

## PERSPECTIVE

## Nano-enabled synthetic biology

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**Biological systems display a functional diversity, density and efficiency that make them a paradigm for synthetic systems. In natural systems, the cell is the elemental unit and efforts to emulate cells, their components, and organization have relied primarily on the use of bioorganic materials. Impressive advances have been made towards assembling simple genetic systems within cellular scale containers. These biological system assembly efforts are particularly instructive, as we gain command over the directed synthesis and assembly of synthetic nanoscale structures. Advances in nanoscale fabrication, assembly, and characterization are providing the tools and materials for characterizing and emulating the smallest scale features of biology. Further, they are revealing unique physical properties that emerge at the nanoscale. Realizing these properties in useful ways will require attention to the assembly of these nanoscale components. Attention to systems biology principles can lead to the practical development of nanoscale technologies with possible realization of synthetic systems with cell-like complexity. In turn, useful tools for interpreting biological complexity and for interfacing to biological processes will result.**

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## Introduction

Understanding the organizing principles of complex systems presents a significant challenge. Whether in the synthetic or biological domain, there is a growing awareness that design, the reiterative process of creating function through the intentional interconnection of components, is an indispensable tool for unraveling complexity. Whereas modeling, simulation, and experimental analyses have a tendency to

focus attention on the details of individual elements, design requires grappling with the trade-offs and compromises needed to enable system function. Along these lines, synthetic biology efforts follow a strategy of constructing deliberately simplified systems to comprehend molecular and cellular regulatory processes from the bottom up (Hasty *et al.*, 2002; Sprinzak and Elowitz, 2005; Andrianantoandro *et al.*, 2006; Guido *et al.*, 2006). Similarly, efforts towards constructing minimal cells either add, subtract or manipulate components to realize simple systems with desired capabilities (Forster and Church, 2006; Luisi *et al.*, 2006). In both cases, iterative design is a fundamental aspect of the approach and represents a major step towards true bottom-up construction of biological complexity. However, these approaches still depend on a platform of an existing cellular environment or the use of biomolecules (nucleic acids, proteins, lipids) to jump start cellular function. Thus, the question remains, what could be learned from a true bottom-up effort to reconstitute cell-like complexity?

Can the deliberate design and assembly of synthetic components lead to systems with cell-like characteristics? The large discrepancy between the functional density (i.e., the number of components or interconnection of components per unit volume) of cells and engineered systems highlights the inherent challenges posed by such a question. A simple example compares *Escherichia coli* (~2- $\mu\text{m}^2$  cross-sectional area) with an equivalent area on a silicon-integrated circuit (Simpson *et al.*, 2001). The *E. coli* cell has an ~4.6-million base-pair chromosome (the equivalent of a 9.2 megabit memory) that codes for as many as 4300 different polypeptides under the inducible control of several hundred different promoters, whereas the same space on a silicon chip could provide only a very small fraction of this memory or a few simple logic gates. Clearly, the operational scale of biological systems is significantly smaller than that of conventionally engineered systems. Beyond just density alone, it is also the drastically different approach to component assembly, interfacing, and organization that differentiates the biological from the synthetic nanoscale system. In the biological substrate, dynamic systems exploit weak interactions, arranged to provide desired specificity, and take place in a fluid environment. These features lead from simply high spatial density to high functional density and the realization of robust, adaptable systems.

As nanoscience and technology advance, the opportunity to match the scale of biological system components becomes feasible. As a first step, nanotechnology presents the ability to directly interface to the working levels of biology, leading to the emergence of new approaches to therapy and diagnostics. Additionally, the emulation of biological design principles using synthetic components becomes feasible. Potentially, as systems of such elements approach biological-scale functional density, they can begin to assume cell-like characteristics including: (1) construction from an inhomogeneous mixture of materials with different properties, modes and strengths of

interactions, and relative abundances; (2) the encoding of information within small populations (e.g., biomolecules or electrons); (3) function emerging from an environment with large stochastic fluctuations (a consequence of (2)); and (4) the efficient transduction of information, energy, and materials that emanates from the molecular scale. It is an intriguing possibility that, as our ability to control the synthesis and direct the assembly of synthetic nanoscale elements increases, we may attempt the bottom-up design and construction of nanosystems with cell-like complexity and capabilities. In turn, the design of such systems will lead to an enhanced ability to understand and interface to biological systems.

