Biologically inspired far-from-equilibrium materials

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Traditional approaches to materials synthesis have largely relied on uniform, equilibrated phases leading to static "condensed-matter" structures (e.g., monolithic single crystals). Departures from these modes of materials design are pervasive in biology. From the folding of proteins to the reorganization of self-regulating cytoskeletal networks, biological materials reflect a major shift in emphasis from equilibrium thermodynamic regimes to out-of-equilibrium regimes. Here, equilibrium structures, determined by global free-energy minima, are replaced by highly structured dynamical states that are out of equilibrium, calling into question the utility of global thermodynamic energy minimization as a first-principles approach. Thus, the creation of new materials capable of performing life-like functions such as complex and cooperative processes, self-replication, and self-repair, will ultimately rely upon incorporating biological principles of spatiotemporal modes of self-assembly. Elucidating fundamental principles for the design of such out-of-equilibrium dynamic self-assembling materials systems is the focus of this issue of *MRS Bulletin*.

Introduction

Complex multifunctionality of materials is seldom achieved with monolithic single crystals. Many of the multifunctional materials found in nature are heterogeneous mixtures of widely disparate components, organized spatially from the nanometer to the micrometer scale and above, and temporally across vastly different time scales.[1,2] A central feature of these systems is that they are all far from equilibrium during their functioning, and can yield (useful, designed) byproducts that are intricately structured and stable (though structurally frozen) approaching equilibrium.

For achieving control over spatial organization, one of the most powerful biosynthetic tools is biomineralization, a stable useful (designed) byproduct of biological activity. Collectively, biomineralization represents a class of synthetic processes in which nucleation, growth, and final morphologies of an ordering component, often inorganic, are directed, constrained, and even frustrated by an intervening "template," typically an organized microphase of organic biomolecules. Some well-known examples are bone, cartilage, and shells.[3] Here, nanoscale (<10 nm) supramolecular assemblies of proteins and lipids template the organization of the inorganic phase (i.e., calcium carbonate, as lamellar aragonites at a much larger scale [<0.5 μm] in single, nanolaminated macroscopic structures). Past efforts focused on understanding the design and biological processing of these biominerals have led to a firm realization that structural hierarchy and kinetic pathways represent critical ingredients transforming otherwise simple materials such as calcium carbonate, silica, and polymers into complex ones displaying extraordinary properties, including strength, toughness, microporosity, and energy dissipation.[1,4]

The control of temporal organization in living systems is most frequently achieved through supramolecular self-assembly.[5] This endows the materials of life with the abilities to sense, respond, and adapt. Living systems generate this dynamic responsiveness by existing far from equilibrium, harnessing energy from their environment and accessing kinetic states in their free-energy landscapes, usually involving various cycles (e.g., the catalysis of adenosine triphosphate [ATP]). Structurally, they organize in modular and hierarchical fashions into a network of interacting functional modules or "interactomes." Different from today's synthetic material systems of interacting components, these networks reconfigure in response to external stimuli through simple recombinations and rearrangements of their constituent "modules" and subnetworks.[6,7]

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An extraordinary example of such temporally flexible, extended modular design is the class of signal transduction machineries. Here, input from an external signal is sensitively recognized by the cell, even from a noisy and chaotic background, and transduced intracellularly to generate a cascade of chemically specific interactions between signaling proteins inside the cell. Needless to say, no single biomolecule (nor any single functional module) is responsible for signal transduction, but rather, the function arises through interactions between tens or even hundreds of modules, such as cytoplasmic protein complexes, acting in concert and with redundancy. This modular organization has proved beneficial to the cell, allowing the properties of individual modules to be robust, insensitive to environmental (or genetic) changes, while at the same time enabling environmentally sensitive dynamic changes not by *de novo* synthesis of new proteins, but by simply modifying the intermodule networks and connections. Thus, "decision-making" living systems are intrinsically endowed with a combination of material flexibility (or deformability), adaptability, and evolvability in their structures on the one hand and highly specific, self-amplifying, and error-correcting capacity for function on the other.

