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Voices

What is the key challenge in engineering microbiomes?

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Deciphering influential control knobs for microbiomes

The microbiome functions that we aim to engineer emerge from a myriad of unknown and dynamic cellular interactions. These functions range from driving biogeochemical cycles in the environment to enabling colonization resistance to invading pathogens in host-associated microbiomes. Depending on the application, these functions can be major contributors to a process or significant minor contributors to a process. In the latter case, even the most effective engineering of microbiomes may only moderately change a given process since other factors beyond the microbiome are the dominant drivers. Therefore, we should prioritize applications in which the microbiome is a major determinant of the target process.

In addition, we should embrace the immense complexity of these multi-scale biological networks shaped by dynamic nonlinear interactions, feedback loops, spatial heterogeneity, and stochastic processes. Constituent community members within microbiomes frequently compete for limited nutrients or produce toxins that inhibit species growth. Members of these communities can also work together to extract energy from chemically complex nutrients and detoxify the environment. In host-associated microbiomes, host-microbiome feedback loops can drive the system into beneficial or deleterious states for the host that are resistant to change. The key challenge is developing the capability to decipher these complex interaction networks that determine the emergent properties of the system. Microbiomes can contain hundreds of species, and these high-richness communities can display resistance to perturbations. Therefore, a major challenge is elucidating the ecological and molecular mechanisms that are influential in steering the system to desired states and developing strategies for precise control.

Harris H. Wang
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Predicting and editing microbiome stability

Even though microbiomes are highly distinct across environments, a defining feature of most microbiomes is that they are compositionally stable over time. Therefore, understanding and predicting microbiome dynamics and stability is key for constructing and engineering them in a variety of applications. Unfortunately, we currently do not have the ability to accurately predict whether an exogenous strain can engraft into a microbiome or whether removing a native strain will disrupt the rest of the community. Overcoming these challenges will require foundational characterizations and new technological advances. First, we need comprehensive profiles of microbes at the strain level to understand their genomic repertoire, transcriptomic regulation, and functional capacity. Second, we need to map the spatial organization of microbes at the micrometer scale and their temporal fluctuations at high resolution in order to understand how microbes spatially co-associate, temporally co-vary, and functionally interact across interspecies metabolic networks. Third, we need precise editing tools that can alter specific members of a microbiome to change their composition, function, and/or stability in the community. Finally, we need better modeling frameworks that can fully integrate high-dimensional spatiotemporal, genomic, and functional microbiome datasets to predict population trajectories and metabolic outputs while also yielding interpretable rules for community assembly and stability. These advances will have a far-reaching impact for microbiome engineering to address global challenges in health and environmental sciences.

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Embracing the complexity of microbiomes using model communities

Natural microbiomes are dynamic, complex, open systems subject to environmental fluctuations such as shifts in nutrients, abiotic stressors, migration events, and host-derived factors. Predicting how they adapt to rapidly changing environments is a major challenge. Precision microbiome engineering will require uncovering and disentangling general principles from system-specific features and extending our ability to sample, measure, and model natural microbiomes. Just as microbiology has been empowered by the development of model organisms, the microbiome field must develop methodologies for generating and characterizing model communities and environments. On one hand, top-down strategies for deriving communities from natural samples result in collections of species representative of the ecosystem of interest, potentially possessing a hidden diversity of rare/undetectable bacteria and phages that can exhibit new dimensions of community response to perturbations. On the other hand, bottom-up synthetic co-cultures of isolated species enable precise control of community membership and species drop-out. Importantly, the two perspectives are complementary, and with improved methods of isolating and culturing low-abundance or fastidious members, a top-down community can be transformed into a synthetic community. These model communities empower high-throughput, systematic, iterative experimentation at a scale unrealizable in animals or natural environments. This wealth of data is empowering development of computational models that incorporate various species' interactions with their abiotic/biotic environment and enable predictive community engineering. By using model communities to embrace the complexity of natural microbiomes, we can create new insights and opportunities to manipulate microbiomes in an open and changing world.

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Engineering microbial communities for bioproduction

Microbiomes or microbial communities are ubiquitous, and their importance is well known in fields like medicine, ecology, agriculture, and food manufacturing. Recently, the potential of using communities in industrial bioproduction—the use of microorganisms to convert feedstocks into commercial products—has attracted a lot of attention. The reasons are 2-fold. One, coordinated communities can outperform the yields of monocultures (e.g., through division of labor). Two, these communities allow us to study consortia rules and dynamics in "simpler" systems and controlled environments, which can shed light on more complex microbiomes (human, plant, soil, etc.). However, while simpler, studying and creating industrial microbiomes is not easy. The challenges span the different steps of the typical synthetic biology workflow: the Design, Build, Test, Learn (DBTL) cycle. "Design" challenges are critical to save time in the implementation steps. Design is informed by both previous knowledge and predictive models, and these two aspects are limited when studying communities. Metabolic models, especially those curated for exchange reactions, and machine learning have the potential to improve the *in silico* design of microbiomes. Challenges in "Building" communities are often due to the lack of molecular tools to efficiently engineer desired behaviors such as cooperation and robustness, and many efforts are being made in this space with encouraging achievements. The "Testing" and "Learning" steps rely on our capacity to track population dynamics and analyze metabolism (e.g., metabolite exchange). Advances in multi-omics, particularly metabolomics, and single-cell techniques will be instrumental to progress. Fortunately, many of us are facing these challenges and developing tools and synthetic microbiomes, which can help shape the sustainable (bio)factories of the future.