The intersection of nanoscale science and technology with biology has figured prominently in even the early stages of envisioning nanoscience research directions and goals (Roco, 2003). In many ways, the biological cell represents an ideal paradigm for nanoscale systems. Being the fundamental unit of biological systems, their function can be extremely diverse, yet uses only a finite, common set of building blocks. Cells operate under a wide range of environmental conditions with efficiencies unmatched by artificial systems. They can be highly specialized and carry out tens of thousands of chemical reactions in parallel. The dimensional characteristics of cells are well conserved and undoubtedly critical for system function (Welch, 1992; Hess and Mikhailov, 1994; Hochachka, 1999; Misteli, 2001; Harold, 2005). Short distances (nm– $\mu\text{m}$ ) enable intra- and intercellular communication using simple diffusion-based mechanisms. Also, the small fluid volume of a cell allows for small fluctuations in numbers of specific molecules to result in dramatic changes in cellular state. Higher order, nanoscale structuring (Welch, 1992; Hochachka, 1999) and excluded volume effects (Hall and Minton, 2003) are also known to be critical to cellular function. In fact, with regard to heredity, the spatial definition of the cell may be as important as the genetic material (Harold, 2005).

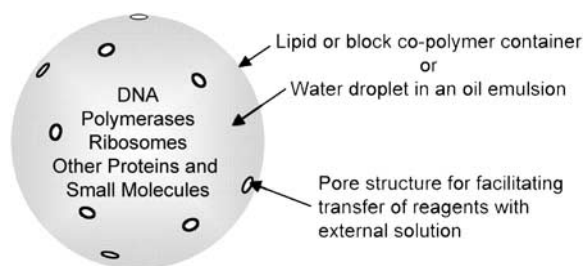
Here, we consider the potential for a nano-enabled synthetic biology that may be derived from the confluence of systems biology and nanoscale science and technology. At this confluence, systems biology provides knowledge of the chemical components that comprise the cell and the spatial and temporal interplay between these components. Initial efforts to mimic cells have followed a path of using soft materials that are similar or identical to cellular materials. However, the continued progress of nanoscale science and technology provides hope that many cellular attributes may be transferred to artificial systems through the control of the synthesis and assembly of hard nanoscale materials at the multiple size scales important to cellular function. In the process, advanced tools for understanding basic questions regarding biological function will be provided. Such developments could benefit both technology and science. Cell-like complexity in nanoscale systems may lead to significantly higher levels of function, whereas also forming an experimental system that would allow a much better examination of cellular organizational principles. Here, we highlight efforts to mimic cell-like systems and the emerging tools of nanoscience that may enable an even more synthetic biology.

## Mimicking cellular systems

The general concept of mimicking cells dates back several decades with the initiation of efforts to make effective blood substitutes (Chang, 2004). More recently, multiple efforts have evolved and are focused on engineering molecular systems and mimicking functional aspects of cells. Additionally, synthetic cell efforts are increasingly integrating synthetic materials and nanotechnologies. A common feature of cell mimic pursuits is containment of a small, aqueous volume. The ability to contain small volumes of liquid (pico- to nanoliter) is a critical aspect of biological cells and enabling for the creation of synthetic cell-like systems. Small volume containers obviate the need for mixing and establish local conditions that are favorable for protein function. Small volumes reduce the number of molecules needed for carrying out a function. Therefore, they are ideal for studying, or exploiting, reactions that involve single molecules. Further, small volume containers can be used for understanding molecular reaction systems and self organization at the cellular scale (Hess and Mikhailov, 1995; Marijuan, 1995; Chiu *et al.*, 1999; Misteli, 2001; Long *et al.*, 2005; Pielak, 2005). They are also valuable for studies related to understanding questions involving the origin of life (Deamer, 2005). On the applied side, miniaturization of the reaction volume can lead to the creation of massively parallel analytical systems (Wolcke and Ullmann, 2001; Heller, 2002; Khandurina and Guttman, 2002), whereas the *in vitro* aspects of the technology allow the use of physical conditions or the synthesis of products that may be toxic to natural cells. New approaches to high throughput screening, chemical sensing, and drug delivery are being enabled. The incorporation of synthetic nanomaterials will be key to realizing these diverse applications. The containing 'membrane' is a distinguishing feature of present approaches to mimic functional aspects of cells (Figure 1). Natural membrane components, synthetic polymers, emulsion systems, and microfabricated structures are being considered. An overview of these different systems is described below.

