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Understanding and Engineering Distributed Biochemical Pathways in Microbial Communities

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Abstract

Microbiomes impact nearly every environment on Earth by modulating the molecular composition of the environment. Temporally changing environmental stimuli and spatial organization are major variables shaping the structure and function of microbiomes. The web of interactions among members of these communities and between the organisms and the environment dictates microbiome functions. Microbial interactions are major drivers of microbiomes and are modulated by spatiotemporal parameters. A mechanistic and quantitative understanding of ecological, molecular, and environmental forces shaping microbiomes could inform strategies to control microbiome dynamics and functions. Major challenges for harnessing the potential of microbiomes for diverse applications include the development of predictive modeling frameworks and tools for precise manipulation of microbiome behaviors.

Graphical abstract

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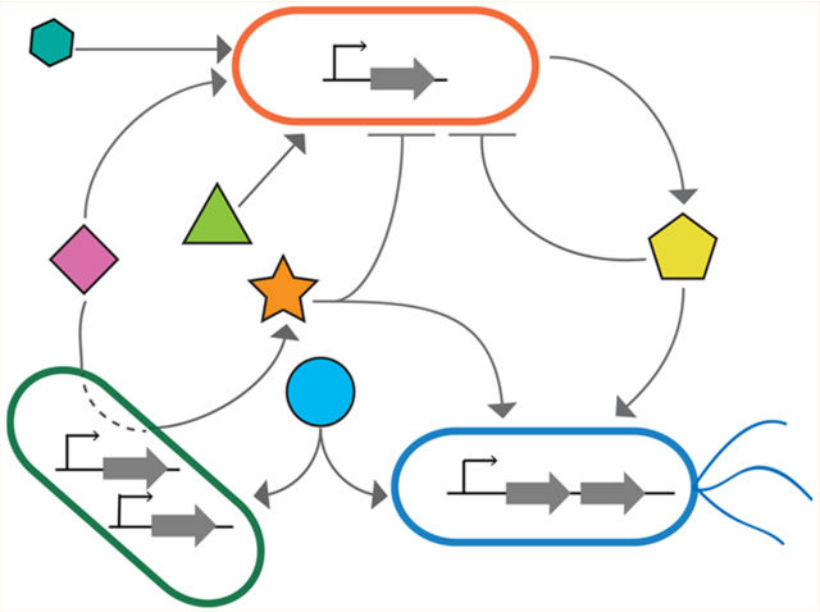

1. MICROBIOMES ARE DYNAMIC NETWORKS

Microbiomes are collections of microorganisms (bacteria, archaea, eukaryotic microbes, and viruses) that occupy and critically impact all environments on Earth by performing diverse biochemical transformations. Microbiomes have been shown to drive biogeochemical cycles;[1] enhance agricultural productivity;[2,3] remediate environmental pollution;[4,5] and impact human health,[6] nutrition,[7] behavior,[8] and development.[9] Microbiomes can also perform metabolic activities that cannot be performed by a single monospecies population. In syntrophic communities, microbes cooperate to perform degradation reactions that would be thermodynamically unfavorable for a single organism.[10] Microbial diversity is a critical variable shaping environmental states. Indeed, a reduction in the taxonomic diversity of microbiomes is associated with human gut microbiome dysbiosis,[11–13] loss of resistance to invasion by pathogens,[14,15] and a decreased level of organic matter deposition and nutrient cycling in soil and oceans.[16,17]

Microbiomes are highly complex and can contain hundreds to thousands of distinct species. These dense and inter-connected networks change as a function of time and spatial organization[18,19] (Figure 1a,b). Environmental factors (physical and chemical parameters) and microbe—microbe interactions combine to shape the properties of microbiomes. In lakes and oceans, microbial community composition is driven by both seasonal and longer-term climate patterns.[20] In the human gut, dietary substrates and drugs modify the growth and biochemical activities of human gut microbiome species.[21–24] In both soils and oceans, climate change has affected microbe—microbe and plant–microbe interactions, contributing to major shifts in the patterns of organism abundance and diversity.[25,26]

Interactions among organisms in microbiomes influence cell growth and biochemical activities, manifesting as positive and negative ecological relationships. Microbial interactions can originate from uptake or secretion of molecules[27,28] or physical contact.

[29–32] The biochemical transformations performed by microbial communities are realized by biomolecular networks distributed among constituent members of the community. For example, the human gut microbiome operates as an anaerobic trophic web wherein metabolic activities are divided among distinct microbial guilds (groups of organisms that exploit the same or related resources). In this system, some organisms degrade complex carbohydrates to simpler sugars, which are fermented by other members of the ecosystem to form short-chain fatty acids.[33] The fermentation products are utilized as substrates by acetogens, methanogens, and sulfate-reducing bacteria.[33,34] Distributed metabolism is a feature of engineered microbiomes as well. In engineered wastewater treatment systems, well-defined guilds are responsible for each step of the nitrogen removal process.[35]

Microbiomes hold significant promise as systems that can be manipulated or designed to address global challenges in medicine, bioenergy, and agriculture.[5] Humans have long used ecological[36,37] and evolutionary[38,39] processes to manipulate microbiomes. However, we currently lack the mechanistic understanding, engineering tools, and predictive modeling approaches necessary to rationally modify the properties of microbial communities and microbiomes. To harness the potential of microbiomes, a major challenge is elucidating the organizational principles of ecological networks that underlie microbiome behaviors. Computational models at different resolutions could enable prediction of microbiome dynamics and functions and inform control strategies. Tools from synthetic biology hold promise to control microbiome activities or design synthetic communities from the bottom up.[40,41]

2. MICROBIAL INTERACTIONS SHAPE MICROBIOME BEHAVIORS AND ARE MODULATED BY SPATIOTEMPORAL PARAMETERS

2.1. Defining Microbial Interactions.

An ecological interaction is defined as the net impact of an organism on the fitness and/or functional activities of a different organism over a given time period. Interactions can be positive (+), negative (−), or negligible (0) and emerge from the cumulative effect of molecules released into the environment and/or physical contact with other microbes as they vary through space and time. For a pair of organisms, there are six types of bidirectional interaction networks: commensalism (0/+), competition (−/−), predation or parasitism (−/+), cooperation (+/+), amensalism (0/−), or no interaction (0/0).[42] The distribution of these pairwise motifs within an interaction network have been shown to influence microbiome stability.[43,44]

