in physics and biology. For reducible phenomena (A) and (B), the macro-scale can be reduced to interactions on the micro-scale and similarly, one can infer or abstract in the opposite direction. (A) The pressure of a gas (high level) can be reduced to collisions of individual particles with the container boundary (lower level). Similarly, one can abstract from the low level to the high level and infer the pressure from the particle velocities, i.e. thermal kinetic energy. (B) The global cell culture growth can often be reduced to reactions of single cells, e.g. cell reproduction and cell death, from which one can in turn abstract the population behavior. (C) and (D) For emergent phenomena, one cannot clearly understand the macro-behavior in terms of the micro constituents and cannot easily infer from the micro to the macro level. (C) In ferromagnetic material, individually independent spins should be able to freely rotate. In a collective interaction, however, ferromagnetism emerges if the temperature is below a critical value. (D) In collective migration, individually non-migrating cell types can interact to collectively migrate [8]. These phenomena are emergent, because they cannot be predicted from the individual constituents.

the distance between Colicin producers and sensitive strains, can influence the strength with which the toxin acts [13, 82].

In contrast to assuming average population behavior, recent studies take into account the phenotypic structure of the C strain and explicitly consider switching to the toxin producing state [82] and reveal optimal values of toxin producer fractions for competition to balance the positive and negative effects of the division of labor [13, 60].

However, only recently a multi-scale approach explicitly highlighted the effects of stochasticity on competition. The study found that the competition outcome is not fully determined by the effective interaction parameters but instead the stochasticity at early time-points and low cell numbers set the stage for the ensuing fixed population dynamics given by the interaction parameters. In the initial phase, by chance, the total C population can decay having no influence in the further population dynamics leading to multi-stability [13]. This observation is in accordance to the considerations from box 1 that showed how the absorbing boundary of the stochastic dynamics can lead to extinction of the C subpopulation.

This stochasticity-induced multi-stability is a great example for cross-scale interactions and shows how a purely reductionist approach breaks down. Events on the single cell level can influence the dynamics at much higher levels of abstraction. In addition, the spatial assortment plays an important role for the interaction. Both aspects, cell individuality and spatial structure, would not have been captured by effective population-level models, such as the ones presented in section 3.1. This underlines the importance of multi-scale approaches to investigate complex bacterial systems.

4. Biofilm formation

4.1. General principles

While eukaryotic biofilms are less studied [105–108], it is generally acknowledged that biofilms are the dominant lifestyle of bacteria [109]. Focusing on bacterial biofilm formation, the term biofilm refers to bacterial communities in which bacteria are embedded in an extra-cellular matrix [110] that can be composed of different exopolymeric substances (EPS) such as proteins, polysaccharides, DNA, or lipids [59, 111]. In general, the formation of biofilms follows a multi-step process including attachment of motile cells, loss of motility function and production of EPS, formation of micro-colonies, phenotypic differentiation and biofilm maturation, and finally dispersal of motile cells to initiate new biofilm formation [112, 113]. Mature biofilms can be considered 'cities of microbes' [114], in which a multitude of different phenotypes form a common population [115] similar to primitive multicellular organisms [116]. Furthermore, mature biofilms feature a distinct "wrinkled" morphology that depends on the production of EPS [112, 117, 118] (figure 5(C)).

The matrix embedding equips the community with new functionalities, such as increased resistance to antibiotics and other chemicals [119–121], protection from high shear forces or other mechanical

