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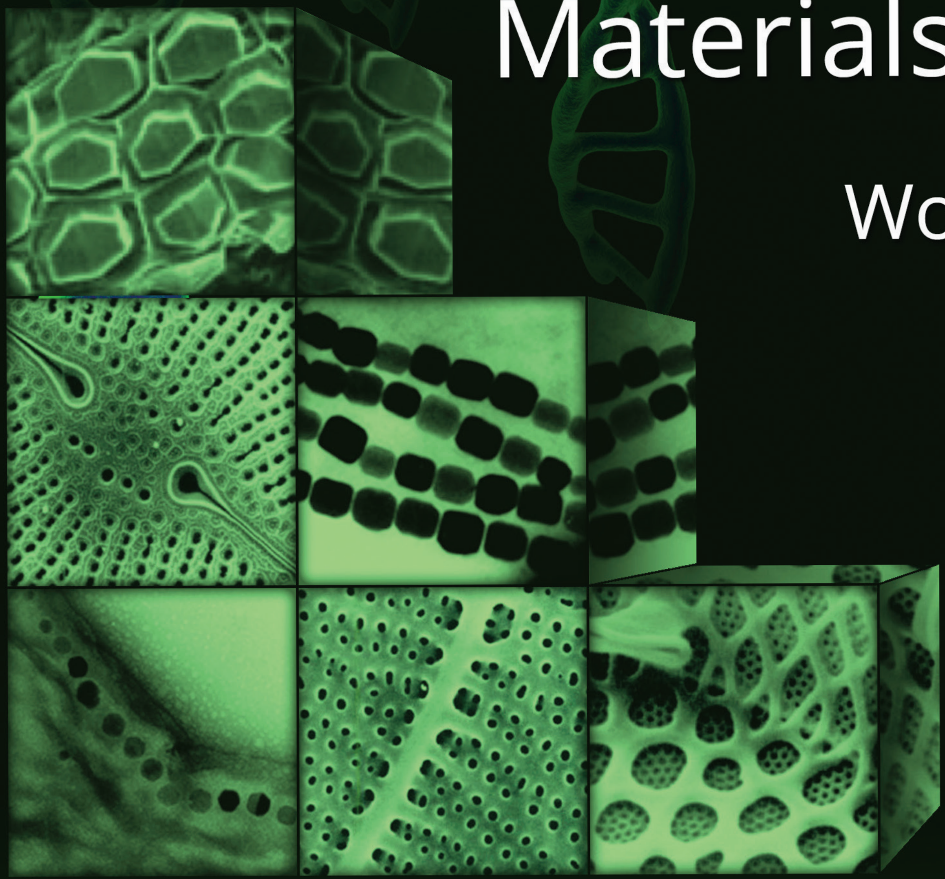
Genome Engineering for Materials Synthesis

Workshop Report



U.S. DEPARTMENT OF ENERGY | Office of Science

Office of Biological and Environmental Research

Genome Engineering for Materials Synthesis Workshop

October 9–11, 2018
Rockville, Maryland

Convened by

U.S. Department of Energy
Office of Science
Office of Biological and Environmental Research

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About BER

The Office of Biological and Environmental Research (BER), within the U.S. Department of Energy (DOE) Office of Science, advances fundamental research and scientific user facilities to support DOE missions in scientific discovery and innovation, energy security, and environmental responsibility. BER seeks to understand biological, biogeochemical, and physical principles needed to predict a continuum of processes occurring across scales, from molecular and genomics-controlled mechanisms to environmental and Earth system change. BER advances understanding of how Earth's dynamic physical and biogeochemical systems (atmosphere, land, oceans, sea ice, and subsurface) interact and affect future Earth system and environmental change. This research improves Earth system model predictions and provides valuable information for energy and resource planning.

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Genome Engineering for Materials Synthesis

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Executive Summary

The Biological Systems Science Division (BSSD) within the U.S. Department of Energy's (DOE) Office of Biological and Environmental Research (BER) funds basic research on plants and microbes relevant to several DOE bioenergy and environmental mission areas. BSSD has a long history of developing and supporting genomic, molecular, and structural characterization of biological systems. This sustained focus has led to important discoveries and increased understanding of these systems, as well as translational pathways to new processes and products.

BSSD research seeks to understand the fundamental genome-encoded properties of plants and microbes that can be harnessed or redesigned for beneficial purposes. Current emphases are leading to the discovery, development, and understanding of numerous plant and microbial species with traits suitable for the production of fuels and chemicals from renewable biomass or light. Additionally, BSSD supports research leading to an understanding of the complex and essential interactions among plants, microbial communities, and the environment.

The processes by which living organisms synthesize intricate and potentially valuable inorganic biomaterials are gaining increasing attention. The application of genomic, molecular, and structural tools offers great potential to better understand and potentially exploit these widespread, yet still poorly understood, genome-encoded biosynthetic processes.

To engage the relevant scientific communities in discussions of this research opportunity, BER convened the Genome Engineering for Materials Synthesis workshop on October 9–11, 2018 (see Appendices 1–3, pp. 45–48). Workshop participants defined opportunities and challenges for future efforts by considering a foundation of existing experimental work relevant to the biosynthesis of three classes of renewable inorganic biomaterials: (1) inorganic biominerals, (2) inorganic-organic hybrids, and (3) composites of inorganic materials and living cells. Participants identified compelling examples for each materials class but recognized gaps in the biological knowledge and technologies needed to enable engineering of biological systems to produce these biomaterials. Also lacking is a molecular-level understanding of the ultrastructure, chemical composition, and bonding within and across interfaces in both composite and hybrid biomaterials, which often have attractive physical, optical, and electromagnetic properties. A more comprehensive systems-level understanding of the genome-encoded mechanisms whereby living organisms create biomaterials with inorganic components will require the following:

1. Strategies to access a fuller taxonomy of species capable of producing biominerals and an expansion of the catalog of biomineral-forming genes and regulatory networks.
2. Improved knowledge of plant and microbial acquisition mechanisms for inorganic materials.
3. Better understanding of the intracellular metabolic processes governing the synthesis, transport, modification, assembly, and storage of inorganic biomaterials.
4. Identification of the genes and biosynthetic pathways controlling the synthesis, transport, modification, assembly, and storage of inorganic biomaterials.
5. Detailed investigation of the assembly mechanisms of specific inorganic biomaterials.
6. Investigation and characterization of engineered pathways, resulting in new inorganic materials.

Although biofuels and bioproducts research has yielded many broadly applicable capabilities in DNA synthesis, genome manipulation, and "omic" approaches, workshop participants recognized that synthesis of inorganic materials requires new technologies, including:

1. New cultivation, single-cell, and omic capabilities directed toward discovery of inorganic

biominerals and the genetic potential underlying their synthesis.

2. Computational systems biology and biodesign tools for a systems-level understanding and forward engineering of inorganic materials synthesis.
3. Biodesign capabilities to manipulate organisms with a breadth of capabilities, including control of transport, spatial patterning, and timing.
4. Technologies to support high-throughput or massively parallel determinations of the function of inorganic biosynthetic pathways.
5. Intentionally aligned structural and functional tools to characterize inorganic biomaterials.

Workshop participants envision that developments in both knowledge and technologies could open opportunities in two broad classes of potential materials: (1) more sustainable production approaches for existing materials of interest and (2) materials with novel performance or function. For example, advances in genome-engineered materials could provide more sustainable syntheses of conducting, semiconducting, and magnetic materials, as well as novel capabilities for assembling lightweight, strong, and multifunctional composite materials and synthesizing self-replicating cell-inorganic composites on demand.

1. Introduction

The most familiar building blocks of life—including proteins, carbohydrates, lipids, nucleic acids, and other macromolecules—are organic molecules rich in carbon. However, life as we know it would not exist without a broad diversity of inorganic and inorganic-organic hybrid biomaterials. Such inorganic and hybrid materials often have mechanical strength or rigidity greater than their organic counterparts. For example, inorganic materials are the primary components of bones, shells, claws, and teeth that allow animals to move, protect themselves, cut, and grind. Additionally, organisms synthesize specialized inorganic materials to sense or focus light and magnetic and gravitational fields and to control buoyancy. While the elemental composition and chemical bonding found in inorganic materials contribute to their size, shape, and orientation, the information encoded in DNA supports the assembly, maintenance, and remodeling of a breadth of genetically encoded inorganic materials, with some examples shown in Fig. 1, this page.

For this report, genetically encoded inorganic biomaterials are those whose synthesis is dictated by an organism's genes and whose function requires an inorganic component. Since organisms construct these materials by scavenging building blocks from the Earth's surface, the elements that predominantly compose these materials are those that are most abundant on Earth's surface: calcium (Ca) in, for example, bones, teeth, and shells; silicon (Si) in diatoms; and iron (Fe) in magnetosomes and magnetite teeth. Each of these elements provides unique capabilities and offers distinct advantages for the organisms that synthesize materials containing them.

Over several billion years, organisms have synthesized inorganic materials that profoundly influenced

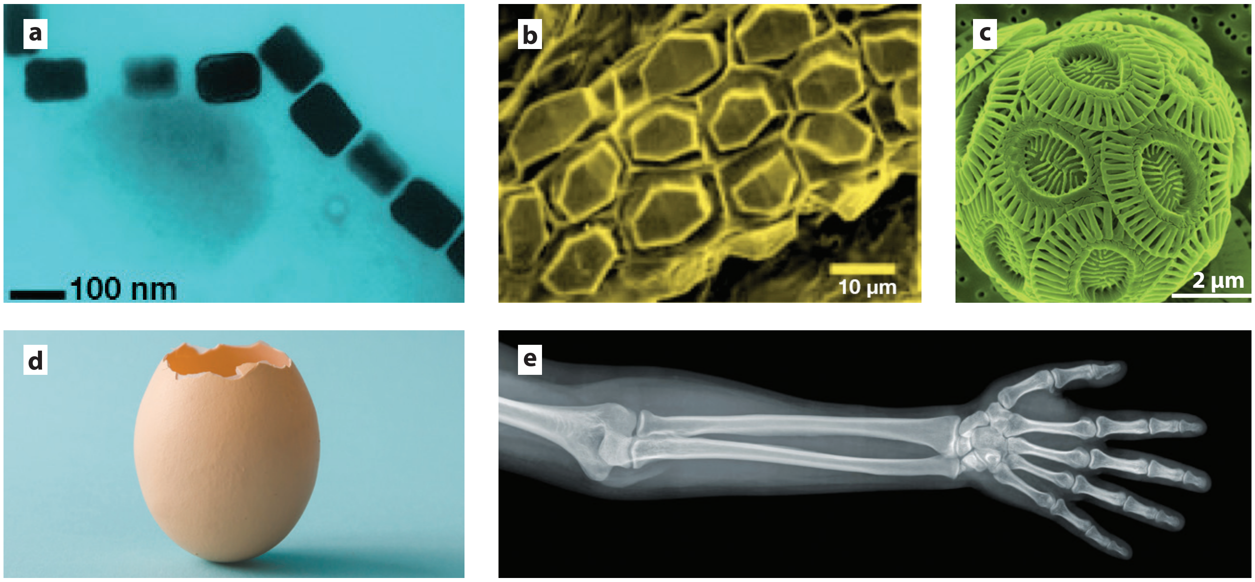


Fig. 1. Examples of Natural Biologically Synthesized Inorganic Materials. (a) Magnetite, **(b)** calcium oxalate crystals, **(c)** coccolithophorid, **(d)** chicken eggshells, and **(e)** human bones.

[Sources: **(a)** Reprinted by permission from (1) Springer Nature: Schüler, D., and R. B. Frankel. 1999. "Bacterial Magnetosomes: Microbiology, Biomineralization and Biotechnological Applications," *Applied Microbiology and Biotechnology* **52**(4), 467–73. Copyright 1999; and from (2) Elsevier: Yan, L., et al. 2012. "Magnetotactic Bacteria, Magnetosomes and Their Application," *Microbiological Research* **167**(9), 507–19. Copyright 2012. **(b)** Reprinted by permission from Springer Nature: McConn, M. M., and P. A. Nakata. 2002. "Calcium Oxalate Crystal Morphology Mutants from *Medicago truncatula*," *Planta* **215**(3), 380–86. Copyright 2002. **(c)** Wikimedia Commons, courtesy Alison R. Taylor, University of North Carolina, Wilmington, via a Creative Commons license (CC BY 2.5). **(d–e)** istockphoto.com.]

terrestrial ecosystems. During the Precambrian eon, cyanobacteria formed calcareous aggregates called stromatolites, which are inorganic structures that retain memory of metabolic processes from the most ancient life on Earth. Microbial oxidation of soluble Fe^{2+} abundant in the Precambrian anaerobic environment led to precipitation as Fe^{3+}, now found in widely distributed, commercially valuable banded iron mineral deposits. Later, protists formed alternative calcareous structures called thrombolites. Viable, contemporary examples of these ancient structures containing living organisms are available and offer potential insight into genetically encoded capabilities of individual species and communities for processing inorganic materials.

More than 50 different biominerals have been identified thus far from a variety of organisms (Lowenstam and Weiner 1989; Weiner and Addadi 2002), and anecdotal evidence from examination of plants and microbes suggests a great wealth of additional natural biominerals (see Table 1, p. 3).

The mechanisms for formation of many biominerals have been investigated, including formation from solution (Poulsen et al. 2003; Sone et al. 2005; Pokroy et al. 2015) and from amorphous transient phases that are precursors to crystalline skeletons such as sea urchin spicules (Beniash et al. 1997), spines (Politi et al. 2004), teeth (Killian et al. 2009), mollusk shell nacre (i.e., mother of pearl; DeVol et al. 2015), and coral skeletons (Mass et al. 2017). Material-toughening mechanisms in biominerals also have been studied (Ritchie 1999; Nalla et al. 2003, 2005; Gao et al. 2003; Peterlik et al. 2006), as have other mechanical properties of specialized biominerals such as chiton teeth (Weaver et al. 2010), mantis shrimp clubs (Weaver et al. 2012), and parrotfish beaks (Marcus et al. 2017). In many biominerals, the first phases deposited are different from the final ones, as demonstrated in Fig. 2, p. 4, which shows amorphous precursors in three distinct phyla.

Many of these materials, primary or transformed, serve as targets or templates for focused research efforts in biomimetic and bioinspired research (U.S. DOE 2017a; Wegst et al. 2015; Yaraghi and Kisailus 2018). Although some of these examples are not wholly inorganic in composition, they are of significant interest within the materials science community due to their strength and durability. Given the breadth of inorganic biomaterials found in nature, significant technological possibilities exist to control the synthesis of genetically encoded inorganic materials, including possibilities for biologically driven, designed synthesis of new inorganic materials with properties that go beyond what exists naturally.

However, limited understanding of natural systems currently constrain capabilities for (1) controlling the synthesis of renewable inorganic biomaterials through manipulation of natural processes encoded in genomes and (2) synthesizing purposely designed biomaterials in engineered organisms or other acellular environments (e.g., cell-free systems). For example, crystallized minerals or nanoparticles are known to be produced by many organisms, but the genetic inputs, operons and gene clusters, transcription, translation, and regulation, if known, have not yet been investigated from the perspective of biomineral design. The lack of mechanistic understanding and details of genetic regulation has impeded efforts to biologically manufacture inorganic biomaterials, keeping this potentially transformative field in its infancy.

New advances in genome science methodologies, biosystems design tools, and characterization approaches have transformed capabilities to read, write, and edit DNA; to design and test assemblies of genes; and to determine structure and function (see Fig. 3, p. 5). The scientific and technological advances listed below offer new opportunities to understand the genome-encoded synthesis of inorganic biomaterials:

- Following completion of the Human Genome Project, new DNA sequencing technologies radically improved the speed, throughput, and accuracy of DNA sequencing. As a result, a vast repository of genomic data is now available in public databases, and the rate of new depositions is accelerating. In the last decade, especially with the advent of new algorithms to assemble metagenomes, the advances have been significant. For example, identifying a single new organism previously took months or years, and now multiple new organisms can be discovered

Table 1. Biologically Formed Minerals

Carbonates	Calcite Aragonite Vaterite Monohydrocalcite	Amorphous calcium carbonate family Hydrocerrusite Protodolomite
Phosphates	Carbonated apatite (dahllite) Francolite Octacalcium phosphate Whitlockite Struvite	Brushite Amorphous calcium phosphate Vivianite Amorphous pyrophosphate
Halides	Fluorite Hieratite	Amorphous fluorite Atacamite
Sulfates	Gypsum Celestite Barite	Jarosite Calcium sulfate hemihydrate
Silicates	Silica (opal)	
Oxides and Hydroxides	Magnetite Goethite Lepidocrocite Ferrihydrite Amorphous iron oxide	Amorphous manganese oxide Amorphous ilmenite Todorokite Birnessite
Sulfides	Pyrite Amorphous pyrrhotite Hydrotroilite Sphalerite	Wurtzite Galena Greigite Mackinawite
Native Element	Sulfur	
“Organic Minerals”	Whewellite Weddellite Manganese oxalate Magnesium oxalate (glushinskite) Copper oxalate (moolooite) Ferric oxalate anhydrous Guanine	Uric acid Paraffin hydrocarbon Wax Calcium tartrate Calcium malate Earlandite Sodium urate

[Source: From Weiner, S., and L. Addadi. 2002. “At the Cutting Edge,” *Science: Biomineralization* **298**(5592), 375–76. Reprinted with permission from AAAS.]

in a day by sequencing DNA extracted from a complex mixture of microbes.

- The cost of DNA sequencing has dropped substantially in the past 10 years while the purity and length of synthesized DNA have continued to increase (see Fig. 3a, p. 5). Consequently, large-scale DNA construction projects are becoming more common, with the ability to automate the *in vitro* generation of combinatorial assemblies of genes and regulatory circuits with optimized codon usage for specific host organisms (Nielsen et al. 2016).
- The development of CRISPR-based technologies, as well as other genome-engineering efforts, has

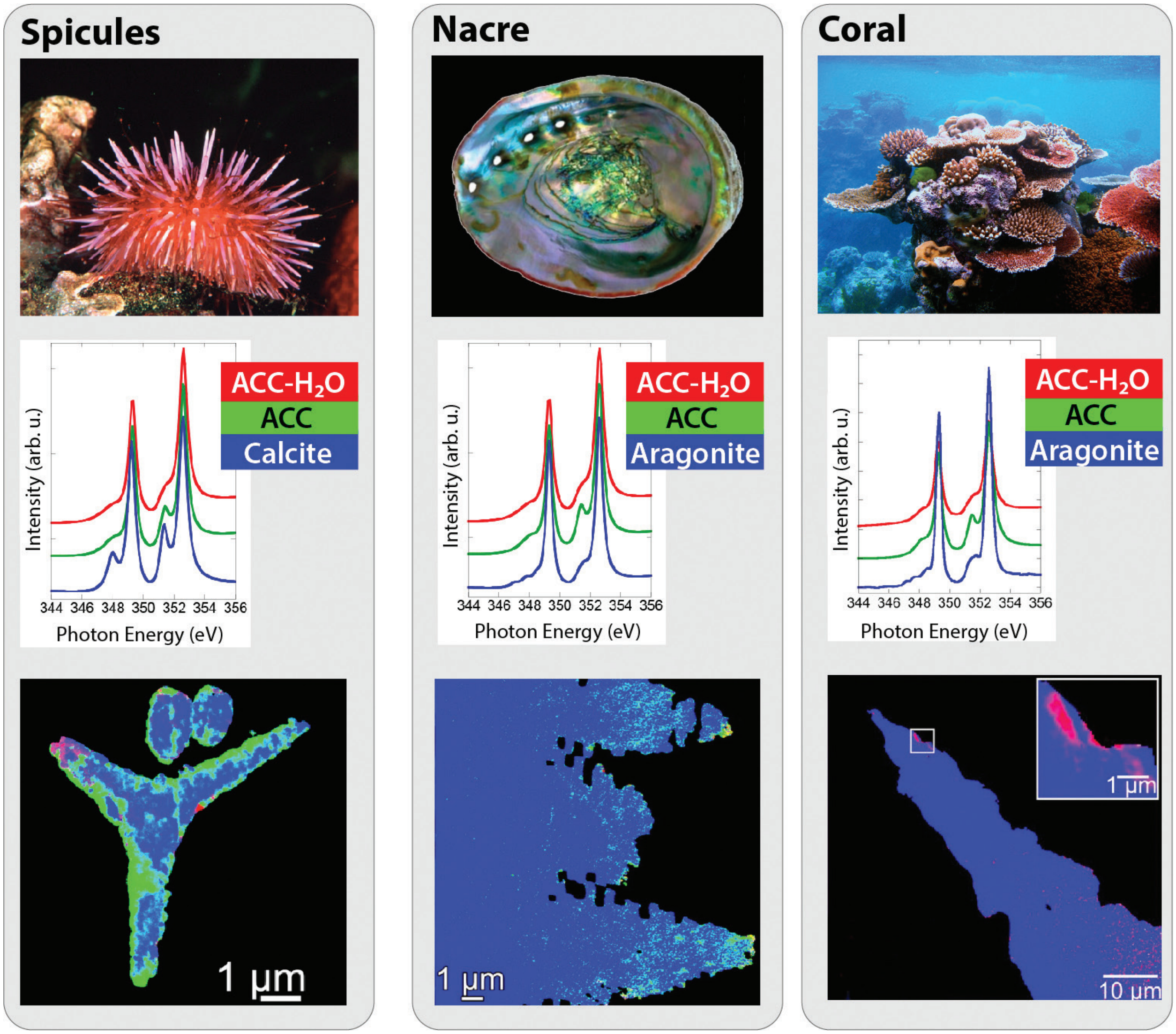


Fig. 2. Example Biominerals Forming from Amorphous Precursors in Sea Urchin Spicules, Nacre, and Coral Skeletons. Hydrated and anhydrous amorphous calcium carbonate (ACC) precursor materials are identified via X-ray absorption near edge structure spectroscopy (XANES) during biomineral formation (middle row). XANES–photoelectron emission microscopy (PEEM) detects calcium mineral phases in biomineral samples (bottom row).

