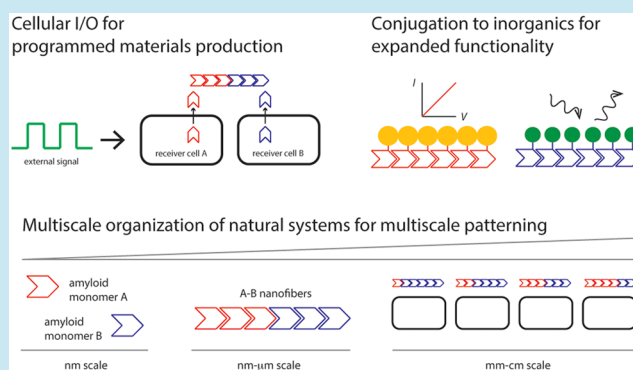


Engineering Living Functional Materials

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ABSTRACT: Natural materials, such as bone, integrate living cells composed of organic molecules together with inorganic components. This enables combinations of functionalities, such as mechanical strength and the ability to regenerate and remodel, which are not present in existing synthetic materials. Taking a cue from nature, we propose that engineered ‘living functional materials’ and ‘living materials synthesis platforms’ that incorporate both living systems and inorganic components could transform the performance and the manufacturing of materials. As a proof-of-concept, we recently demonstrated that synthetic gene circuits in *Escherichia coli* enabled biofilms to be both a functional material in its own right and a materials-synthesis platform. To demonstrate the former, we engineered *E. coli* biofilms into a chemical-inducer-responsive electrical switch. To demonstrate the latter, we engineered *E. coli* biofilms to dynamically organize biotic-abiotic materials across multiple length scales, template gold nanorods, gold nanowires, and metal/semiconductor heterostructures, and synthesize semiconductor nanoparticles (Chen, A. Y. *et al.* (2014) Synthesis and patterning of tunable multiscale materials with engineered cells. *Nat. Mater.* 13, 515–523.). Thus, tools from synthetic biology, such as those for artificial gene regulation, can be used to engineer the spatiotemporal characteristics of living systems and to interface living systems with inorganic materials. Such hybrids can possess novel properties enabled by living cells while retaining desirable functionalities of inorganic systems. These systems, as living functional materials and as living materials foundries, would provide a radically different paradigm of materials performance and synthesis—materials possessing multifunctional, self-healing, adaptable, and evolvable properties that are created and organized in a distributed, bottom-up, autonomously assembled, and environmentally sustainable manner.



An overarching goal of synthetic biology is to design biological systems for useful applications. In exploring the space of possibilities, one logical approach is to consider the characteristic properties of living systems, how they may be useful and how synthetic biology tools can engineer them. Three such properties are evolvability, self-organization, and responsiveness to environment. We propose that (1) these properties of living systems have been productively applied in synthetic biology, (2) these characteristics are useful to materials science by enabling new functionalities and synthesis capabilities, and (3) synthetic biology is well-positioned to harness these features to create novel living functional materials and materials foundries (Figure 1).

Protein engineering and heterologous expression have allowed protein domains to be borrowed from nature and modified or fused in various combinations to generate useful products, such as soft materials.¹ For example, pH and temperature-sensitive leucine zipper domains fused to hydrophilic domains have led to polymers that reversibly form a hydrogel in response to their environment.² Spider silk protein has also been recombinantly

expressed and spun into fibers, with processing of this aggregation-prone protein facilitated by extracellular secretion.³

Traditionally, when engineered proteins are used as building blocks for materials, only the first step in creating the materials (i.e., heterologous expression of subunits) is performed by cells. Assembly of subunits into higher-order structures is performed *in vitro* after subunit purification. However, living systems are capable of much more than heterologous protein expression. As a result of advances in synthetic biology, there is the opportunity to evolve or design materials subunits and to engineer cells to assemble subunits into higher-order materials, rather than depend solely on *in vitro* assembly.⁴ Having cells perform assembly, or even be incorporated as part of the final material, would open up many new capabilities for novel materials, such as self-healing, remodeling, hierarchical organization, and other

