



# Engineering Living Functional Materials

The Harvard community has made this article openly available. [Please share](#) how this access benefits you. Your story matters

Citation	Chen, Allen Y., Chao Zhong, and Timothy K. Lu. 2015. "Engineering Living Functional Materials." ACS Synthetic Biology 4 (1): 8-11. doi:10.1021/sb500113b. <a href="http://dx.doi.org/10.1021/sb500113b">http://dx.doi.org/10.1021/sb500113b</a> .
Published Version	<a href="https://doi.org/10.1021/sb500113b">doi:10.1021/sb500113b</a>
Citable link	<a href="http://nrs.harvard.edu/urn-3:HUL.InstRepos:24984023">http://nrs.harvard.edu/urn-3:HUL.InstRepos:24984023</a>
Terms of Use	This article was downloaded from Harvard University's DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at <a href="http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA">http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA</a>

## Engineering Living Functional Materials

Allen Y. Chen,<sup>†,‡,§,⊥,#</sup> Chao Zhong,<sup>‡,§,⊥</sup> and Timothy K. Lu<sup>\*,†,‡,§,⊥,||</sup>

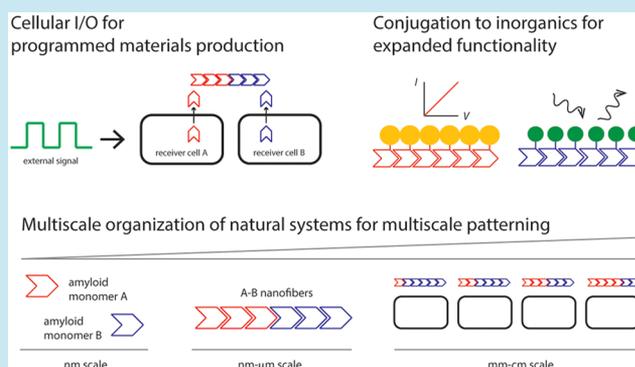
<sup>†</sup>Biophysics Program, Harvard University, Cambridge, Massachusetts 02138, United States

<sup>‡</sup>Department of Electrical Engineering & Computer Science, <sup>§</sup>Department of Biological Engineering, and <sup>||</sup>Microbiology Program, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, Massachusetts 02139, United States

<sup>⊥</sup>MIT Synthetic Biology Center, 500 Technology Square, Cambridge, Massachusetts 02139, United States

<sup>#</sup>Harvard–MIT Health Sciences and Technology, Institute for Medical Engineering and Science, 77 Massachusetts Avenue, Cambridge, Massachusetts 02139, United States

**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.<sup>1</sup> 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.<sup>2</sup> Spider silk protein has also been recombinantly

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

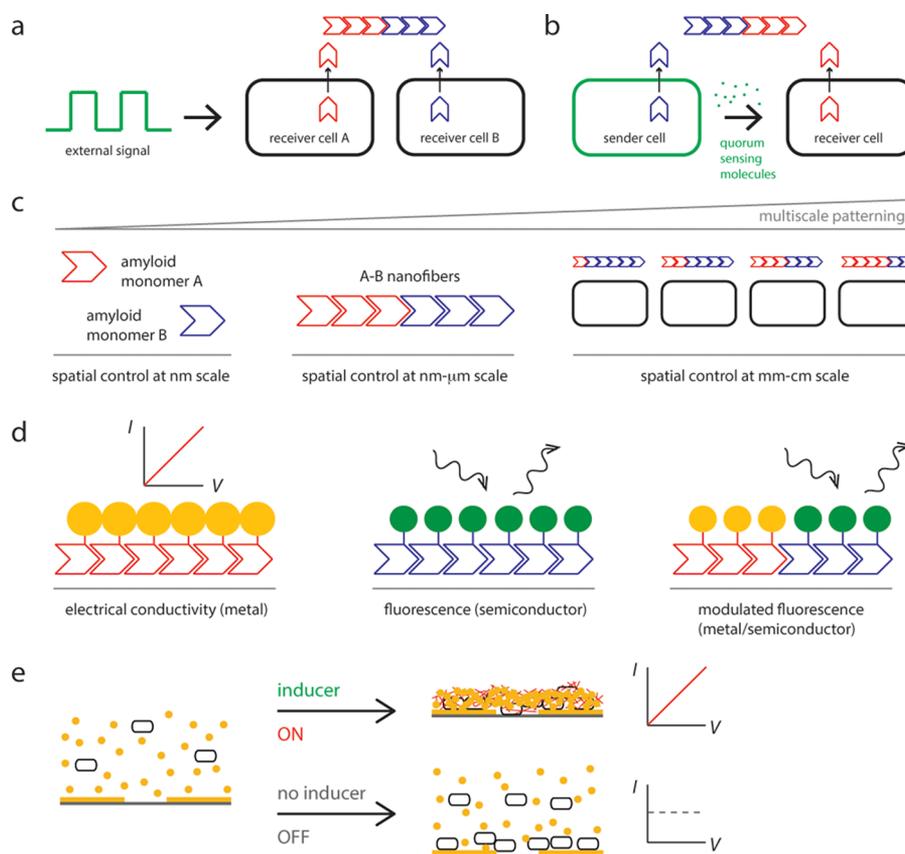
Traditionally, when engineered proteins are used as building blocks for materials, only the first step in creating the materials (i.e., heterologous expression of subunits) is performed by cells. Assembly of subunits into higher-order structures is performed *in vitro* after subunit purification. However, 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.<sup>4</sup> 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

**Special Issue:** SEED 2014

**Received:** March 6, 2014

**Published:** January 16, 2015





**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.<sup>18</sup>

### ■ 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 materials<sup>19</sup> (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 biofilms<sup>19</sup> 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.