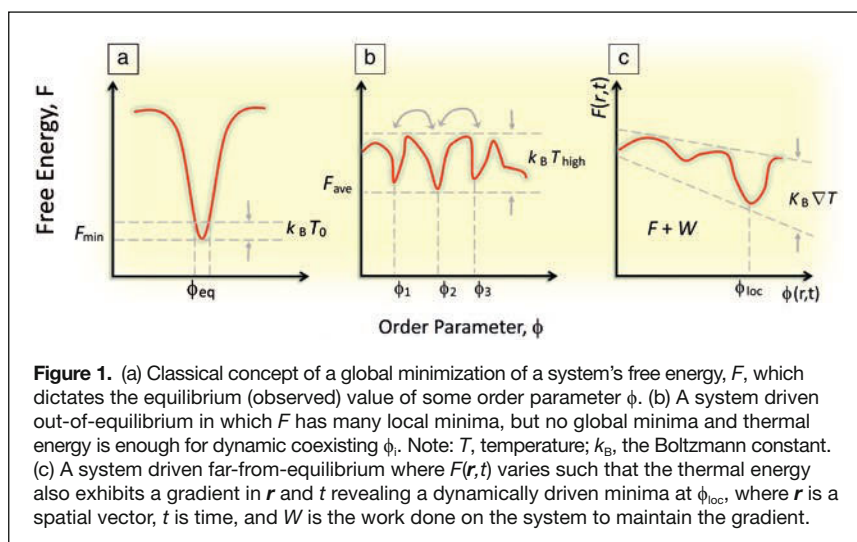## Far-from-equilibrium self-organization

From the vantage of materials science, far-from-equilibrium spatiotemporal control over self-organization during material syntheses challenges the traditional *modus operandi* in the synthesis of materials, which seeks to create equilibrated phases of consolidated matter—through preparation of uniform, equilibrated phases (e.g., monolithic single crystals) devoid of heterogeneities, interfaces, defects, and fluctuations—residing at the deep thermodynamic minima of their free energies and exhibiting long-term thermodynamic stability. In a sense, traditional materials science concerns the fabrication of materials that are "context-free" (having properties that are intrinsic to the material and independent of their boundaries or histories). Rather, the biological inspiration offers a rich new approach to "synthesis with design," that is, materials whose properties depends on their "context" (how and by how far are they driven from equilibrium). Implicit in this approach is a shift in emphasis from thermodynamics to kinetic regimes in which equilibrium structures (global energy minima) based on single length scales (e.g., unit-cell dimensions in single crystals) are replaced by higher-order, nonequilibrium organizational states (**Figure 1**). In terms of energetics, these organized states become accessible through three generic routes.[5]
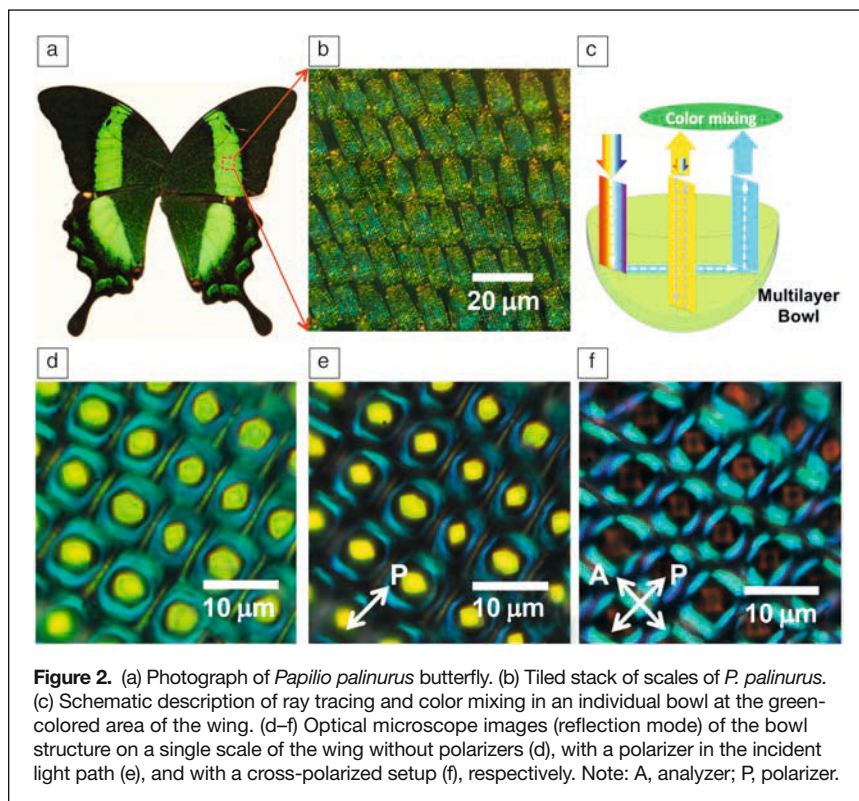
First, an assemblage of biomolecules comprising functional living systems exploits its compositional heterogeneity and fluctuations producing energy landscapes consisting of multiple local minima separated only by weak activation energy barriers (comparable to thermal fluctuations). These systems display temporal control of organization where the local context of the system biases the energy landscape in a feedback loop. That is, functional processes are driven by energy gradients that, in turn, help establish other energy gradients. These gradients are both temporal as well as spatial.