Positive interactions can arise when one organism secretes metabolites that can be utilized as a substrate by another[45] or removes molecules that inhibit another's growth (such as through degradation of antibiotics[46]). Negative interactions can arise because of resource competition,[47] production of antimicrobial substrates,[48] or toxic metabolic byproducts.[49] In multispecies communities, pairwise interactions can be altered by other members of the community, representing a pairwise interaction that changes as a function of community context.[50] For example, a negative pairwise interaction mediated by antibiotic production can be weakened by a third organism that exhibits antibiotic-degrading activity.[51]

Nevertheless, pairwise interactions are major parameters shaping the dynamics of multispecies consortia. For example, a recent study demonstrated that time-resolved measurements of single-organism growth and all pairwise communities could be used to train a dynamic model that accurately predicted the assembly of a 12-member human gut microbiome community and all single-species dropouts (all 11-member consortia) in vitro.[44]

2.2. Changes in Microbial Interactions Across Physiological or Evolutionary Time Scales.

Microbial interactions are context-dependent and can change as a function of environmental inputs and spatial positioning[52,53] (Figure 1). On shorter time scales, microbes respond to the environment by sensing specific environmental signals and adjusting intracellular network activities accordingly. A microbe's internal state can be altered in response to abiotic or biotic (biomolecules produced by living organisms) parameters. Organisms grown in coculture have been shown to activate biosynthetic gene clusters for secondary metabolite production that are otherwise silent in monoculture.[54,55] The response of an organism to coculture growth can be quite specific: one study showed that *Streptomyces coelicolor* produced a unique set of molecules in coculture with each of five different actinomycetes, as revealed by mass spectrometry imaging of *S. coelicolor* colonies growing near each actinomycete species.[56] Changes in internal network activities can also alter microbial interactions and community-level functions. For example, *S. cerevisiae* was shown to secrete amino acids in a nitrogen-rich environment, allowing *Lactobacillus plantarum* and *Lactococcus lactis* to grow by utilizing these subtrates.[57] While these studies have illuminated changes in intracellular network activities in simple microbial communities, the network-level mechanisms through which biotic and abiotic factors combine to alter community-level functions in more complex microbiomes remain less understood.

On longer time scales, microbes can acquire and lose functional activities via horizontal gene transfer (HGT) and/or random (vertical) mutations. Evolutionary adaptations to grow and survive in microbial communities have impacted the acquisition and loss of microbial traits, including secondary metabolites such as antibiotics and signaling molecules.[58,59] In particular, the evolution of antibiotic production has been shown to occur via both horizontal and vertical mutation. In an instance of horizontal gene transfer, genomic analysis showed that, when evolved in coculture with *Streptomyces padanus*, *Rhodococcus fascians* acquired a large pathway from *S. padanus* for the production of rhodostreptomycins,[60] a class of antibiotics targeting a set of Gram-positive and Gram-negative bacteria. In an instance of vertical mutation, *Streptomyces clavuligerus* evolved to produce an antibiotic compound holomycin in the presence of *Staphylococcus aureus*[61] because of several mutations affecting secondary metabolite biosynthesis.

Living in microbial communities also enables evolutionary adaptations that are not possible for organisms that live in isolation. These adaptations include (i) cross-feeding, where one species provides a molecule such as nutrient or cofactor that enables a second species to grow or survive;[62,63] (ii) syntrophy, or obligate mutualism, wherein each organism performs a biochemical function essential for survival of a partner or community;[64] (iii) division of labor, where different organisms specialize in specific tasks that are integrated at the community-level such as secreting virulence factors for elimination of competitive

organisms,[65] metabolizing available substrates,[66] or biofilm formation;[67] and (iv) Black Queen evolution (also called genome streamlining) where mutants lose “leaky” nonrequired functions (e.g., those that produce public goods or compounds that can benefit the community, such as extracellular cellulase) and rely on other community members to perform those functions.[68]

In turn, these adaptations can significantly change community-level functions, stability, diversity, and response to disturbances. For example, loss-of-function mutations have been shown to promote diversity in iron-limited communities.[69] In iron-limiting conditions, some organisms produce energetically costly siderophores to scavenge iron from their environment. In these conditions, the siderophore-producer *Pseudomonas aeruginosa* outcompetes *Burkholderia cenocepacia.*[69] However, when *P. aeruginosa* cheaters (those which do not produce siderophores but can scavenge them) are introduced into the population, competition between *P. aeruginosa* cheaters and noncheaters reduces the fitness advantage of noncheating *P. aeruginosa*, enabling coexistence with *B. cenocepacia*.[69] A second study revealed the coexistence of cheater and noncheater *P. aeruginosa* populations in natural communities, suggesting cheating shapes the diversity of natural communities as well.[70] In addition, community complexity can greatly alter adaptive evolution in ways that cannot be predicted from single-species evolution. For example, species in a synthetic community evolved to use waste products generated by other species, an evolutionary adaptation that may be less accessible for the same organisms grown in isolation.[71]

2.3. Spatial Organization Impacts Microbial Community Properties.

The spatial distance separating microbial populations is a major variable shaping the biochemical transformations that can be realized by microbiomes, as well as the metabolic efficiency of these processes (for example, in biofilms[72]). Spatial structuring at multiple scales influences the functions and stability of microbiomes. For example, in a mouse gastrointestinal tract, the spatial distribution of organisms in a synthetic human gut microbiome community exhibited distinct patterns at different length scales.[73] On micrometer scales, species were intermixed, whereas spatial positioning was heterogeneous over longer length scales. These spatial distributions can significantly influence the functional capabilities of the gut microbiota.[73] The density of cells and the level of mixing of different organisms in the environment can alter the frequency and strength of interspecies interactions (Figure 1b),[53,74] and interspecies interactions may alter the spatial organization of microbiomes.[75]

Spatial organization can also be used to alter the outcome of long-term community dynamics. For example, spatial organization and low resource availability can prevent cheaters from dominating in the community.[76,77] The precise mapping between the degree of spatial organization and community-level properties such as stability, diversity, and resilience depends on specific molecular and ecological mechanisms. For example, community stability was maximized in a synthetic consortium of soil isolates when the constituent community members were spatially separated by intermediate distances.[53] Such optimal distance responses can arise because of trade-offs between positive or negative interactions that vary as a function of spatial separation.[53] Exploiting this principle, bacterial

signaling circuits programmed to activate only at intermediate distances from a signal sender population can generate spatial patterns.[78] The quantitative contributions of spatial parameters and temporally changing inputs to microbial community behaviors can be studied using microfabrication techniques.[79]

3. COMPUTATIONAL MODELING OF MICROBIAL COMMUNITIES

Developing the capability to predict the response of micro-biomes to environmental inputs will allow rational control and bottom-up design of microbial communities. This section will describe modeling approaches for prediction of microbiome behaviors at different temporal scales and levels of biological organization (Figure 2).