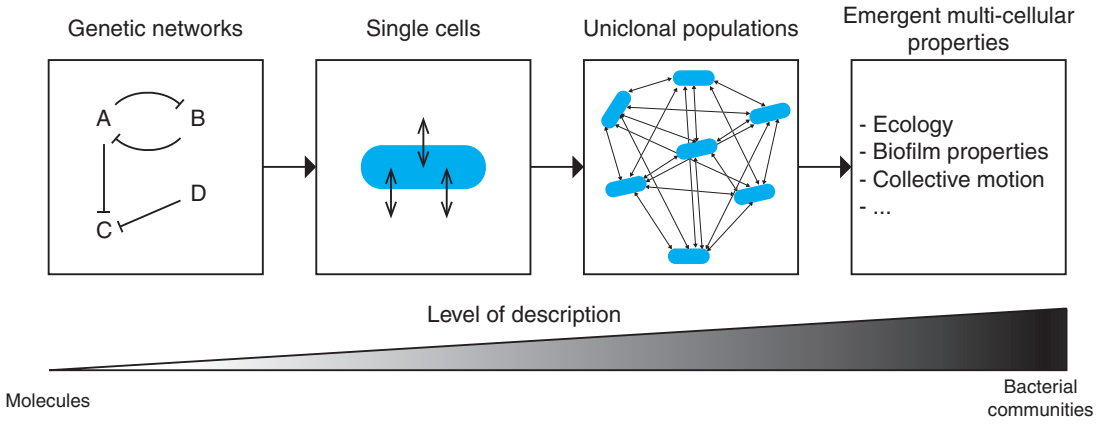

Figure 2. Abstraction levels in complex microbial systems. Microbial systems can be described on different levels of abstraction. In going from smaller to larger scales, many details of smaller scales can be neglected, e.g. molecular details of the gene network can be averaged to an effective single cell response (*reductionist* view). However, in other cases, interactions of many individual constituents give rise to qualitatively new *emergent* properties, such as collective motion or biofilm formation in multi-cellular communities.

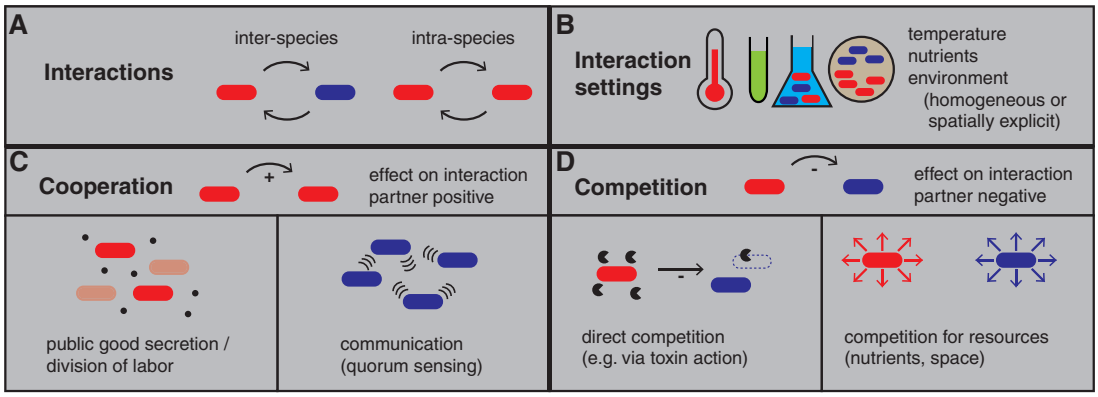

Figure 3. Ecological interactions. (A) Interactions in general can occur between microbes of the same (intra) or of different (inter) (sub-) species. (B) The interaction strongly depends on the interaction settings such as temperature, nutrients, and the interaction environment. The interaction environment can be spatially homogeneous (e.g. in well-mixed liquid cultures) or spatially explicit (e.g. on solid agar plates). (C) The term cooperation describes interactions that have positive effects on the interaction partner ($\alpha_{ij} > 0$ in equation (1)) and comprise division of labor or effective communication, for example. (D) Competition induces negative effects on the interaction partner ($\alpha_{ij} < 0$ in equation (1)) and can be direct (e.g. via toxin action) or indirect (e.g. via occupation of nutrients or space).

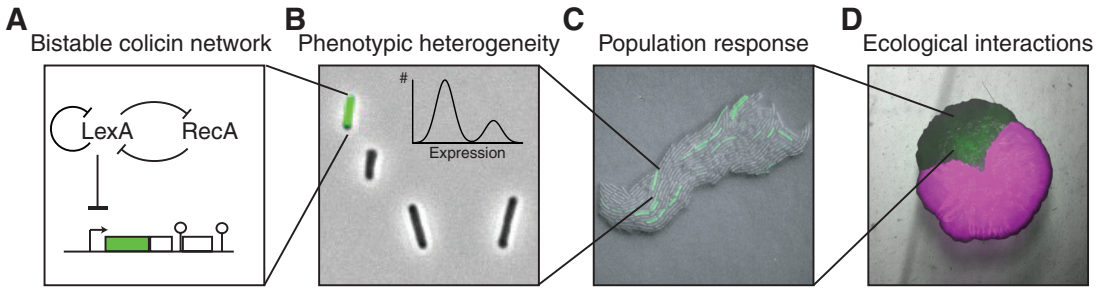

Figure 4. Hierarchy of interactions. (A) The ColicinE2 operon is transcriptionally regulated via the SOS response, whose regulatory proteins LexA and RecA exhibit bistable behavior and consequently heterogeneous expression. (B) Heterogeneous expression of the ColicinE2 system is illustrated by fluorescence reporter protein expression. Two-states are evident, a highly expressing state (ON) of 'producer cells' and a low expressing state (OFF) of 'non-producer cells'. (C) On the population level, the two expression states generate an average population response (also see box 1). (D) In ecological competition, the population response often reduces to effective interaction parameters (Image D reused from [13] under creative commons license 4.0).