[Sources: Adapted from (1) Gong, Y. U. T., et al. 2012. "Phase Transitions in Biogenic Amorphous Calcium Carbonate," *Proceedings of the National Academy of Sciences USA* **109**(16) 6088–93; (2) DeVol, R. T., et al. 2015. "Nanoscale Transforming Mineral Phases in Fresh Nacre," *Journal of the American Chemical Society* **137**(41), 13325–33; and (3) Mass, T., et al. 2017. "Amorphous Calcium Carbonate Particles Form Coral Skeletons," *Proceedings of the National Academy of Sciences USA* **114**(37), E7670–78.]

transformed the ability to edit the genomes of organisms in simple, precise, fast, and scalable ways (Knott and Doudna 2018).

- Recent developments in technologies for bioprospecting; single-cell omics; multimodal spectroscopy, microscopy, and crystallography; and tools for *in vivo* characterization have enabled previously unattainable discoveries and mechanistic insights (U.S. DOE 2017b).

The positive developments described above suggest a timely opportunity for application of genomic, molecular, and structural biology tools to better understand

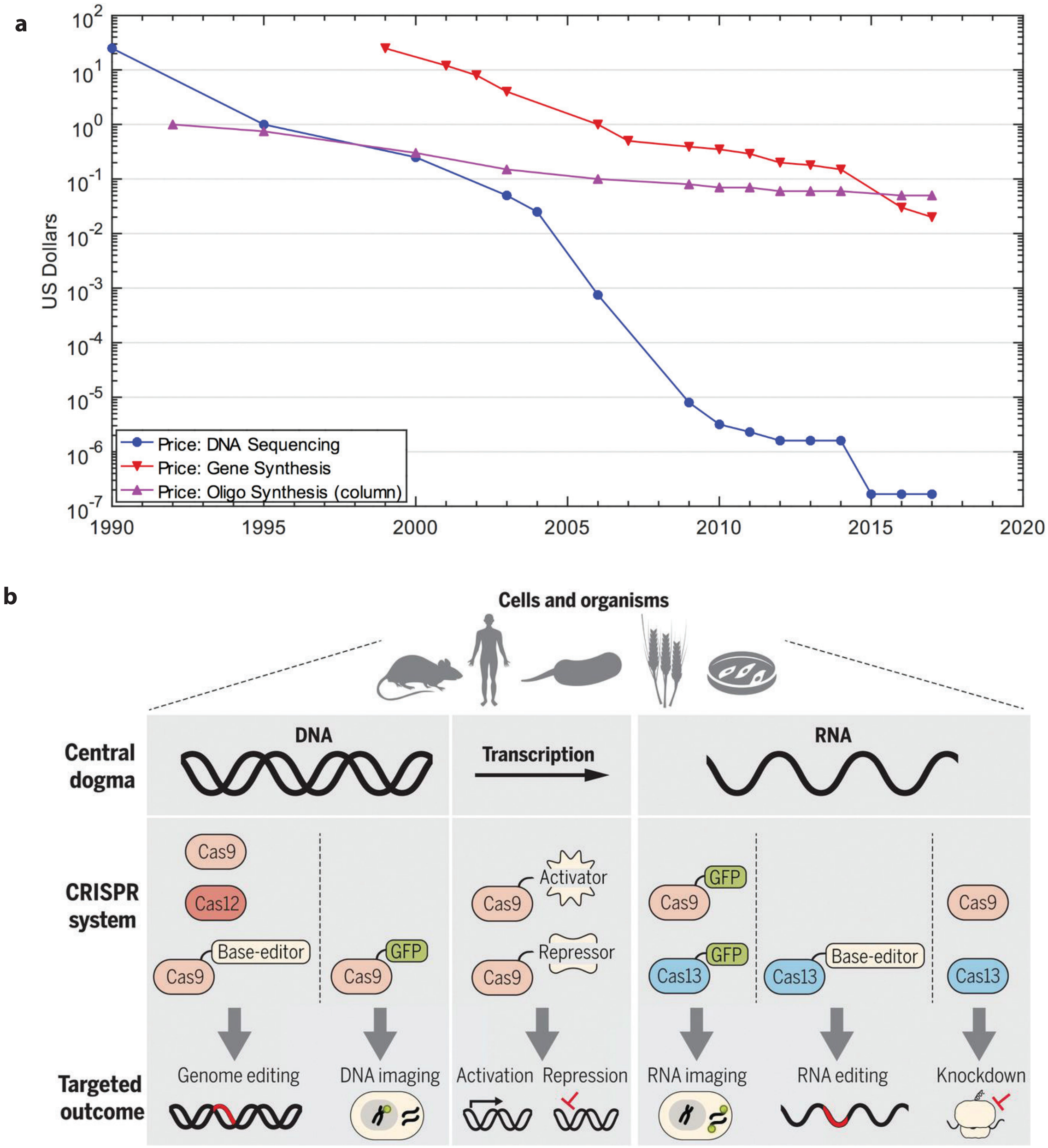


Fig. 3. Advances in Genome Sciences that Support Research on Genome-Encoded Inorganic Materials. (a) Decreases in the cost per base of DNA sequencing and improvements in the quality and length of synthesized DNA have accelerated biological discovery and design of biosystems. **(b)** CRISPR-Cas methods allow precise modifications of the genome, supporting the breadth of discovery and technology development.

[Sources: **(a)** Bioeconomy Capital, www.bioeconomycapital.com/bioeconomy-dashboard/, via a Creative Commons license (CC BY-ND 4.0). **(b)** From Knott, G. J., and J. A. Doudna. 2018. "CRISPR-Cas Guides the Future of Genetic Engineering, *Science* **361**(6405), 866–69. Reprinted with permission from AAAS.]

and potentially exploit these widespread, yet still poorly understood, genome-encoded processes for producing renewable inorganic biomaterials.

To engage the relevant scientific communities in discussions of this research opportunity, BER convened the Genome Engineering for Materials

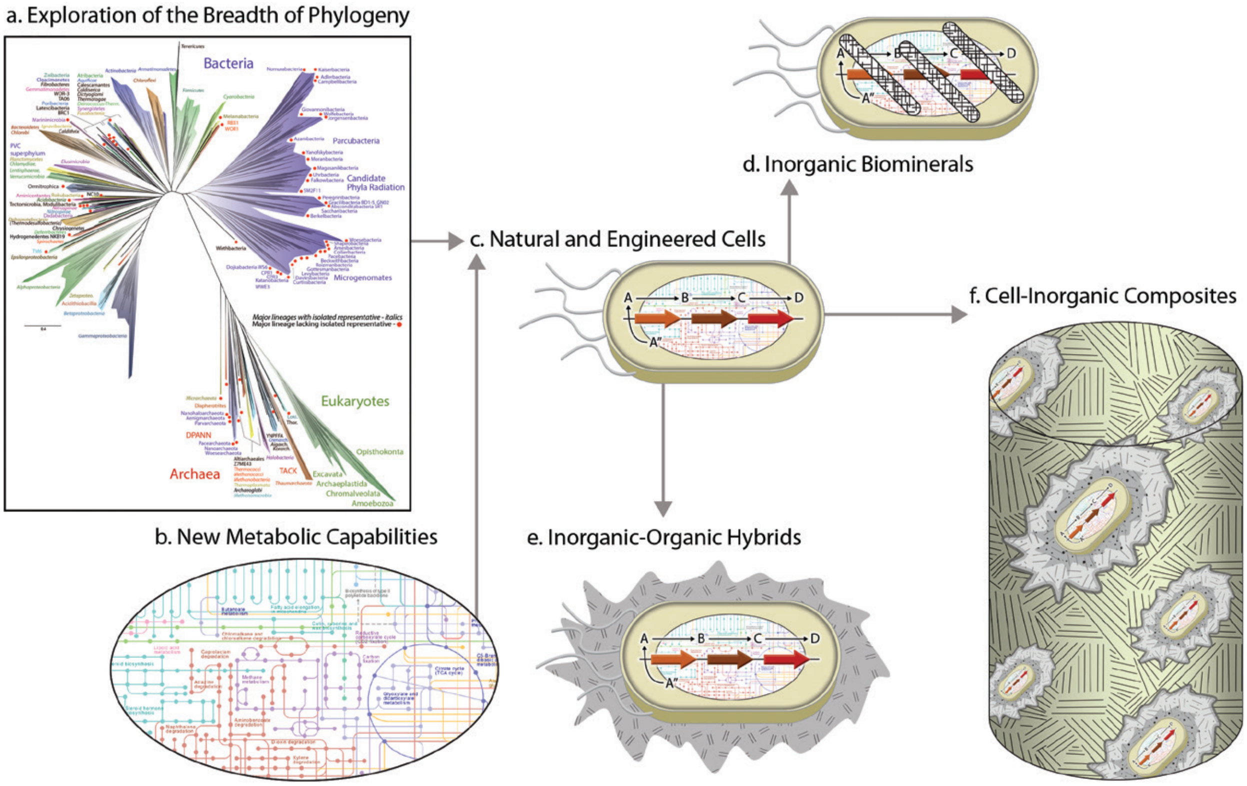

a. Exploration of the Breadth of Phylogeny
Bacteria
Parcubacteria
Candidate Phyla Radiation
Microgenomates
Major lineages with isolated representative - *italics*
Major lineage lacking isolated representative - ●
Eukaryotes
Opisthokonta
Excavata
Archaeplastida
Chromalveolata
Amoebozoa
DPANN
TACK
Archaea
b. New Metabolic Capabilities
c. Natural and Engineered Cells
d. Inorganic Biominerals
e. Inorganic-Organic Hybrids
f. Cell-Inorganic Composites
A
B
C
D
A"

Fig. 4. Overview of Natural Inputs to the Biosynthesis of Inorganic Materials. (a) Organisms from all phylogenetic space are known to make inorganic materials. **(b)** Compared to the biosynthesis of organic compounds, relatively little is known about the metabolic pathways used to make inorganic materials. **(c)** Advances in the understanding of the biosynthesis of inorganic materials will require identification of new organisms and development of chassis organisms to support biodesign and synthetic biology approaches. Inorganic materials can be classified as **(d)** inorganic biominerals, **(e)** inorganic-organic hybrids, and **(f)** composites of inorganic materials and living cells.

[Sources: **(a)** From Hug, L. A., et al. 2016. "A New View of the Tree of Life," *Nature Microbiology* **1**, 16048, via a Creative Commons license (CC BY 4.0). **(b)** Metabolic map: DOE KBase, narrative.kbase.us/narrative/ws.15253.obj.1 and KEGG Database, www.genome.jp/kegg/. See also Edirisinghe, J. N., et al. 2016. "Modeling Central Metabolism and Energy Biosynthesis Across Microbial Life," *BMC Genomics* **17**(568).]

Synthesis workshop on October 9–11, 2018 (see Appendices 1–3, pp. 45–48). This workshop gathered researchers from academia and national laboratories with expertise in synthetic biology, materials science, genome science, and metabolic engineering to discuss the opportunities and challenges of using genome-enabled approaches to discover and design new inorganic biomaterials.

The workshop included discussions on a broad set of topics related to gaining a better understanding of biominerals produced by organisms and the possibilities for designing organisms to produce new biomaterials (see Fig. 4, this page). The ability to synthesize inorganic biomaterials is apparently widely distributed across phylogeny, offering opportunities to systematically understand the breadth of species that have this capability and the diversity of biomaterials that are made. Relatively little is known about the pathways used to synthesize these materials; therefore, the comprehensive study of promising organisms potentially can reveal new biological capabilities and metabolic processes. Studies of both natural organisms and their communities as well as the development of new chassis organisms will be needed to understand the function of new pathways and to establish the foundations for efforts in biodesign and synthetic biology to produce sustainable inorganic biomaterials.

Three classes of genome-encoded inorganic biomaterials were discussed in the workshop: (1) inorganic biominerals, (2) inorganic-organic hybrids, and (3) composites of inorganic materials and living cells. A wide variety of inorganic biominerals in the first class are formed inside cells, including the magnetite found in magnetotactic bacteria. An example of the second class is the siliceous cell wall of diatoms, one of many extracellular inorganic materials produced by different types of organisms. Known examples of cell-inorganic composites within the third class include cyanobacterial stromatolites. All these materials often are complex in structure and exhibit multiple types of inorganic materials assembled by living organisms (or communities of cells or specialized cell types) to achieve spatially heterogeneous materials with remarkable breadth of functional properties. The opalescent property of nacre and the strength of bone are well-known examples.

To achieve the promise of designed control of genome-encoded inorganic biomaterials, BER recognizes the need for basic research that integrates biology, biotechnology, and genome science with engineering, materials science, and physics to address this challenge. By focusing on the diversity of biominerals and other inorganic materials produced by plants, microbes, and other organisms, this report outlines the scientific and technical gaps in the discovery, characterization, and engineering of these genetically encoded materials; describes new opportunities for applying biosystems design and synthetic biology approaches to make these advances; and gives examples of genetically encoded biomaterials of practical interest.

2. Genetically Encoded Materials

As the world population grows toward 10 billion people in 2050, demand for energy and raw materials will also grow, along with strong economic and technological imperatives to meet those demands.

Use of the Elements in Biology

U.S. Geological Survey data suggest that natural resources such as transition metals, rare earth elements, and other inorganic materials will face peak production followed by declines in field production within this century (Kerr 2014). Phosphorus provides a useful example (Vaccari and Strigul 2011). Although phosphorus is the eleventh most abundant element in the Earth's crust, its commercially viable sources are projected to be depleted within 40 to 80 years (MIT 2016; see Fig. 5a, this page).

Phosphorus is essential for life, and living organisms have evolved many ways to extract, store, and use it from overall dilute, highly abundant natural sources that are unlikely to ever be commercially viable for conventional mining. For example, some microbes can produce polyphosphate as a storage polymer (Achbergerová and Nahálka 2011; see Fig. 5b), and efforts to control and improve this capability have brought some success. Moreover, many periodic table first-row transition metals (see Fig. 6, p. 10) are readily trafficked by living organisms, and biological systems capable of extracting, concentrating, and reassembling rare earth elements are now emerging (Jahn et al. 2018; Nakagawa et al. 2012; Skovran and Martinez-Gomez 2015; Wehrmann et al. 2018). Therefore, in addition to providing a sustainable approach for producing novel bioinorganic materials, the use of biological systems for producing biominerals offers the potential to extract and concentrate the necessary raw materials from dilute sources.

Elemental analysis shows that hydrogen (H), carbon (C), nitrogen (N), and oxygen (O) account for 97% of the weight of living organisms (see Fig. 6, p. 10, *grey*

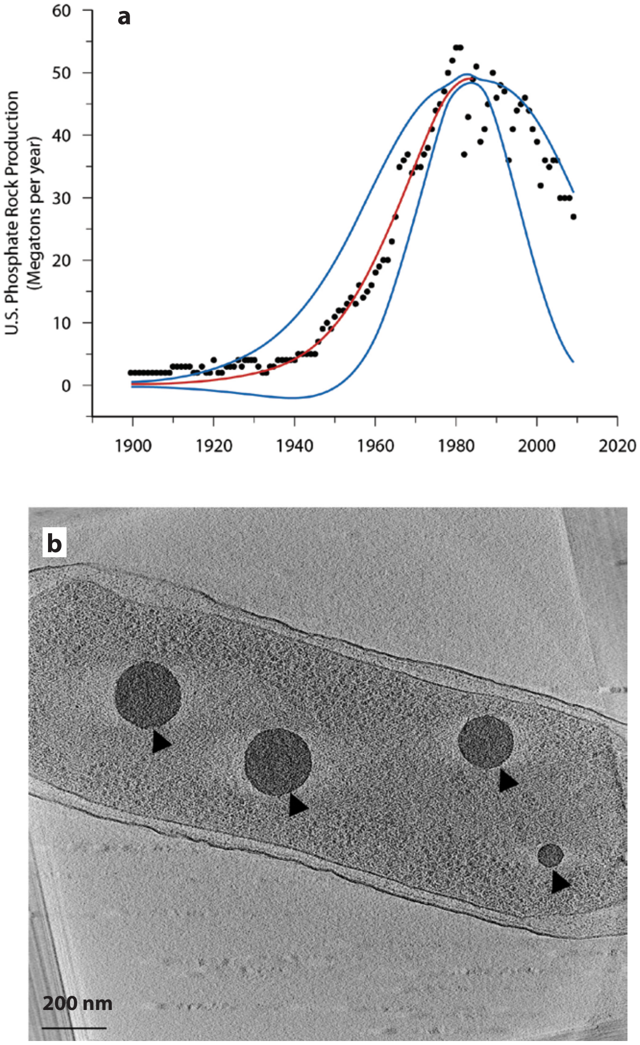
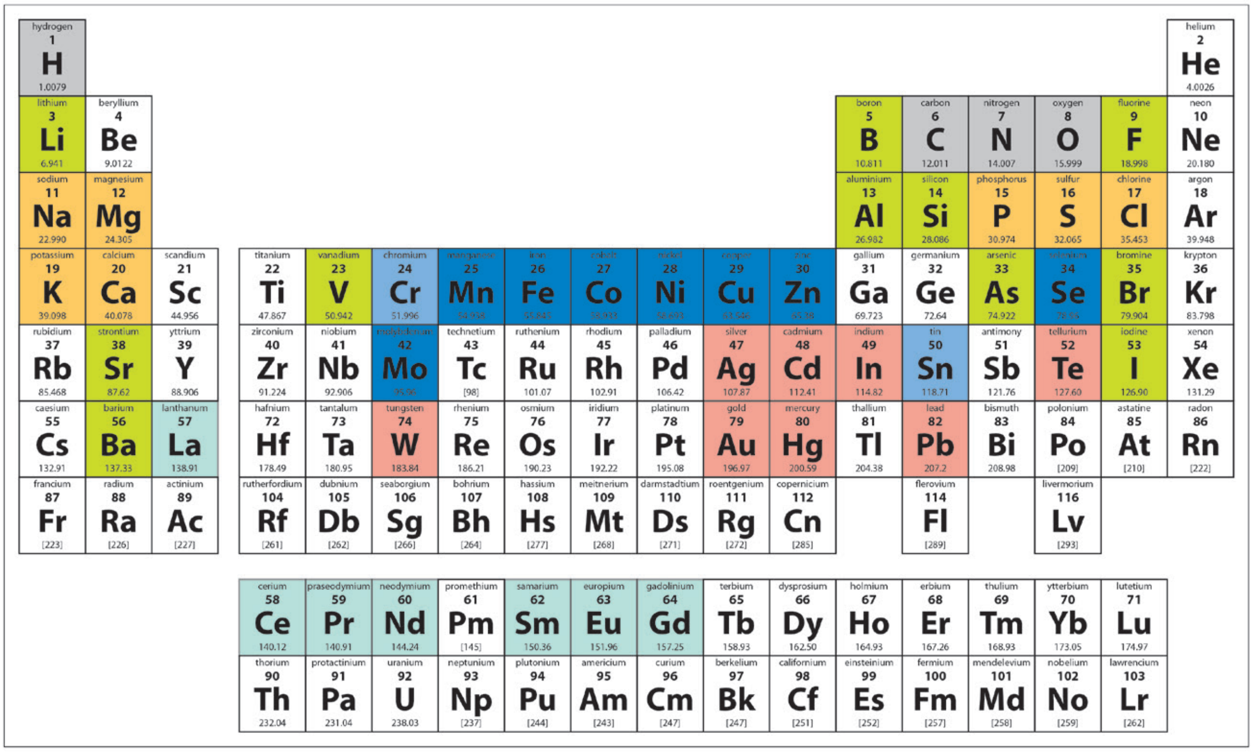


Fig. 5. U.S. Phosphate Rock Production over Time and Bacterial Biogenesis. (a) Logistic curve fitting of annual U.S. phosphate rock production (black dots) from 1985 with extrapolation to 2015. Red line represents the fitted model, and blue lines show the confidence intervals around the regression. **(b)** Transmission electron micrograph showing polyphosphate storage granules (black triangles) formed in *Pseudomonas aeruginosa* under nutrient limitation conditions.