### Vesicle-based systems

The most widely studied biomimetic containment systems are based on vesicles prepared from amphiphilic molecules. These



**Figure 1** Overview of a genetic-based synthetic cell. The membrane of synthetic cells can be created from a variety of materials, including natural membrane components, synthetic polymers, and micro- or nano-fabricated materials. Alternatively, a water-in-oil emulsion is used to define the 'cell'. Typical cell volumes are on the order of picoliters to nanoliters and contain genetic material and appropriate transcription and translation machinery. The incorporation of pore structures into the membrane facilitates transfer of appropriate reagents.

self-assembling structures can be formed from lipids, creating liposomes, or from synthetic molecules such as block copolymers, which are often referred to as polymersomes (Vriezema *et al*, 2005). They are also considered to be ideal biomimetic nanoscale reaction containers (Karlsson *et al*, 2004). Liposomes have long been used to encapsulate enzymes and can be prepared using a variety of techniques (Walde and Ichikawa, 2001). Their potential application as delivery vehicles for therapeutics has garnered much attention. Liposomes can protect enzymes from degradation, effect slow release of a reagent, or contain chemical reactions. For example, enzymes entrapped in the interior of the liposome can be used for diagnostic applications (Ho *et al*, 1987), for metabolizing toxic reagents (Petrikovics *et al*, 1999), or as catalysts (Yoshimoto *et al*, 2003). Further, multi-component systems have been designed that allow for targeting and stimuli-dependent release of encapsulated reagents (Guo and Szoka, 2003).

Gene-based reactions systems are also being developed. Reactions involving nucleic acids and polymerases have been described (Walde and Ichikawa, 2001; Monnard, 2003). Further, simple genetic constructs, involving a promoter and gene sequence, and cell-free extract (Spirin *et al*, 1988; Shimizu *et al*, 2001) can be pooled in liposomes to produce the corresponding protein. The expression of green fluorescent protein enables easy assessment of the reaction (Yu *et al*, 2001; Oberholzer and Luisi, 2002; Nomura *et al*, 2003; Ishikawa *et al*, 2004; Sunami *et al*, 2006). More complex reactions have also been demonstrated. For example, Ishikawa *et al* (2004) demonstrated a two-stage genetic network, where the protein product of the first stage is necessary for driving protein synthesis of the second stage. Other multi-stage reaction systems have been described leading to the possibility of constructing cell-free genetic circuits (Noireaux *et al*, 2003; Noireaux and Libchaber, 2004).

The use of natural lipids facilitates biocompatibility, including the use of membrane proteins to facilitate material exchange with the enclosed volume. However, the long-term stability of these structures can be problematic. Nanotechnologies can effectively address this shortcoming. Related efforts have investigated the use of synthetic polymers to create polymersomes. Block copolymers are finding multiple applications in nanotechnology. These polymers are composed of at least two parts of differing solubility and can self assemble into a variety of structures (Forster and Antonietti, 1998; Klok and Lecommandoux, 2001; Park *et al*, 2003). They can also be formed into vesicles. Vesicles with a broad range of chemistries and physical properties that are based on the choice of polymer type, block ratio, and molecular weight, can be constructed (Discher and Eisenberg, 2002). As with liposomes, applications in chemical sensing, reagent delivery and reaction containment are pursued. For example, enzyme activity can be preserved when encapsulated within polymersomes and such systems can be used for sensing and can be made stimulus responsive (Napoli *et al*, 2004a, b). Facilitating the use of polymersomes for incorporation into biological systems is the discovery that natural membrane spanning proteins can incorporate into block copolymer shells. Nardin *et al* (2001) demonstrated that the *E. coli* porin protein OmpF can form a stable protein/polymer hybrid membrane and act as a size

selective filter. In this case, protein incorporation occurs even though the polymer membrane is two- to threefold thicker than a conventional lipid bilayer. Subsequent work has demonstrated the ability to use other block copolymer systems and incorporate other proteins (Ho *et al*, 2005; Ranquin *et al*, 2005). For example, Ho *et al* (2005) have incorporated the energy-transducing membrane proteins bacteriorhodopsin and cytochrome *c* oxidase into block copolymer vesicles. This system was shown to generate transmembrane pH gradients and highlights the potential use of hybrid nanosystems for harnessing the energy conversion processes of natural systems.

## Emulsion-defined systems

Small volume reaction containers can also be created by water in oil (w/o) emulsions (Tawfik and Griffiths, 1998; Griffiths and Tawfik, 2000, 2003; Ghadessy *et al*, 2001; Pietrini and Luisi, 2004). Enclosed, femtoliter scale volumes can be defined through simple shaking, stirring or extrusion of a mixture containing an aqueous solution, oil and appropriate surfactants to stabilize the emulsion. The typical size of the water droplets is on the order of a few microns, comparable to that of a microbial cell. Even smaller droplets can be prepared using ultrasonication (Musyanovych *et al*, 2005). The simplicity and ability to create  $\sim 10^{10}$  containers/ml volume has enabled a variety of applications. For example, complete genomic libraries can be created, amplified and characterized using w/o techniques (Margulies *et al*, 2005; Shendure *et al*, 2005). Alternatively, genetic variation within individual alleles can be quantified (Dressman *et al*, 2003). In these applications, the DNA is diluted such that, on average, a single template is contained within an aqueous droplet. These individual DNA strands can then be amplified by the polymerase chain reaction in separate aqueous volumes within a single tube.