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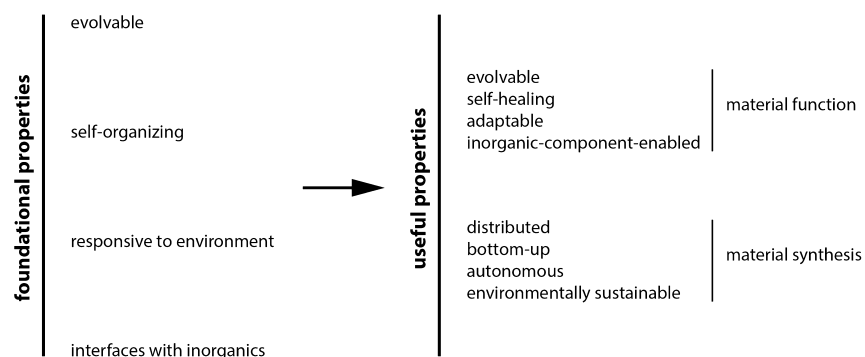


Figure 1. Foundational properties of living systems give rise to useful properties for materials function and synthesis. Integrating nonliving and living systems enables the creation of 'living functional materials'.

properties characteristic of living systems. Furthermore, such materials could be grown in bottom-up, energy efficient processes, thus enabling distributed manufacturing. We envision that these living functional materials could revolutionize the fundamental ways in which our world makes and uses materials.

■ EVOLVABILITY

Living systems are able to generate heritable diversity in the form of genetic variation that manifests as phenotypes that can be acted upon by selective pressures. This property has been applied in synthetic biology to create directed evolution platforms⁵ that have been used to optimize metabolic pathways, create novel enzymes, and evolve protein domains with useful binding properties.

An early directed evolution platform, phage display, has proven useful to materials science as a way to select for peptides that bind and nucleate inorganic materials. This approach eventually allowed fabrication of genetically engineered batteries⁶ and other devices. New directed evolution platforms leveraging cells could be adapted to optimize pathways for assembling materials. Such a platform could allow tuning of not only the peptide sequence and subunit composition of materials such as collagen, polyesters, and polysaccharides but also how the subunits are assembled—an important determinant of material properties.

■ SELF-ORGANIZATION

Living systems can perform impressive feats of self-organization that are hierarchical and coordinated in space and time, a prime example being embryogenesis. This property has been studied using synthetic-biology approaches, as well as applied broadly for patterning based on intercellular communication and motility,^{7,8} for synchronization of cell populations, and for intracellular organization to achieve cell polarization.

Nature's solutions for creating hierarchal self-organized structures⁹ (biomolecules → biomolecular assemblies → organelles → cells → tissues → organs → organisms) may be mapped onto the problems that materials scientists face in fabricating multiscale patterned materials. Self-organization in biology is carried out by genetic programs and aided by physical forces; one-dimensional strings of letters in DNA, encoding genes and regulatory elements, can somehow direct the fabrication of complex three-dimensional structures in cells and organisms.

Understanding and modifying these programs through synthetic biology may allow us to harness them for making materials that assemble autonomously. Early steps have been

taken in this direction at various scales. Work in biomolecular self-assembly uses the physical components that store and carry out genetic programs to create nanoscale materials. Intricate static and dynamic structures may be precisely assembled by using DNA as a structural material as well as the carrier of instructions for its own assembly;¹⁰ similar feats may be achieved with designer RNA and proteins. Self-organization at larger scales has also been harnessed to create materials, some examples being active self-assembled matter based on microtubules and kinesin,¹¹ self-organized tissues¹² and organoids,¹³ and self-organized robot swarms¹⁴ (inspired by social insects) that create user-defined structures.

■ RESPONSIVENESS TO ENVIRONMENT

Living systems sense inputs from the environment, integrate them, and respond with appropriate outputs. Environmental responsiveness is a property that is one of the building blocks of evolvability and self-organization, and is useful in its own right. The sense-and-respond property of living systems has been applied broadly in synthetic biology,^{15,16} some examples being optogenetic control of cell behavior, light-dark boundary detection, sensing-and-destruction of pathogenic microbes via detection of quorum sensing molecules, sensing-and-destruction of cancer cells via detection of microRNA signatures, sensing-and-amelioration of high uric acid levels, and multicellular computation in which cells implementing simple logic gates are wired together by chemical signaling. Cells have also been incorporated into materials as sense-and-respond modules that, for example, produce urate oxidase in response to uric acid or produce insulin upon activation by light.

