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Synthetic biology at all scales

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Most of life on Earth exists at the micro scale. In many respects, these microscopic bacteria are an ideal design platform for synthetic biology – hardy, inexpensive, quick to reproduce, and existing in a vast array of species with unique properties. An expanding future landscape of integrated synthetic biology will utilize the native networks of diverse microbial species in concert with our own engineered gene circuits to execute novel biotechnology [1,2]. To utilize the rich diversity of the microbial world, we must bridge the gap between the microscopic and the macroscopic, designing biological function at all scales.

Through a process known as self-assembly, local interactions (biological or otherwise) lead to complex structure much beyond the length scales of individual components [3]. As single-cell organisms, bacteria display a surprising degree of multicellularity through similar means, with one clear example being the biofilm [4,5]. Here, individual bacteria collaborate to produce macroscopic behavior reminiscent of higher organisms – division of labor, spatial localization of metabolism, and protection against invasion. These complex structures continue to evolve in response to changes in the environment through cell–cell signaling, disassembling when the cost to maintain them is no longer justified.

With an expanding list of microfabrication technologies, we are following the leadoff Nature provides in engineering spatial structure in bacterial communities. Perhaps even further, we can cultivate a snapshot of the community in a desired state – cell density, spatial arrangement, or species composition can be specified using technologies like microfluidics, patterning, scaffolding, and soon perhaps 3D printing – that may otherwise be unstable due to growth or migration. This advantage is significant because it allows us to provide constraints in the spatial dimension distinct from that experienced during normal community development.

In a recent example, a liquid crystal display (LCD)-like array of bacterial 'biopixels' was used to sense arsenic by synchronizing genetic oscillations across centimeter distances – about 10 000 times the length of an individual cell (Figure 1) [6]. The key to bridging length scales was the combination of spatially ordered microcolonies, or biopixels, and two synergistic modes of communication: quorum sensing (correlated to population density within a colony) that can produce *N*-acyl homoserine lactones (AHLs) as signaling molecules, and redox signaling (H_2O_2 vapor) between colonies. The synergy hinges on the issue of scale. As a dissolved species, AHL moves slowly and strongly

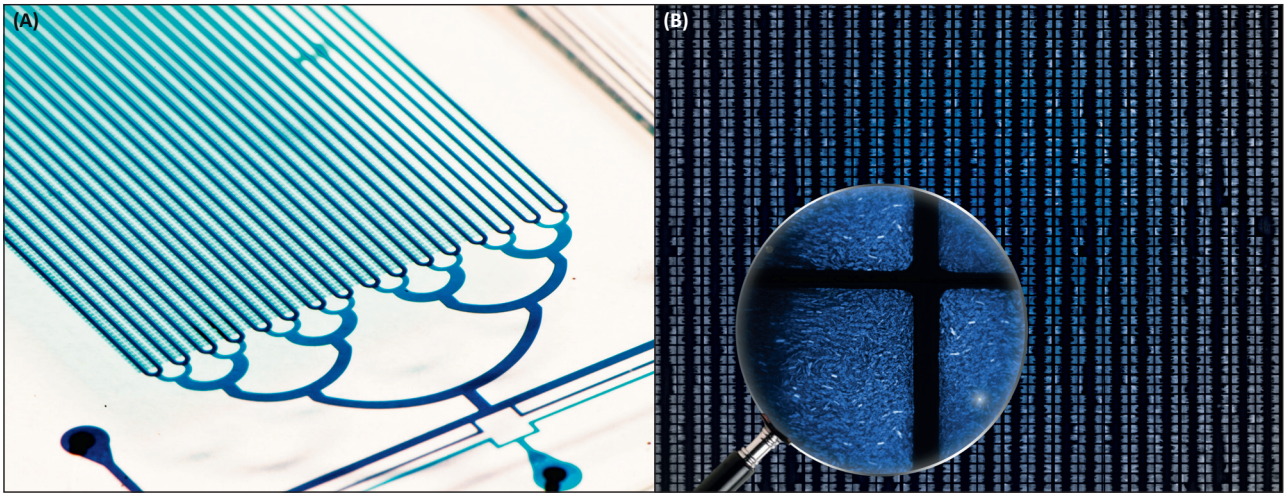

Figure 1. Synthetic biology at all scales. **(A)** A variety of microfabrication technologies, such as the microfluidic device pictured, can be utilized to pattern microbial species in precise spatial arrangements. **(B)** In a liquid crystal display (LCD)-like 'biopixel' array, genetic synchronization is achieved at all scales – single-cell, colony, and multicolony array across centimeter distances, about 10 000 times the length of an individual cell – by arranging thousands of bacterial microcolonies in close proximity. Critically, this spatial arrangement enables the genetic circuit to execute its synchronization program.

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affects transcription, yet it does not reach very far. H_2O_2 vapor moves quickly and only weakly affects transcription, yet it can travel much longer distances. Together, locally initiated bacterial sensing can be propagated across great distances.

In trying to engineer biology, it is often important to stay close to what biology does best – self-assembly via local sensing and response. The previous study rewired quorum sensing, probably the most common form of bacterial cell–cell signaling, to generate spontaneous colony-level oscillations once enough cells have accumulated within a biopixel. Furthermore, by controlling the spacing between biopixels, multicolony synchronization was achieved via the stress response network – one of the fastest, most intricate, and highly adapted response modules in the cell – through the diffusion of H_2O_2 vapor produced by adjacent biopixels.

In addition to sensors, metabolic networks have emerged as a key target for spatial ordering. At the intracellular scale, these organelle-inspired technologies are serving to increase reaction efficiencies by sequestering toxic intermediates and avoiding unwanted side reactions [7,8]. The emergence of next-generation sequencing technologies – particularly through a focus on the human microbiome – may push future technology development toward the intercellular ordering of multiple species; often described as synthetic ecology or microbial consortia [9]. Recent examples of multicellular studies involving spatial arrangement have thus far included the stabilization of multispecies ecosystems, multicellular computation across chemical 'wires', and the emergence of antibiotic resistance in ordered microenvironments [10–12].

Although still preliminary, these approaches populate the exciting frontier of our ability to engineer biological systems by manipulating their spatial arrangement. In the future, distributed manufacturing by microbial assembly lines may leverage sequential metabolic operations by different species. In the inverse process, microbial disassembly lines might be designed to break down complex input molecules for bioremediation or energy harvesting. Although one goal may be to scale-up synthetic biology; perhaps equally appealing is the ability of the microscopic to serve as a tractable model for complex, macroscopic phenomena such as community-level evolution and drug resistance. Central to these efforts is the dream of using natural substrates to design new biological function – exploring the biology that could be [1].

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