A notable feature of the w/o emulsion technique is the ability to link genotype with phenotype in a small volume reactor. An *in vitro* compartmentalization system has been described that is useful for high-throughput screening and selection (Aharoni *et al*, 2005b). In this approach, a gene sequence, along with the appropriate reagents for transcription and translation, is contained within the aqueous compartment. As a large number of compartments can be created and tested simultaneously, entire libraries of genetic variants can be assessed. This enables 'directed evolution' of protein function by selection for the appropriate activity. For example, DNA polymerases (Ghadessy *et al*, 2001) and methyl transferases (Tawfik and Griffiths, 1998; Lee *et al*, 2002) have been selected for by simply breaking the emulsion and identifying the remaining gene sequences. Other approaches exploit a physical connection between the gene and its product (Doi and Yanagawa, 1999; Sepp *et al*, 2002; Griffiths and Tawfik, 2003) or novel approaches (Aharoni *et al*, 2005a; Mastrobattista *et al*, 2005) to allow for subsequent sorting.

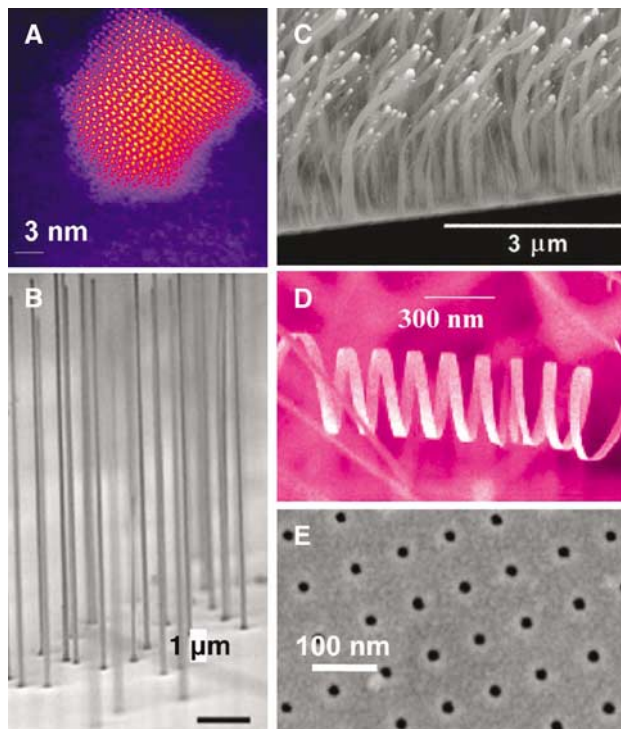
Enhancing the ability to transport reagents into and out of w/o emulsion-based reaction vessels is still under investigation. In general, reaction extent inside the vessel is limited by the availability of precursor reagents, as transport within the oil phase is unlikely. Some reagent exchange is believed to take place upon contact between individual compartments

(Ghadessy *et al*, 2001). Fusion of compartments is also a potential mechanism for exchanging or feeding reagents to reaction vessels (Bernath *et al*, 2004; Pietrini and Luisi, 2004). A related approach exploits microfluidics technology for creating aqueous droplets of defined size and composition (Dittrich *et al*, 2005). Specific reagents can be mixed and compartmentalized by the merging of microfluidic flow streams. This approach allows facile manipulation of the droplet and can be combined with sensitive detection techniques.

## Nanomaterials: from individual elements to cell-like complexity

As described above, most efforts to mimic cells have relied on the self-assembly properties of organic materials. However, many applications have benefited from small scale and high parallelism afforded by advanced microfabrication techniques, and these techniques have become better integrated with biological materials to enable greater functionality. One use of these fabrication techniques is for creating robust reaction containers of defined volume and contents. Various etching, drilling, embossing or molding techniques can be used to create containers of a range of sizes (z1– $\mu$ l). The integration of such structures with fluids and biological materials is enabling multiple applications. For example, small volume reaction containers are being considered for high-throughput screening, (Grosvenor *et al*, 2000; Angenendt *et al*, 2005), single molecule enzymology (Rondelez *et al*, 2005; Rissin and Walt, 2006), and analyses of single cells (Cooper, 1999; Johannessen *et al*, 2002). These reaction containers are also being developed for the cell-free synthesis of proteins (Nojima *et al*, 2000; Tabuchi *et al*, 2002; Yamamoto *et al*, 2002; Angenendt *et al*, 2004; Kinpara *et al*, 2004; Mei *et al*, 2005). In addition to creating a new approach to the parallel production of various proteins, these structures are permitting novel functional assays (Angenendt *et al*, 2005; Mei *et al*, 2005). The use of microfabricated structures allows for the controllable exchange and mixing of reagents (Nojima *et al*, 2000; Wang *et al*, 2005) and the integration of sensitive techniques for sampling and analysis of reaction products.