[Sources: **(a)** Reprinted from Vaccari, D. A., and N. Strigul. 2011. "Extrapolating Phosphorus Production to Estimate Resource Reserves," *Chemosphere* **84**(6), 792–97 with permission from Elsevier. Copyright 2011. **(b)** Racki, L. R., et al. 2017. "Polyphosphate Granule Biogenesis is Temporally and Functionally Tied to Cell Cycle Exit During Starvation in *Pseudomonas aeruginosa," Proceedings of the National Academy of Sciences USA* **114**(12), E2440–49.]



hydrogen 1 H 1.0079																	helium 2 He 4.0026
lithium 3 Li 6.941	beryllium 4 Be 9.0122											boron 5 B 10.811	carbon 6 C 12.011	nitrogen 7 N 14.007	oxygen 8 O 15.999	fluorine 9 F 18.998	neon 10 Ne 20.180
sodium 11 Na 22.990	magnesium 12 Mg 24.305											aluminium 13 Al 26.982	silicon 14 Si 28.086	phosphorus 15 P 30.974	sulfur 16 S 32.065	chlorine 17 Cl 35.453	argon 18 Ar 39.948
potassium 19 K 39.098	calcium 20 Ca 40.078	scandium 21 Sc 44.956	titanium 22 Ti 47.867	vanadium 23 V 50.942	chromium 24 Cr 51.996	manganese 25 Mn 54.938	iron 26 Fe 55.845	cobalt 27 Co 58.933	nickel 28 Ni 58.693	copper 29 Cu 63.546	zinc 30 Zn 65.38	gallium 31 Ga 69.723	germanium 32 Ge 72.64	arsenic 33 As 74.922	selenium 34 Se 78.96	bromine 35 Br 79.904	krypton 36 Kr 83.798
rubidium 37 Rb 85.468	strontium 38 Sr 87.62	yttrium 39 Y 88.906	zirconium 40 Zr 91.224	niobium 41 Nb 92.906	molybdenum 42 Mo 95.96	technetium 43 Tc [98]	ruthenium 44 Ru 101.07	rhodium 45 Rh 102.91	palladium 46 Pd 106.42	silver 47 Ag 107.87	cadmium 48 Cd 112.41	indium 49 In 114.82	tin 50 Sn 118.71	antimony 51 Sb 121.76	tellurium 52 Te 127.60	iodine 53 I 126.90	xenon 54 Xe 131.29
caesium 55 Cs 132.91	barium 56 Ba 137.33	lanthanum 57 La 138.91	hafnium 72 Hf 178.49	tantalum 73 Ta 180.95	tungsten 74 W 183.84	rhenium 75 Re 186.21	osmium 76 Os 190.23	iridium 77 Ir 192.22	platinum 78 Pt 195.08	gold 79 Au 196.97	mercury 80 Hg 200.59	thallium 81 Tl 204.38	lead 82 Pb 207.2	bismuth 83 Bi 208.98	polonium 84 Po [209]	astatine 85 At [210]	radon 86 Rn [222]
francium 87 Fr [223]	radium 88 Ra [226]	actinium 89 Ac [227]	rutherfordium 104 Rf [261]	dubnium 105 Db [262]	seaborgium 106 Sg [266]	bohrium 107 Bh [264]	hassium 108 Hs [277]	meitnerium 109 Mt [268]	darmstadtium 110 Ds [271]	roentgenium 111 Rg [272]	copernicium 112 Cn [285]		flerovium 114 Fl [289]		livermorium 116 Lv [293]		
			cerium 58 Ce 140.12	praseodymium 59 Pr 140.91	neodymium 60 Nd 144.24	promethium 61 Pm [145]	samarium 62 Sm 150.36	europium 63 Eu 151.96	gadolinium 64 Gd 157.25	terbium 65 Tb 158.93	dysprosium 66 Dy 162.50	holmium 67 Ho 164.93	erbium 68 Er 167.26	thulium 69 Tm 168.93	ytterbium 70 Yb 173.05	lutetium 71 Lu 174.97	
			thorium 90 Th 232.04	protactinium 91 Pa 231.04	uranium 92 U 238.03	neptunium 93 Np [237]	plutonium 94 Pu [244]	americium 95 Am [243]	curium 96 Cm [247]	berkelium 97 Bk [247]	californium 98 Cf [251]	einsteinium 99 Es [252]	fermium 100 Fm [257]	mendelevium 101 Md [258]	nobelium 102 No [259]	lawrencium 103 Lr [262]	

Fig. 6. Use of the Elements in Biology. Bulk elements such as hydrogen (H), carbon (C), nitrogen (N), and oxygen (O) account for ~97% of the weight (grey boxes) across all living organisms. Other bulk elements include sodium (Na), magnesium (Mg), phosphorus (P), sulfur (S), chlorine (Cl), potassium (K), and calcium (Ca) (*orange* boxes). Trace elements (*blue* boxes) with established biological function include manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), selenium (Se), and molybdenum (Mo); chromium (Cr) and tin (Sn) are provisionally included in this group. Numerous other elements are accumulated in certain species (*green* boxes), including rare earth elements that have been shown recently to have catalytic function in enzymes (*cyan* boxes). Additional elements recently have been incorporated into nanomaterials synthesized by *Escherichia coli* co-expressing metallothionein and phytochelatin synthase (*pink* boxes).

[Sources: Periodic chart template obtained from blog.coudert.name/post/2014/09/10/Periodic-table%3A-PDF-and-Illustrator-template/; Information derived from Choi et al. 2018 and Jahn et al. 2018.]

boxes). Seven other elements account for ~3% of the remaining weight: sodium (Na), magnesium (Mg), phosphorus (P), sulfur (S), chlorine (Cl), potassium (K), and calcium (Ca) (*orange* boxes). Many other elements are present in trace amounts that serve essential functional roles in metabolic processes or as highly accumulated materials in certain classes of organisms (*blue* boxes). Rare earth elements also are frequently found in living organisms, with recent discoveries (Jahn et al. 2018; Skovran and Martinez-Gomez 2015; Wehrmann et al. 2018) defining new roles in enzyme function and gene regulation (*cyan* boxes).

Many different intracellular minerals lacking an organic component have been found in plants and microbes (see Table 1, p. 3; Lowenstam and Weiner 1989; Weiner and Addadi 2002). The diversity of biominerals observed in living organisms generally follows the organisms' expected elemental composition. However, recent work indicates that microbes can synthesize a wide variety of new nanomaterials and also incorporate elements not commonly found in living organisms. For example, an *Escherichia coli* strain was engineered to express the metal-trafficking proteins metallothionein and phytochelatin synthase that resulted in the synthesis of crystalline nanomaterials (Choi et al. 2018), including manganese oxide (Mn_3O_4), iron oxide (Fe_3O_4), copper oxide (Cu_2O), molybdenum (Mo), silver (Ag), indium(III) hydroxide [$In(OH)_3$], tin(IV) oxide (SnO_2), tellurium (Te), gold (Au), cobalt ferrite ($CoFe_2O_4$), nickel ferrite ($NiFe_2O_4$), zinc manganate ($ZnMn_2O_4$), zinc ferrite ($ZnFe_2O_4$), silver sulfide (Ag_2S), silver tellurite (Ag_2TeO_3), silver tungstate (Ag_2WO_4), mercury tellurate (Hg_3TeO_6), lead molybdenum oxide ($PbMoO_4$), lead tungsten oxide ($PbWO_4$), and lead vanadium oxide hydroxide [$Pb_5(VO_4)_3OH$] (*pink* boxes in Fig. 6, p. 10). Cell-free extracts prepared from this strain were also able to synthesize nanomaterials. The contributions of metallothionein and phytochelatin synthase to the formation of those nanomaterials provide an important validation of the potential for genome-encoded biological synthesis.

Organisms have the ability to synthesize many types of materials containing a breadth of elements as described above, but they also face common challenges in doing so. To synthesize biomaterials containing inorganic elements, organisms may need to (1) extract the desired elements from the environment, (2) exchange ligands between environmentally stable configurations and others that are compatible with biological transport and storage, (3) control redox states, and (4) carry out chemical modification to produce biominerals or other materials. Biodesign and synthetic biology efforts will need to consider these challenges to gain control of biomineral synthesis.

There are also thermodynamic considerations regarding how genetically encoded systems might be used to synthesize inorganic biomaterials. For example, aluminum (predominantly Al^{3+}) makes up ~8% by weight of the Earth's crust and is the third most abundant element after oxygen and silicon. It is abundant in aluminosilicate minerals such as feldspars; in commercially useful ores such as bauxite; and in valuable crystalline materials such as garnet, ruby, sapphire, and others. Although organisms that accumulate Al^{3+} do exist, genetically encoded systems that support biological conversion of Al^{3+} are unknown, with the strength of covalent bonding between aluminum and oxygen representing a thermodynamic challenge. Another example is concrete. Containing variable proportions of oxides such as calcia, silica, and alumina, concrete has become the most abundantly used industrial material worldwide. Production of concrete is energy intensive due to the high temperature needed to produce the anhydrous oxides. Over geologic time, living organisms have also used calcia and silica to make a variety of durable structural materials at ambient temperature from dilute solution that are now recognized as vast deposits of aragonite (i.e., limestone, $CaCO_3$) and diatomite (i.e., various compositions of silicates, alumina, and iron). However, production rates currently are not compatible with industrial needs.

Three Classes of Genome-Encoded Inorganic Minerals

As noted in the Introduction, p. 1, workshop discussions were organized around three classes of genome-encoded inorganic materials: (1) inorganic biominerals, (2) inorganic-organic hybrids, and (3) cell-inorganic composites. Highlighted below for each category are examples of known compositions, synthesis mechanisms, and functions.

Examples of Inorganic Biominerals in Microbes and Plants

Biominerals have a variety of perceived functions. Within microbes, one widely adopted function is to concentrate and sequester molecules of interest into storage granules for fabrication of inorganic and inorganic-organic hybrid materials and to cope with the presence of toxic ions. Two notable examples are phosphorus storage in polyphosphate granules (see Fig. 5b, p. 9) and iron storage in ferritin and encapsulin compartments. Mineral formation also combats toxicity arising from accumulation of free ions. For instance, when ferrous iron is enzymatically oxidized and mineralized during storage in ferritin cages and encapsulin shells, the generation of reactive oxygen species is circumvented (Andrews 1998). Nanoparticles of silver (Klaus et al. 1999), cadmium, and selenium (Debieux et al. 2011) formed in various bacterial species also provide opportunities for detoxification.

Magnetotactic bacteria are one of the best understood examples of biologically controlled mineral formation. This phylogenetically diverse and cosmopolitan group of microbes form highly ordered intracellular

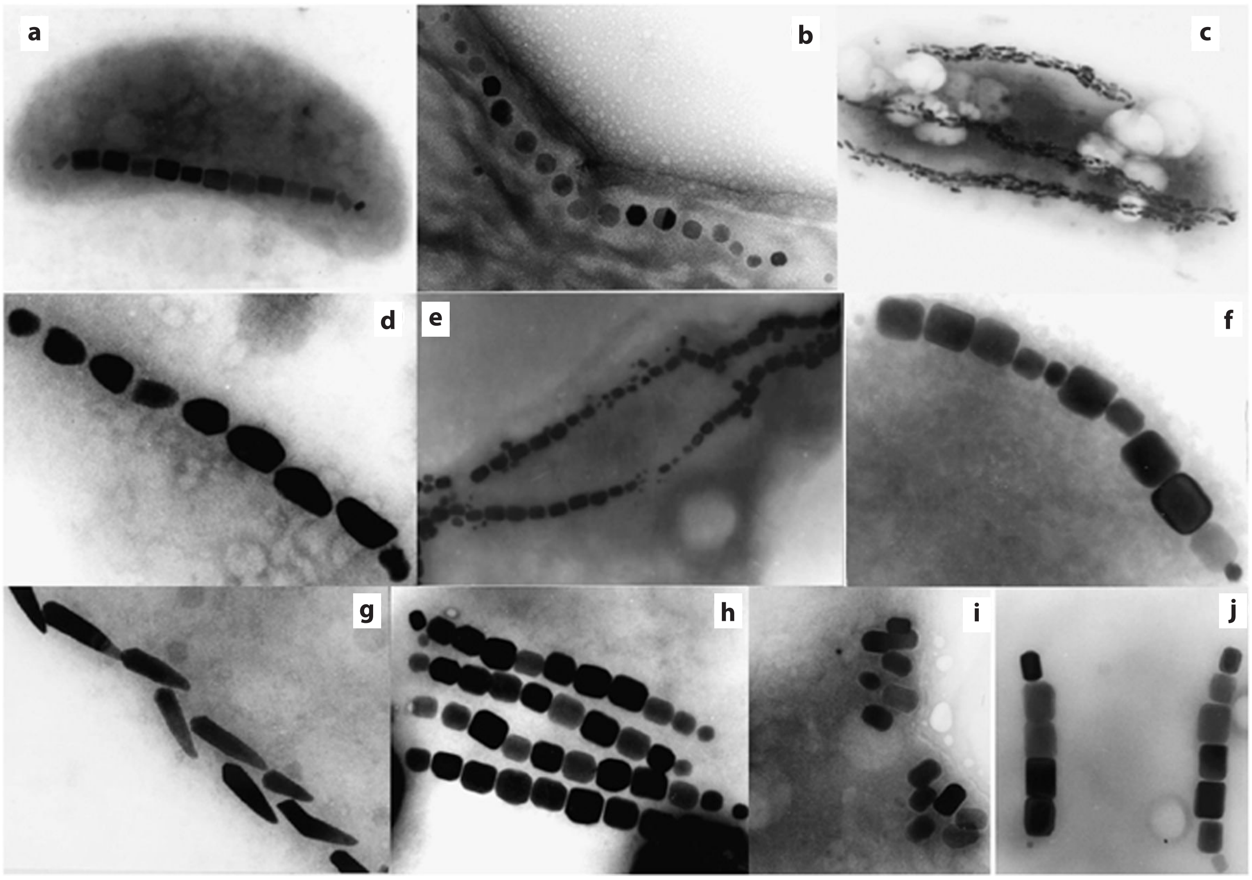
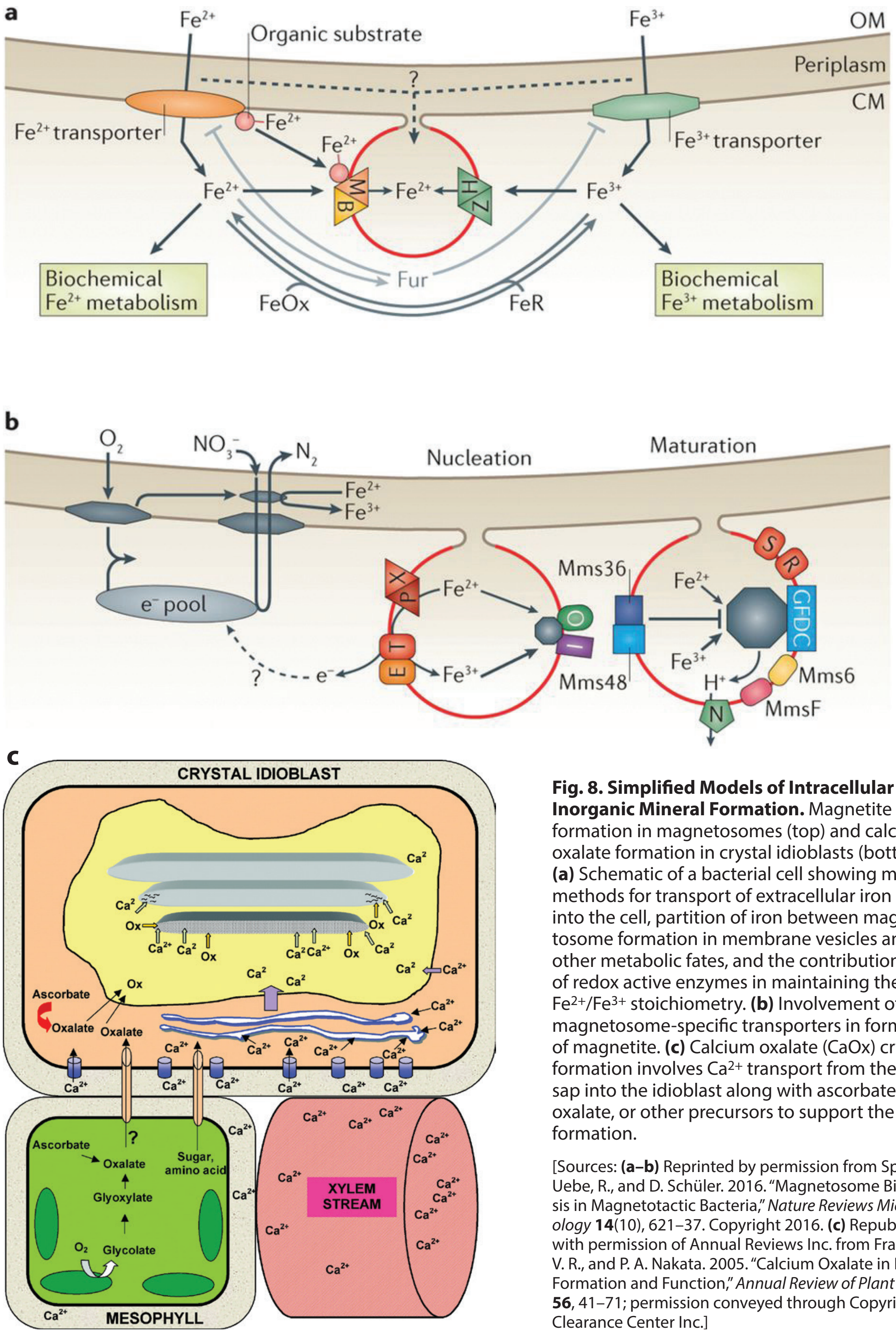


Fig. 7. Diversity of Magnetosome Crystal Morphologies and Arrangements in Various Magnetotactic Bacteria. Characteristic crystal morphologies include elongated prism **(a, e, f, h, i,** and **j)**, cubo-octahedral **(b)**, and bullet-shaped **(c, d,** and **g)**. Crystals can be arranged in single or multiple chains.

[Source: From Schüler, D. 2008. "Genetics and Cell Biology of Magnetosome Formation in Magnetotactic Bacteria," *FEMS Microbiology Reviews* **32**(4), 654–72. Reproduced by permission of Oxford University Press on behalf of the Federation of European Microbiological Societies.]

nanometer-sized crystals (see Fig. 7, this page) of either magnetite $[Fe^{2+}(Fe^{3+})_2O_4]$ or greigite $[Fe^{2+}(Fe^{3+})_2S_4]$ within specialized lipid-bounded organelles called magnetosomes (Uebe and Schüler 2016). Different crystal morphologies are observed among the magnetotactic bacteria; the underlying mechanisms leading to these differences have not been identified. Individual nanocrystals in magnetosomes are precisely aligned into one or more chains via a dedicated cytoskeletal network to create an intracellular device that can orient the cell and promote navigation along a magnetic field. This mode of navigation is thought to simplify the search for the desired redox setting in the environment because magnetic field direction carves a predictable path through natural gradients of oxygen and other molecules.

To stimulate nucleation and intracellular growth of an inorganic mineral (see Fig. 8, p. 13), a separate compartment, usually defined by a lipid bilayer, is used as a biologically assembled crystallization chamber. Figures 8a and b summarize the steps needed to form magnetosomes. Extracellular iron (Fe^{2+} or Fe^{3+}) must be captured from the environment and brought into the cell using specialized siderophores and ion transporters. Specialized vesicles derived from the cytoplasmic membrane form a compartment for accumulation of substrate precursors (iron and either water or sulfide, depending on microbial species) and


a
Fe2+
Organic substrate
Fe3+
OM
Periplasm
CM
?
Fe2+ transporter
Fe2+
Fe2+
Fe3+ transporter
Fe2+
M
B
Fe2+
H
Z
Fe3+
Biochemical Fe2+ metabolism
Fur
FeOx
FeR
Biochemical Fe3+ metabolism
b
O2
NO3−
N2
Nucleation
Maturation
Fe2+
Fe3+
e− pool
X
P
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Mms36
Fe2+
S
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GFDC
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?
Oxalate
Sugar, amino acid
Glyoxylate
O2
Glycolate
Ca2+
MESOPHYLL
XYLEM STREAM
Ca2+

Fig. 8. Simplified Models of Intracellular Inorganic Mineral Formation. Magnetite formation in magnetosomes (top) and calcium oxalate formation in crystal idioblasts (bottom). **(a)** Schematic of a bacterial cell showing multiple methods for transport of extracellular iron (Fe) into the cell, partition of iron between magnetosome formation in membrane vesicles and other metabolic fates, and the contributions of redox active enzymes in maintaining the Fe^{2+}/Fe^{3+} stoichiometry. **(b)** Involvement of magnetosome-specific transporters in formation of magnetite. **(c)** Calcium oxalate (CaOx) crystal formation involves Ca^{2+} transport from the xylem sap into the idioblast along with ascorbate, oxalate, or other precursors to support the crystal formation.

[Sources: **(a–b)** Reprinted by permission from Springer: Uebe, R., and D. Schüler. 2016. "Magnetosome Biogenesis in Magnetotactic Bacteria," *Nature Reviews Microbiology* **14**(10), 621–37. Copyright 2016. **(c)** Republished with permission of Annual Reviews Inc. from Franceschi, V. R., and P. A. Nakata. 2005. "Calcium Oxalate in Plants: Formation and Function," *Annual Review of Plant Biology* **56**, 41–71; permission conveyed through Copyright Clearance Center Inc.]

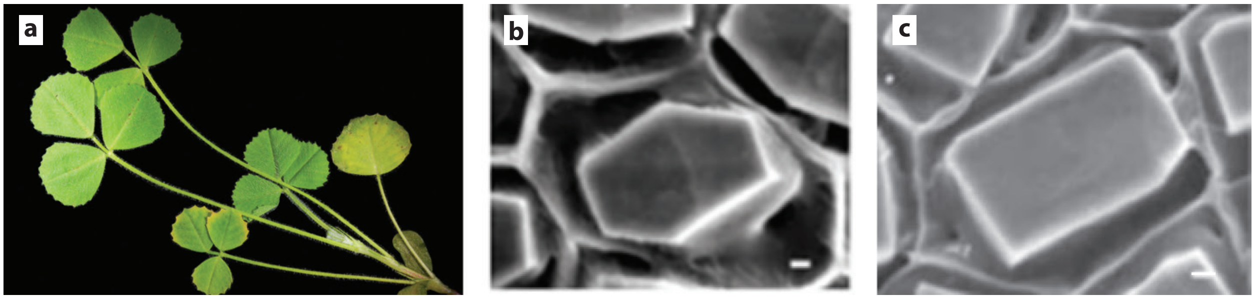


Fig. 9. Inorganic Mineral Production in Plants. *Medicago truncatula* **(a)** produces CaOx crystal whose native morphology **(b)** can be impacted by mutagenesis **(c)** as demonstrated by scanning electron microscopy. Bars = 1 µm.

[Sources: **(a)** Wikimedia Commons, courtesy Ninjatacoshell, via a Creative Commons license (CC BY-SA 3.0). **(b–c)** Reprinted by permission from Springer Nature: McConn, M. M., and P. A. Nakata. 2002. "Calcium Oxalate Crystal Morphology Mutants from *Medicago truncatula*," *Planta* **215**(3), 380–86. Copyright 2002.]

subsequent formation of the biomineral. These vesicles also have specialized ion transporters needed to drive the concentration of crystallization precursors toward supersaturation. Redox enzymes also are used to maintain the proper stoichiometry of Fe^{2+} and Fe^{3+} required to form the magnetic mineral. Furthermore, because of the potential for deleterious reactions with oxygen, microbes have evolved elaborate regulatory mechanisms to overcome the toxicity of reactive oxygen stress (not shown in figures). Similar approaches for uptake, sequestration, and control of redox state and toxicity may be used by microbes to assemble other biominerals and serve as examples of the complexity of the biosynthetic machinery that might be productively harnessed or redesigned in efforts to control biomineral formation.