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Robustness in uncertain environments

Our desire to construct new microbial communities and manipulate existing microbiomes has exploded over the last decade. This desire has coincided with, and has perhaps been facilitated by, two great strides in science: metagenomics has allowed us to better understand the ubiquity and complexities of microbiomes, and synthetic biology has turbo-charged our ability to engineer biological systems. Advances in both of these areas are needed to make microbiome engineering routine. We need methods that can cheaply elucidate microbiome dynamics over short timescales alongside more comprehensive libraries for engineering community interactions. These challenges are technical and, with some effort, will be overcome.

There is, however, a more complex challenge in microbiome engineering. Whether we want to treat disease with live bio-therapeutics, produce valuable chemicals in a bio-reactor, or bio-remediate polluted ecosystems, the environment is uncertain yet fundamental. Resources flow in and out and are produced, consumed, modified, and degraded. Competitors come and go, grow and move, and adapt and die. Temperatures change, forces are applied, gases diffuse, and liquids evaporate. While some of this may be predictably periodic, much of it will occur unpredictably in time and space. Although these same challenges are present for monocultures, the environment is an intrinsic part of a microbiome—it is, after all, the arena within which all interactions are mediated. This uncertainty requires us to adapt our thinking when working with microbial communities. Our designs must be able to bend to external pressures—within reason—and to fail safely and predictably when those pressures are too great.

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Identifying the culprits

It has been more than two decades since publication of the first sequence-based microbiome study that the US Food and Drug Administration (FDA) recently approved, the first fecal microbiota product to treat recurrent *C. difficile* infection (rCDI). While this represents a huge success for the field, microbiome-directed therapies for other diseases are still far from clinical implementation. Identifying the "missing microbes" in low complex or severely disrupted microbiomes and supplementing them by fecal microbiota transplantation (FMT) or more defined microbiome therapeutics is low-hanging fruit compared to correcting the microbiome in inflammatory bowel diseases or cancer, where a broad spectrum of microbes promote the disease in many different ways. Although strain-specific therapies have shown promising results to eliminate individual culprits, development of more broadly applicable tools for microbiome engineering is critically needed. But above all, identifying the disease-driving entities of human microbiomes in the first place still constitutes another major challenge in the field. One reason is that the functional characterization of our microbes is only at the beginning, and most of their genomic content is still "dark matter" to us. Furthermore, there is an urgent need to uncover microbial (metabolic) interaction networks, which constitute the functional basis of microbial communities, and implement them into next-generation microbiome-engineering approaches. Curiously, FMT "works" broadly and reproducibly against rCDI, even though we do not fully understand the mode of action. In the case of more complex diseases, we will only make progress if we can identify the disease-causing entity, implement specific diagnostic tools, and develop tailored and personalized approaches for patient treatment.

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Systematic prediction of microbiome function

While tools to construct and manipulate microbiomes are improving, predicting the desired functional outcome of a microbiome based on its membership and environmental conditions remains a key challenge. This is driven by the high complexity of microbiome interactions and calls for a systematic approach to guide engineering. Genome-scale models are one promising approach, given their ability to represent community metabolic networks and interactions. However, obtaining accurate network reconstructions for microbiomes with tens to thousands of poorly defined strains or species is difficult and can result in poor predictions due to incorrect reactions, missing regulatory information, and insufficient constraints.

An alternative approach is to instead use machine learning to map the interaction of individual community member functions (or loss of functions) to microbiome function. A major challenge here is the massive amount of data required, demanding a rethink on bench experiments. Lab automation, such as liquid-handling robots and microfluidic devices integrated with real-time functional readouts (e.g., metabolite and cell concentrations), can provide the needed throughput to construct and test microbiomes, although advances in cultivation methods, on-line analysis, solid/gaseous substrate handling, and building spatial gradients are needed. Defining microbiome composition around functional capabilities (or guilds) rather than strain taxonomy may also help reduce data requirements for searching the enormous microbiome design solution space. With new possibilities to create datasets where microbiome composition can be precisely manipulated through bottom-up assembly or emerging *in situ* genome editing tools, systematic prediction of microbiome function driven by lab automation and machine learning may be possible. This would also generate novel hypotheses that can lead to deeper understanding of microbial interactions.