However, the use of microfabrication technology to achieve or interface to cell-like complexity is ultimately limited by the shortcomings of top-down synthesis processes that require layer-by-layer definition of structure through a very well-controlled series of deposition, lithography, and etching steps. Instead, synthetic systems must exploit characteristics similar to natural components, and nanoscale materials are especially suited to this challenge as they reside on the same size scale as the components of biological processes, whereas exhibiting electrical, magnetic, optical, thermal, and chemical properties conducive to the construction of complex networks of functional parts. By definition, nanoscale materials have a limited extent (nominally defined as less than 100 nm) in at least one of the three spatial dimensions. Zero-dimensional (0-D) materials include semiconductor quantum dots (QD), colloidal metal particles, and atomic or molecular clusters that are confined in all three spatial dimensions; 1-D structures (which we refer to collectively as nanowires) are confined in



**Figure 2** Collage of synthetic nanoscale materials. (A) 0-d nanoscale material shown in a Z-STEM image of 655 Qdots™ (CdSe/CdS core/shell, Quantum Dot Corporation; image courtesy of Professor S Rosenthal, Vanderbilt University (adapted with permission from McBride *et al*, 2006)). (B–D) 1-d nanoscale materials: (B) silicon nanowires grown on a silicon wafer (image courtesy of Professor P Yang, University of California, Berkeley (adapted with permission from Goldberger *et al*, 2006)); (C) carbon nanofibers with orientation controlled by an electrical field that was switched during the growth process from 90 to 45° with respect to the plane of the substrate (Merkulov *et al*, 2002c); (D) ZnO nanospring resulting from a polar surface-induced growth phenomena (Image courtesy Professor ZL Wang, Georgia Tech (adapted with permission from Kong and Wang, 2003)). (E) A 50-nm thick silicon nitride membrane (2-d material) with a regular array of 25-nm diameter holes (image courtesy of Dr HD Tong, Nanosens, and Dr HV Jansen, University of Twente (adapted with permission from Tong *et al*, 2004)).

two spatial dimensions; and 2-D structures (thin films such as silicon nitride or lipid bilayer membranes) are confined in only one spatial dimension (Figure 2).

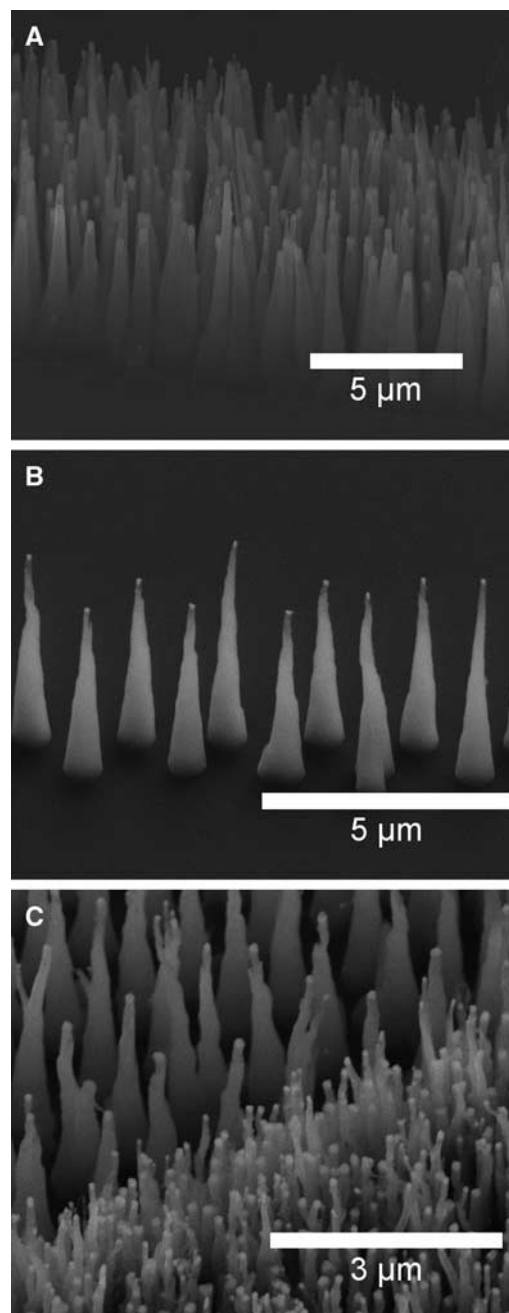
Much of the early effort in nanoscience has focused on the synthesis and characterization of individual or homogeneous arrays of nanoscale elements, and numerous techniques have been developed for the synthesis of a variety of nanoscale materials: QDs composed of periodic groups II–VI (e.g., CdSe) or III–V (e.g., InP) materials are synthesized by injecting liquid precursors into hot (300°C) coordinating organic solvent (Murray *et al*, 1993; Peng *et al*, 1998); semiconductor 1-d nanowires can be grown in a vapor-liquid-solid process (Wagner and Ellis, 1964) in which a liquid metal cluster or catalyst acts as the energetically favored site for absorption of gas-phase reactants; and carbon nanowires, which include carbon nanotubes (Ajayan and Ebbesen, 1997) and carbon nanofibers (CNFs) (Rodriguez, 1993; Melechko *et al*, 2005), are synthesized in numerous processes including laser-vaporization (Kroto *et al*, 1985), arc discharge (Kratschmer *et al*, 1990; Iijima, 1991), catalytic chemical vapor deposition, and catalytic plasma-enhanced chemical vapor deposition (C-PECVD).