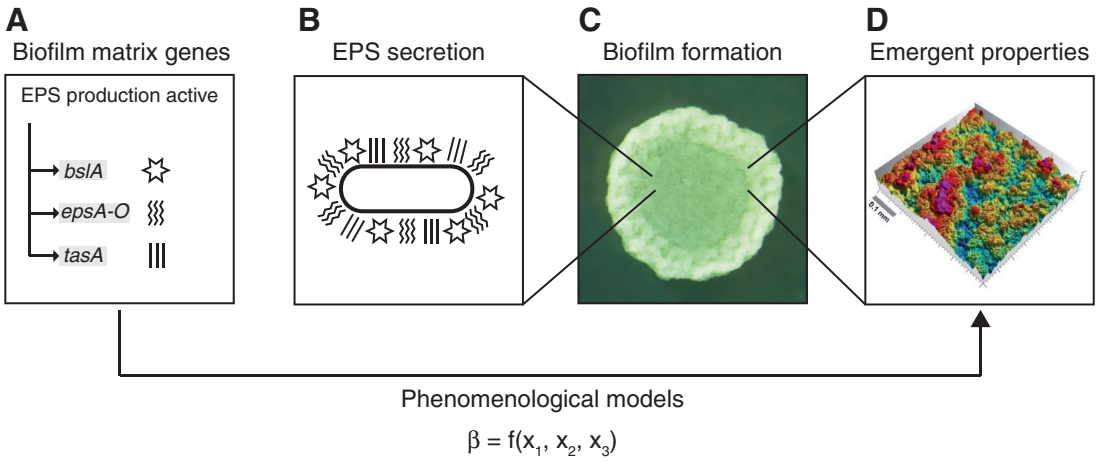

Figure 5. Biofilm formation. (A) *B. subtilis* cells can differentiate into various cell types. In case of biofilm matrix producing cell types, three genes in the NCIB 3610 strain are active: *bslA*, the operon *epsA-O*, and *tasA*. (B) Matrix producing cells secrete the EPS biopolymers and embed themselves in it. (C) Cells surrounded by the biofilm matrix form morphologically distinct 'wrinkled' communities. (D) In mature biofilms, new properties and functions emerge through the community structure and composition. Surface properties such as surface roughness being one of the new features. (Image (D) reused from [134]—published by The Royal Society of Chemistry under creative commons license 3.0).

stresses [122, 123] and increased invasion resistance [124]. These properties can be beneficial for industrial applications such as waste water treatment [125], but they pose a large threat in the medical context. Biofilms growing on heart valves or catheters cause serious infections and device failure [126, 127].

Being a health care problem only increased the necessity to understand how biofilms gain their emergent functionalities. Recent studies underline the idea that an holistic understanding of biofilm biology needs to cover a hierarchy of description levels [59, 128]. At the fundamental level, the intracellular production machinery for the EPS polymers and its control set the stage for understanding biofilm formation. In the past years, research has successfully revealed key matrix components of several biofilms, e.g. of those formed by bacterial strains from the *Bacillus*, *Pseudomonas, Staphylococcus* and *Streptococcus* families [113, 129–131]. But only recently, technical advances in high-resolution optical microscopy [132] and scanning electron microscopy [133] allowed the investigation of how specific matrix components affect biofilm structure.

4.2. Biofilm formation with focus on *B. subtilis* NCIB 3610

As described above, extracellular matrix components are the key determinants for biofilm structure and function. However, detailed information about the function of the individual matrix molecules and their contribution to the physical properties of biofilms is still sparse and quantitative composition-function studies are lacking. Here, we use the example of *B. subtilis* to illustrate how the consideration of multiple scales occurs naturally in biofilm formation.