3.1. Genome-Scale Metabolic Modeling.

Genome-scale models (GEMs) can be used to predict mechanisms of metabolic interaction (Figure 2, red box).[80] From a mathematical representation of an organism's metabolic reactions, these models predict the rates and yields of metabolites consumed and secreted by each monospecies, as well as between the community and the environment.[81,82] GEMs are analyzed using techniques from mathematical optimization, in which physiochemical constraints and optimality principles (i.e., maximization of an objective function, such as total community biomass) are applied to predict rates and yields.[82] Although GEMs assume an intracellular steady state, they can still be used for predicting temporal dynamics, provided the steady-state assumption holds.[83] These dynamic models predict metabolite and biomass concentrations, commonly by embedding steady-state models inside kinetic models of cellular growth and uptake.[83] Steady-state models have been extensively used to simulate microbe—microbe and host-microbe interactions.[82,84–90] Dynamic methods have been used to study interactions in the subsurface,[91,92] as well as to predict emergent biosynthetic capacity,[93] defined here as community production of a metabolite not produced by any of its constituent organisms growing alone.[93] For example, a model of competition between the acetate-oxidizing metal reducers *Rhodoferax* and *Geobacter* correctly predicted the relative abundance of these two species under different geochemical conditions and forecasted that *Geobacter* is responsible for enhanced uranium reduction under conditions of low organic nitrogen.[91]

Despite their widespread adoption and accessibility,[94,95] GEMs have a number of limitations. First, GEMs do not include regulatory information, and predicted metabolic states may not be achievable because of cellular regulation. Nonetheless, methods for incorporating regulatory networks into GEMs are emerging.[96] Second, methods to analyze GEMs have an implicit requirement of evolutionary optimality.[97] Optimal strategies may be unknown, change through time, or trade off with one another.[98] In some cases, evolutionary optimality may not hold at all, such as in synthetic consortia composed of organisms that have not evolved in the same environment and thus have not encountered one another. Third, GEMs have many degrees of freedom and may require knowledge of the microbiome interaction network for accurate predictions.[88,99] For dynamic GEMs, knowledge of growth-limiting substrates and their kinetic parameters may also be required for accurate predictions.[100]

3.2. Differential Equation Modeling.

Differential equation models are a broad class of models that can be applied to predict population dynamics, multisubstrate utilization, and/or intracellular network activities in microbial communities. Generalized Lotka–Volterra (gLV) models are commonly used to capture community dynamics (Figure 2, blue box). In gLV models, the abundance of an organism is a function of its intrinsic growth rate plus a term accounting for the sum of pairwise interactions with all members of the community, including self-interactions.[101,102] Using techniques from dynamical systems theory, gLV models can also be used to evaluate community properties such as stability (the rate at which a community returns to an equilibrium point following a disturbance).[103] Apart from interspecies interactions, gLV models have been used to simulate diverse aspects of microbial community behaviors, including assembly,[104] stability,[105] coexistence,[44] and history-dependent dynamics.[44] gLV equations have also been extended to include environmental inputs, including antibiotic administration[105] and temperature and nutrient concentration.[106]

Despite their utility, gLV models have several limitations. First, microbes can interact both positively and negatively, and these interactions can change through both space and time. In a gLV model, the single, fixed interaction term represents the inferred cumulative impact across all of these mechanisms over a period of time. Second, some studies have shown that pairwise interactions are insufficient to fully explain community dynamics,[107] meaning that gLV models may need to be extended to include higher-order interactions. Furthermore, standard gLV models may require a mechanistic representation of metabolite exchange to capture some types of pairwise interactions.[107,108]

Mechanistic differential equation models provide an alternative approach to overcoming some of the limitations of gLV models (Figure 2, purple box). In a mechanistic model, molecular-level details of the interaction are explicitly represented, and such models have been used to model 3-and 4-member communities with known mechanisms of molecular interaction.[107,109,110] However, because of a need to characterize the mechanisms of interspecies interaction, determine the appropriate model structure, and identify model parameters, mechanistic models are significantly more difficult to develop than gLV models. As a result, mechanistic models typically describe only a few metabolic reactions performed by a small number of organisms, although some genome-scale mechanistic models have been developed.[111–113]

3.3. Modeling Cellular Heterogeneity Using Individual-Based Models.

Heterogeneity within microbial communities can arise from variation in microenvironments and/or variation within individuals. To capture spatial heterogeneity, partial differential equation models (PDEs) are used to model the underlying processes, such as heat and mass transfer.[114] Heterogeneity within populations can be modeled using individual-based models (iBMs), which represent the activities of individual cells (or populations of cells) (Figure 2, green box). In these frameworks, system-level properties emerge from the combined activities of individuals.[115,116] In many environments (such as biofilms[117]), microbial communities exhibit both spatial and individual heterogeneity, and PDEs and IBMs must be combined to model community behaviors.[116]

Recently, GEMs have been combined with IBMs. Using this approach, GEMs are used to simulate cells or populations on a lattice (a structured arrangement of points representing a surface),[118–120] with differential equations describing molecular diffusion on the lattice. These models have been used to study the emergence of competition and cooperation in 2-or 3-member consortia,[118] niche formation in the human microbiome,[119] and spatial variation in colony size.[121] A mechanistic, individual-based model of the cyanobacteria *Anabaena* has also been developed, wherein nitrogen metabolism within each cell was mechanistically modeled via differential equations.[122]

As with other modeling approaches, both PDEs and IBMs have limitations.[115,116] PDE models typically do not account for phenotypic heterogeneity within members of a population.[115,116] For example, the nonlinear Droop equation describes the growth rate of phytoplankton as a function of their intracellular phosphorus (P) content. Because of this nonlinearity, the growth rate of a population with average P content is greater than the average growth rate of the individual cells with a distribution of P contents.[123] In addition, differential equation models assume that populations of discrete individuals can be approximated using continuous functions.[115,116] In contrast, IBMs explicitly model individual organisms, enabling them to capture population heterogeneity as well as interactions between individual cells and between individual cells and their local environment.[115,116] However, IBMs may result in complex and nonlinear dynamics that make parameter estimation difficult.[116] Finally, integration of PDE models and IBMs poses its own challenges, such as representing processes occurring on different spatial or temporal scales.