Plants contain a variety of minerals whose complexity of biosynthesis, crystal morphologies, and function vary among different species (e.g., phytoliths and cystoliths). The genetic pathways of biomineral formation in plants are less explored compared with inorganic materials produced in microbes, and the function of these plant minerals remains largely unknown (Markovich et al. 2017). Plant-synthesized minerals principally include silica, calcium oxalate (CaOx), and calcium carbonate ($CaCO_3$; Arnott 1982), although calcium phosphate [$Ca_3(PO_4)_2$] and other calcium salts also have been reported in some species (Arnott and Pautard 1970). Like microbes, plants can create biominerals to avoid toxicity. For example, silica biomineralization is believed to reduce Al^{3+} toxicity (Hodson 2002). The formation of silica *in vitro* can be induced by the cell wall polymer callose, (a β-1,3-glucan), which is also implicated in silicification in the model plant *Arabidopsis* and the "living fossil" *Equisetum*, also known as horsetail rush (Guerriero et al. 2018).

Biominerals composed of metals such as potassium, aluminum, sodium, cadmium, iron, manganese, strontium, barium, and zinc have also been observed in some plant species (He et al. 2014). Some research suggests that these biominerals play a role in metal detoxification and protection against herbivory. In Australia, plants in the *Acacia* species (comprising shrubs and trees), which exhibit biomineralization, are favored for use in land restoration and detoxification of metals after mining operations (He et al. 2012).

Plants make biominerals to carry out other specific functions. For example, although formation of $CaCO_3$ minimizes Ca^{2+} toxicity (Webb 1999), some studies propose that production of CaOx crystals also provides mechanical support, mineral balance, waste sequestration, and protection against herbivores (Coté 2009). CaOx crystals found in the anthers of chili pepper (*Capsicum annuum*) potentially could play a role in the facilitation of pollen release and germination (Horner and Wagner 1992). Higher plants such as *Medicago truncatula* (see Fig. 9a, this page), *Dieffenbachia seguine* (Araceae), and others produce crystals of CaOx (as the monohydrate) or $CaCO_3$, and the morphology of the crystals can be influenced by mutation of genes involved in their synthesis. CaOx crystals also may be coated in reactive proteins (e.g., proteases and glucosidases), leading to their designation as "microscopic poison darts" (Coté 2009).

Calcium oxalate formation does not occur as a random physical-chemical precipitation of endogenously synthesized oxalic acid and environmentally derived calcium. Rather, specialized cells called idioblasts and biforines are used to create and store crystals of specific shapes and sizes under hydrostatic pressure, leading to forceful ejection when the cells are disrupted. These properties imply a complex network of genetic control, which also has differentiated among CaOx-producing species (Franceschi and Nakata 2005).

Certain steps are needed to form CaOx crystals in plants (see Fig. 8c, p. 13). Calcium present in the xylem sap must be transferred by Ca^{2+}-selective ion transporters into the idioblast and subsequently into the specialized vacuole that serves as a crystallization chamber (e.g., biforine). Similarly, oxalate or suitable precursors synthesized in the cytoplasm (e.g., ascorbate, glycollate, and glyoxalate) must be transferred into the idioblast and then into the crystallization chamber. Ultimately, the control of the concentrations of Ca^{2+}, oxalate, and presumably other co-crystallization additives will be achieved via location-specific expression of enzyme and protein isoforms with binding and catalytic properties needed to achieve the appropriate crystallization conditions and breadth of observed CaOx crystal morphologies. Since cell growth will commonly proceed at the same time as crystal growth, cell expansion and crystal growth must also be coordinated (Franceschi and Nakata 2005). Indeed, mutations that affect protein, lipid, and polysaccharide synthesis have been shown to contribute to alterations in crystal size and shape (McConn and Nakata 2002; see Figs. 9b and c, p. 14).

As described, research has identified the overall processes for producing CaOx in plants and magnetosomes in magnetotactic bacteria, with a basic understanding of sources of substrates, their transport, compartmentalization, and some of the genes involved. However, considerable additional genome-encoded information will be required to productively harness the formation of these paradigmatic biominerals, as well as others, for rational redesign. To start, strategies are needed to access a fuller taxonomy of species capable of producing biominerals and an expansion of the catalog of genes and regulatory networks used to form biominerals.

Examples of Inorganic-Organic Hybrid Materials

Inorganic-organic hybrid materials such as bone and nacre have been studied intensively for decades. Patterning, templating, and self-organization of proteins and other organic molecules are also key for producing many inorganic-organic hybrid materials by cellular systems. Well-known examples include the nacre (aragonite) of mollusks, the intricate silica shells of diatoms, the silica spicules of sea urchins, and the aragonite skeletons of corals and human bones (apatite). The combination of inorganic and organic components ordered at multiple length scales (see Fig. 10, p. 16) produces hybrid materials that have extraordinary multifunctionality. For example, nacre combines iridescence, strength, and toughness with elasticity and light weight that cannot be easily replicated at the same scale with synthetic approaches. Currently, the scientific community is just beginning to investigate and exploit spatial organization mechanisms found in nature.

In addition to these macroscale composite minerals, many eukaryotic algae produce microscale composite materials that are important in global biogeochemical cycles and applied technological settings. Diatoms are one of the most ecologically significant classes of eukaryotic microbes, accounting for about 20% to 25% of primary biomass production in oceans and freshwater environments. The sheer abundance of these organisms and their incredible capability to concentrate and transform silica translate to an estimated annual total production of 240 teramoles (Tmol) of biogenic silica worldwide (Tréguer et al. 1995).

Diatoms, with an estimated 100,000 species, have the potential to produce a vast diversity of porous, amorphous silica cell wall structures called frustules (see Fig. 11, p. 17), which are thought to protect the

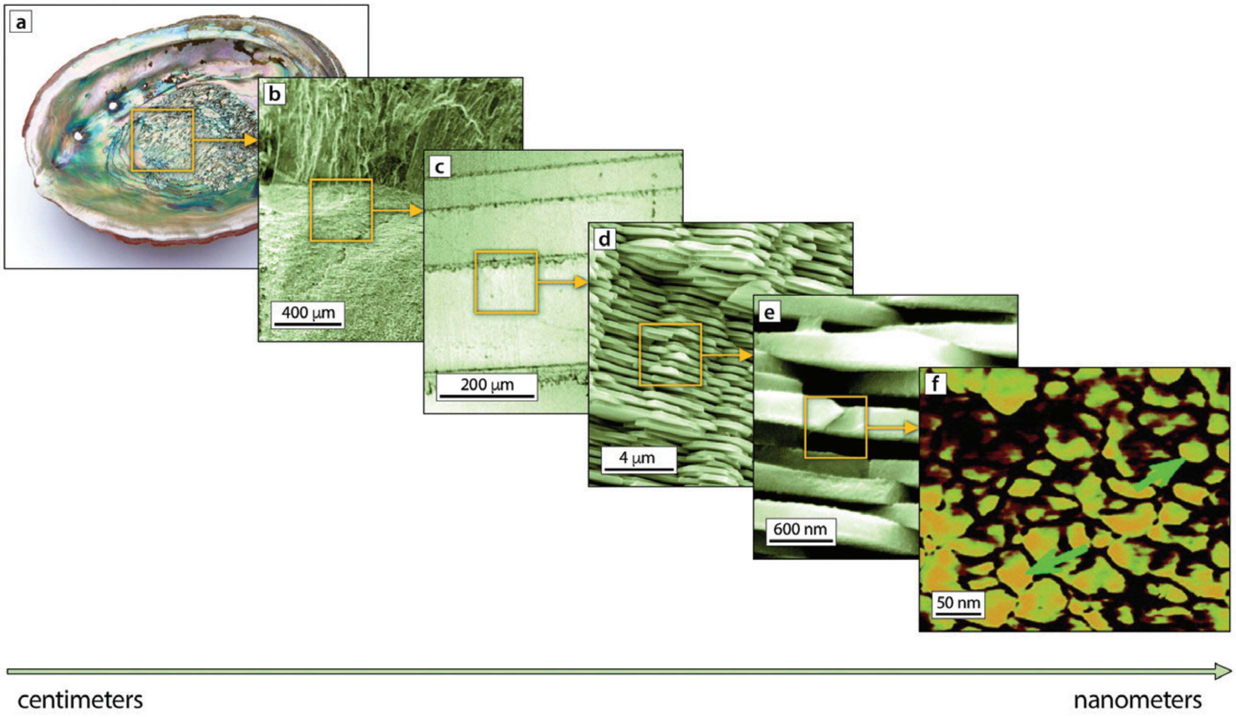


Fig. 10. Hierarchical Structure of Mother-of-Pearl (Nacre) from Abalone. These six different structures, spanning the nanometer to centimeter length scales, together make nacre a multifunctional material that is simultaneously iridescent, strong, and stiff.

[Sources: **(a)** iStockphoto. **(b, d–e)** Adapted with permission from Li, X., et al. 2004. "Nanoscale Structural and Mechanical Characterization of a Natural Nanocomposite Material: The Shell of Red Abalone," *Nano Letters* **4**(4), 613–17. Copyright 2004 American Chemical Society. **(c)** Reprinted with permission from Elsevier from Menig, R., et al. 2000. "Quasi-Static and Dynamic Mechanical Response of *Haliotis rufescens* (Abalone) Shells," *Acta Materialia* **48**(9), 2383–98. Copyright 2000. **(f)** Adapted with permission from Li, X., et al. 2006. "In Situ Observation of Nanograin Rotation and Deformation in Nacre," *Nano Letters* **6**(10), 2301–304. Copyright 2006 American Chemical Society.]

organisms from grazing and ultraviolet (UV) damage. The material properties of diatom silica also are quite interesting, spanning numerous applications including uses such as lenses, coarsening agents, biopurification templates, and drug delivery vehicles. Better exploration of the applied landscape of diatom silica requires a thorough understanding of the genetic and biochemical basis of frustule formation.

The formation of a silica frustule is a complicated multistep process that begins with the transport and intracellular accumulation of silica to concentrations that are several orders of magnitude higher than the extracellular environment. A specialized membranous organelle, the silica deposition vesicle, creates the proper chemical environment to form the silica precipitates that are then extruded to the outside of the cell and, presumably, attached to an organic matrix. Many of the specific proteins and organic molecules that mediate silica precipitation, transport, and anchoring remain in the completed frustule, creating an inorganic-organic hybrid material. A fascinating video of a diatom carrying out this assembly and extrusion has been obtained by Taylor et al. (2007).

Other early and notable advances in understanding the molecular mechanisms of silica biomineralization were made through identification of proteins and organic components intimately associated with frustules. One prominent class of proteins tightly associated with silica are the silaffins (Kröger 2007; see Fig. 12, p. 18).

Silaffins are a class of proteins highly enriched (Kröger 2007) in serine and lysine decorated by a variety of post-translational modifications such as methylation and phosphorylation. Silaffins, as well as biomimetic

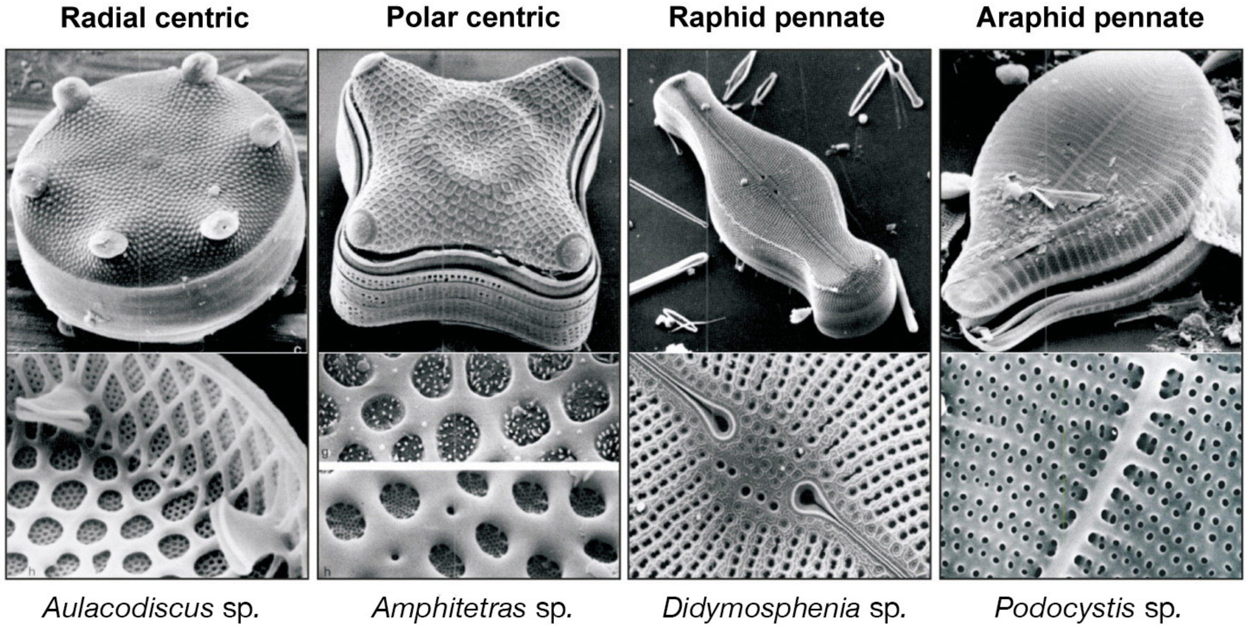


Fig. 11. Examples of Structural Diversity Among Diatom Frustules. Upper panels show a full image of the frustule, classified by overall morphology (~20 to 200 µm); lower panels show magnifications of unique nanostructural features of different diatom genera.

[Sources: Republished with permission of Annual Reviews, Inc., from Kröger, N., and N. Poulsen. 2008. "Diatoms—From Cell Wall Biogenesis to Nanotechnology," *Annual Review of Genetics* **42**, 83–107. Copyright 2008. Permission conveyed through Copyright Clearance Center Inc. Images originally from Round, F. E., et al. 1990. *The Diatoms: Biology and Morphology of the Genera.* Copyright Cambridge University Press, Cambridge, UK. Reproduced with permission through PLSclear.]

peptides bearing their general features, are sufficient to promote biomineralization of silica from inorganic components *in vitro*. Other protein classes, including silacidins, pleuralins, and silaffin-like cingulins, also have silica precipitation activity and may participate in localized connections and assembly of the various components of the frustule. Other organic molecules also play significant roles in formation of diatom silica. For instance, long-chain polyamines constitute a major organic component of frustules and can direct silica precipitation *in vitro*. Chitin, a cell wall polysaccharide, is also intimately associated with frustules, although it is thought to act as a template for biomineral assembly rather than play a direct role in biomineralization. Different combinations of these components give rise to the stunning diversity of silica frustules observed in nature and offer potential for systematic investigation of their combinations, uncovering rules that will enable controlled changes in engineered versions.

The accumulated knowledge of biomolecules and cellular components of diatom silica paint a complex picture of a biomineralization process that is coordinated in time and space. Future challenges in diatom research include the use of genetic and genomic approaches to disentangle the biological networks that control frustule formation and uncover the function of silica biomineralization factors in a cellular context. The prospects of deep molecular investigation of biosilica formation have been boosted by recent development of transcription activator-like effector nucleases (TALEN) and CRISPR-based tools for genome editing in diatoms. Additionally, efforts to develop a variety of model organisms for genomic and genetic work will shed light on the mechanisms that control the diverse patterning of silica biomineralization in diverse diatoms. These tools also should enable more rational engineering in model diatom chassis species so that functionalized frustules and larger multicellular and self-organizing assemblies of diatoms can be tailored for specific applications.

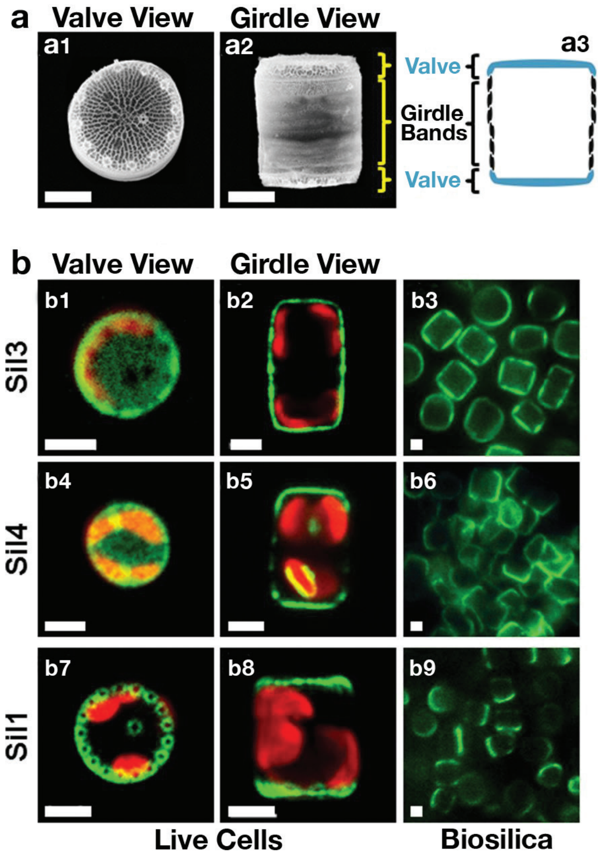


Fig. 12. Localization of Silaffin-GFP Fusion Proteins in *Thalassiosira pseudonana*. (a) Scanning electron micrographs (**a1** and **a2**) and schematic (**a3**) of single biosilica cell walls in valve orientation (**a1**) and girdle band orientation (**a2** and **a3**). These *T. pseudonana* cell walls exhibit cylindrical shapes, appearing as circles in valve view and as rectangles in girdle view. (**b**) Confocal fluorescence microscopy images of individual live cells in valve view (**b1**, **b4**, and **b7**) and girdle view (**b2**, **b5**, and **b8**) of *T. pseudonana* transformant strains expressing green fluorescent protein (GFP) fusion proteins (green color) of the indicated silaffins. Red color is caused by chloroplast autofluorescence. For each clone, the valve and girdle view images are from different cells. Epifluorescence microscopy images of isolated biosilica from multiple cells of the same transformant strains (**b3**, **b6**, and **b9**) are shown. White bars: 2 μm.

[Source: From Poulsen, N., et al. 2013. "Pentalysine Clusters Mediate Silica Targeting of Silaffins in *Thalassiosira pseudonana*," *Journal of Biological Chemistry* **288**, 20100–109. Copyright the American Society for Biochemistry and Molecular Biology.]

Examples of Cell-Inorganic Composites

Cell-inorganic composites are widespread in nature and include the exoskeletons of microbial cells and mineralized tissues (e.g., bones). Moreover, a large fraction of the carbonate rock on Earth's surface arose from biogenic mineralization facilitated by bacteria, algae, fungi, and other metazoans.

Stromatolites are well-known large structures formed from layered calcium carbonate (calcite) deposition driven by the photosynthetic activity of cyanobacteria in microbial mats. Fossilized stromatolite structures date back more than a billion years, and microbialites (layered stromatolites and less-structured thrombolites) still form in certain environments (see Fig. 13, p. 19; White et al. 2015). Yellowstone National Park hot springs and other sites commonly contain silica-encrusted cyanobacteria (see Fig. 13; Smythe et al. 2016), while in other environments iron phosphates or iron hydroxides or oxides are biomineralized by microbes (Phoenix and Konhauser 2008).

Precipitation of minerals on the outer surface of microbial cells can be induced by a chemical change in the local environment that switches the mineral saturation phase in favor of precipitation. Metabolic processes of bacterial cells may change the local pH and calcium concentration, which together may induce mineral precipitation. Furthermore, extracellular polymeric substances (EPS) produced by bacteria as well as their cell walls provide charged or polar sites that bind mineral cations or silica and can serve as nucleation sites for crystallization. Well-known metabolic processes involved in microbially induced biomineralization include (1) alkalization and Ca^{2+} secretion associated with photosynthesis in cyanobacteria; (2) alkalization and CO_2 production associated with urea metabolism in *Bacillus*, *Sporosarcina*, and other ureolytic bacteria; (3) anaerobic denitrification by halophiles such as *Halomonas*; (4) oxidative deamination of amino acids by *Myxococcus*; and (5) production of bicarbonate by sulfate reduction and methane oxidation (Zhu and Dittrich 2016).

For these inorganic materials, it is generally thought that the microbe has little or no control over the mineralization process itself, unlike the functional structures formed by diatoms or other examples described herein. Although extracellular mineralization may be an inadvertent side effect of metabolism or cell-surface compositions for most microbes, some organisms may

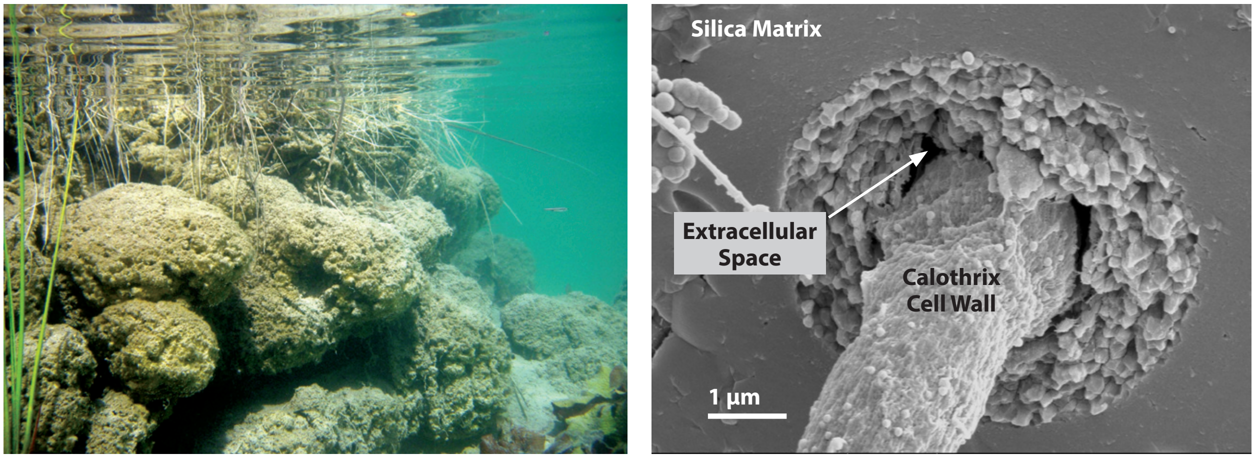


Fig. 13. Living Cell–Inorganic Composites. (Left) Modern freshwater microbialite and **(right)** silica-encrusted *Calothrix* cyanobacteria from hot springs in Yellowstone National Park.

