Perspective

Nanoscale Process Systems Engineering: Toward Molecular Factories, Synthetic Cells, and Adaptive Devices

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Introduction

Research in nanoscale science and engineering[1] has been primarily directed toward the design and manufacturing of (a) *materials with passive nanostructures* (e.g., nanostructured coatings, dispersion of nanoparticles, and bulk nanostructured metals, polymers and ceramics), and (b) *active devices with nanostructured materials* (e.g., transistors, amplifiers, targeted drugs and delivery systems, actuators and adaptive structures). Research on the design, fabrication and operation of integrated "nanoscale factories", that is, processes with unit operations and materials movement among these units at the nanoscale, along with the requisite energy supply system and monitoring and control infrastructure, is lagging seriously behind. It is progress at this frontier that will enable the research visions of molecular factories, synthetic cells and adaptive devices[1] (e.g., artificial tissues and sensorial systems, nanosystem biology for health care and agricultural systems,[2] scalable plasmonic devices, chemico-mechanical processing, targeted cell therapy and nanodevices, human-machine interfaces at the tissue and nervous system level) to become reality.

Process systems enginering (PSE), as an area of academic chemical engineering research, has effectively solved all the major technological problems associated with simulation, design, control, diagnosis, scheduling and planning of operations for large-scale continuous and batch chemical processes. As the focus of research moved in scale from cubic meters to cubic mm, the design, simulation, control and programmed operation of "plants or labs on a chip" [3,4,5] benefited from the accumulated PSE technologies, since the underlying physicochemical phenomena could still be handled under the same assumption of effective continuous media. Thus, while novel deployments of fabrication techniques (e.g., photolithographic pattern definition, etching, deposition, diffusion) have been implemented for the construction of microprocesses, the scope, theory and tools for "microscale process engineering" have remained essentially unchanged.

Current basic research though has pushed the scale of processing operations to molecular and supramolecular levels of a few nanometers. Creative exploitation of hydrogen bonding, π-stacking, electrostatic and/or hydrophobic-hydrophilic interactions has led to deliberate and purposeful *molecular tectonics,*[6,7] yielding a fast growing repertory of supramolecular structures with exquisite precision and functionalities, which can and have been used as *molecular reactors, separators, molecular tubes, motors, shuttles* or *pumps* for the transport of materials, *molecular gates* or *channels* for the selective propagation of molecules, etc. Two recent special issues[8,9] offer an informative panorama of research trends in designing molecular architectures and synthesizing functional nanostructures.

Integration of functional nanoscale units into a coherent process with specific overall functionality and behavior has not yet started in earnest, and has only been mentioned in passing as a future goal and justification of current research. The absence of a concise systems theory for the engineering of such integrated supramolecular factories compounds the inherent physical and chemical difficulties of their design and fabrication (to be discussed later on in this article) and deprives current research efforts of purposeful direction. Construction of different configurations of nanotube-vesicle networks[10] are among the first examples of integrated nanoscale units. While they do not fit the profile of a nanoscale process, as described in subsequent paragraphs, they indicate the feasibility of the proposition. When appropriately designed, these networks can function as nanofluidic systems to study single-molecule dynamics, enzyme-catalyzed reactions, single-file diffusion, single-molecule sequencing and synthesis, as well as gain an understanding of materials transport and reactions in biological systems.

With the proposition of "nanoscale factories" as the next frontier of processing scales, PSE must offer new theories and tools to handle the design, simulation, operation and control of active processing systems with the following distinguishing features: (a) The "unit operations" are self-assembled supramolecular structures at the scale of a few nanometers, (b) the spatial topology of the "process flowsheets" is guided by molecular scaffolds and the unit operations are positioned in space through directed self-organization mechanisms of independent units, and (c) the operation of such "supramolecular factories" is driven by preprogrammed information encoded in the design of the system itself, and is robustly controllable through local feedback loops with no evidence of centralized coordination mechanisms.

Self-assembly of molecules into supramolecular structures[11] and their guided organization into an integrated

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processing system are at the core of the needed new theories and methodologies. Design issues arising from the ensuing complexity[12,13] and the looming threat of computational irreducibility,[12] must be addressed. Information encoded into the judicious design of molecular structures and networks,[11] is leading to a convergence of chemistry, biology and computer science, with *Systems Biology* being its most visible manifestation. *Molecular computers*[14,15] have opened the possibility of preprogrammed operations at the local level of nanoscale unit operations and the global scale of an integrated process. What is the role of purposeful engineering, as exemplified by the tradition of PSE, in shaping these developments?