Although these materials synthesis efforts have been foundational for nanoscale science, taken alone they do not provide the means to construct or interface to cell-like complexity. It is the collective behaviors of interacting nanoscale components, where scale and complexity lead to 'entirely new properties' (Anderson, 1972). The question then is not only of the synthesis of nanomaterials, but also that of how these materials should be organized into ensembles that exhibit new levels of functionality. Addressing this question shifts the focus from synthesis of individual elements to the controlled synthesis and directed assembly of systems of nanoscale components that are capable of assuming cell-like organization. By controlled synthesis, we mean a process of mass nanostructure growth, where the pertinent attributes (location, size, orientation, composition, electrical, mechanical, and thermal properties, etc.) of the individual elements can be selected *a priori* by the choice of the growth conditions or the preparation of the growth substrate. Much like biological materials (e.g., silica biomineralization (Morse, 1999; Hildebrand, 2003; Hildebrand *et al.*, 2006)), or embryogenesis (Carroll *et al.*, 2004)), directed assembly in this context may more appropriately be thought of as hierarchical assembly, as each stage in the process forms the template for the next layer of added complexity. We illustrate these concepts in a synthetic system using the example of CNFs below. In this example, we emphasize the use of self-organization, hierarchical assembly, and the emergence of functional order from stochastic processes.