Living sense-and-respond systems can be useful as "smart" environmentally responsive factories for materials or "smart" environmentally responsive materials (if cells themselves are incorporated as part of the material and endow it with function). A specific example of a responsive material would be one that senses damage and autonomously self-heals. Such a system would be capable of sensing and repairing more diverse types of damage compared to current nonliving self-healing materials, which largely respond to mechanical and thermal damage with relatively simple fixes.¹⁷

■ BRIDGING THE FUNCTION GAP WITH INORGANIC MATERIALS

While living matter made of organic molecules offers exciting prospects for novel functional materials, it can be deficient in certain useful properties, such as the ability to conduct electrons and the ability to interact with magnetic fields. This gap can be

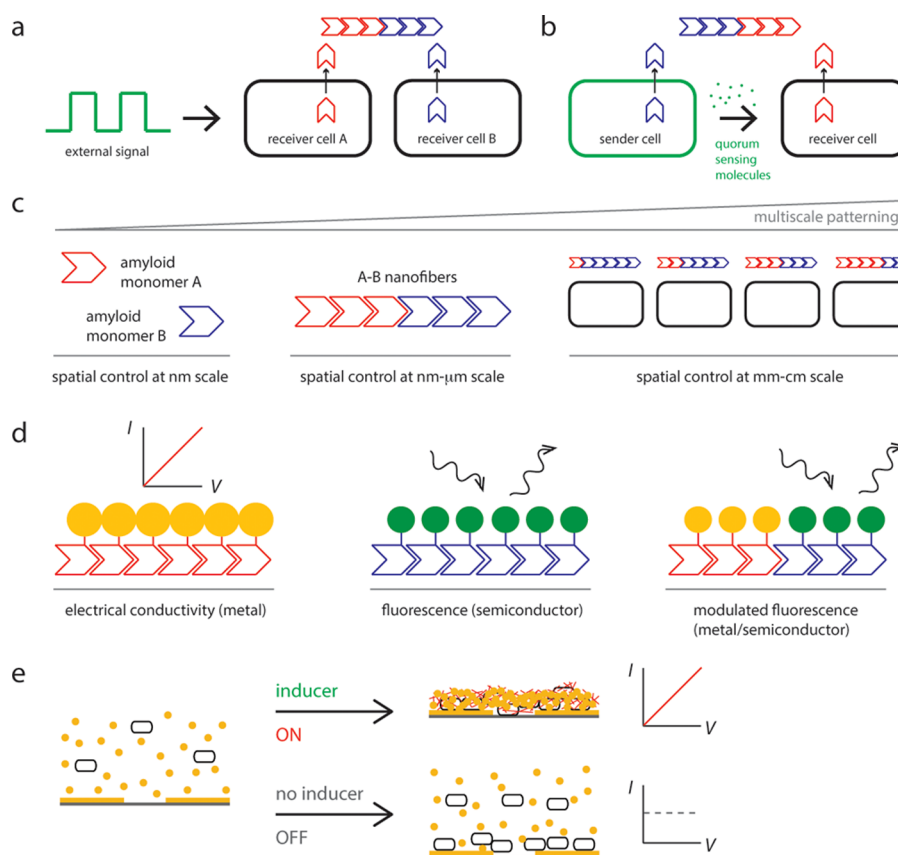


Figure 2. Living materials-synthesis platform and living functional materials based on *E. coli* biofilms. This strategy is able to (a) pattern materials in response to external inputs, (b) organize materials autonomously via cell-to-cell communication, (c) pattern materials across multiple length scales, (d) interface with inorganic components to enable electrical conductivity and modulated fluorescence, and (e) implement a chemically responsive electrical switch based on biofilms.

bridged by incorporating inorganic components that possess such properties, creating multifunctional materials that combine the capabilities of living systems with those of inorganic materials. An example from nature is magnetotactic bacteria, which are able to navigate magnetic field lines based on their ability to biomineralize ferromagnetic crystals and organize them into chains (magnetosome chains). In a synthetic context, interfacing biology with inorganic materials can be accomplished via biomineralization, which may be engineered with synthetic biology, or via bioconjugate chemistry. As an example of the former, gene clusters encoding the biosynthetic pathway for magnetosome chains in *M. gryphiswaldense* have been transferred to a foreign microbial host to form functional, heterologous magnetosomes. As an example of the latter, semiconductor devices were incorporated into the extracellular matrix of engineered tissues to give hybrid, or “cyborg” tissues.¹⁸