A particularly striking example can be found on the wing scales of butterflies,[8] where the intricate structures lead to stunning optical effects that have fascinated scientists for centuries since the time of R. Hooke[9] and Newton.[10] For example, the green color on the wings of the *Papilio palinurus* butterfly (**Figure 2**)[11] originates from the hierarchical microstructure of individual wing scales that are tiled on the wing. Each wing scale is about 100-μm long and composed of 4–10-μm-diameter bowls (Figure 2b–c), which in turn are lined with a multilayer stack of 11 alternating layers of air and chitin, each 75-nm thick. The distinct green color of the wings is the result of additive color mixing of yellow and blue, involving a combination of two reflections off the multilayer in the bowl; the yellow color is due to the reflection from the bottom of the bowl at normal incidence and the blue results from two 45° reflections at the edge of the bowl. The polarization of the ray reflecting from the edge is changed upon each reflection and thus the edge is visible in a reflection mode microscope under crossed polarizers (Figure 2f).

Significant advances have been made in understanding the developmental underpinnings that generate wing patterns over the past couple of decades. Such studies have primarily focused on the control of early pattern elements on the wing genes that control variation in color patterns between species, and mathematical or optics-based models of how some structural colors are produced. What is not understood in our current picture of wing patterning is the developmental process that gives rise to an individual wing scale cell, a necessary primary unit for butterfly wing color, its particular shape, and the exquisitely intricate structures that pattern the wing surface and modulate or create its individual (structural) color.

Figure 1. (a) Classical concept of a global minimization of a system's free energy, F, which dictates the equilibrium (observed) value of some order parameter ϕ. (b) A system driven out-of-equilibrium in which F has many local minima, but no global minima and thermal energy is enough for dynamic coexisting ϕ_i. Note: T, temperature; k_B, the Boltzmann constant. (c) A system driven far-from-equilibrium where $F(r,t)$ varies such that the thermal energy also exhibits a gradient in r and t revealing a dynamically driven minima at ϕ_{loc}, where r is a spatial vector, t is time, and W is the work done on the system to maintain the gradient.

Figure 2. (a) Photograph of *Papilio palinurus* butterfly. (b) Tiled stack of scales of *P. palinurus*. (c) Schematic description of ray tracing and color mixing in an individual bowl at the green-colored area of the wing. (d–f) Optical microscope images (reflection mode) of the bowl structure on a single scale of the wing without polarizers (d), with a polarizer in the incident light path (e), and with a cross-polarized setup (f), respectively. Note: A, analyzer; P, polarizer.

Dinwiddie et. al.[12] provide evidence that nonequilibrium processes or far-from-equilibrium processes lead to the formation of these intricate structures, which are eventually frozen to produce the beautiful optical effects. In the pupal stage of the scale development, each scale template produces a cytoplasm-filled extension surrounded by an active cell membrane extending out to the wing epithelium. It is also known that these begin as cylinders that become flattened during the growth phase. The authors utilized confocal microscopy to provide a detailed look at dynamic organization of F-actin (filamentous protein essential for cellular function) during the development of the scales of a butterfly. During the growth process, as the scale elongates, actin bundles become thicker and fewer in number. It should be noted that F-actin is a necessary requirement to elongate the butterfly wing scale cells, as well as for the development of the finger-like projections at the tip of the scale cells. The researchers also demonstrated that inhibiting the polymerization of actin can have disastrous effects on the development of the scale properties.