[Sources: **(Left)** Mya Breitbart, University of South Florida. **(Right)** Smythe, W. F., et al. 2016. "Silica Biomineralization of *Calothrix*-Dominated Biofacies from Queen's Laundry Hot-Spring, Yellowstone National Park, USA," *Frontiers in Environmental Science* **4**. Copyright 2016, authors; reprinted via a Creative Commons license (CC BY 4.0).]

gain advantage by controlling the thickness of mineral encapsulation, a capability which may protect viable cells against UV radiation and dehydration (Phoenix and Konhauser 2008).

Calcium carbonate ($CaCO_3$) precipitation by microbial cells is increasingly exploited to heal cracks in concrete surfaces and engineer self-healing concretes. In the simplest case, suitable bacterial cells are applied to surfaces to induce $CaCO_3$ precipitation. Fabrication of self-healing concrete involves the introduction of bacteria into the cement, where they need to remain viable during cement curing and for a long time afterwards until crack formation occurs. A range of strategies has been explored to ensure viability, including selection of robust, spore-forming bacteria and encapsulation of spores (Lee and Park 2018).

Immobilization of microbial cells by entrapment or attachment to inorganic materials has a long history in biotechnology and is widely used for biotransformation. More advanced cell-inorganic composite materials have been engineered by encapsulating cells in a silica gel to generate robust materials for biosensing or biotransformation reactions where the cells may remain metabolically active for extended periods but do not grow or divide (Meunier et al. 2010; Mutlu et al. 2016). Cationic hydroxide layers represent another widely used strategy for the fabrication of advanced, functional cell-inorganic composites (Forano et al. 2018).

Although numerous examples of natural cell-inorganic composites are known, the exquisite mechanisms determining the architecture, properties, and replication of these materials in living organisms are not well understood. Harnessing the genetic building blocks and metabolic events that trigger extracellular biomineralization may provide new opportunities for assembly of designed cell-inorganic composites with new properties and function.

3. Basic Science Opportunities for Genetically Encoded Materials: Knowledge Gaps

Several major knowledge gaps must be overcome to enable facile synthesis of genetically encoded materials. Foremost among these gaps is the lack of understanding of (1) the phylogenetic and genetic diversity of organisms producing biominerals, (2) the mechanisms that control the morphology and composition of biominerals, and (3) the transferability of capacity for biomineralization between hosts. Workshop participants suggested that an approach linking bioprospecting, omics, and bioinformatics could expand knowledge of these organisms, including their physiology, genetics, genomes, and the genes responsible for producing sustainable biomaterials. Additionally, biophysical studies could improve understanding of the mechanisms responsible for biomineralization.

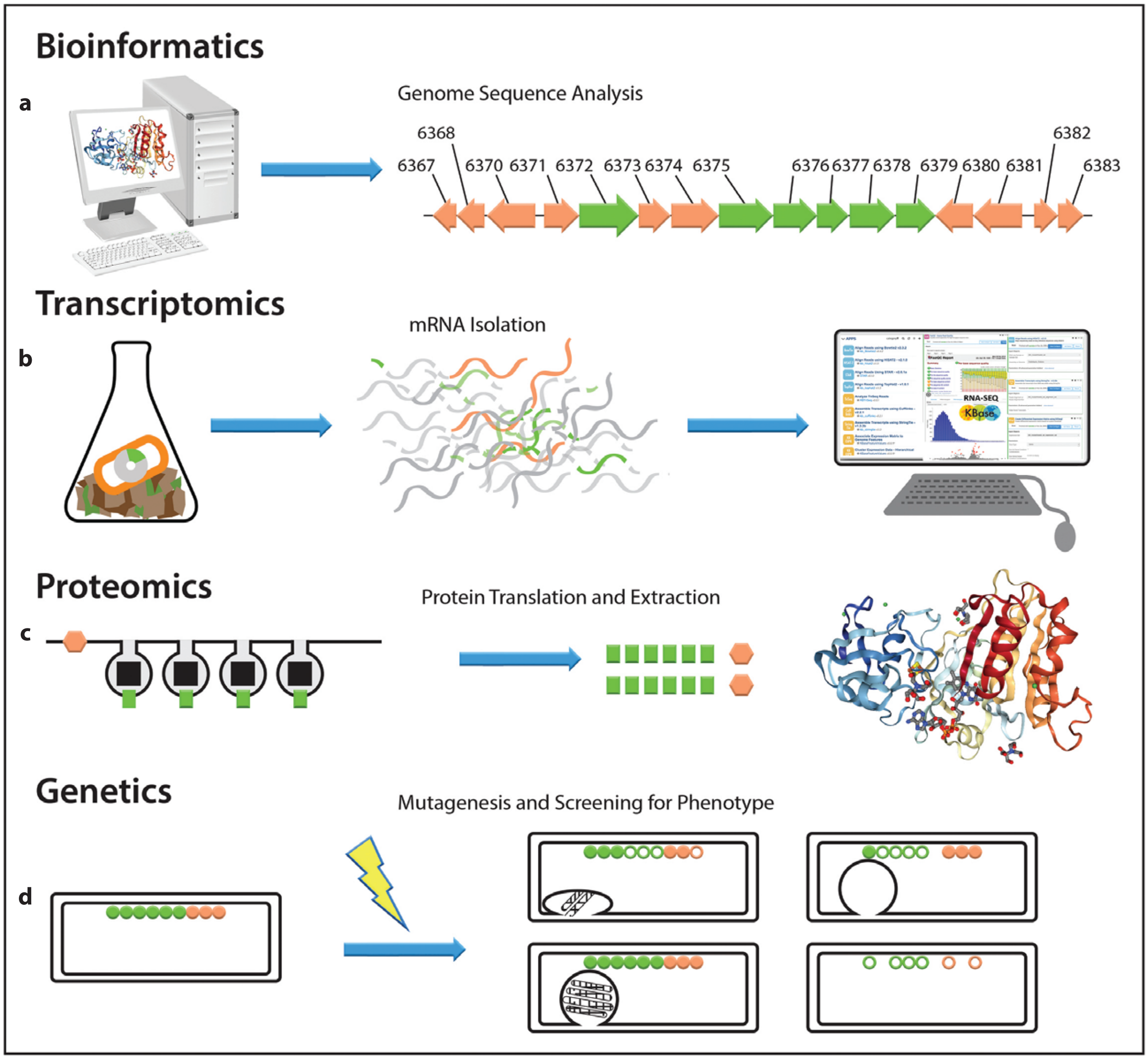
Organisms, Genomes, and Genes Responsible for Inorganic Material Biosynthesis

In marked contrast to understanding natural product synthesis, knowledge of the underlying genetic basis for biomineralization is scarce because relatively few systems have been investigated in detail. The abundance of electron micrographs of organisms showing electron-dense intracellular materials suggests there is a larger diversity of biominerals and biomineral-forming plants, fungi, and microbes than currently identified. Exploration of the composition and patterning of biominerals from different branches of the tree of life (see Fig. 4, p. 6) could address this knowledge gap.

Coordinated efforts spanning bioinformatic analyses to experimental validation (see Fig. 14, p. 22) can significantly broaden understanding of the phylogenetic diversity of biomineral-forming organisms and reveal best opportunities for future research. Grouping organisms that make the same or similar minerals can provide a useful additional hierarchy for future research. Bioinformatic tools can identify sets of genes from across phylogeny that are potentially associated with formation of different types of biominerals. These tools also can analyze potential clusters of biomineralization genes and sort their potential gene products into functional classes such as ion transporters, redox enzymes, compartmentalization-specific genes, ATPases, ABC transporters, and regulatory proteins. Genes of unknown function may also be of interest.

Transcriptomic and proteomic comparisons between different species, between individuals from the same species, or among cells from different tissues or developmental stages that alternately display or lack biominerals will lend additional confidence to the identification of participating genes. Transcriptomic approaches have advantages of high throughput and the potential to provide a breadth of clarifying leads for additional investigation. Proteomic approaches can directly identify proteins associated with biomineral formation. Genetic approaches such as CRISPR-Cas9 can be used to precisely delete individual genes to test the role of the corresponding protein (or mRNA) products in biomineralization. Additional experimental validations of function can be carried out in natural organisms, alternate expression hosts, or cell-free systems, presuming that proper substrates and products can be identified and that sufficiently sensitive and specific technologies can be deployed for their detection.

Similar to the BioBricks™ approach already in successful use by the synthetic biology community, a catalog of genetic parts with verified roles in biomineralization would catalyze new understanding of biomineralization principles across organisms and facilitate engineering of biomineralization processes (Endy 2005; Galdzicki et al. 2011; Boyle et al. 2012). A long-term goal will be to create a list of individual genes or genetic modules with defined functions. These genes or modules could be assembled in


Bioinformatics
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Genome Sequence Analysis
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Transcriptomics
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mRNA Isolation
RNA-SEQ
KBase
Proteomics
c
Protein Translation and Extraction
Genetics
Mutagenesis and Screening for Phenotype
d

Fig. 14. Identifying Genes and Proteins Involved in Biomineralization. (a) Bioinformatic approach to identify genes by alignment with known genes or by mapping the frequency of the appearance of related genes in organisms that produce similar inorganic materials. **(b)** Transcriptomic approach to identify genes that are differentially expressed by growth conditions that promote biomineral formation. **(c)** Proteomic approach to extract translated proteins from organisms producing biominerals and also from the biominerals themselves and to identify them by mass spectrometry or other approaches. **(d)** Genetic approach of mutagenesis followed by detection of changes in phenotype.

[Source: Protein structure DB ID: 4WQM. Acheson, J. F., et al. 2015. "Structure of T4moF, the Toluene 4-Monooxygenase Ferredoxin Oxidoreductase," *Biochemistry* **54**, 5980–88.]

different combinations to engineer novel organisms with desired mineral composition and patterns. If successful, designer biominerals could perhaps be created with a range of properties, structures, hierarchical architectures, and newly embedded functionalities. Adoption of the modular approach to engineer biomineralization also would facilitate the interchange of biosynthetic pathways between different hosts to enable large-scale production of biomaterials.

Biomineralization Mechanisms

It is well understood that organisms create special compartments and transport ions, molecules, and particles into these compartments (see Fig. 8, p. 13) in a complex temporal sequence to form biominerals. Although the elements composing biominerals have been trafficked for billions of years, there are many gaps at the genomic level in understanding how these compartments are assembled and how transport is carried out. Details of transport processes and the chemical identity of key intermediates remain unclear, and overcoming these knowledge gaps will be necessary to enable synthesis of novel biomaterials.

A largely unexplored area is how organisms alter the shape and size of their biomineral storage compartments to achieve a given size, morphology, or polymorphic nature of a biomaterial. Furthermore, while inorganic material biosynthesis is genetically encoded, in the sense that these materials result from coordinated transport and enzymatic catalysis, their biosynthesis apparently is not template driven like that of nucleic acids or proteins. The structure of these molecules is determined by pre-existing DNA or RNA and then catalyzed by polymerases or ribosomes that lead to a large structural diversity. Greater insight is needed into the chemical bonding and speciation properties that contribute to the diversity of biominerals.

Overcoming these knowledge gaps is essential for enabling synthesis of genetically encoded biomaterials. Developing appropriate assay conditions and proper application of measurement and imaging technologies will be necessary. Because new biomaterials likely will first be studied in the natural organisms producing them, investigators will have to identify methods to reproducibly induce the organism to produce the biomaterial. Doing this may require new methods to culture organisms or specifically detect them in natural environments and simultaneously monitor production of the desired biomaterial as a function of changes in physical conditions, prospective substrates, or genetic disruption of predicted genes.

Biosystems Design for Engineered Biominerals

Many studies have characterized biominerals or biomineralization mechanisms, but few have attempted to manipulate them (Söllner et al. 2003; Komeili et al. 2004, 2006). Developing selection criteria that support genetic engineering of specific steps of biomineralization pathways will enable isolation and identification of additional steps along the pathway and thus control or improvement of biomineral synthesis. Examples include the role of specific proteins (Metzler et al. 2010) or polysaccharides (Chan et al. 2004) in biomineral production, which previously could be identified only in isolation and *in vitro* but now can be explored within the living organism or along the entire pathway. Recent advances in capabilities to engineer the polysaccharides and proteins that compose the bacterial biofilm extracellular matrix and to genetically control biofilm formation and patterning at length scales much larger than individual cells (Nguyen et al. 2018) will open novel avenues to design programmable pathways and organisms for the synthesis of inorganic-organic hybrid materials. Furthermore, up- or down-regulating expression of the N16 protein in the nacre layer of the Japanese pearl oyster *Pinctada fucata* is expected to enhance or suppress assembly of aragonite ($CaCO_3$), while the presence of alginate may influence the precipitation of iron oxyhydroxide (FeOOH) into akageneite [β-FeOOH] in bacteria that form this mineral. It is not known, however, whether the specific mineralization outcomes would be directly caused by (1) changes in protein or polysaccharide, (2) gene regulation in response to exposure to inorganic ions or precursors, (3) consequences for protein or polysaccharide biosynthesis, or (4) secondary effects. Answers to these questions will greatly clarify and quantify the role of these polymers in biomaterial formation and elucidate how polymers may participate in other stages of biomineral formation. Applying genome-engineering approaches to address these challenges in biomaterials synthesis will accelerate the understanding of biomineralization pathways and the ability to design new renewable materials.

Successful identification of pathways and mechanisms for biosynthesis of inorganic materials will need to incorporate efforts to identify the substrates and intermediates used to form the desired end products. Substantial challenges in achieving these goals include insufficient knowledge of the specific inorganic precursors, potential lack of spectroscopic or other unique signatures that can be measured and correlated with time-dependent product formation, and low solubility of the substrates or products. New approaches that overcome these issues will be needed to achieve fuller understanding of the mechanisms and processes used to produce inorganic biomaterials.

Engineering and characterization of biomineralization mechanisms for producing genetically encoded materials in many cases will require transfer and redesign of the biosynthetic machinery from the native producer into different, genetically tractable organisms that eventually can be cost-effectively grown at an industrially relevant scale. New chassis organisms for biomaterial design will require secondary functions that exist in the progenitor wild-type strain. Such functions include, for example, the capacity and machinery to secrete proteins and organic molecules involved in biomineralization, compartmentalization, sequestration, and concentration of minerals, as well as inorganic molecule transporters, co-factors, chaperones and accessory proteins, and post-translational modifications that alleviate stress or toxicity imposed by the biomineral or bioproducts. Successful implementation of these processes in non-native organisms also will require detailed understanding of the modularity of the discovered primary biomineralization mechanisms as well as secondary functions required for successful operation of these mechanisms if new chassis organisms are to be developed. This comprehensive knowledge of the biomineralization process is essential to facilitate the bottom-up design of new biomaterials. Addressing the modularity and plasticity of particular biomineralization processes will require dividing these complex processes into different functional subtasks or modules that differ in related organisms and could, therefore, enable mixing and matching to obtain improved performance or produce new materials—an important elaboration of the BioBrick™ concept. Another opportunity arises from identification of genes involved in biomineralization that also may have promiscuous activities or functions that can be swapped or further engineered to change material properties or improve productivity. A comprehensive, systematic understanding of the key biological parts and their impacts in a biomineralization process has the potential to enable creation of a design framework for the production of a huge diversity of non-natural genetically encoded inorganic materials with tailored material properties and biological functions.

4. Basic Science Opportunities for Genetically Encoded Materials: Technology Gaps

To advance understanding of the synthesis of genetically encoded inorganic materials, new technologies will be needed to gain mechanistic insight on biomineralization and to support engineering of pathways and creation of new functionalities. In this section, several technology gaps are described, along with innovations needed to overcome current limitations in technologies and instrumentation. Strategies and tools to enable discovery, characterization, and engineering of improved or new genetically encoded inorganic materials are considered.

Strategies to Discover, Cultivate, and Understand Organisms from the Environment

There is a large amount of untapped biosynthetic potential contained across the breadth of phylogeny (see Fig. 4a, p. 6). With the advent of next-generation sequencing and open access to these data in public repositories, scientists estimate that less than 1% of the biosphere can be cultivated inside the laboratory (Vartoukian et al. 2010). Many reasons exist for organisms remaining unculturable, including (1) low prevalence in the environment; (2) especially slow growth; (3) special growth conditions or growth factors that are required but not well understood; (4) their need to live in a consortium (i.e., organisms that cannot survive on their own because they require beneficial interactions and signals); or, perhaps, (5) phenotypes that are indistinguishable from other known organisms (even if they have different genotypes). Addressing these challenges requires new strategies and technologies to discover, characterize, and culture organisms that are not yet identified and perhaps cannot be cultured.

High-Throughput Cell Culturing

An emerging trend in cell culturing centers on the use of droplet technologies, where growth chambers can be reduced to a volume that is a thousand to a million times smaller than that in a microtiter plate well. Culture of individual cells in droplets can enable ultrahigh-throughput methods to isolate and grow individual cells. Moreover, the combination of advanced cell isolation technologies with combinatorial assembly of growth media components can be used to more efficiently identify optimal conditions for cell growth. For example, methods to screen hundreds of cultivation conditions (i.e., culturomics) can greatly increase the portion of culturable microbes (Lagier et al. 2016), thus providing greater access to natural diversity for subsequent analysis.

The many combinations of cells and growth media will need to be interrogated for changes in gene expression, metabolic activities, and product formation. To obtain maximum advantage, these analytical measurements also need to be carried out in small volumes with high sensitivity and in potentially complicated background matrices. Integrating technologies to work in a correlative way could transform capabilities for isolating organisms and determining their potential for biosynthesis of genetically encoded inorganic materials.

To date, most single-cell analysis has been performed on mammalian cells because of their large size. Analyzing microbes and their communities requires more-sensitive and higher-resolution technologies. Although commercial solutions for single-cell genomics and transcriptomics already exist, measurement of proteins and metabolites in individual cells is still quite challenging because these molecules cannot be amplified like genomes and transcripts can. Consequently, mass spectrometry is one of the most promising analytical modalities because of its sensitivity, specificity, label-free nature, and opportunities for automation to achieve increased throughput (Comi et al. 2017). To make impactful contributions, other methods will have to balance needs for small volume, high throughput, and the sensitivity and specificity of detection.

Accuracy in Genome Annotation

Recent advances in DNA sequencing technologies have dramatically decreased the cost for whole-genome sequencing (see Fig. 3, p. 5). However, the increasing number and complexity of genomic sequences available have created new challenges for genome annotation (Furnham et al. 2012). Assisted by the advances in omic technologies and computer science, modern genome annotation pipelines are able to predict encoded functions more precisely and deeply (Tatusova et al. 2016). For example, the boundaries of coding regions (genes) and the organization of domains can now be specified with considerable accuracy, while regulatory elements and functional RNAs are also routinely identified (Alexander et al. 2010). A well-annotated genome can significantly improve the prediction and characterization of additional genes involved in biosynthetic pathways as well as facilitate their engineering. For organisms that do not have genes organized into clusters or operons, making these higher-order annotations is more difficult. Moreover, predicting the function of a family of genes is challenging if an experimental validation of the function of even a single gene is not available. Efforts to make experimental assignments of new genes putatively involved in synthesis of inorganic materials will need to be undertaken so that genome annotations can have maximum impact and reliability.

Correctly assigned genes from across many genomes, particularly those with potentially novel function, provide additional insight into the validity of annotation and assignment of biological function. Furthermore, well-curated families of related genes become an ideal resource for construction of new gene circuits and pathways for synthesis of materials with new or improved functions. To study new genes involved in inorganic materials synthesis, improvements will be needed in strategies to assemble genetic elements, such as j5 (Hillson et al. 2012), Raven (Appleton et al. 2014), and Cello (Nielsen et al. 2016). In addition, simulation of gene interaction networks at a genome scale, which has an important role in the engineering of new chassis organisms and improvement of product yield, is also dependent on the quality of genome annotation (Henry et al. 2010).

Computational Systems Biology Tools that Correlate Genotype to Phenotype

The rational design of cell factories for synthesis of genetically encoded inorganic materials is a challenge for many reasons. For example, differentially expressed genes may provide targets for forward engineering, but phenotypic responses often are not directly linked to transcriptional profiles. Moreover, difficulties in both quantitatively measuring and integrating system-wide measurements across different functional levels (e.g., mRNAs, proteins, metabolites, and fluxes) limit the ability to dissect regulatory mechanisms and rewire control elements. This includes, for example, the ability to understand trafficking of inorganic materials into cells. In addition, there often is a lack of experimental and theoretical information describing regulatory and kinetic responses upon which cellular responses are based. Furthermore, data-driven methods commonly used to uncover hidden correlations between genotype and phenotype typically can explain cellular responses only in light of existing biological knowledge.

Taken together, these challenges motivate the need for new systems biology approaches to integrate data for predictive design. Already, integrated systems biology studies have been important in gaining a quantitative understanding of complex biological systems (Sauer et al. 2007; Yamada and Bork 2009). For example, such studies have revealed (1) DNA damage response pathways (Workman et al. 2006), (2) novel biosynthetic control mechanisms in amino acid metabolism (Moxley et al. 2009), and (3) the functional landscape of a genome-reduced bacterium (Güell et al. 2009; Kühner et al. 2009; Yus et al. 2009). The wealth of information generated from these studies emphasizes the importance of integrating data across the different functional levels along with protein interaction networks (Feist and Palsson 2008).

Recent advances in systems biology have enabled researchers to investigate the genome, transcriptome, proteome, and metabolome of a target microorganism with a very high resolution, leading to an abundance of data. Machine learning has now emerged as a powerful method for analyzing such high-dimensional data and is being applied in a variety of disciplines to identify patterns among complicated datasets and to build predictive models. Two paradigms of machine learning, supervised and unsupervised, can be leveraged to infer relevant biological insights.