Compared to the relatively simple two-phenotype system of Colicin production considered above (in section 3.2), the gene regulatory network of *B. subtilis* is more complex and controls the differentiation between motile, competent, sporulating, and biofilm matrix-producing phenotypes [112, 118, 135]. For the NCIB 3610 strain, three main EPS molecules are produced when matrix genes are activated: a surface layer protein BslA, a fiber protein TasA, and the exopolysaccharides EpsA [112, 135].

Intuitively, the biofilm life cycle begins at the single cell level with the attachment to a surface [112] and the switch from motility to cell clustering [136]. Already at this early step, the EPS molecules produced can influence attachment [137]. Continued cell growth leads to the formation of a highly diverse labor dividing population of motile, matrix producing and sporulating cell types [118, 138]. Interestingly, also dead cells play an important role and were found to facilitate the mechanics of wrinkle formation [139].

Initially, the relations between biofilm formation and the matrix composition were derived from knock-out mutants in which the genes for the production of specific EPS molecules were deleted and the biofilm structure, i.e. the 'wrinkliness' of the biofilm was assessed qualitatively [112]. However, newer studies try to quantify biofilm formation of a variety of biofilm forming strains (not only *B. subtilis*) in terms of the biofilm's physical properties. Recent efforts investigated biofilm structure (height, width, surface roughness [134, 140, 141]), its mechanical properties (shear strength [122, 141–144], erosion stability [145], elasticity [141, 145–148], surface stiffness [141], tensile strength [148], indentation [141, 149–151], and adhesion [137, 152]), its physiochemical properties (wettability/hydrophobicity [146, 149, 153, 154]), or microscopic organization [146, 149] and try to link the properties to the composition of the biofilm matrix (Figure 6). In yeast biofilms, the spatial scales of wrinkle morphology have been quantified by Fast Fourier Transformation of colony images [155].

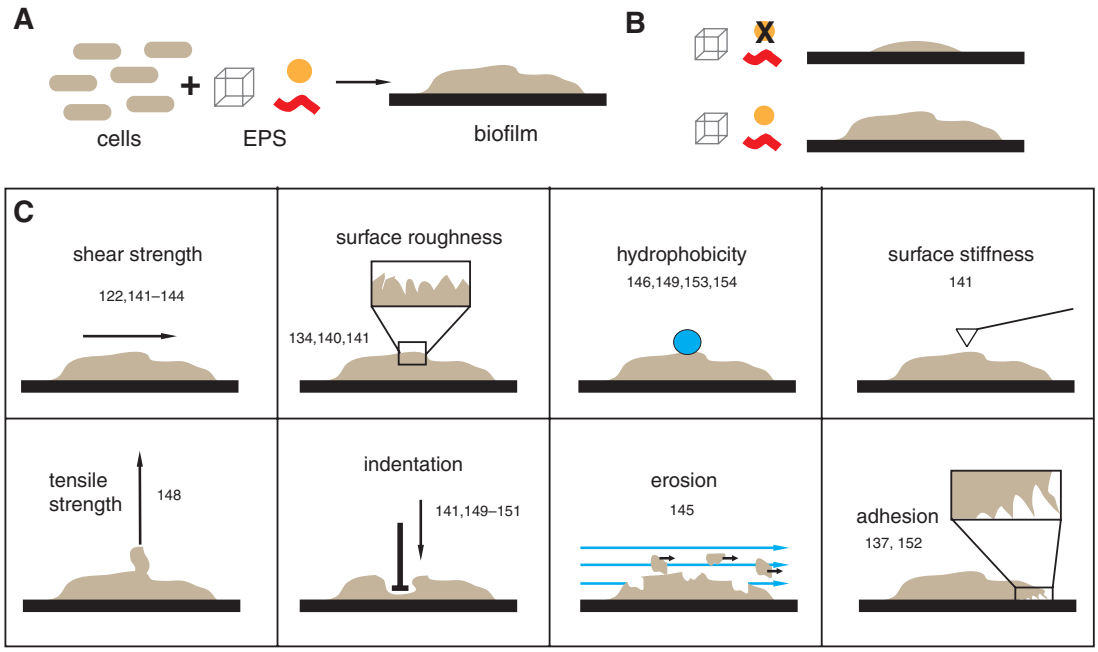

Figure 6. The biofilm matrix composition determines the biophysical properties of bacterial biofilms. (A) The biofilm matrix surrounding the cells is composed of DNA, proteins, sugars and lipids (exopolymeric substances (EPS)). (B) A change in matrix composition changes biofilm properties. (C) Examples of experimentally accessible biofilm properties with reference numbers. Changes in matrix composition and environmental conditions can induce changes in biofilm properties. Several research groups are currently quantifying physical properties of bacterial biofilms, but direct correlations to the underlying matrix compositions are scarce.