### Controlled synthesis and hierarchical assembly

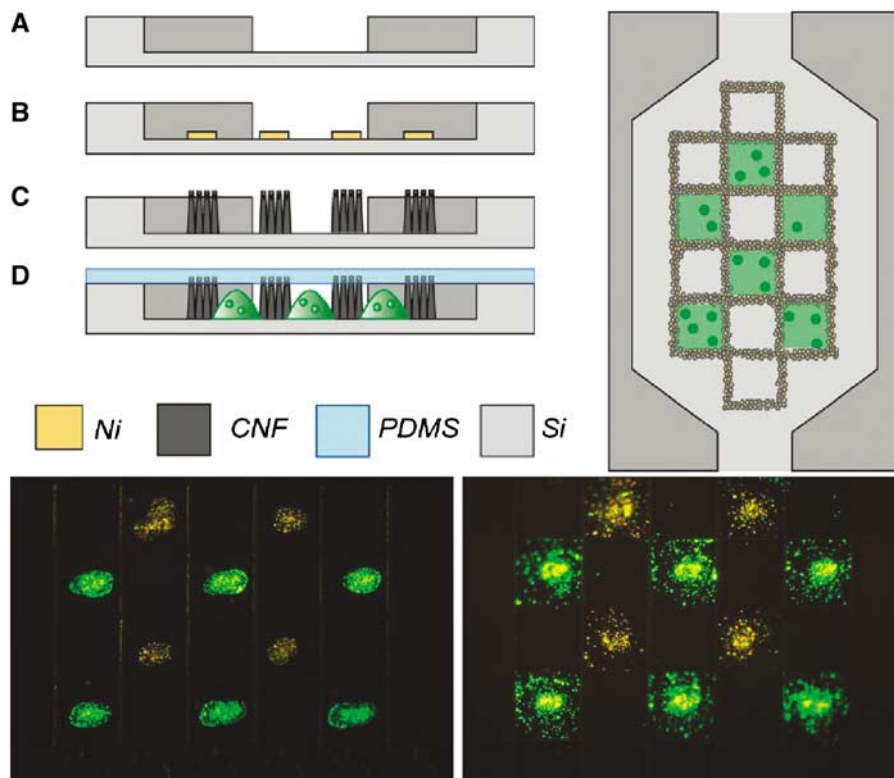
Carbon nanofibers are grown in a PE-CVD process from metal catalyst materials supported on substrates of various types (Melechko *et al.*, 2005). The complex plasma environment can be manipulated to produce changes in nanofiber aspect ratio, diameter, orientation, shape, and chemical composition (Merkulov *et al.*, 2001, 2002a,b,c; Melechko *et al.*, 2002). Likewise, CNF morphology, crystalline structure, and composition can be varied through manipulation of the growth substrate (Klein *et al.*, 2005; Fowlkes *et al.*, 2006b). For example, the patterning of the catalyst material allows the selection of either randomly spaced forests or deterministically placed isolated fibers (Figure 3); the selection of the plasma source gases control nanofiber composition; and nanofiber shape can be controlled by selection of the catalyst material crystallographic orientation (Fowlkes *et al.*, 2006b). The forest morphology is particularly interesting, as it is the result of a self-organization process initiated by the plasma-induced fragmentation and reordering of the initial microscale catalyst pattern followed by nanofiber growth from each of the nanoparticles. Although the placement of individual nanofibers exhibits a high degree of stochasticity, the distributions of interfiber spacing and fiber size are strongly influenced by the choice of catalyst material, thickness, and crystallographic orientation.

A concurrent self-organization is the emergence of nanoscale pores that form between the randomly spaced nanofibers. These structures can act as passive membrane mimics in microfluidic devices (Zhang *et al.*, 2002; Fletcher *et al.*, 2004). Whereas the pores are relatively large (e.g., 150–250 nm) in as



**Figure 3** Micrographs of carbon nanofibers. (A) Vertically aligned carbon nanofibers can be prepared from a continuous catalyst stripe yielding an array of CNFs that are randomly arranged. (B) Individual CNFs can be precisely positioned using electron beam lithography to define catalyst sites for growth of individual nanofibers (Melechko *et al.*, 2005). (C) These patterning techniques can be combined on a single substrate to yield both ordered and disorder arrays of CNFs.

grown forests, hierarchical processing that adds to the diameters of the nanofibers (e.g., conformal SiO<sub>2</sub> coating or electroplating of conductive polymers) leads to the formation of planar or 3-D pore networks (Fowlkes *et al.*, 2005). Within these forest structures, diffusive transport is a strong function of the excluded volume (i.e., space taken up by the nanofibers) and the placement of nanofibers. The stochastic nature of the



**Figure 4** Fabrication of a cell mimic array. The device is created using (A) contact photolithography and ICP-RIE etching of silicon to define a fluidic channel approximately 10  $\mu\text{m}$  in depth. (B, C) Metal lift-off of Ni catalyst is followed by PECVD growth of CNF forests that rise slightly above the channel ceiling. (D) Structures are sealed with a PDMS lid, following selective ink-jet introduction of reagents (15  $\mu\text{l}$ ) into mimic cells, as diagrammed in the upper right and seen in the fluorescent micrograph at bottom left. The image at bottom right shows the structure after sealing and wetting of the device. The materials are contained, but fluid diffuses between the chambers (Fletcher *et al.*, 2004).

interactions between diffusing molecules and nanofibers leads to regions of anomalous diffusion (i.e., time-variant diffusion coefficient), whereas a large excluded volume leads to significantly reduced molecular mobility (Fowlkes *et al.*, 2006a). The net effect is that the diffusive transport properties of these membrane mimics may be controlled through self-organization of stochastic nanofiber forests and hierarchical processing to define the structure of a nanoporous network.

These membrane structures can be patterned in arbitrary shapes and used for creating cell mimic structures (Fletcher *et al.*, 2004; Fowlkes *et al.*, 2005). As shown in Figure 4, advanced, multiscale (nano to macro) fabrication techniques allow for the integration of nanomaterials, fluids, and biological reagents to create structures that mimic functional characteristics of a cell. In this example, the enclosed volume can be tuned to closely match those of a natural cell. Such a container would be useful for experimentally characterizing reaction systems and material organization in a contained fluid environment. Further, this structure allows for controlled transport between the contained volume and the local environment through design of the membrane properties.