3.4. Game-Theoretical Evolutionary Models.

The long-term outcome of changing interactions in microbial communities can be analyzed via evolutionary game theory (Figure 2, orange box).[124] In this modeling framework, evolving entities (players) alter their reproductive fitness by adopting life-style strategies, or adaptations (such as cheating).[124] These adaptations alter the fitness of the players, in a manner that depends on the behavior of other players in the game, as well as their relative abundance in the population.[124] Apart from simulating evolutionary dynamics, evolutionary game theory can also be used to identify evolutionarily stable strategies, defined as combinations of strategies that are resistant to invasion (that is, there are no other strategies that would provide increased fitness if adopted). Additionally, evolutionary games can be transformed into gLV models,[124] enabling techniques from dynamical systems theory to be applied to microbial communities on evolutionary time scales.

Evolutionary game theory was able to explain snowdrift dynamics, defined as the stable coexistence of cheaters and cooperators in a population.[125] In a second example, game theory was combined with GEMs to predict evolutionarily stable interactions mediated by the exchange of specific amino acids.[126] Finally, IBMs have been combined with trait-based models, in which fitness emerges from trade-offs among traits (measurable aspects of an organism’s phenotype, such as cell size or temperature sensitivity).[127] Such models have been used to study the factors shaping cell size in oceanic phytoplankton[128] and to highlight the importance of interspecies interactions in evolutionary outcomes.[129]

Despite these successes, evolutionary game theoretical models have a number of significant limitations. Using these models requires a priori knowledge of potential evolutionary strategies and their context-dependent effects on fitness.[124] Furthermore, because ecological strategies and their effects on fitness are determined in advance, evolutionary game theory cannot predict the adoption of novel strategies or predict how their fitness changes in the face of a dynamic environment. Additionally, the probability that a particular strategy arises depends on the mechanism of adaptation, suggesting that evolutionary models will benefit from incorporating mechanistic information.[130] Finally, evolutionary games with two players and two strategies have been studied in great detail,[131] whereas microbial communities contain numerous organisms and strategies. This additional complexity can result in a breakdown of classical results from two-player games.[131]

4. MANIPULATING THE FUNCTIONS OF NATURAL AND SYNTHETIC COMMUNITIES

Traditional approaches for modifying the properties of microbial communities involve iterative evolution by selecting for specific phenotypes.[132] Microbial communities used for wastewater treatment are a classical example: starting with a sample containing the organisms of interest, reactor engineering and environmental selection are used to enrich for strains with the desired traits,[39] and metagenomic assembly/binning and metatranscriptomic profiling can be used to identify organisms potentially responsible for a function.[133] However, selection-based approaches have notable limitations, including challenges in reverse engineering the mechanisms underlying changes in target function activity.[134,135] In addition, because evolutionary optimization requires selection on existing traits, selection-based approaches cannot install novel capabilities. Here, we discuss how tools from synthetic biology hold promise to manipulate microbial communities or design microbial communities from the bottom up.

4.1. Harnessing Synthetic Biology to Manipulate Microbial Communities.

A core challenge in microbiome engineering is precisely altering the abundance of specific microbial populations to steer communities to desired states. Methods based on RNA-guided nucleases have been used to target specific organisms in multispecies communities. For example, CRISPR-Cas circuits delivered by bacteriophage or conjugation were used to target DNA sequences associated with antibiotic resistance or virulence, leading to selective killing of strains harboring these sequences.[136,137] The host range of bacteriophage has also been modified for population editing using a modular tail fiber swapping method[138] (Figure 3a). A third approach involves engineering controller organisms to selectively sense a pathogen and produce and deliver antimicrobials that inhibit the growth of the pathogen. This strategy has been used to sense and target pathogens *including* Salmonella, Pseudomonas aeruginosa, *and* Vibrio *cholerae.* [139–142] In CRISPR-Cas-based methods, efficient delivery and expression of the circuit components in target organisms can present challenges. Challenges in phage engineering include high mutation rates and programming phage host range to target specific populations.[143] Finally, engineering a controller strain to sense and target specific organisms has a number of challenges, including ensuring the ecological and functional stability of the organism in the community for a period of time,

developing tools for selective sensing, and targeting of specific organisms in the community.[144,145]

The introduction of non-native strains (such as probiotics) to microbiomes also presents several challenges. The ecological and functional stability of a non-native strain is dependent on regulatory adaptation to the target environment, ability to secure a niche, and could potentially destabilize the resident community.[146] For example, a recent human trial showed a strong negative association between colonization ability of *Bifidobacterium longum* AH1206 and the abundance of other *B. longum* strains and specific carbohydrate utilization genes that contribute to the ability of *B. longum* AH1206 to secure a niche in this environment.[147]

An engineered organism could be introduced into a microbial community to alter community-level outputs (Figure 3b). The abundance of the engineered organism could be modulated by an external parameter, such as nutrient availability. Engineering unique niches could allow stable integration of non-native organisms in microbiomes. For example, porphyran-degrading *Bacteroides* species were able to colonize mice fed a diet containing porphryan,[148,149] and the abundance of these organisms was altered by administering different concentrations of porphyran or a pulsed diet. These studies also show that abundance of porphyran-degrading *Bacteroides* could be more precisely controlled in mice with fewer species in their microbiota, suggesting that community context can affect the controllability of species.[148,149]

An alternative to niche engineering involves genetic manipulation of the biochemical activities of resident organisms of microbiomes. For example, the microbiome-produced metabolite indolepropionic acid (IPA) is present in human serum and is associated with reduction of intestinal permeability, radical-scavenging, and neuroprotective functions.[150] When a mutant symbiont *Clostridium sporogenes* deficient in IPA production was introduced into mice colonized with a defined, IPA-deficient microbial community, IPA levels in serum were not detected and intestinal permeability was increased compared to control mice harboring wild-type *C. sporogenes.*[150]