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The ever-changing landscape of the gut

The luminal environment of the gut is constantly changing, with fluctuations in factors such as nutrient availability, oxygen levels, pH, and motility. This dynamic nature is reflected in the gut microbiome, which can change by more than 80% within a 24 h period. Understanding these fluctuations is important for maintaining host physiological homeostasis, and recent studies suggest that similar dynamics occur in the human gut microbiome. However, when designing engineered microbiomes, it is important to consider not only inter-individual variability among patients but also intra-individual variability within a single patient over time. How will an engineered microbiome impact the dynamic structure of the gut microbiome? Additionally, will the effects of an engineered microbiome differ at different times? The luminal microbes have evolved to thrive in the dynamic environment of the gut by preventing competitors, such as those found in engineered microbiomes, from colonizing or surviving. As a result, the design of engineered microbiomes should consider the use of native bacteria or a deeper understanding of how native organisms have adapted to the constantly changing luminal landscape to provide similar advantages for survival. Moreover, specific fluxes in key microbial metabolites, of which short chain fatty acids and bile acids are the best studied, provide important environmental timing cues that can affect processes as diverse as metabolism, circadian rhythms, and inflammation. Understanding the synergistic and antagonistic relationships of microbial members over time can help design more effective engineered microbiomes.

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Genetically engineering the non-model microbiome

The microbiome is an integral part of the human "pan-genome." Decades of studies using multi-omics have demonstrated significant associations between microbiota and human health, yet the molecular mechanisms behind these associations are largely undefined. The functional and mechanistic dissection of microbiome-host interactions is expected to uncover more microbiome therapeutic targets. Therefore, genetically engineered microbiomes will have significant therapeutic potential, resulting in increased efficacy of existing treatments that are microbiome-based (like FMT) or mediated (like immunotherapy) or even novel microbiome-based therapies.

One challenge in microbiome engineering is that the human microbiome is composed primarily of non-model microorganisms and is highly complex. Gene transfer methods or genetic toolsets are not readily available for many of these non-model human isolates. Microbiome complexity and heterogeneity make precise editing challenging. Engineering targets may be redundant in complex microbiomes or encoded by genetically intractable and even unculturable microorganisms. While gain-of-function engineering might be more accessible, sometimes loss of function is necessary and more difficult to accomplish.

Further development of technologies integrating novel gene-editing methods (like CRISPR), new approaches enabling site-/cell-specific delivery of the engineering machinery, or new toolsets to manipulate the native microbiome *in vivo* will make precision editing of the human microbiome feasible. These technologies will enable us to better understand how the microbiome impacts human health at the molecular level and unlock the true potential of engineering the microbiome for therapeutic purposes.

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The importance of spatial organization

Microbes in their natural environments, such as animal guts, soil, and the ocean, live in multi-species communities. Understanding species interactions, co-existence, and evolution in these communities will increase our understanding of microbial processes in nature and our ability to construct bespoke "synthetic" communities or engineer natural ones.

A key challenge for both these fundamental and applied research directions is to account for spatial organization in microbial communities. Spatial organization can lead to diverse microbial structures such as biofilms, granules, and mats and is shown to directly influence onset and outcome of species-species interactions, community stability and function, and evolutionary dynamics within the community. Spatial organization can emerge from a combination of externally imposed factors and internal microbial activity. As a spatial structure emerges, it facilitates formation of metabolic gradients, which impacts microbial physiology, which can give rise to new metabolic gradients. This feedback process can give rise to new species interactions and open new routes for co-existence in ways that are not possible in well-mixed environments.

Studying microbial spatial organization is not trivial. We need better tools to monitor species (and strain) distribution over space and track movement and physiology of cells both in space and time. The resulting data need to be combined with spatiotemporal models, which are harder to develop and analyze compared to spatially homogeneous differential equation models. Model systems will help with these individual challenges, and progress has already been made with engineered biofilms of mutant strains. This progress needs to expand to multi-species, spatially structured model systems.

Better understanding the dynamics of spatial organization will open new ways for engineering and constructing communities. Facilitating spatial organization can allow for new chemical conversions based on metabolic gradients and interfaces (e.g., combining oxic/anoxic processes) or more metabolically efficient communities. Disrupting spatial structure might allow certain species or processes in a community to be specifically engineered out. In short, understanding spatial dynamics can literally give us a new dimension in which we can explain and manipulate microbial communities.

DECLARATION OF INTERESTS

H.H.W. is a scientific advisor for SNIPR Biome, Arranta Bio, Kingdom Supercultures, and Fitbiomics. H.H.W. is a co-inventor on patent applications related to technologies in microbial spatial metagenomics and microbiome editing filed by The Trustees of Columbia University in the City of New York.