Engineering nanoscale processes is in essence the engineering of complex systems, whose hallmarks are[16]: self-assembly (at a small scale), self-organization (at a larger scale), self-replication, adaptation and self-regulation. As Ottino[16] has observed, ". . . this is where the conceptual conflict with engineering arises. Engineering is not about letting systems be. Engineering is about making things happen, about convergence, optimum design and consistency of operation. Engineering is about assembling pieces that work in specific ways - that is, designing complicated systems." The challenge is to attenuate and resolve this conflict, through methodological reconciliation of the two choices[16]: (1) Design everything at the outset, or (2) let systems design themselves.

In the following sections we will focus on two points: First, we will argue that the existing scope, theory and tools of PSE are insufficient for understanding the design principles and operational behavior of a nanoscale process, e.g. a eukaryotic cell or an artificial supramolecular factory. In order to set the background for this argument, we will briefly review (a) the structure and components of a nanoscale process, (b) the features that drastically differentiate them from processes at the macro- and microscale, and (c) the research trends in constructing nanoscale unit operations, material transport mechanisms, energy support systems, and molecular signaling networks for monitoring and control. Second, we will offer a series of research propositions that need to be addressed before *Nanoscale PSE* can tackle the deliberate engineering of living cells or the design of new classes of materials and devices based on active processing systems at the scale of a few nanometers.

Nanoscale, Supramolecular Factory

The biological cell is the prototypical model of a "supramolecular factory", inspiring current biomimetic research, primarily by synthetic organic chemists, to create supramolecular structures imitating the functionality of particular cellular operations. In a eukaryotic cell, the plasma membrane defines the boundaries of the "factory", allowing for selective flow of molecules in and out of the cell. The cell's organelles (e.g., nucleus, endoplasmic reticulum, Golgi complex, mitchondria, lysosomes, endosomes, peroxisomes) represent the "supramolecular unit operations", whose relative positions in the cytosol are guided by the 3-dimensional (3-D) structure and functionality of the cytoskeleton scaffold. Thermal energy within the cell allows for molecules and organelles to move in random directions for very short distances (i.e., Brownian motion). Effective concentration gradients and other mechanisms also apply. However, for materials that require motion in specific directions and/or for longer distances,[17] mechanical work is transferred from an energy source (e.g., ATP or GTP hydrolysis) by motor and membrane proteins. Membrane-limited vesicles, for instance, travel from the endoplasmic reticulum to the cis-Golgi network, from one Golgi cisterna to another cisterna, and from the trans-Golgi network to the plasma membrane or to the late endosome. The transport vesicles bud from one membrane-limited unit and, with the aid of motor proteins, travel along the tracks of the cell's cytoskeletal scaffold structure before finally fusing with the next unit in sequence.

Chemical energy fuels many cellular processes, and certain unit operations (e.g., mitochondria) produce energy-storing molecules for use in other units. Furthermore, information signals, produced either in parallel or in sequence by distinct molecular conformations, molecular concentrations (with or without spatial directionality), cascades of molecular charge distributions, and other mechanisms, are used to (1) convey information about the operating states within organelles, and (2) manipulate the state of controlling actuators, thus, yielding effective decentralized control of cellular processes.

Figure 1 shows the secretory and endocytic pathways of protein sorting, a typical example of a nanoscale factory.

The cell as a system parallels in many respects current macro- and microscale continuous manufacturing processes. Furthermore, as biologists have been uncovering the fundamental mechanisms of cellular processes, many others, primarily chemists, have been synthesizing novel supramolecular structures with specific functions, mimicking biological prototypes, such as the following:

Unit Operations: (a) *Reactors* of various conformational shapes (rings, spheres, open vessels, tubes) and reactive functionalities (including catalysis), (b) *Separators* (nanoporous membranes, molecular sieves and sorters, molecular channels, ion gates) which selectively transport ions, molecules, or stereoisomers. (c) *Molecular mixers and splitters.* (d) *Energy producing and dissipating units.*

Material Transporters: (a) *Nanotubes* made of various materials and using several types of transport-driving potentials (e.g., chemical, charge distributions along the tube) for the transport of ions, molecules, or isomers, and (b) *Molecular pumps, motors, shuttles, actuators.*

Control Elements: (a) *Signal carriers* (molecular electrical wires, cascades of molecular conformational changes, directional "effective" concentrations of surface charges, ions, or molecules), and (b) *Actuators* (molecular switches, gates, valves, motors, pumps, shuttles).