Finally, tools from synthetic biology can be used to engineer microbiomes by introducing novel functions. When engineering principles are applied, organisms can be designed to sense specific environmental signals (e.g., pH, temperature, and light), perform computation on these signals, and produce specific output responses[151,152] (Figure 3c). Harnessing synthetic biology approaches to manipulate microbiomes presents several challenges. First, synthetic circuits must be designed to operate robustly in the presence of environmental variability and selectively respond to specific environmental inputs.[153] A recent study demonstrated that a genetic circuit encoding memory of the transient inflammation signal tetrathionate in a murine *Escherichia coli* strain retained memory for long periods of time, as evidenced by detection of activated circuits in fecal samples six months after inflammation was induced in mice.[154] A second challenge in engineering genetic circuits is minimizing fitness burden to the host cell due to competition for limited intracellular resources between the host cell and the synthetic circuit.[155,156] If the metabolic costs are significant, nonfunctional mutants could rapidly outcompete the functional subpopulation.[157,158]

Although many advances have been made to design circuits to minimize metabolic burden,[159] it is still difficult to predict the interactions between the host cell and the synthetic circuit and use this information to inform circuit design.[160,161] Finally, biocontainment strategies must be developed to prevent engineered organisms from escaping into other environments.[162] To implement biocontainment, toxic genes have been used to induce cell death in response to specific signals processed by multilayered logic gates.[163] However, the killing efficiency of the circuits declined over time and these circuits have not been characterized in complex environments.[163]

4.2. Design of Synthetic Communities from the Bottom Up.

In natural microbial communities, biochemical transformations are partitioned among diverse organisms, a phenomenon known as division of labor (DOL)[164] (Figure 3d). DOL is thought to increase productivity by enabling community members to specialize at certain subtasks.[165] In fact, several studies have demonstrated that synthetic consortia engineered for DOL have potential advantages compared to engineering a single strain, including modularity, the ability to exploit the unique capabilities of different strains,[166] and the ability to control product rates and yields by altering community composition.[167] Nevertheless, engineering DOL also has potential disadvantages, such as competition for shared resources and challenges maintaining stable coexistence of community members over time.[168,169] The trade-offs between advantages and disadvantages of DOL have been recently explored using a theoretical framework.[170] By analyzing 24 common architectures of metabolic pathways in which DOL can be implemented, a recent study concluded that DOL is advantageous when the increase in productivity due to DOL (as measured by product titer) is larger than the decrease in productivity arising from resource competition and limitations in cellular transport rates. The shape of the trade-off among these factors varies with metabolic pathway architecture.[170] Furthermore, the authors suggest that DOL increases productivity when it reduces metabolic burden or toxicity. Metabolic burden and toxicity could be caused by expression of multiple genes in a pathway or the production of toxic intermediates.[171] However, methods to predict the metabolic burden or toxicity of engineered pathways are lacking.

A core challenge for microbiome engineering is achieving stability as a function of time in response to perturbations. Equilibrium stability is a concept from dynamical systems and is defined as the rate at which a system returns to its original state (equilibrium point) following a perturbation. However, this definition does not apply to systems operating away from equilibrium. Microbial communities are highly dynamic and may not be operating at equilibrium because of temporally changing environmental stimuli. In fact, fluctuations in population sizes driven by external inputs to the system can promote species diversity over time.[172] In microbial community engineering, rewiring of interactions among constituent community members may be required to achieve stability. Currently, the types (positive/negative/negligible), molecular mechanisms, and distributions of required interactions to realize stability and resilience to perturbations remain unresolved. A theoretical study showed that stability is enhanced by limiting positive interactions or globally weakening interactions.[43] Bidirectional positive interactions can be destabilizing because of positive feedback loops, which renders the network sensitive to fluctuations in the abundance of

organisms that are positively coupled in the network. Globally weakening interactions in the ecological network will result in a smaller change in organism abundance in response to variations in the abundances of other community members. A balance between negative and positive interactions in an ecological network can promote system stability: network topologies consisting of positive/negative (+/−) interactions can exhibit robust species coexistence by establishing a negative feedback loop.[44] A different theoretical study showed that competitive interactions are stabilizing only when species exclusively consume but do not produce resources.[173] The same study predicted that positive interactions are stabilizing when all pairwise interactions are reciprocated, or when production rates are sufficiently small.[173]

These predictions could be tested using engineered microbial communities. In addition to exploiting well-characterized mechanisms including bacterial signaling systems, cross-feeding of amino acids and metabolic byproduct utilization, microbial interactions can also be constructed by rewiring biosynthetic pathways.[110,174–176] In one study, the authors used modular genetic pathway reconfiguration to design pairwise consortia with distinct interaction topologies.[110] Experimental results from these pairwise consortia were used in model-guided design of three-and four-strain communities with predictable behaviors.[110] In addition, spatial organization can augment community stability by excluding cheaters and invaders[76,177] (Figure 2b) and could thus be harnessed as a design parameter for achieving desired community-level functions.

5. PERSPECTIVES

5.1. Challenges in Identifying Mechanisms of Ecological Interaction over Short and Long Time Scales.

Experimental approaches to determine ecological relationships and molecular mechanisms in microbial communities have largely focused on simple communities under well-defined conditions. Fabricated ecosystems that mimic the physical and chemical structure of natural environments (such as the gut-on-a-chip) can be used to determine environmental effects on interaction networks by providing investigators precise control over environmental variables.[178,179] Once interactions have been identified, high-throughput genetic approaches such as Tn-Seq[180] or genome-scale CRISPRi libraries[181] could identity genes that enhance or weaken an interaction. Furthermore, metabolomic techniques such as mass spectrometry imaging could be used to identify molecules that may underlie the interaction.[182] Isotope-labeling techniques such as ^{13}C-metabolic flux analysis could be used to quantify intracellular fluxes,[183,184] thereby linking intracellular metabolic states to interaction networks.

Long-term dynamics of microbial communities are ultimately determined by evolution. We currently have a limited understanding of ecological and evolutionary time scales of microbial communities and lack an effective strategy to predict or control the long-term evolution of microbiomes. One experimental evolution study showed that replicate communities followed a cluster of evolutionary trajectories measured by community structure, suggesting that community evolution may be partially determinstic.[185] A second study showed that evolutionary outcomes of pairwise growth experiments followed simple

qualitative rules, although the predictions were limited to a small set of closely related species with competitive interactions.[186] In sum, these studies suggest that replication of laboratory evolution experiments is a useful tool for quantifying the forces shaping microbiome evolutionary dynamics. However, a core challenge is extracting evolutionary design principles that can be generalized across different systems. Similar experiments should be undertaken to predict community evolution patterns in more complex communities, in the presence and absence of spatial structure, and over evolutionary time scales.