## AUTHOR INFORMATION

### Corresponding Author

\*Email: timlu@mit.edu.

### 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.

## ACKNOWLEDGMENTS

We thank R. Citorik, Z. Deng, M. Mimee, O. Purcell, N. Roquet, J. Rubens, and E. Tham from the Lu lab, S. Keating from the lab of Neri Oxman (Media Lab, MIT) for close readings of the manuscript. The Lu lab acknowledges the Office of Naval Research, the Army Research Office, the Presidential Early Career Award for Scientists and Engineers, and the MRSEC Program of the National Science Foundation (DMR-0819762) for support of this work. Furthermore, the authors thank the

Hertz Foundation, the Department of Defense, and the National Institutes of Health (T32GM007753, 1DP2OD008435) for funding.

## REFERENCES

- (1) Zhong, C., Gurry, T., Cheng, A. A., Downey, J., Deng, Z., Stultz, C. M., and Lu, T. K. (2012) Strong underwater adhesives made by self-assembling multi-protein nanofibres. *Nat Nanotechnol.*, DOI: 10.1038/nnano.2014.199.
- (2) Petka, W. A., Harden, J. L., McGrath, K. P., Wirtz, D., and Tirrell, D. A. (1998) Reversible hydrogels from self-assembling artificial proteins. *Science* 281, 389–392 DOI: 10.1126/science.281.5375.389.
- (3) Widmaier, D. M., Tullman-Ercek, D., Mirsky, E. A., Hill, R., Govindarajan, S., Minshall, J., and Voigt, C. A. (2009) Engineering the *Salmonella* type III secretion system to export spider silk monomers. *Mol. Syst. Biol.* 5, 309 DOI: 10.1038/msb.2009.62.
- (4) Rice, M. K., and Ruder, W. C. (2014) Creating biological nanomaterials using synthetic biology. *Sci. Technol. Adv. Mater.* 15, 014401.
- (5) Wang, H. H., Isaacs, F. J., Carr, P. A., Sun, Z. Z., Xu, G., Forest, C. R., and Church, G. M. (2009) Programming cells by multiplex genome engineering and accelerated evolution. *Nature* 460, 894–898 DOI: 10.1038/nature08187.
- (6) Lee, Y. J., Yi, H., Kim, W.-J., Kang, K., Yun, D. S., Strano, M. S., Ceder, G., and Belcher, A. M. (2009) Fabricating genetically engineered high-power lithium-ion batteries using multiple virus genes. *Science* 324, 1051–1055 DOI: 10.1126/science.1171541.
- (7) Basu, S., Gerchman, Y., Collins, C. H., Arnold, F. H., and Weiss, R. (2005) A synthetic multicellular system for programmed pattern formation. *Nature* 434, 1130–1134 DOI: 10.1038/nature03461.
- (8) Payne, S., Li, B., Cao, Y., Schaeffer, D., Ryser, M. D., and You, L. (2013) Temporal control of self-organized pattern formation without morphogen gradients in bacteria. *Mol. Syst. Biol.* 9, 697 DOI: 10.1038/msb.2013.55.
- (9) Weiner, S., and Wagner, H. D. (1998) The material bone: Structure mechanical function relations. *Annu. Rev. Mater. Sci.* 28, 271–298 DOI: 10.1146/annurev.matsci.28.1.271.
- (10) Seeman, N. C. (2010) Nanomaterials based on DNA. *Annu. Rev. Biochem.* 79, 65–87 DOI: 10.1146/annurev-biochem-060308-102244.
- (11) Sanchez, T., Chen, D. T., DeCamp, S. J., Heymann, M., and Dogic, Z. (2012) Spontaneous motion in hierarchically assembled active matter. *Nature* 491, 431–434 DOI: 10.1038/nature11591.
- (12) Liu, J. S., and Gartner, Z. J. (2012) Directing the assembly of spatially organized multicomponent tissues from the bottom up. *Trends Cell Biol.* 22, 683–691 DOI: 10.1016/j.tcb.2012.09.004.
- (13) Lancaster, M. A., Renner, M., Martin, C.-A., Wenzel, D., Bicknell, L. S., Hurler, M. E., Homfray, T., Penninger, J. M., Jackson, A. P., and Knoblich, J. A. (2013) Cerebral organoids model human brain development and microcephaly. *Nature* 501, 373–379 DOI: 10.1038/nature12517.
- (14) Werfel, J., Petersen, K., and Nagpal, R. (2014) Designing collective behavior in a termite-inspired robot construction team. *Science* 343, 754–758 DOI: 10.1126/science.1245842.
- (15) Benenson, Y. (2012) Biomolecular computing systems: Principles, progress, and potential. *Nat. Rev. Genetics* 13, 455–468 DOI: 10.1038/nrg3197.
- (16) Brophy, J. A., and Voigt, C. A. (2014) Principles of genetic circuit design. *Nat. Methods* 11, 508–520 DOI: 10.1038/nmeth.2926.
- (17) Wool, R. P. (2008) Self-healing materials: A review. *Soft Matter* 4, 400–418 DOI: 10.1039/b711716g.
- (18) Tian, B., Liu, J., Dvir, T., Jin, L., Tsui, J. H., Qing, Q., Suo, Z., Langer, R., Kohane, D. S., and Lieber, C. M. (2012) Macroporous nanowire nanoelectronic scaffolds for synthetic tissues. *Nat. Mater.* 11, 986–994 DOI: 10.1038/nmat3404.
- (19) Chen, A. Y., Deng, Z., Billings, A. N., Seker, U. O. S., Lu, M. Y., Citorik, R. J., Zakeri, B., and Lu, T. K. (2014) Synthesis and patterning of tunable multiscale materials with engineered cells. *Nat. Mater.* 13, 515–523.