■ A PLATFORM FOR ENGINEERING LIVING FUNCTIONAL MATERIALS

As described above, a large body of literature has developed around engineering living systems that evolve, self-organize, and compute. A complementary literature has developed around engineering inorganic nanomaterials with diverse, tunable properties. Leveraging these capabilities, we recently presented a novel platform for integrating living cells with inorganic components into living functional materials¹⁹ (Figure 2). We engineered *E. coli* biofilms with artificial gene circuits to control the synthesis of extracellular curli amyloid nanofibers. These

nanofibers were functionalized with peptide domains to interface them with inorganic materials. This platform enables multiple novel materials properties derived from the combination of synthetic biology and materials science. Specifically, these engineered *E. coli* biofoundries can be used to manufacture materials with controllable nanoscale-to-microscale structure and composition by controlling either the timing or magnitude (e.g., frequency modulation or amplitude modulation, respectively) of external inducer inputs. Furthermore, by using cell–cell communication circuits, the synthetic biofilms generated materials whose nanoscale-to-microscale composition and structure were varied in a dynamic and autonomous fashion, with no requisite user input.

Moreover, by incorporating synthetic gene circuits with other forms of patterning, such as spatial inducer gradients and protein engineering, we were able to achieve self-organization of materials across multiple length scales with biofilms. The ability to hierarchically organize materials across multiple length scales is a major challenge for materials science but is one that can be readily addressed with biological systems. Specifically, by combining synthetic gene circuits with spatial inducer gradients to control curli nanofiber production, engineered biofilms were able to implement nanoscale-to-millimeter-scale patterning. By integrating synthetic gene circuits with protein engineering of the curli amyloid subunits themselves, the artificial biofilms were able to more finely control the nanoscale-to-microscale patterning of the curli nanofibers. With further integration of top-down patterning strategies (such as inputs of patterned light or 3D

printing) with bottom-up organization (such as rational design of protein self-assembly or directed evolution to achieve desired morphologies) and synthetic gene circuits, the precise, dynamic, and autonomous control of multiscale structure should be achievable by living functional materials.

We also demonstrated that living functional materials and living materials-synthesis platforms based on biofilms can organize and synthesize biotic-abiotic hybrid structures with novel functions. For example, gold nanorods, nanowires, and chains were templated on biofilm-synthesized curli nanofibers. This allowed the implementation of electrically conductive biofilm switches whose conductivity could be toggled on with the addition of external chemical signals. In addition, semiconductor quantum dots were synthesized or organized on biofilm-fabricated curli nanofibers, and coassembled with metal nanoparticles. The resulting metal/semiconductor heterostructures exhibited modulated fluorescence properties. Thus, we have shown that biofilm-based living functional materials can be used to synthesize and organize biotic-abiotic systems, as well as modulate their properties. Since biofilms are robust and abundant living systems that are easy to grow and scale up, they are promising substrates for the distributed, environmentally friendly manufacturing of materials and devices. We envision that related platforms could enable the creation of cell-synthesized batteries and solar cells, self-healing living-cell glues, self-regenerating catalytic membranes and filters, enzymatic scaffolds for tunable reaction rates, and dynamic extracellular matrices for tissue engineering.

OUTLOOK

Living system properties of evolvability, self-organization, and responsiveness to the environment, in conjunction with tools to interface cells with inorganic components, constitute a foundation for synthesizing materials with novel functionalities. Our recent work with engineered biofilms¹⁹ establishes a proof-of-concept that living cellular systems can act not only as foundries for new materials but can also be incorporated into hybrid materials systems. Man-made materials are often made by centralized, top-down, energy-intensive processes, and are limited in their ability to respond and adapt. The integration of engineered living systems with inorganic components to create 'living materials-synthesis platforms' and 'living functional materials' has the potential to dramatically change the fabrication and use of materials.

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Notes

The authors declare the following competing financial interest(s): T.K.L. and A.Y.C. have filed a patent application with the U.S. Patent and Trademark Office.

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