5.2. Challenges in Computational Modeling of Microbiomes.

Computational models of microbes and microbial communities are often optimized for processes occurring at distinct spatial or temporal scales. However, developing a quantitative and mechanistic understanding of how molecular-level changes alter long-term evolutionary outcomes will require multiscale frameworks that can link processes occurring at different temporal or spatial scales. Some efforts are already underway, such as frameworks that embed constraint-based models of metabolism inside spatial[118] or evolutionary models[187] (Figure 2). Model-reduction techniques could be used to simplify mechanistic multiscale models. In addition, combining time-resolved measurements of community structure and/or function (e.g., metabolomics) with machine learning could be used to identify model structures underlying community dynamic behaviors. Such an approach has recently been used to predict metabolic pathway dynamics in engineered *E. coli.*[188] Regardless of the modeling approach used, computational models must be rigorously validated with properly collected data in order to be useful. Validation may require new tools for spatial and temporal monitoring of metabolites, environmental parameters, and absolute abundance quantification of species abundance. Furthermore, the number of system parameters that could be modeled far exceeds the number that may be practical to infer from data. Combined experimental and computational studies could elucidate the types and frequency of data to collect, as well as the most informative way to use these data to accelerate predictive modeling.

5.3. Engineering Microbiomes or Designing Microbial Communities.

When introducing novel functions into natural microbiomes, model organisms such as *E. coli* may be preferred because of the availability of genetic tools. However, to persist stably in a new environment, laboratory strains must first be adapted to the abiotic and biotic properties of the target environment. If the key parameters of the target environment are known, microbes could be adaptively evolved in the lab for increased fitness before introduction to the new environment. Additionally, engineered organisms can be rapidly adapted to new environments using functional metagenomic libraries containing the genetic sequences from the resident bacterial community.[189] Alternatively, microbes native to the target environment may be more suitable as an engineering chassis because of pre-existing adaptations. Thus, further development of genetic tools and parts libraries for nonmodel microbes should be encouraged, such as the promoter and ribosome-binding site libraries that have been developed for the human gut commensal *Bacteroides thetaiotaomicron.*[190]

When constructing synthetic microbial communities, a major challenge is optimizing division of labor (DOL) for community-level functions. DOL is determined in part by the metabolic burden an engineered pathway imposes on a cell across different environments. Indeed, the trade-off between engineered circuit activity and host-cell fitness depends on a changeable and uncertain environment. Coarse-grained kinetic models or genome-scale metabolic models could be used to simulate the fitness costs associated with different DOL schemes and predict functional activities associated with different configurations. Communities could be constructed and characterized, and the results used to identify new constraints and trade-offs that impact DOL.

Finally, control of microbiome structure and function requires advances in our understanding of how to manipulate complex networks of microorganisms that consist of numerous interconnected feedback loops. A control-theoretical frame-work should be developed for microbial communities to determine a minimum set of influential organisms and/or environmental inputs that need to be manipulated to steer the system to specific states. To implement such control architectures, we need modular platforms for rapidly designing microbiome editing tools to control system behaviors.

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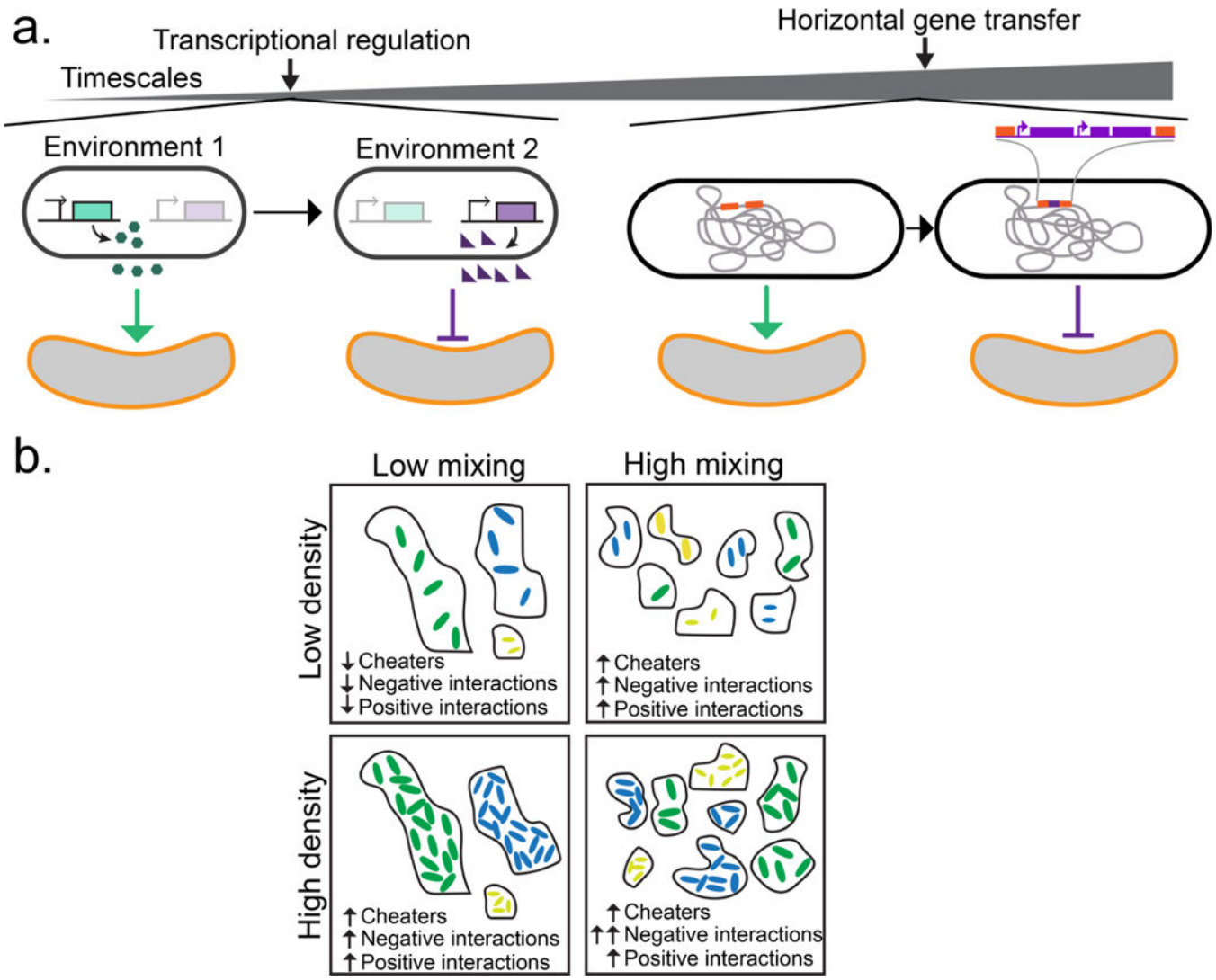

Figure 1.