These structures can advance from being simple, passive structures that control transport based on physical size to more sophisticated, active nanostructures. For example, additional control of the membrane properties is possible through the application of chemical coatings (Fowlkes *et al.*, 2005; Fletcher *et al.*, 2006; McKnight *et al.*, 2006). Such coatings can bestow

chemical specificity in addition to size-selective transport. Polymeric coatings on the CNFs can be exploited to create active interfaces. Polypyrrole can be selectively patterned onto CNF-based electrodes (Chen *et al.*, 2001; Nguyen-Vu *et al.*, 2006; Fletcher *et al.*, 2007). Such coatings can be reversibly actuated to expand or contract with the application of an electrical signal (Smela, 2003). Other polymers are responsive to chemical or physical stimuli (Gil and Hudson, 2004; Yoshida *et al.*, 2006).

## Realizing a nano-enabled synthetic biology

Nano-enabled synthetic biology is in its early stages. For biological systems, functionality at any scale begins at the cellular level. The efforts described above illustrate the different approaches involved in attempting the bottom-up construction of simple cellular-like structures. Nanotechnologies are becoming increasingly involved. Nanoscale science has delivered the ability to synthesize a variety of nanoscale components that provide the means to dramatically increase the density of elements. Controlled synthesis and hierarchical assembly allow these components to mimic passive, and even active, cell-like behaviors. As described, carbon-based nanostructures and block co-polymers match many of the design requirements of synthetic membranes. Future efforts will

likely see the integration of other nanomaterials for potentially transducing energy, conveying signals, or controlling the arrangement of biological and synthetic structures.

Understanding and improving the interface between natural and synthetic structures represents a key next step for nano-enabled synthetic biology. Related challenges in controlling synthesis across the multiple length scales relevant to biology and in the development of tools that are useful in characterizing interactions at these scales also need continued attention. Effective emulation, interpretation, or control of biomolecular events will depend on this interface. As stated at the beginning of this article, it is both scale and complexity (interconnectivity of the elements) that lead to higher levels of functionality, and further progress hinges on increases in the latter. Perhaps paradoxically, increasing complexity requires a renewed, albeit redirected, focus on synthesis. Nanoscale materials have been pursued with an eye on unique properties that emerge usually due to electron confinement, the increased ratio of surface area to volume, or the physical properties that result from precise molecular arrangements. Increased attention towards control of surface properties to allow site-specific functionalization of nanomaterials will be required. The binding affinity between natural and synthetic structures will need to be carefully prescribed. Nanoelements will need to be multifunctional, possibly requiring a mix of soft/hard material functionality and hybrid synthesis techniques that are not yet commonplace. Synthetic nanostructures will need to become active participants in the feedback mechanisms of biological networks for effective interfacing.

Ultimately, the development of nanotechnology-based tools will enable hybrid systems that will substantially enhance the synthetic biology toolbox. Practical biomedical devices will also result. Of course, interfacing to biological systems is not a requirement for systems composed of synthetic nanoscale components. As nanostructured materials take on the characteristics of biological materials, synthetic systems of high functional density and cell-like complexity may also be realized. Learning how to assemble these components into functional networks will require a close coupling with systems biology efforts. Such bio-inspired nanomaterial systems would not be restricted to operation in aqueous environments or a narrow range of physical conditions. Considering the diversity observed in biology and the commonality of their system architecture, even simple synthetic systems have the potential for addressing multiple applications.

## Conclusion

The initial focus in nanoscience on the synthesis of nanomaterials closes the gap between the scales of biological and synthetic systems. The next steps in nano-enabled synthetic biology will be about closing the complexity gap, which is especially challenging, as there is no general theory to guide the organization of nanoscale materials into highly interactive collectives. Instead, this field is most likely to advance through the transfer of biological principles of organization into the bottom-up synthesis of complex synthetic nanoscale materials systems. Undoubtedly, this will require the adoption of design paradigms quite different from usual engineering practice, and

instead will embrace the organizational forces of excluded volume effects, stochastic modulation of nonlinear processes, scale-free networking of elements, and interconnectivity through weak and malleable interactions. Yet, these issues faced on the scale of collective behavior come full circle to present a challenge at the scale of individual nanoscale elements: can the synthesis of nanoscale materials be controlled to enhance the self-organization of highly interconnected networks? This question will drive a new emphasis on nanomaterial synthesis and the next steps in nano-enabled synthetic biology. With the continuing integration of nanoscience and technology with biological systems, a nano-enabled synthetic biology emerges and provides the tools to use the time-honored practice of design as a tool to understand complexity.

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