In addition to the physical properties of biofilms, the formation of biofilms leads to an emergent fitness advantage of the biofilm forming community [59] and their presence within mixed communities strongly affects community composition. Likewise, yeast biofilm formers have been found to out-compete knock-out mutants [155]. Biofilm formation itself can be evolutionary stable even under conditions that select for the purely planktonic state [156]. Exopolymeric substances have been found to be shared with non-producing cells, such that a mixture of a mutant strain missing the *bslA* gene and a mutant strain unable to build the exopolysaccharide and the protein TasA reconstituted wild type behavior [157]. A recent study found that division of labor between genetically engineered specialized BslA and TasA producers can outperform naturally occurring phenotypic specialists [158]. This underlines the function of biofilm formation as a cooperative interaction.

Although many biofilms can be assessed experimentally, it is challenging to unravel how the macroscopic properties emerge from the heterogeneous microscopic units, such as EPS molecules or cells of different phenotypes (figures 6(A) and (B)).

The importance of EPS is increasingly acknowledged in various types of models. They include individual-based models that were able to predict spatial structure and composition [88, 159, 160], metabolic phenotypes [65], and also detachment [161] of microbial colonies. Furthermore, continuum [162], cellular automata [163], and finite element models [164, 165] incorporate EPS alongside bacteria to characterize biofilms. There are even polymer network models that are solely based on EPS matrix structure [166].

However, a complete understanding of how the macroscopic properties of the biofilm emerge through the properties of the specific produced EPS molecules is still missing. In order to bridge the gap between microscopic scale of the EPS molecules and large-scale biofilm properties, phenomenological models can help to determine the effects of a specific matrix component on the physical property of interest. In general, a biofilm property β can be formulated mathematically as a function $f(x_1, x_2, \ldots, x_n)$ with the x_i representing the biofilm building blocks. Given a feasible parametrization of f, experiments comparing different knock out mutants can quantify the influence of a given building block x_i on the biofilm property β. A recent study used a multiplicative model to assess the influence of the individual matrix components (EpsA, TasA, BslA) on biofilm structure (height, area, roughness) and could thereby quantify each component's influence [134]. Similar models can easily be formulated for other problems.

In order to generate a more fundamental understanding of biofilm properties, approaches from other physical fields may be helpful. Material properties are often described at the continuum scale for idealized homogeneous materials, which in fact are only effective properties of highly inhomogeneous constituents that are structured at the micro-scale [167]. These microstructures can include and are not restricted to voids, cracks, inclusions, or grain boundaries. Still, the use of micromechanics enables to determine aggregate

properties of the heterogeneous materials [168]. Recent advances in experimental methods allowed investigation of biofilm formation from the single-cell scale [132] and probing of the micromechanical structure of biofilms [169]. This could pave the way for a micromechanical understanding of biofilms.

5. Conclusion

Here, we reviewed complex microbial system across different description levels and in doing so focused on two model systems: ecological competition by toxin release and biofilm formation. We demonstrated that, with increasing scale, details of the microscopic description level can be often abstracted to average or typical macroscopic properties. However, we also showed, that emergent properties and cross-scale interactions impede naïve extrapolations and necessitate comprehensive investigations.

More and more studies appreciate this necessity and typically combine experimental and theoretical approaches to study large-scale properties in terms of its microscopic constituents. These include, among others, active matter properties of mutually killing bacteria [170], architectural structure of biofilms [132], morphology driven patterning in bacterial colonies [171], multi-stability in stochastic bacterial competition [13], coordination of multi-cellular behavior by secrete-and-sense communication in yeast [61, 172], cell-level driven multi-cellular evolution in yeast [173], and phenotypic heterogeneity that is driven by coupling of ecological and population dynamics through quorum sensing [174].

Studies like these can add to the overall understanding of the emergence of multi-cellular properties in complex biological systems that are determined by the underlying microscopic interactions and can aid in developing or refining new frameworks for their understanding [175, 176]. Such efforts could eventually lead to a 'statistical biology' in reference to physics' statistical mechanics [55, 177] that enables cross-scale understanding of biological phenomena.

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