Interactions among organisms within microbiomes change through time and space. (a) Changes in intracellular network activities or changes in genotype via random mutation and/or horizontal gene transfer (HGT) can lead to changes in microbial interactions in microbiomes. Left: On short time scales, regulatory networks govern how microbes respond to their environment. In one environment, one microbe may produce molecules that lead to a net positive impact on a second microbe (green circles and green arrow), while in a second environment the same microbe may produce molecules that lead to a net negative impact on a second microbe (purple triangles and purple arrow) due to changes in transcriptional activity. Right: On longer time scales, genetic changes due to HGT can alter microbial intracellular networks and contribute to changes in ecological relationships. In natural competence (one mechanism of HGT), extracellular DNA can be taken up by a recipient organism and integrated into the chromosome via homologous recombination. When this occurs, the functional activities of a microbe can change, manifesting as a change in the ecological network from a net positive (green arrow) to a net negative interaction (purple

arrow). (b) Schematic highlighting a hypothesis about how spatial mixing (*x*-axis) and population density (*y*-axis) can alter the frequency of cheating (wherein the benefit from cooperating is disproportional to the contribution toward cooperation) and the distributions of positive/negative interactions in a microbial community. Downward arrows indicate a low frequency, and upward arrows indicate a high frequency. When population densities and spatial mixing are low, microbes are less likely to encounter one another, leading to infrequent interactions and a low frequency of cheating due to low concentrations of public goods ("low density, low mixing" box). As population densities and/or spatial mixing increase, both the frequency of interactions and frequency of cheating increase ("low density, high mixing" and "high density, low mixing" boxes). In well-mixed, dense communities, negative interactions will be especially frequent because of resource competition ("high density, high mixing" box).

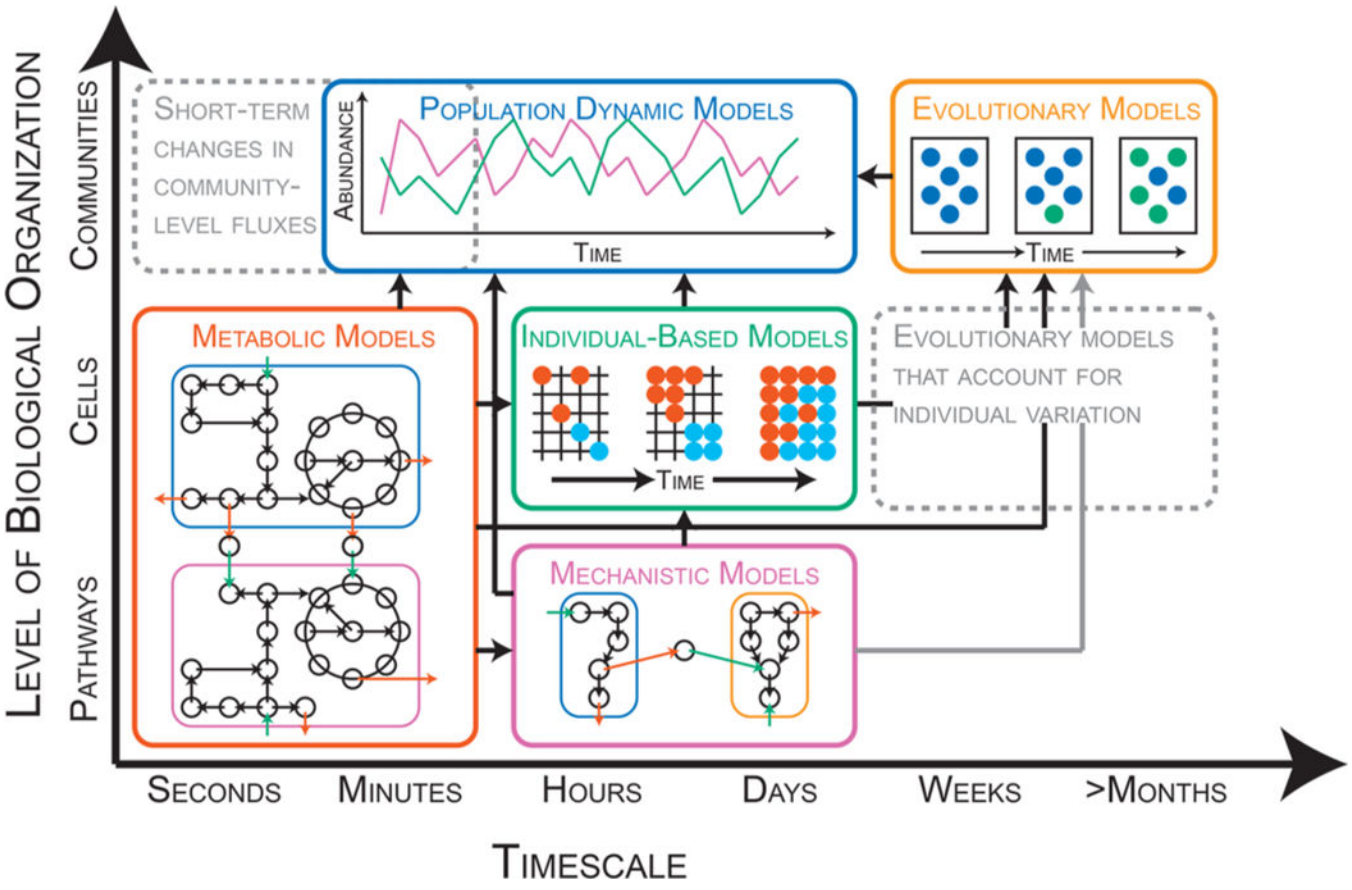

Figure 2.