Second, spatiotemporal organization arises in many biomolecular (and nonbiomolecular) assemblies through a continuous consumption or flow of energy through the system. Called dissipative self-assembly,[13] these systems maintain ordered states as long as an external "fuel" is available. A striking example is a recent demonstration of how a simple cell-like compartment responds to sudden changes in its surroundings, such as a sudden drop in the concentration of dissolved molecules in the water that bathes the cell. This perturbation could result in a rapid flow of water into the cell through osmosis, causing it

to swell, rupture, and die. To avoid this catastrophic death, even single bacterial cells have evolved mechanosensitive channel proteins, which allow them to release excess water from the cell. But how might primitive protocells handle such as assault from the environment?

Recently, Oglęcka et al.[14] used giant lipid vesicles to investigate how a rudimentary cell-like compartment devoid of proteins and cytosolic components responds to a sudden drop in the amount of dissolved molecules (e.g., sugar) in water. Using a synthetic, protocell-like system, consisting of only three-component mixtures of lipids, they found that these vesicular compartments respond to the osmotic assault by reorganizing the molecules at the membrane boundary and by opening a pore, which allows it to release dissolved molecules and lose excess water from the interior, thus decreasing the pressure of the encapsulated cargo. The action of this "pressure-release valve" is not all or nothing. The valve (or hole) opens for less than a second, releasing some of the internal sugar several times, producing a pulsating, breathing pattern in the size and molecular texture of the vesicle (**Figure 3**).[15] This autonomous capability of simple vesicles to manage an external osmotic perturbation by a coordinated and cyclical sequence of physical mechanisms, which allow vesicles to sense (by lateral reorganization of membrane components and by domain formation) and regulate (by solute efflux) their local environment in a negative feedback loop, suggests a primitive form of a dissipative self-assembly in a synthetic material system (i.e., a simple microemulsion produced from just lipids, water, and sugar). From the vantage point of
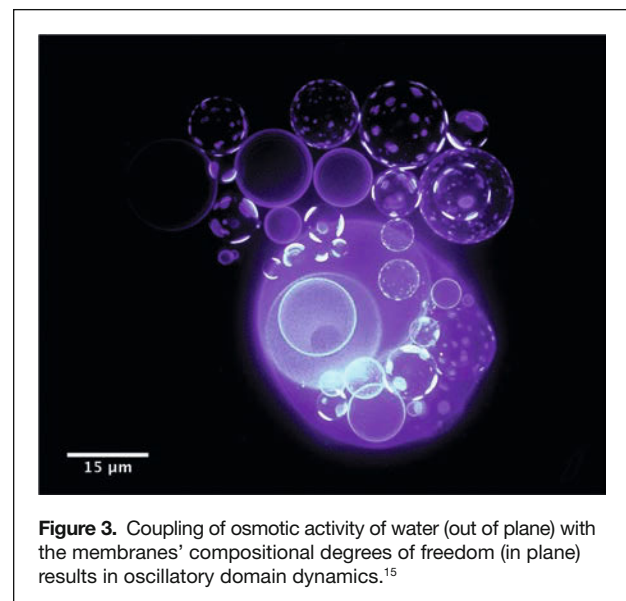

Figure 3. Coupling of osmotic activity of water (out of plane) with the membranes' compositional degrees of freedom (in plane) results in oscillatory domain dynamics.[15]

designing far-from-equilibrium materials, the findings suggest how chemical energy stored in concentration gradients might be controllably dissipated by cell-like compartmentalized morphologies to drive structural reorganizations, which can be exploited for new functions.

Third, a novel mode of material organization involves what is now called active organization.[16] Here, the "fuel" or energy flow used to access a higher-order organized state is neither external nor uniform. Instead, individual building blocks of the assemblages independently consume local chemical energy (e.g., ATP) resources (establishing internal energy gradients) achieving ordered states through widely disparate, and as yet incompletely understood, mechanisms. These systems are not perpetual, and ultimately, they rely on a net energy flow through the entire system.