In supervised learning, a labeled training dataset is used to establish parameters of a statistical model, which are then used to make predictions for scenarios not included in the training dataset. Recently, a supervised learning approach was implemented to predict *in vivo* enzyme turnover rates (Heckmann et al. 2018). Unsupervised learning methods classify unlabeled data into clusters according to similarity features and enable the recovery of biologically relevant characteristics. A principal component analysis (PCA)–based method was applied on targeted proteomic datasets, suggesting pathway enzymes for balancing expression; as a result, two terpene compounds showed a 40% improved production (Alonso-Gutierrez et al. 2015). Advancing machine-learning approaches, including democratizing them to all researchers, could be transformative for advancing the synthesis of genetically encoded materials.

Computational Biodesign Tools for Forward Engineering of Inorganic Materials Synthesis

Many computational tools have been developed for design and forward engineering of biological systems on the molecular level in the past decade (Chao et al. 2017). For example, on the DNA level, the ribosome binding site (RBS) calculator is used to design synthetic RBSs to precisely control gene expression (Salis et al. 2009), while Gene Designer is used for synthetic gene design such as codon optimization, restriction site insertion and removal, and oligonucleotide design (Richardson et al. 2010). On the protein level, Rosetta, the most widely used protein modeling and design tool, has been used to design proteins with new functions (Das and Baker 2008). Although continued improvements to these approaches will be necessary, more complex tools also are needed for the pathway and cellular level to advance a new paradigm of genetically encoded materials synthesis.

Pathway and Cellular Biodesign

On the pathway level, genome-scale metabolic models link genotype to phenotype through the reconstruction of the complete metabolic reaction network of an organism. This technique can be used to define theoretical production limits and design and to test new microbial strains *in silico*. This approach has been especially effective for predicting and improving metabolite production rates in heterologous biosynthetic pathways. Flux balance analysis (FBA), flux variability analysis (FVA), and minimization of metabolic adjustment (MOMA) have been successfully used, in combination with genome-scale metabolic models, to predict cell growth, flux distribution, and product synthesis, as well as to guide strain design for product synthesis. A MATLAB® toolbox called COBRA provides a convenient framework to simulate and analyze the phenotypic behavior of a genome-scale stoichiometric model (Schellenberger et al. 2011), and retrobiosynthesis tools such as Biochemical Network Integrated Computational Explorer (BNICE) and RetroPath are used to design new or improved biochemical pathways (Medema et al. 2012). In these design tools, software identifies novel metabolites, reactions, and whole pathways by predicting promiscuity based on classifications of enzymes according to their chemical action (i.e., a given enzyme can perform similar chemistry, such as oxidation of an alcohol to a carbonyl, on chemicals similar to the native substrate).

On the cellular level, a wide variety of strain design tools have been developed for identifying gene targets for knockout, overexpression, or downregulation; introduction of non-native enzymatic reactions; and elimination of competing pathways to improve cellular phenotypes (Long et al. 2015). Pathway and strain improvements achieved from these design tools are often nonintuitive and nonobvious. Although genome-scale metabolic models have been important for metabolic engineering efforts with organic compounds, they will need to be adapted to inorganic components and mechanisms used for inorganic materials assembly. This or similar toolboxes also will need modification to support biosynthesis of inorganic materials.

At present, these types of tools have not been applied to biosynthesis of inorganic materials. Knowledge gaps include ways to account for (1) compartmentalization of processes needed to permit crystallization of inorganic minerals; (2) templating of the structure of inorganic materials (e.g., frustule assembly); and (3) the spatial complexity, temporal discontinuities, and differential localization of the complete biosynthetic apparatus needed to make cell-inorganic composite materials.

Computational Approaches Applied to Materials

Computational tools are also being used to predict the properties of chemicals and other materials (Barthelat 2007; Foster et al. 2019; Neugebauer and Hickel 2013). Of note, density functional theory (DFT) allows calculation of electronic and other properties of materials. Other approaches such as molecular mechanics (MM) and combined quantum mechanical (QM) and MM (QMMM) can be used to gain insights about electronic and molecular structure coupled to molecular dynamics. One example would be modeling the structure and properties of magnetosomes or other inorganic minerals with altered composition of elements. Application of these methods has potential to provide deeper insight into the properties of genetically encoded inorganic materials and to support predictions of how these materials might be modified to obtain desired changes in properties. These approaches are facilitated by access to atomic coordinates available from crystal structures. Therefore, less regular inorganic materials found in inorganic-organic hybrid materials or cell-inorganic composite materials may present challenges.

Genetic Tools for Enabling Biosystems Design

Over the past two decades, the utility of model organisms such as *Escherichia coli* and *Saccharomyces cerevisiae* for synthetic biology applications and bioengineering has greatly advanced. This largely has occurred by improvements in DNA design, assembly, and sequencing, as well as deployment of methods to carry out automated high-throughput strain engineering and selection for desired phenotypes. However, even the most refined design-build-test cycles for optimizing a given biosynthetic pathway still take weeks to months to complete, and process-based challenges associated with using model organisms for manufacturing materials still exist (e.g., limited substrate range, susceptibility to contamination, and genetic instability). Additionally, *E. coli* and *S. cerevisiae* intrinsically lack certain cellular traits, thus limiting the diversity of biosynthetic capabilities and, ultimately, products that can be targeted. These limitations support the need to identify new model organisms and expand the number and capabilities of existing chassis organisms to support production of inorganic materials. This technology need also is recognized in the National Academy of Sciences *Roadmap for Industrialization of Biology*, which proposes how to accelerate the advanced manufacturing of chemicals (NRC 2015).

Most organisms involved in the synthesis of inorganic materials are genetically intractable. However, with recent advances in systems biology, next-generation sequencing, and genome engineering, the genetic manipulation of these nonmodel organisms has become increasingly feasible. For example, low transformation efficiency is a typical obstacle to genetic manipulation in nonmodel organisms. However, efficiency may be improved by optimizing protocols (e.g., buffers, recovery times, voltages, and DNA–competent cell ratios; Kawai et al. 2010). In addition, because promoters, terminators, replication sequences, reporters, and selection markers can be identified from genome sequencing (Liachko et al. 2010), RNA-Seq (Gao et al. 2017), and flow cytometry (Cao et al. 2017), subsequent foundational challenges arise in the development of stable episomal vectors and precise genome-editing tools.

In prokaryotes, a plasmid requires only a specific antibiotic selection marker and an origin of replication to maintain its stability; in eukaryotes, however, both autonomously replicating sequences and centromeres play an important role in plasmid replication and segregation (Vernis et al. 2001).

Precise genome-editing tools are essential to enable construction of sophisticated genomic circuits. CRISPR-Cas9, multiplexed automated genome engineering

(MAGE; Wang et al. 2009), conjugative assembly genome engineering (CAGE; Isaacs et al. 2011), and CRISPR-Cas9 and homology-directed-repair (HDR)–assisted genome-scale engineering (CHAnGE; Bao et al. 2018), among others, have facilitated gene editing in model hosts because of their ease of reprogramming with simple genetic elements (e.g., a single guide RNA or sgRNA). Though the strategy for Cas9 engineering is similar for almost all organisms, a variety of strategies for sgRNA expression have been developed, including RNA polymerase III promoter, synthetic RNA polymerase III promoters, and RNA polymerase II promoter with ribozymes flanking the sgRNA (Löbs et al. 2017). Zinc finger nucleases (ZFN), TALEN (Gaj et al. 2013), and Base Editor (Rees and Liu 2018) are also potential genome-editing methods. Furthermore, CRISPR-dCas9 fused with tags of VPR [VP64-p65-Rta (VP64 = viral protein 64, Rta = R transactivator)], KRAB (Kruppel associated box), Mxi1 (MAX interacting protein 1), and HAT/HDAC (histone acetyltransferase and deacetylase; Xiao et al. 2019) enables the transcriptional regulation and epigenetic control of gene expression in nonmodel organisms. Such systems also could be optimized in nonmodel hosts suitable for inorganic materials synthesis.

The conventional approaches for plant genetic transformation include *Agrobacterium tumefaciens*, particle bombardment (Christou 1992), homologous recombination (HR), and, more recently, genome editing using engineered nucleases including CRISPR-Cas9 (Hansen and Wright 1999), ZFN, and TALEN. Comparatively, CRISPR-Cas9 carries the advantage because it is more efficient and can edit multiple target genes simultaneously.

Cell-free systems provide attractive opportunities to validate libraries of genetic parts and inducible tools (e.g., promoters, terminators, ribosome binding sites, and insulators) and to prototype designs including standardizing and building model genetic circuits (Moore et al. 2018; Wang et al. 2018). To date, there exists only a limited number of sufficiently large datasets to allow comparison of the performances of cell-free and cellular (*in vivo*) translation platforms, so this approach is an opportunity.

Frontiers for Enabling Transformative Biosynthesis Capabilities

The genome-engineering tools described in this report are important for a range of host organisms. However, there are many opportunities that could be enabled by addressing technology gaps in engineering plant-microbe consortia, genomically recoded organisms, and cell patterning.

Plant-Microbe Consortia

Root-associated microbial communities play important roles in plant performance by improving mineral availability (Müller et al. 2016). Furthermore, some soil bacteria are able to multiply inside roots as benign endophytes and directly modulate plant growth and development. Decoding the chemical dialogues between plants and microbes is essential to understanding the complex interactions below ground.

In addition to overcoming the difficulties associated with genetic engineering of plants, the exploration of root-associated microbial communities (e.g., understanding root exudates) has implications beyond potential biological fabrication of novel inorganic materials, ranging from enhanced crop productivity (Mei and Flinn 2010) to phytoremediation (Weyens et al. 2009). Although the application of advanced sequencing technologies has allowed precise identification of microbes present in the soil, including those associated with a specific plant, current sequence analyses explore only the taxonomical composition of a plant microbiome. Currently, little is known about contributions of individual microbial strains to the trafficking of inorganic elements and materials and how nutrient availability affects the composition of the rhizospheric microbiome and the health of the plant. Unusual precursors synthesized by root-associated microbes in the root exudate or as endophytes may potentially be used by the plant to create new inorganic materials. Identifying whether and how individual microbial species contribute to biomineral synthesis in plants would provide a basis for engineering these species to modulate the chemical composition and morphology of inorganic materials.

Genomically Recoded Organisms

The design and construction of genomically recoded organisms (GROs; Lajoie et al. 2013) could facilitate production of new classes of inorganic, hybrid, or composite materials with an expanded range of genetically encoded chemistry (Arranz-Gibert et al. 2018). Indeed, GROs, in which codons have been reassigned to create an alternative genetic code, offer an exciting new direction on multiple levels. First, GROs allow for novel biocontainment strategies, enabling safe application of engineered organisms (Mandell et al. 2015; Rovner et al. 2015). Second, alternative genetic codes broadly obstruct horizontal gene transfer of genetic elements (e.g., multivirus resistance) to stabilize biosystems in the environment. Third, recoded organisms establish orthogonal translation systems to build buffered systems inside cells capable of encoding noncanonical amino acids (ncAAs) into proteins or exotic biopolymers. This ability could make possible new classes of sequence-defined polymers that span vast structural and functional diversity and yet are unattainable through synthetic chemistry or natural biological processes. For example, synthesis of sequence-defined polymers with multiple ncAAs that can bind to metals, or template hierarchical assembly, could lead to new classes of materials with new genetically encoded chemistry.

Despite the promise of GROs, there are many technology gaps to be addressed to enable this frontier. To date, researchers have been successful in fully recoding only one codon in an *E. coli* cell, the amber codon. Thus, construction of GROs with two and then three or more completely open coding channels (including sense codon reassignment) is needed. One ongoing effort seeks to reduce the number of codons in the *E. coli* genetic code from 64 to 57 by removing instances of the UAG stop codon and excising two arginine codons, two leucine codons, and two serine codons (Ostrov et al. 2016). However, challenges exist in learning genome design rules to avoid fitness impairments, especially given the interconnectedness of the translation apparatus (e.g., ribosomes, tRNAs and aminoacyl-tRNA synthetases). Although genome recoding efforts have only been pursued in *E. coli* and, more recently, in yeast (syntheticyeast.org), recoding efforts in organisms that might naturally produce inorganic materials are a reasonable extension. Also needed is the development of orthogonal translation systems that support the introduction of multiple, distinct ncAAs into a single biopolymer (Soye et al. 2015). This need includes new orthogonal tRNA-synthetase pairs as well as strategies to engineer ribosomes that can accommodate new non-α–amino acid substrates (d'Aquino et al. 2018). One potential opportunity is to create full, parallel, and independent translation systems in a cell (Liu et al. 2018). In the long term, the construction of GROs and orthogonal translation systems could open the way to the design of inorganic materials in a rational and knowledge-based way by developing an empirical and perhaps even model-based connection between sequence composition and properties of polymers incorporated into inorganic-organic hybrid materials.

Hierarchical Spatial Ordering

One striking feature of biominerals is the spatial patterning that can occur across nanometer to micrometer scales (see Fig. 11, p. 17). Currently, there are no top-down or bottom-up manufacturing methods—for example, three-dimensional (3D) printing—that can bridge across these multiple length scales (Wegst et al. 2015). However, plants and microbes contain structures that open the opportunity to mimic the hierarchical assembly more typically found in metazoans. Indeed, genetic strategies based on quorum sensing, which were some of the earliest successes in biodesign, can pattern populations of cells in 2D on the 1- to 10-mm scale (see Fig. 15a–d, p. 31; Basu et al. 2005). At the other end of the length scale, self-assembling proteins have been developed to pattern materials on the surface of microbes over the 1- to 10-nm length scale in both 2D (see Fig. 15e; Charrier et al. 2019) and 1D (see Fig. 15f; Chen et al. 2014; also see Nussbaumer et al. 2017; Seker et al. 2017).

While these approaches set the important precedent that living plants and microbial cells can pattern materials at various length scales, several gaps need to be overcome to enable genome-engineered materials synthesis across scales. First, there are a dearth of approaches to pattern structures and materials at the

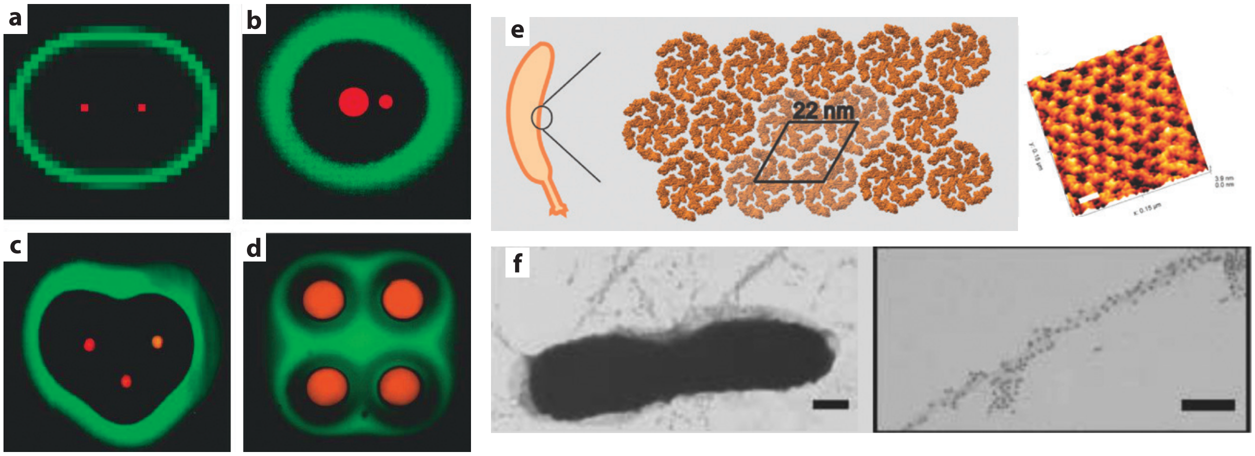

Fig. 15. Biodesign Tools Developed for Patterning at Different Length Scales. (a–d) Quorum sensing–based 1D patterning of *Escherichia coli* colonies (Basu et al. 2005). **(e)** 2D patterning of the cell surface of *Caulobacter crescentus* using its surface layer protein (adapted from Charrier et al. 2019). **(f)** 1D patterning of curli fibers in *E. coli* (Chen et al. 2014).

[Sources: **(a-d)** Reprinted by permission from Springer Nature: Basu, S., et al. 2005. "A Synthetic Multicellular System for Programmed Pattern Formation," *Nature* **434**(7037), 1130–34. Copyright 2005. **(e)** Adapted with permission from Charrier, M., et al. 2019. "Engineering the S-Layer of *Caulobacter crescentus* as a Foundation for Stable, High-Density, 2D Living Materials," *ACS Synthetic Biology* **8**(1), 181–90. Copyright 2018 American Chemical Society. **(f)** Reprinted by permission from Springer Nature: Chen, A. Y., et al. 2014. "Synthesis and Patterning of Tunable Multiscale Materials with Engineered Cells," *Nature Materials* **13**(5), 515–23. Copyright 2014.]

1- to 100-micrometer (μm) scale. A second major gap is to integrate these genetic tools to enable a single system to achieve hierarchical patterning over multiple length scales. Since synthesis of both quorum-sensing and self-assembling systems is resource intensive, integration of multiple systems poses a challenging burden to the cell.

Regulation of Compartmentalization, Ion Transport, and Mineral Templating

As illustrated in the examples of magnetite synthesis in magnetosomes and CaOx in idioblasts, natural biomineralization proceeds by a complex orchestration of events. Specialized compartments are formed, ions and molecules are selectively transported to those compartments, redox chemistry is performed, and templated mineralization is initiated in a highly regulated temporal process to produce biominerals with uniform composition and size (see Fig. 16, p. 32). The relatively small number of well-characterized and transferrable genetic parts with these functions represents a major gap in knowledge and technology needed to manipulate or refactor biomineralization. Gene clusters that create bacterial microcompartments or membrane-bounded organelles have been identified and can be transferred to heterologous organisms. Genetic parts that are focused on ion transport or redox processes exist. However, the known parts are limited in their chemical versatility; that is, they usually are focused on iron trafficking, and how proteins are targeted to these unique microcompartments and organelles is poorly understood. Also needed is establishment of regulatory mechanisms that control the timing of these various steps. Thus, developing the genetic parts and strategies to perform the unique processes of biomineralization will be essential to advancing genome-engineered materials.

Precision Synthesis of Post-Translational Modifications for Accelerating Inorganic Biomaterials Design

Many inorganic-organic hybrids feature proteins that are highly post-translationally modified (e.g., glycosylation and phosphorylation), and these post-translational modifications (PTMs) are important for the function of the inorganic-organic hybrid. For example, because of the extraordinary

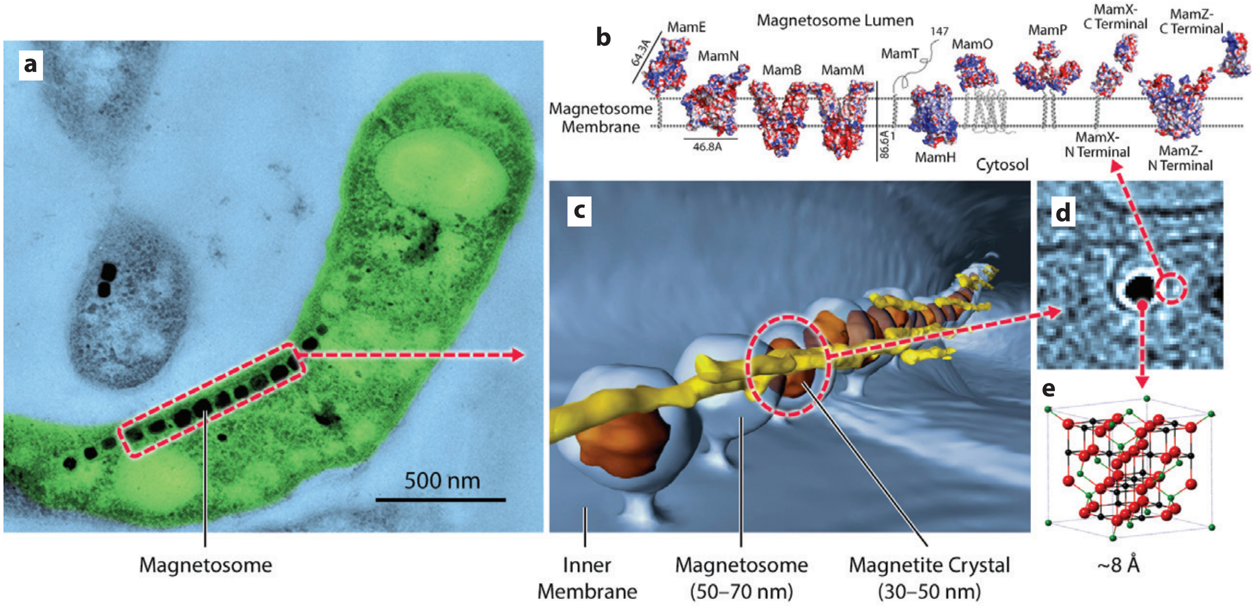

Fig. 16. Multiscale View of Magnetic Particle Formation in Bacteria. The associated proteins and hypothesized compartmentalization involved in magnetite formation in magnetotactic bacteria are shown.

[Sources: **(a)** Komeili, A., et al. 2004. "Magnetosome Vesicles Are Present Before Magnetite Formation, and MamA Is Required for Their Activation," *Proceedings of the National Academy of Sciences of the USA* **101**(11), 3839–44. Copyright 2004 National Academy of Sciences. **(b)** Nudelman, H., and R. Zarivach. 2014. "Structure Prediction of Magnetosome-Associated Proteins," *Frontiers in Microbiology* **5**, 9, available via a Creative Commons license (CC BY 3.0). **(c)** Komeili, A. 2007. "Molecular Mechanisms of Magnetosome Formation," *Annual Review of Biochemistry* **76**, 351–66. **(d)** Komeili, A. 2006. "Magnetosomes Are Cell Membrane Invaginations Organized by the Actin-Like Protein MamK," *Science* **311**(5758), 242–45. Reprinted with permission from AAAS. **(e)** Adapted from Wu, W., et al. 2015. "Recent Progress on Magnetic Iron Oxide Nanoparticles: Synthesis, Surface Functional Strategies and Biomedical Applications," *Science and Technology of Advanced Materials* **16**(2), 023501, via a Creative Commons license (CC BY 3.0).]

utility of L-3,4-dihydroxyphenylalanine (DOPA) as an adhesive, a metal chelator, and redox amino acid, DOPA-containing peptides and proteins have served as a basis for a variety of nanostructured materials. Additionally, metalloproteins are a key class of post-translationally modified proteins that are essential for the biosynthesis of many genome-encoded inorganic materials. While PTMs serve as a mechanism for enormous diversification in the function and molecular recognition of proteins, understanding the role of site-specific PTMs remains a significant challenge due to several technological limitations. First, the chemical heterogeneity and diversity of PTM states on a single protein make it difficult to predict a particular PTM's effect on the biochemical and biophysical properties of a protein to which it is attached. Second, most proteins isolated from cells and tissues are complex heterogeneous mixtures of different chemical structures, owing to their mode of biosynthesis, subcellular distribution, and diversity of PTMs. Third, extensive crosstalk between protein networks in cells expands the spatiotemporal diversity of PTMs within the cell, confounding genetic approaches to decode the relationship between a specific PTM event and its biological function. These challenges notwithstanding, the lack of tools and technologies to produce useful quantities of proteins with a defined PTM state for biochemical, mechanistic, and structural studies is perhaps the most critical barrier to understanding the role of site-specific PTM events and how they might guide biomineral formation.