Schematic representation of modeling frameworks for processes occurring at different temporal (*x*-axis) and spatial (*y*-axis) scales. Metabolic, population-dynamic, individual-based, mechanistic, and evolutionary models are represented by the red, blue, green, purple, and orange boxes, respectively. Dashed gray boxes indicate temporal/spatial scales where modeling frameworks are currently missing. Black arrows between boxes indicate existence of publications that link modeling frameworks. Gray arrows between boxes indicate opportunities to link modeling frameworks. Metabolic models: Steady-state flux of metabolites (black circles) in and out (green arrows and red arrows, respectively) of two cell populations (blue and purple boxes) as predicted by a genome-scale metabolic network (black circles and arrows). Population-dynamic models: Change in abundance of two populations (green and purple) over time. Individual-based models: Emergence of spatial partitioning of two populations of cells (red and blue circles) on a discrete lattice, a structured arrangement of points representing a surface (black gridlines). Mechanistic models: Dynamic flux of metabolites (black circles) in and out (green arrows and red arrows, respectively) of two cell populations (blue and orange boxes) as predicted by a model of a biochemical pathway (black circles and arrows). Evolutionary models: Change in community population structure through evolutionary time. Initially, the population is homogeneous (blue circles, left-most rectangle). One cell acquires a mutation that provides a fitness advantage (green circle, middle rectangle). As a result, the community population structure changes over time (green and blue circles, right-most rectangle).

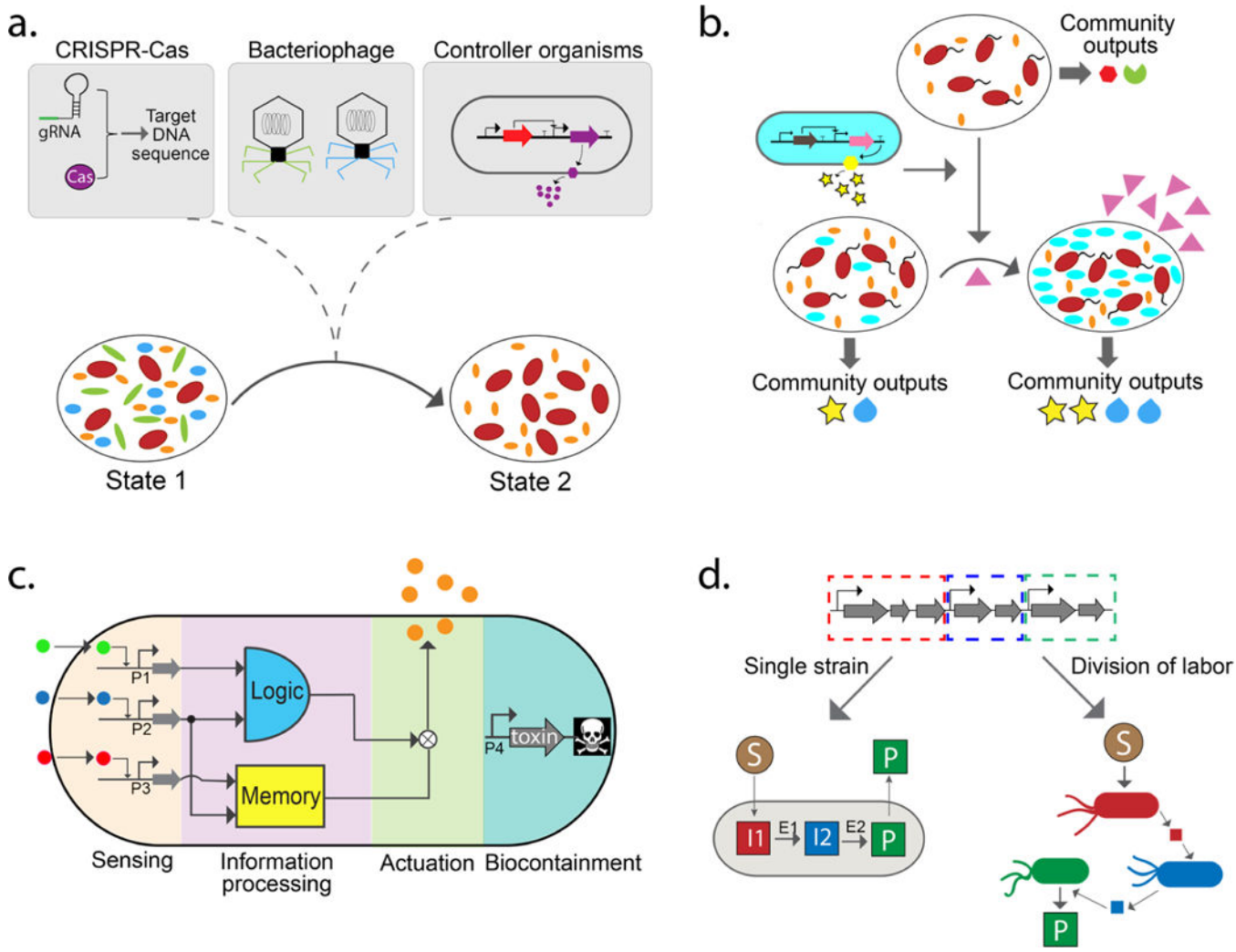

Figure 3.

Strategies for engineering natural and synthetic communities. (a) Precise inhibition of specific organisms in natural communities via (i) CRISPR-guided nucleases that target specific DNA sequences, (ii) engineered bacteriophages, and (iii) microbes designed for production of antimicrobial compounds. (b) Introduction of an engineered organism (cyan) into a microbial community. The engineered organism can stably integrate into the community and alters community-level outputs (represented by yellow stars and blue teardrops). The abundance of the new species could be controlled through niche engineering, wherein a non-native organism is engineered to utilize a unique substrate (pink triangles) that cannot be utilized by other members of the community. (c) Design of cellular control organisms using engineered biomolecular networks to perform novel functions in complex environments. Functional modules include environmental sensors, information processing (e.g., memory and logic circuits), actuators (e.g., antimicrobial compounds), and growth control circuits for biocontainment. (d) Construction of synthetic communities from the bottom up to perform desired functions through division of labor (DOL). Top: A single biochemical pathway can be conceptually decomposed into three subpathways (red, blue, and green). Left arrow: The biochemical pathway for synthesizing a target molecule can be

introduced into a single cell population. Right arrow: The biochemical pathway is partitioned among different organisms, reducing the metabolic burden on individual populations but requiring stable coexistence as a function of time. S, substrate; P, product; I, intermediate; E, enzyme.