An example of this third mode is the assembly and coordinated motion of individual living units such as birds, fish, bacteria, and humans, among others. (**Figure 4**).[17] Each member is a self-contained unit responding to simple signaling among the members that confer directed organized motion or activity to the whole. While these assemblies do require flow of energy through them overall, as in the second mode (each member has to eat), the activity is entirely driven by the use of internal energy and uses gradients to even dictate roles for members on the outward facing surfaces different from those in the interior. This mode has potential far-reaching implications in understanding the early evolution of single cell organisms into multicellular organisms with specialized functions distributed among its members. While it is clear that self-assembly is occurring, it is an open question how to describe the transition from a collection of individual automata to that of a single new organism.

In this issue
All of the articles in this issue provide, in one form or another, a take on how biology produces the amazing structures with

Figure 4. Example of flocking behavior of starlings in response to a threat to the group. Note the specialization of different groups of birds within the flock to respond to the predatory bird (seen in the upper center of the image).[17]

various functionalities that have garnered the attention of scientists starting with the studies of R. Hooke, who documented the intricacies of the structures found on the bodies of a variety of insects. Since that time, tremendous progress has been made in the understanding of the structures as well as their function, whether that was the intended purpose or not. In this issue, we solicited what we perceive to be at the cutting edge of the area of "bioinspired far-from-equilibrium materials."

When one searches for materials with improved properties or entirely new functionalities or properties, the process often involves an iteration that is designed to bridge the gap between the initial starting point and the desired target. In their article, Murugan and Jaeger[18] believe that this can be viewed as an optimization problem with a vast search space. However, they take a different tack and point to more recent strategies exploiting knowledge about the material configuration statistics as well as highlighting the advantages of considering time-varying environments, as is often the case in biological structures.

Wilts et al.[19] provide a fascinating view of the development of functional nanostructures, which are structurally frozen during development by out-of-equilibrium processes aided by actin filaments, as was documented by R. Hooke in his monograph *Micrographia*,[9] which dealt with the microstructure found on the wings of bodies of insects. The brilliant colors of many animals are a consequence of the physical interaction of light with the nanostructures that are precision-built utilizing biomaterials under ambient conditions. They note that the formation processes of nature use both elements of equilibrium self-assembly and of far-from-equilibrium and growth processes. In their article, they provide a toolbox of natural multifunctional nanostructures as well as current knowledge about the understanding of their far-from-equilibrium assembly processes.

Taking a slightly different point of view, Hess et al.[20] discuss the potential of molecular motors, which are motors that utilize chemical energy or light to perform work in a cyclic process, thus serving as central components for what are currently termed "active materials." It should be noted that chemists (Sauvage, Stoddart, and Feringa) were recognized with the 2016 Nobel Prize in Chemistry for their work with the citation "for the design and synthesis of molecular machines."[21] It should be no surprise then that nature has been there before man, as is often the case. In this article, the authors highlight the machinery that nature has evolved over more than a billion years, operating close to the fundamental limits with respect to efficiency and for generation per unit mass.

In a similar vein, the article by Ross[22] provides a broad overview of what she refers to as "autonomous materials from biomimicry," where she imagines materials or autonomous systems that can sense, compute, and react to external stimuli as well as being robust. Continuing with this theme, Zocchi[23] provides an intriguing look at phenomena occurring at various length scales from the nanometer scale to the mesoscopic scale—these length scales have specific definitions and we

prefer to leave it that way. In this case, Zocchi presents a nice tour of events from the sub-5-nm length scale to about 100 nm. He discusses processes in context, spanning molecules to neural networks, addressing basic science questions and in particular paying attention to dissipation at the atomic scale.

Finally, the article by Chatterjee and Iannacchione[24] addresses a well-studied system at macroscopic length scales, on the order of 20 cm with a thickness of about 7 mm. This system is the well-known, and well-studied Rayleigh–Bénard convection cell, where a thin liquid layer is subjected to a vertical temperature gradient, which results in the emergence of convection cells that are ordered. In this article, the authors explore how nonequilibrium fluctuations differ from equilibrium fluctuations.

Conclusion

Examples of synthetic materials fabricated deliberately using far-from-equilibrium synthetic strategies previously discussed are only beginning to emerge, suggesting the coming era of a new science of materials synthesis. We anticipate that understanding and exploiting nonequilibrium routes for materials synthesis will lead to new biologically motivated approaches for predictable and scalable syntheses with design, which will lead to advanced material functionalities such as self-sensing, self-healing and error-correcting, and self-replicating properties for advanced functions.

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