Consequently, we can envision the creation of artificial nanoscale processing systems, far more active than the vesicles-nanotubes networks developed by Shimizu et al.[10], such as the one shown in Figures 2 and 3.

Macro- through Micro- to Nanoscale Manufacturing Systems

To delineate the frontier of scientific and technological challenges faced by Nanoscale PSE, let us compare the essential features of processes at the macro-, micro-, and nanoscales.

Scale of Manufacturing, Topological Configuration and Positional Accuracy. For macroscale processes, the size of unit

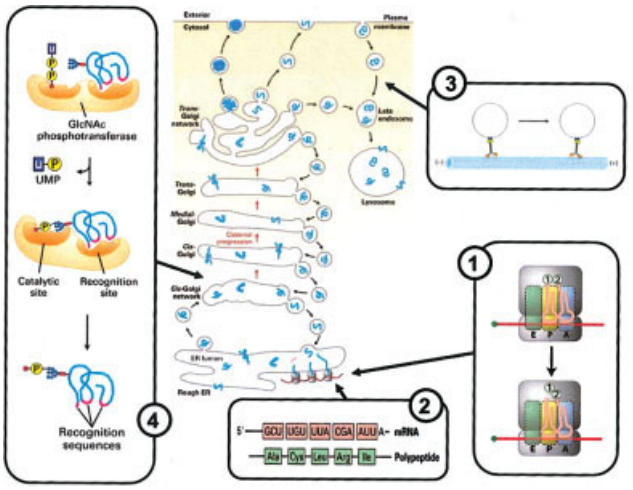

Figure 1. The secretory and endocytic pathways of protein sorting: A biological "nanoscale factory" (from Lodish et al.[17]).

(1) Protein synthesis on ribosomes bound to the rough ER represents a synthesis unit operation, with the ribosome acting simultaneously as a molecular reactor and catalyst for the reaction. (2) The mRNA being translated acts as a means of data storage and retrieval, encoding the instructions necessary for a molecular machine (the ribosome) to synthesize a particular peptide. (3) Material movement is accomplished via motor proteins that use energy to carry cargo-bearing units (vesicles) along a molecular scaffold (microtubules) that both facilitates transport and provides structure. (4) Control can be achieved through a network of molecular signaling mechanisms, like modification by the GlcNAc enzyme in the *cis*-Golgi in order to target a protein to the lysosome. Images reprinted with permission. Copyright 2004 WH Freeman and Company.

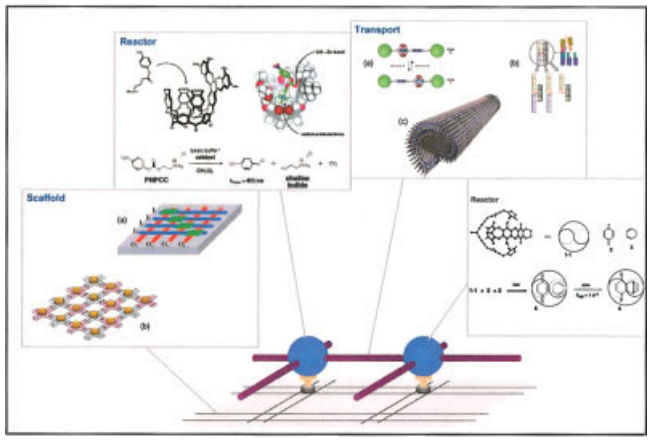

Figure 2. A nanoscale process with supramolecular unit operations, scaffold and transport mechanisms.

The two units are nanoreactors (*top left* — Richeter and Rebek[87] (Images reprinted with permission. Copyright 2004 American Chemical Society); *bottom right* – Kang and Rebek[88] Images reprinted with permission. Copyright 1997 Nature Publishing Group.). The structural scaffold can be made of (a) crossed semiconductor nanowires (Zhong et al.[29] (Image in Figure 2 reprinted with permission. Copyright 2003 American Association for the Advancement of Science)), or (b) DNA tiles (Park et al.[25] (Image reprinted with permission. Copyright 2005 American Chemical Society)). Between the supramolecular unit operations, molecules may be transported selectively through (a) shuttles (Balzani et al.[89]. (Image reprinted with permission. Copyright 2000 Wiley), (b) motors (Sherman and Seeman[53] (Image reprinted with permission. Copyright 2004 American Chemical Society)), and (c) nanotubes (Blau and Fleming[90]. (Image reprinted with permission. Copyright 2004 American Association for the Advancement of Science.)

operations and their positional accuracy in a flowsheet is at the scale of meters, and the topological configuration of units in space is driven by cost and safety considerations. In microscale processes, operations take place in units at the cubic mm scale, their positional accuracy is in mm (micrometer accuracy is also possible), and effective space utilization and process efficiency are the guiding considerations in determining the topology of the resulting flowsheet. For nanoscale processes, the size of supramolecular unit operations is at a scale from a few to a few hundred nanometers, and as evidence from biology suggests, overall-system functionality is the overriding consideration in defining the topological structure of the flowsheet and the positional accuracy (several Angstroms to a few nanometers) of the units in it.