As a result, new tools and technologies should be advanced for precision proteoform synthesis (i.e., the ability to produce useful quantities of proteins with defined PTMs) and their analysis (i.e., proteomics). In one approach, researchers could develop orthogonal translation systems that co-translationally incorporate

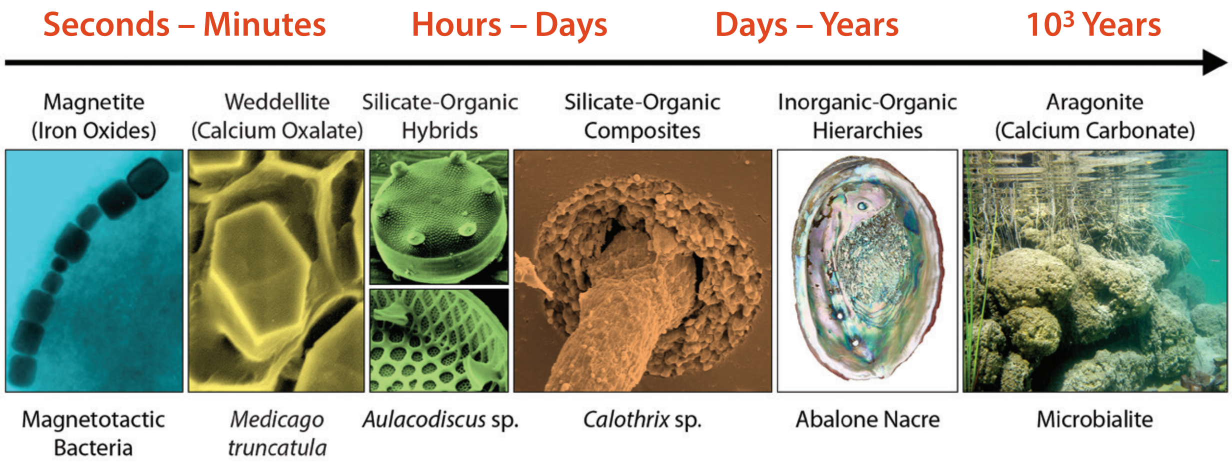

Fig. 17. Overview of Size and Time Scales Relevant to Materials and Processes Involved in Biosynthesis of Genetically Encoded Inorganic Materials.

[Sources: From left, Fig. 7, Fig. 9, Fig. 11, Fig. 13, iStockphoto, Fig. 13.]

such monomers to provide new ways to study them. In another approach, high-throughput efforts, including cell-free methods, should be improved to better control enzyme translation and the extent and specificity of PTMs. Continued efforts to create a paradigm shift in understanding how PTM structure-function relationships perform their critical and versatile roles in cellular regulation hold promise to significantly impact biosynthesis of genetically encoded materials.

Characterization Capabilities for Advancing Synthesis of Genetically Encoded Materials

Chemical analysis at the nanoscale is critical to progress in the various fields of biology. Complicated processes like cellular signal transduction and trace element characterization in biological systems require nanometer-resolved multimodal chemical and physical analysis. This need subsequently drives the requirement for novel analytical tools offering higher sensitivity, as well as detailed chemical information coupled to high–spatial resolution modes. There is a clear need for the development of workflows that allow co-registration between established stand-alone techniques currently used for studying biological systems, such as electron microscopy (EM), fluorescence and atom probe microscopies, and secondary ion mass spectrometry (SIMS). Advancements in chemical, morphological, and physical characterization capabilities at a number of DOE scientific user facilities and national laboratories are further driving the discovery of genome-linked biological processes, providing a more comprehensive understanding of genome-level details, and linking gene to function. These capabilities include resources and instrumentation at the Environmental Molecular Sciences Laboratory (Pacific Northwest National Laboratory), Nanoscale Science Research Centers, structural biology capabilities at the Advanced Light Source (Lawrence Berkeley National Laboratory) and Advanced Photon Source (Argonne National Laboratory), and dedicated biological cryo-EM facilities at SLAC National Accelerator Laboratory and Brookhaven National Laboratory. Workshop participants discussed existing capabilities and identified gaps and the need for adaptation of some capabilities for understanding the genes, metabolic pathways, and processes that control the synthesis of inorganic biomaterials in microbes and plants. Key needs include technologies capable of analyzing both soft tissues and hard inorganic biomaterials (and the interface between them) and tools that enable fundamental understanding of the dynamic processes that span length scales from nanometers to micrometers in a biological system (see Fig. 16, p. 32, for example).

Figure 17, this page, gives an overview of the size range of representative materials encountered in research on the biosynthesis of inorganic materials. The *Technologies for Characterizing Molecular and Cellular Systems Relevant to Bioenergy and Environment* workshop report (U.S. DOE 2017b) provides a full analysis of the capabilities for microscopy, spectroscopy, crystallography, and other biophysical characterization approaches relevant to the development of a robust workflow with genetically encoded inorganic materials.

Technical capabilities needed in the arena of inorganic materials are framed by the approaches anticipated to advance understanding (see Fig. 14, p. 22) and the challenges suggested by experimental results in other figures of this report. The importance of obtaining high spatial resolution coupled with high elemental specificity must be emphasized, along with the need to detect changes in chemical bonding and molecular composition associated with changes in the hierarchy of inorganic-organic hybrids and diverse materials contained in cell-inorganic composites. Automated systems that overcome the challenges of handling biologically generated inorganic materials while also meeting the technical demands of the selected characterization method are needed. Making these measurements in complex biological samples will also be advantageous.

Success with transcriptomic, proteomic, and genomic approaches described in Fig. 14, p. 22, rely on having associated methods to detect cell growth and induction of pathways required for synthesis of an inorganic material. There is a compelling need for capabilities to detect consumption of substrates and accumulation of intermediates and final products over time and to quantitate formation of inorganic materials. These enabling measurements need to be made across physical dimensions from nanometers to meters and over time spans from seconds and minutes to years.

High Throughput and High Sensitivity in Single-Cell Analysis

Cells are the basic functional unit of life. For multicellular organisms, such as plant tissues (Shulse et al. 2018) and microbiota (Terekhov et al. 2018), the phenotypes and dynamics of an ensemble and its members are dictated by cellular heterogeneity, spatial organization, and molecular communications. Such information is lost when bulk measurements are performed using traditional means. By correlated study of the genome, transcripts, proteins, and metabolites of an individual cell, single-cell biology promises to provide quantitative descriptions of stochastic measurements of different constituents, a capability which is critical for developing predictive models for interactions of complex biosystems.

Current single-cell approaches usually start with dispersion of multicellular ensembles into individual cells. In this way, spatial information is lost and cellular states may change during sample preparation as they are removed from their natural context. Therefore, the desirable approach is to develop *in situ* technologies to analyze single cells in their natural environment. Combining droplet technologies that allow isolation of single cells with microscopy (Chang et al. 2017), electrochemistry (Grime et al. 2008; Schulte and Schuhmann 2007), and *in vivo* chemical spectroscopy (Wu et al. 2011) has enabled single-cell measurements while maintaining natural metabolic context. In addition to measurement capabilities, also necessary are ways to precisely perturb living cells using physical, genetic, and biochemical means at single-cell levels within a plant tissue or a microbial community. Alternatively, bottom-up construction of synthetic, multicellular models can be used to understand community structure and potentially mimic interactions with the environment in a controlled manner. The technical needs for this latter approach are enabled by engineering advances in nanotechnology, microfluidics, and acoustic and 3D printing (Biteen et al. 2016).

Methods that allow elemental analysis in the dimension of a cell, or at the subcellular level, are needed to distinguish the locations of elements in desired materials relative to their distribution across all cellular constituents, which often have similar ligation environments (see Figs. 7, p. 12, and 9, p. 14). Since many intracellular minerals are formed from elements that are also major constituents of cells, a combination of positional specificity and dynamic range of detection will also be important.

Table 2. Methods to Characterize Biological Specimens Containing Inorganic Materials

Method	Spatial Resolution	Temporal Resolution	Comments
Microscopy			
Transmission electron microscopy (TEM)	1 – 50 nm	100 ps – 100 ms	Cryo-TEM and phase plate detection brings resolution to ~2 Å; use with thin samples
Scanning electron microscopy (SEM)	1 – 10 nm	minutes	Generates an image of the surface of an object
Confocal microscopy	500 – 1000 nm	1 µs – 100 ms	Widely available
Tomography	1 nm – 1000 nm 5 – 100 nm	1 – 100 ms; seconds to minutes	3D reconstructions facilitated by use of focused ion beam (FIB) milling; resolution enhancements from cryo-TEM tomography
X-Ray Methods			
Macromolecular X-ray crystallography (MX)	0.01 – 0.05 nm; 0.01 – 50 nm	100 ps – 1 s; 10 fs – 10 ps	Additional resolution range and time scale from X-ray free electron laser (XFEL)
Wide angle X-ray scattering (WAXS)	1 – 10 nm	100 ps – 1 ns	Utility in determining degree of crystallinity in samples
Small angle X-ray scattering (SAXS)	1 – 10 nm	100 ps – 1 ns	Utility in determining size and shape distributions in solution samples
X-ray absorption (XAS, EXAFS, XANES)	500 – 1000 nm	1 – 10 s	Identity, local structure, ligation, and redox state of transition metals

Breadth of Characterization Methods

A few technologies offer promise for structural characterization of biological specimens containing inorganic materials (see Table 2, this page). DOE investments in user facilities provide community-wide access to this instrumentation and supporting scientific expertise, and additional information is provided in the 2017 workshop report focusing on characterization technologies for molecular and cellular systems (U.S. DOE 2017b).

Microscopy Imaging

Microscopy provides images of biological materials across the scale from angstrom (Å) to µm (0.1 to 1000 nm), spanning much of the scale relevant to knowledge gaps in the biosynthesis of inorganic materials (see Fig. 10, p. 16). Imaging methods have great promise to elucidate the ultrastructure of complex inorganic-organic hybrid materials, including boundary and phase changes. Prominent modes of electron microscopy offer access to thin samples (transmission electron microscopy, TEM) and surfaces of materials (scanning electron microscopy, SEM), while focused ion beam–SEM (FIB-SEM) can be used to create successive images of the surface of thick milled materials that are used to create a 3D reconstruction.

Synchrotron microscopies such as photoelectron emission (PEEM), scanning transmission X-ray microscopy (SXTM), and ptychography also provide unique tools to analyze synthetic and biogenic materials chemically, morphologically, and functionally, in 2D and 3D.

Other forms of microscopy are widely available (e.g., confocal), albeit with lower resolution. Adoption of the strategy of correlated light-electron microscopy (Hampton et al. 2017) to problems in biosynthesis of inorganic materials, facilitated by new labeling and molecular registration strategies, will serve as a bridge from lower to higher resolution.

X-Ray Methods

X-ray crystallography has supported advances in chemistry and structural biology for more than 50 years and will continue to be impactful, particularly when high-resolution structures (and information on elemental heterogeneity) of biominerals and relevant proteins and enzymes are needed. For example, a program to control biosynthesis (Lefèvre et al. 2011) of either magnetite [$(Fe^{2+}Fe^{3+})O_4$] or greigite [$(Fe^{2+}Fe^{3+})S_4$] versus other natural, nonbiogenic minerals such as tetrahedral pyrite (FeS_2), orthorhombic marcasite (also FeS_2), chalcopyrite ($CuFeS_2$), and mackinawite $(NiFe)_9S_8$ will benefit from structural characterization of the biosynthesized minerals. Moreover, researchers increasingly will be able to take advantage of the photon intensity and time resolution provided by the X-ray free electron laser (XFEL) at the Linac Coherent Light Source facility located at SLAC National Accelerator Laboratory to carry out more extensive structural investigations. A necessary condition of access to this powerful technology will be development of methods to produce samples of biologically generated inorganic materials that are compatible with the technical demands of XFEL.

Other X-ray methods termed "spectromicroscopies" have proven extremely productive to investigate cells, tissues, minerals, materials, and inorganic biomaterials. These methods include PEEM, which is surface sensitive (Frazer et al. 2003), and STXM, which works in transmission for samples less than 200 nm in thickness (Obst et al. 2009). A newer, extremely promising method that will be able to investigate both organic and inorganic tissues and their interfaces is ptychography, which also works in transmission at various X-ray energies and thicknesses in 2D (Giewekemeyer et al. 2010) and in 3D (Dierolf et al. 2010).

Other Approaches

Materials containing transition metals have been studied using various approaches, including electron paramagnetic resonance (EPR) spectroscopy, Mössbauer spectroscopy, and X-ray absorption spectroscopy (XAS). With respect to the challenges of sample preparation, as well as the confounding effects of heterogeneity arising from multiple ligation environments, these methods can provide important information on quantitation, ligation environment, and redox state. Owing to the tunability of synchrotron radiation, XAS also can provide opportunities for elemental analysis. With knowledge of substrates and products, methods that enhance sensitivity such as specific isotopic enrichment (Zachleder et al. 2018) and dynamic nuclear polarization (Rogawski and McDermott 2017) can be incorporated into solid-state nuclear magnetic resonance (NMR) approaches to study inorganic materials.

Methods to perform correlative light or fluorescence and electron microscopies, perhaps using genetically encoded pathway proteins or enzymes that have been modified to contain diagnostic light or fluorescence probes, will also be advantageous (Hampton et al. 2017).

Atom probe tomography (APT) is an especially powerful technique to interrogate complex materials (Gordon et al. 2012). APT dissects a sample atom by atom and can detect and identify chemically up to 80% of all atoms in a specimen, with no chemical bias, at subnanometer spatial resolution, and independent of crystallinity.

Characterization in Complex Background Matrices

Specific labeling of biomolecules via isotopic substitution (Zachleder et al. 2018) or attachment of fluorescence or other high-sensitivity probes (Ritchie et al. 2013) has greatly facilitated studies of their assembly and properties, particularly in complex backgrounds such as heterogeneous tissues (see Fig. 12, p. 18) or environmental samples. While this approach is well developed for labeling proteins, nucleic acids, and lipids, new methods will be required to specifically label inorganic materials and account for the more complex background given by mixtures of cells and heterogeneity in the materials produced. Appropriately designed, labeling methods can provide an additional advantage by supporting correlations between characterization techniques. For example, attachment of ^{19}F-containing fluorescence probes can facilitate correlation of distance measurements made by solid-state NMR relaxation methods with fluorophore localization given by optical and electron microscopies, thus covering three orders of magnitude in distance or greater (Sakamoto et al. 2018; Tanaka et al. 2011), and potentially also can provide redox-state sensitive switching of fluorescence responses (Tanaka et al. 2009).

For biosynthesis of inorganic materials, relevant measurements include ion concentrations, pH, local concentrations of organic metabolites that may serve as co-substrates in the biosynthesis, and redox potential. Methods to measure these properties in small scale are needed, preferably being carried out under conditions containing living organisms. Combining approaches such as single-cell microelectrochemistry and laser scanning confocal microscopy also has future promise (Grime et al. 2008; Schulte and Schuhmann 2007).

5. Scientific Opportunities: Materials Needed and Why

This report identifies knowledge and technology gaps that, once addressed, would transform the ability to make new classes of genetically encoded materials. Emphasis has been given to three classes of inorganic biomaterials: (1) inorganic biominerals, (2) inorganic-organic hybrid biomaterials, and (3) cell-inorganic composites that include inorganic biomaterials and living cells. Making these molecules with biological systems holds promise to open an entirely new area of science at the interface of materials chemistry and synthetic biology. Several types of materials, from magnetic nanoparticles to lightweight, strong composites, are described as examples. The scientific opportunities for manufacturing renewable materials with biological systems can help build sustainable, national-scale capabilities, including strategies to make materials directly in a decentralized manner where they are needed.

Genome-Encoded Bioinorganic Materials for Enabling New Functional Materials

Workshop attendees envisioned that biodesigned systems could synthesize materials with new properties. In particular, natural biomaterials have several unique characteristics compared to fabricated or human-made materials. Natural biomaterials can grow themselves in place and, if they contain living cells, can self-repair (e.g., bone). Additionally, the hierarchical structure of biominerals gives rise to their multifunctionality. These design principles could, in theory, be co-opted to make new classes of materials with structures and functions that are currently unmatched by chemically synthesized materials. Efforts to monitor, interrogate, and understand the process of biomaterials synthesis in cells are expected to reveal how to diversify, evolve, and repurpose the cells to generate new classes of bioinorganic materials. The breadth of functions in these materials is limited only by the research community's collective imagination, but which types of materials might these be? And why should they be made? Below are several examples of these classes of materials (see Fig. 18, p. 40).

Functionalized Nanoparticles

Nanoparticles are an exciting class of materials that could be enabled by genome-encoded approaches (see Fig. 18a). Conducting, semiconducting, and magnetic nanoparticles are already used in a variety of energy, health, and entertainment applications [e.g., energy-efficient light-emitting diodes (LEDs)]. However, current production approaches present several challenges. First, these nanomaterials typically are synthesized at high temperature, at high pressure, and in organic solvents with reagents and capping ligands that are often toxic or hazardous for the environment (Duan et al. 2015). Second, solubility constraints often require that nanoparticle surfaces are modified after their synthesis, possibly altering their functional properties. Third, materials of defined atomic sequence, exact monodisperse length, and programmed stereochemistry remain difficult to make using traditional chemical approaches. These challenges have hindered development of human-made materials with complex functional properties that rival the complexity of those made in biological systems.

Harnessing biological systems offers a new direction. For example, magnetotactic bacteria synthesize magnetic nanoparticles (single-domain magnetite) with control of shape and size that is equal to or exceeds state-of-the-art colloidal syntheses (Schüler 2008; see Fig. 18a, p. 40). These magnetic nanoparticles are chemically pure and stable single-domain magnets. Moreover, there is exquisite species-specific variation in geometry and subcellular arrangement arising from anisotropic control of crystal growth and elaborate manipulation of the supporting cytosolic architecture leading to multiple nanoparticle variations. Additionally, the biological membrane around magnetosomes is a natural interface that produces an

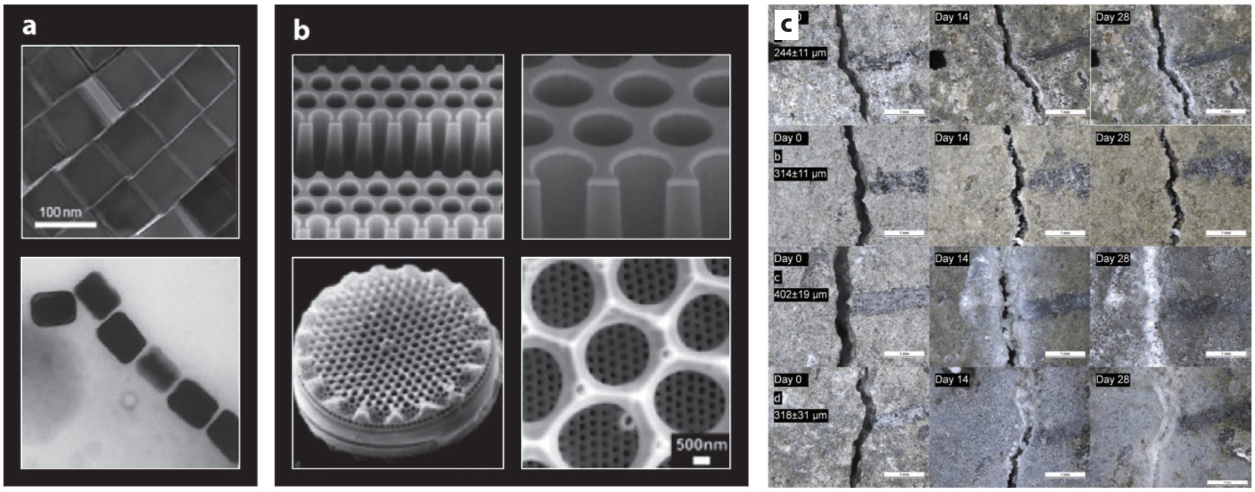

Fig. 18. Examples of Potential Genome-Encoded Materials. (a) Magnetite particle fabricated via (top) colloidal synthesis (Ling et al. 2015) and (bottom) magnetite particle in chain synthesized by magnetotactic bacteria (Yan et al. 2012). **(b)** Top down–fabricated photonic crystal (Mocella et al. 2009) and diatom with similar hierarchical porosity (Kröger 2007). **(c)** Self-healing concrete (Çağatay et al. 2016).

[Sources: **(a) Top image:** Reprinted with permission from Ling, D., et al. 2015. "Chemical Synthesis and Assembly of Uniformly Sized Iron Oxide Nanoparticles for Medical Applications," *Accounts of Chemical Research* **48**(5), 1276–85. Copyright 2015 American Chemical Society. **Bottom image:** Reprinted by permission from (1) Springer Nature: Schüler, D., and R. B. Frankel. 1999. "Bacterial Magnetosomes: Microbiology, Biomineralization and Biotechnological Applications," *Applied Microbiology and Biotechnology* **52**(4), 467–73. Copyright 1999; and from (2) Elsevier: Yan, L., et al. 2012. "Magnetotactic Bacteria, Magnetosomes and Their Application," *Microbiological Research* **167**(9), 507-19. Copyright 2012. **(b) Top images:** Reprinted with permission from Mocella, V., et al, 2009. "Self-Collimation of Light Over Millimeter-Scale Distance in a Quasi-Zero-Average-Index Metamaterial," *Physical Review Letters* **102**(13), 133902. Copyright 2009 by the American Physical Society. **Bottom images:** Reprinted with permission from Elsevier from Kröger, N., 2007. "Prescribing Diatom Morphology: Toward Genetic Engineering of Biological Nanomaterials," *Current Opinion in Chemical Biology* **11**(6), 662–69. Copyright 2007. **(c)** Reprinted with permission from Elsevier from Çağatay, Y., et al. 2016. "Enhanced Crack Closure Performance of Microbial Mortar Through Nitrate Reduction," *Cement and Concrete Composites* **70**, 159–70. Copyright 2016.]

ideal chamber for controlled growth of functionalized magnetic nanoparticles. As a result, biological production of these nanoparticles is an exciting opportunity, especially since the process is genetically controlled. Sulfate-reducing bacteria can provide biological routes to the production of nickel- and platinum-containing nanoparticles, which could be interesting targets for development (Capeness et al. 2015). Looking forward, the development of functionalized nanoparticles could lead to novel catalysts, new energy generation methods (e.g., by electromagnetic induction), and novel bioremediation or element extraction strategies.

Photonic Crystals and Metamaterials

These metamaterials, arising from designer biological structures, are another emerging opportunity for biomanufacturing. Photonic crystals and optical metamaterials serve as antireflection coatings, often with directional or wavelength selectivity, solar energy harvesters, and so-called invisibility cloaks. The functional properties of these 2D and 3D inorganic materials, fabricated by top-down lithographic methods, arise from periodic structures on the order of the wavelength of light. As with the described nanoparticles, however, current approaches to making such materials are often constrained by environmentally hazardous synthesis conditions and imperfections in the crystal patterns that impact functionality. The exquisite control provided by biological systems can address these constraints. For example, naturally occurring photonic crystals, such as opals and some butterfly wings, have unmatched molecular precision. Moreover, diatoms and stromatolites make periodic structures that resemble photonic crystals (see Fig. 18b, this page). Although outstanding challenges remain, such as the fact that the optical properties of diatom materials (e.g., $CaCO_3$ or SiO_2) do not have the transparency or refractive indices in the relevant wavelength range required to make photonic crystals or metamaterials, opportunities exist to harness biosynthesis of new photonic crystals and metamaterials.

Self-Healing Cell-Inorganic Composites

These composites offer a third category of genome-encoded materials with powerful new applications. Key among these is the ability to design and construct materials that regenerate or self-heal in response to damage. These composites could be impregnated or coated with an organism that would detect damage, for example, by sensing changes in light, oxygen, or moisture, which then would trigger re-synthesis or repair of that material while in place. A recent example that has captured the imagination of scientists and the public alike is the development of self-healing concrete (see Fig. 18c, p. 40). In this work, several strains of bacteria have been developed to induce the precipitation of calcium carbonate when sufficient nutrients and calcium sources are provided to self-heal cracks in concrete.

Lightweight, Strong Composite Materials

These materials, such as biominerals, are exceedingly strong for their weight. Examples include nacre or bone. Engineered composites that mimic this combination could be used to reduce the weight of automobiles and airplanes, in turn, saving energy. If these lightweight, strong biomaterials can be synthesized using biological systems that operate at near room temperature and pressure, they also might be produced using less energy than traditional materials and without the need for critical metals.

Ion-Specific Chelators, Transporters, and Carrier Proteins

Incorporation of a broader suite of elements into biologically synthesized, inorganic material systems will require new options for their extraction, storage, and trafficking. Genetically encoded chelators, transporters, and carrier proteins that achieve reversible uptake and specific delivery properties will be useful in supporting engineering pathways to produce inorganic biomaterials.

Novel Classes of Sequence-Defined Polymers for Inorganic-Organic Hybrid Materials

Polymers synthesized by biological polymerases could open new opportunities for making inorganic-organic hybrid materials, especially those that arise from hierarchical assembly. For example, in nature peptidoglycan is an essential, hierarchical polymer. This multifunctional material is built from sugars joined together into parallel chains, which are further cross-linked with polypeptides to build a complex and highly functional network. Building on this theme, hierarchical assemblies of biologically synthesized polymers may be used to support the synthesis of inorganic-organic hybrid materials. Design efforts may lead to incorporation of unique, complex, and highly useful properties such as biomechanical and catalytic properties, as well as selectivity for incorporation of inorganic elements and self-assembly arising from the control of surface characteristics. At each of multiple length scales is also the possibility of varying surface properties to be positively or negatively charged, hydrophilic, hydrophobic, or any combination of these traits. Taken together, efforts to make new sequence-controlled polymer scaffolds could enable new classes of genetically encoded biomaterials that go beyond the chemistry of living systems for a variety of applications.

Genome-Encoded Synthesis of Inorganic Materials for Enabling More Sustainable and Decentralized Manufacturing

Biological synthesis of inorganic materials will lead to new properties and applications and also has the potential to enable more sustainable and decentralized manufacturing practices. As the world population grows to an estimated 10 billion people by 2050, the demand for materials is also expected to grow by 50% to 100% relative to 2006 levels (Allwood and Cullen 2009), especially those materials that can be sourced sustainably. This increased demand will occur in the face of decreasing availability of raw materials such as phosphorus (Vaccari and Strigul 2011), rare earth elements (Zhou et al. 2017), and copper (Kerr 2014), as well as the increasing pressure to use less energy–intensive approaches. Additionally, the United States is facing an infrastructure crisis that will require an estimated $3.6 trillion investment that includes repair of bridges, dams, levees, and roads (ASCE 2013). Investing in the basic science of genome-encoded inorganic materials synthesis can help address these national-scale challenges by building the capabilities to make materials in a more sustainable manner and to make multifunctional materials that are not currently accessible via state-of-the art chemical synthesis or materials science fabrication methods.

Another benefit of biologically based materials syntheses is that biological systems offer the ultimate decentralized manufacturing capability, potentially enabling new opportunities in deployable materials. Unlike plastics or other human-made materials, biomaterials are unique in that molds generally are not needed to produce the final structure; the structure information is carried in the DNA sequence of the producing organism. Imagine being able to encode all the needed information for making any given biomaterial (natural or unnatural) in an easily transportable and deployable manner. Workshop participants discussed how—through use of microbes, fungi, plants, or even cell-free biosystems—all the needed information for making a biomaterial can be contained and then introduced into the right conditions for materials synthesis in the appropriate place. Traditional manufacturing and transportation of precursors to their desired location can take weeks to months, jeopardizing the timely delivery of needed supplies. Furthermore, preparing materials in advance of an anticipated need can result in wasted energy, labor, and money when that need is not realized. By moving manufacturing from the factory to locations where materials are needed, the need for stockpiling and complex supply-chain logistics can be redefined. As an added bonus, organisms, or cell-free systems, engineered to produce deployable biomaterials could be valuable in areas where resources are constrained or are not abundant.

6. Summary and Conclusions

Although there is increasing awareness that living organisms can extract, accumulate, and assemble inorganic elements into a myriad of intricate and potentially valuable biomaterials, additional systematic work is needed to fully understand the natural breadth of capabilities to biosynthesize inorganic materials.

To start, strategies are needed to access a fuller taxonomy of species capable of producing biominerals and an expansion of the catalog of genes and regulatory networks used to form biominerals. Further needs will require considerable effort to develop genomic, molecular, functional, and structural tools appropriate to the experimental challenges inherent in defining genetic, transcriptional, compartmental, and catalytic mechanisms for assembling renewable inorganic biomaterials.

The three classes of inorganic biomaterials and their compelling examples described in this report represent an organizational framework. An overview presents distinct opportunities and significant challenges inherent in advancing the understanding of each (see Figs. 4, p. 6; 14, p. 22; and 17, p. 33).

The simplest model for an inorganic biomaterial is plausibly a biomineral formed inside an individual microbial cell, such as magnetite formed in the magnetosome, or calcium oxalate formed in a plant idioblast. This model is attractive because the entire process is encoded in a single genome and the biosynthesis can take place within a single organism.

This apparent simplicity belies a more complex reality, however, and represents only a minimal outline of the complex network in operation: (1) distinct genetically encoded systems for uptake of all necessary precursors and their intracellular trafficking, (2) creation of specialized compartments to provide storage or function as more specialized crystallization chambers, (3) concentration of precursors in these chambers, (4) control of the redox state of transition metals, (5) prevention of elemental toxicity, (6) control of coordination chemistry to promote ligand exchange versus thermodynamic stability, and (7) coordination of the genetic regulation of the proteins and enzymes participating in each of these distinct biological systems. This complexity needs to be defined, modeled, and then engineered to ultimately achieve full control of a biomineral synthesis. This type of biosynthesis happens inside a cell, and, though the dimensions of products obtained will likely be restricted to the nanometer scale, they may have great utility when coupled to high compositional and structural specificity.

A second model for an inorganic biomaterial is an inorganic-organic hybrid such as the elaborate silica-based frustules built inside diatoms and then extruded to the outside. Beyond the complex biological network described above for internal biomineral formation, extracellular biosynthesis also must overcome constraints imposed by the external environment. One way that living organisms achieve control of external biosynthesis is by use of molecular scaffolds provided by proteins or polysaccharides. These scaffolds have a profound influence on the shape and quality of extracellular structures. The promise of being able to genetically manipulate those scaffolds is obvious, but the full details of the diversity, structure, and function of potential scaffolds and how they might be manipulated to yield new inorganic materials in dimensions of micrometers (and perhaps larger) have not yet been established.

A third model for an inorganic biomaterial is a composite of an inorganic material and living cells. This model extends the complexity presented by the diatom frustule by recognizing the advantages provided by a heterogeneous, self-replicating biomaterial produced by either single cells or a community. Production of mammalian bone represents an unparalleled example of the relationship between an inorganic biomaterial and several supporting cell types, with valuable properties of strength relative to weight, ability to self-repair, and provision of a mobilizable pool of valuable inorganic elements. These high-order capabilities are hallmarks of living organisms and have not been successfully replicated to date in a test tube. The combination of genomic information and synthetic biology approaches to create new types of chassis organisms that can produce inorganic biomaterials not observed in nature provides a great challenge with immense future promise.

Biosynthesis of inorganic materials in each of these classes is strongly dependent on the ability of living organisms to selectively extract inorganic elements from the environment, traffic them to appropriate cellular locations, and, finally, exquisitely direct their assembly into a myriad of materials, either inside or outside the cell. The expanding knowledge of genetic diversity arising from high-throughput genome and metagenome sequencing is demonstrating the likelihood that organisms having a breadth of capabilities to produce inorganic biomaterials may already exist in nature. Study of their most compelling capabilities can provide new basic insights. Also needed is the application of omic technologies and precise genetic manipulations in nonstandard organisms to support effective engineering and redesign efforts.

High-throughput or massively parallel determinations of the function of biosynthetic pathways and the structures of inorganic biomaterials and biocatalysts will be essential to advance this research. Comprehensive understanding of composite and hybrid biomaterials, which often have attractive physical, optical, and electromagnetic properties, will require atomic- and molecular-level definition of their ultrastructure, chemical composition, and bonding, both within a single material and across biologically synthesized interfaces between materials. The re-deployment of existing technologies or creation of new ones will be needed to carry out functional assays that can overcome the anticipated low concentration of substrates and the expected insolubility of polymerized inorganic products. Technology development efforts will have to address a lack of optical, fluorescence, or other spectroscopic signals in both substrates and products to achieve high-throughput or massively parallel assessments of function. These signals will be needed to identify new organisms, measure differences in functional properties among many gene variants, and carry out effective pathway engineering.

Potential translational outcomes from systematic efforts to understand and extend the biological synthesis of inorganic materials may include improved sustainability and atomic specificity of magnetic, conducting, semiconducting, and optically active nanoparticles; assembly of lightweight, strong, and perhaps functionally differentiated composite materials; and the creation of self-replicating cell-inorganic composites, which may provide new levels of specificity, functionality, and versatility, while also providing a pathway to increased dimension and amount of materials that can be made in a sustainable way.

Appendix 1. Workshop Agenda

Genome Engineering for Materials Synthesis

October 9–11, 2018
Rockville, Maryland

Tuesday, October 9

7:30 a.m. – 8:00 a.m.	Breakfast
8:00 a.m. – 8:30 a.m.	Welcome, introductions, and overview, Dr. Todd Anderson, DOE Office of Biological and Environmental Research
8:30 a.m. – 9:15 a.m.	"*GEMS: Potential Scientific Opportunities*" presentation and agenda outline for Day 1, workshop co-chairs
9:15 a.m. – 9:45 a.m.	Science presentation: "*Biomineralization of Nacre and Sea Urchin Spicules*," Dr. P.U.P.A. Gilbert, University of Wisconsin, Madison
9:45 a.m. – 11:45 a.m.	**Breakout Session 1: Designer Inorganic Materials**
11:45 a.m. – 12:45 p.m.	Lunch break
12:45 p.m.– 1:15 p.m.	Breakout session report preparation
1:15 p.m. – 2:15 p.m.	Summary and discussion of Breakout Session 1(15 minutes for each group, 15 minutes Q&A)
2:15 p.m. – 2:30 p.m.	Coffee break
2:30 p.m. – 3:00 p.m.	Science presentation: "*Synthetic Biology with Protist Biominerals: The Diatom Paradigm*," Dr. Nils Kröger, University of Dresden (Germany)
3:00 p.m. – 3:30 p.m.	Science presentation: "*Towards Genetically Programmable Biocomposites with Controllable Architectures, Mechanical Properties, and Bio-Functionalities*," Dr. Claudia Schmidt-Dannert, University of Minnesota
3:30 p.m. – 5:30 p.m.	**Breakout Session 2: Designer Hybrid Soft-Hard Materials**
5:30 p.m. – 6:00 p.m.	Breakout session report preparation
6:00 p.m. – 7.00 p.m.	Summary and discussion of Breakout Session 2 (15 minutes for each group, 15 minutes Q&A)
7:00 p.m. – 7:30 p.m.	Group discussion on Topics 1 and 2
7:30 p.m.	Adjourn (dinner on your own)

Wednesday, October 10

8:00 a.m. – 8:30 a.m.	Breakfast
8:30 a.m. – 8:45 a.m.	Agenda outline for Day 2, topics, groups, and breakout schedule, workshop co-chairs
8:45 a.m. – 9:15 a.m.	Science presentation: "*Exploring and Exploiting Bacterial Compartments for Synthetic Biomineral Production*," Dr. Arash Komeili, University of California, Berkeley
9:15 a.m. – 9:30 a.m.	Coffee break
9:30 a.m. – 11:30 a.m.	**Breakout Session 3: Designer Cell-Inorganic Materials**
11:30 a.m. – 12:00 p.m.	Breakout session report preparation
12:00 p.m. – 1:00 p.m.	Lunch break

1:00 p.m. – 2.00 p.m.	Summary and discussion of Breakout Session 3 (15 minutes for each group, 15 minutes Q&A)
2:00 p.m. – 2.30 p.m.	Science presentation: "*Towards Material Farming: Where Plant Biology Meets Material Sciences,*" Filipe Natalio, Weizmann Institute of Science
2:30 p.m. – 3:00 p.m.	Science presentation: "*Next-Generation Synthetic Biology Tools,*" Huimin Zhao, University of Illinois, Urbana-Champaign
3:00 p.m. – 5:00 p.m.	**Breakout Session 4: Enabling Characterization Technologies**
5:00 p.m. – 5:30 p.m.	Breakout session report preparation
5:30 p.m. – 6:15 p.m.	Summary and discussion of Breakout Session 4 (15 minutes for each group, 15 minutes Q&A)
6:15 p.m. – 7:00 p.m.	Group discussion on Topics 3 and 4 (and overall)
7:00 p.m.	Adjourn (dinner on your own)

Thursday, October 11

Attendance by co-chairs and writing team only

8:00 a.m. – 8:30 a.m.	Breakfast
8:30 a.m. – 10:30 a.m.	Writing team organization and summaries
10:30 a.m. – 10:45 a.m.	Coffee break
10:45 a.m. – 12:30 p.m.	Working lunch
12:30 p.m. – 1:30 p.m.	Summary preparation, additional writing assignments
1:30 p.m.	Adjourn

Appendix 2. Breakout Session Assignments

Questions serving as general guides for breakout discussions:

1. What kinds of inorganic or inorganic-organic hybrid materials can be made now?
2. What other materials could you synthesize biologically?
3. Why would you want to do this? What for?
4. How would you do it? What would you need to do it?

Breakout Session 1: Designer Inorganic Materials

1.1 (Eisenhower)	Jay Keasling,* Sanat Kumar,* Derk Joester, Arash Komeili, Olga Ovchinnikova, Laurie Gower, Brian Fox
1.2 (Jackson)	Farren Isaacs,* Arpita Bose,* Filipe Natalio, Yasuo Yoshikuni, Lance Stewart, Nils Kröger, John Shanklin, Caroline Ajo-Franklin
1.3 (Monroe)	Huimin Zhao,* Claudia Schmidt-Dannert,* Wil Srubar, Philippe Noirot, P.U.P.A. Gilbert, Kevin Morey, Oleg Gang

Breakout Session 2: Designer Hybrid Soft-Hard Materials

2.1 (Eisenhower)	Derk Joester,* Laurie Gower,* Filipe Natalio, Yasuo Yoshikuni, Huimin Zhao, Claudia Schmidt-Dannert, Philippe Noirot, Farren Isaacs
2.2 (Jackson)	P.U.P.A. Gilbert,* Arash Komeili,* Oleg Gang, Sanat Kumar, Jay Keasling, Arpita Bose, Lance Stewart
2.3 (Monroe)	Brian Fox,* Nils Kröger,* Caroline Ajo-Franklin, Wil Srubar, John Shanklin, Kevin Morey, Olga Ovchinnikova

Breakout Session 3: Designer Cell-Inorganic Materials

3.1 (Eisenhower)	Filipe Natalio,* Wil Srubar,* Huimin Zhao, Sanat Kumar, Yasuo Yoshikuni, Nils Kröger, Olga Ovchinnikova
3.2 (Jackson)	Caroline Ajo-Franklin,* John Shanklin,* Arash Komeili, Oleg Gang, Jay Keasling, P.U.P.A. Gilbert, Arpita Bose, Laurie Gower
3.3 (Monroe)	Philippe Noirot,* Kevin Morey,* Brian Fox, Derk Joester, Lance Stewart, Claudia Schmidt-Dannert, Farren Isaacs

Breakout Session 4: Enabling Characterization Technologies

4.1 Instrumentation (Eisenhower)	Olga Ovchinnikova,* Oleg Gang,* Derk Joester, Arash Komeili, Filipe Natalio, Sanat Kumar, Claudia Schmidt-Dannert, P.U.P.A. Gilbert, Philippe Noirot, Kevin Morey, Caroline Ajo-Franklin
4.2 Biotools/Methodology (Jackson)	Yasuo Yoshikuni,* Lance Stewart,* Wil Srubar, Nils Kröger, Arpita Bose, Laurie Gower, Huimin Zhao, Jay Keasling, Brian Fox, John Shanklin, Farren Isaacs

* *Breakout session leads and note-takers*

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Appendix 5. Acronyms and Abbreviations

1D, 2D, 3D	one, two, three dimensional
ABC	ATP binding cassette
ACC	amorphous calcium carbonate
APT	atom probe tomography
BER	DOE Office of Biological and Environmental Research
BNICE	Biochemical Network Integrated Computational Explorer
BSSD	Biological Systems Science Division (BER)
CAGE	conjugative assembly genome engineering
CHAnGE	CRISPR/Cas9 and homology-directed-repair assisted genome-scale engineering
CRISPR	clustered regularly interspaced short palindromic repeats
CRISPR-Cas	targeted genome editing system using engineered nucleases (e.g., Cas9, dCas9)
DFT	density functional theory
DOE	U.S. Department of Energy
DOPA	L-3,4-dihydroxyphenylalanine amino acid
EPR	electron paramagnetic resonance
EPS	extracellular polymeric substances
FBA	flux balance analysis
FIB-SEM	focused ion beam-scanning electron microscopy
FVA	flux variability analysis
GRO	genomically recoded organism
HAT/HDAC	histone acetyltransferase and deacetylase
HDR	homology-directed repair
KRAB	Kruppel associated box
LCLS	Linac Coherent Light Source (at SLAC National Accelerator Laboratory)
MAGE	multiplexed automated genome engineering
MATLAB	matrix laboratory (multipurpose numerical computing environment; MATLAB is proprietary programming language)
MM	molecular mechanics
MOMA	minimization of metabolic adjustment
mRNA	messenger RNA
MX	macromolecular X-ray crystallography
Mxi1	MAX interacting protein 1
ncAA	noncanonical amino acid
NMR	nuclear magnetic resonance
PCA	principal component analysis
PEEM	photoelectron emission microscopy
PTM	post-translational modification

QM	quantum mechanics
QMMM	quantum mechanics and molecular mechanics
RNAseq	RNA sequencing
SAXS	small angle X-ray scattering
SEM	scanning electron microscopy
sgRNA	single guide RNA
SIMS	secondary ion mass spectrometry
SXTM	scanning transmission X-ray microscopy
TALEN	transcription activator-like effector nucleases
TEM	transmission electron microscopy
UV	ultraviolet
VPR	VP64-p65-Rta (VP64 = viral protein 64, Rta = R transactivator)
WAXS	wide angle X-ray scattering
XANES	X-ray absorption near edge structure spectroscopy
XAS	X-ray absorption spectroscopy
XFEL	X-ray free electron laser
ZFN	zinc finger nucleases

A G C G T C G C T G T T G A T A C A
C C G C A G C A C T G G A G G C G G
G T T T C C T T G G C C T C C A C C
A A G A T A G C C C T C G T G T T
T G C A C T T T C A G A A C A A
C T A G A A T C C T T C
T A C T A T G G A
A C G A C C T
C T T C C
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A