Characterization of Processing Materials. Bulk characterization (averages over an appropriate length scale, e.g. meters to mm) of processing materials for macro- and microscale processing systems is sufficient; the manufactured fluid or solid products have desired features (type of material, concentration, shape) at similar scales. In nanoscale processes the continuum assumption is still operational for the liquid media in which the process is immersed, as long as its critical size is of order 10 molecular layers[18]. However, the characterization of the supramolecular unit operations, material transporters and associated molecular control structures cannot be based on averaged bulk properties, but on specific atomic and molecular configurations.

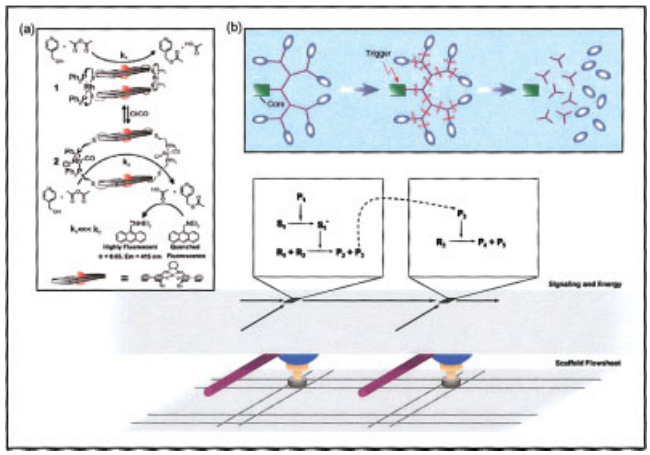

Figure 3. Nanoscale signaling and energy use.

In the first nanoreactor unit, a signaling network similar to (a) Gianneschi et al.[70] (Image reprinted with permission. Copyright 2005 American Chemical Society) occurs, where a signal, P_1, triggers a conformation change in S_1. This change catalyzes the reaction of R_1 and R_2 to form P_2 and P_3. P_3 is then transported to the second nanoreactor unit, where it is a trigger for a reaction similar to (b) Meijer and van Genderen[36] (Image reprinted with permission. Copyright 2003 Nature Publishing Group. P_3 triggers the dendrimer to self-destruct and release its cargo, P_5. One can envision the dendrimer, R_3, being an energy storage unit that releases energy-providing P_5 molecules when they are needed in the system.

(Area/Volume) Ratio, A/V, and the Scaling of Continuum-Based Phenomena. The (Area/Volume) ratio increases substantially as we move from macro- to microscale processes and becomes effectively "infinite"for the nanoscale. Effects scaled with volume diminish rapidly and those which scale with area dominate[19]. Mass transport is dominated by viscous dissipation and inertial effects are negligible. The impact of surface tension is greatly amplified, and capillary forces can have a determining effect in fluid manipulation through modification of solid/liquid surface tension, or inducement of gradients in the liquid/particle surface tension. Electrokinetic effects are of particular significance, and their application on systems with designed surfaces (e.g., patterned surfaces with nonuniform charge, active control of surface charge) offers a broad range of potential designs. Effects scaled with area will be essential in guiding the self-organization of nanoscale processes.

Design Philosophy and Fabrication. Macro- and microscale processes can be designed at the outset with a top-down, hierarchical approach.[20] Their fabrication is also through *top-down, man or machine controlled construction.* Existing evidence from biology and organic synthesis research suggests that the elements of a nanoscale process can be constructed and their relative positioning achieved with requisite accuracy (Angstroms to a few nanometers) only through a free and/or guided *bottom-up self-organization of molecules and supramolecular structures*.

Operational Control Structures. For macroscale processes characteristic time-constants are in minutes, with operating cycle times from a few to many hours. In microscale processes control occurs at time-constants of a few seconds, and operating cycle times are in minutes. The control design principles for both macro- and microscale processes remain the same: multivariable centralized control, or centralized coordination of local, decentralized control loops. For nanoscale processes, the time-constants of operating control are in milliseconds, and the manufacturing cycle times are in the range of seconds. *Self-regulation,* both for the local supramolecular unit operations and the overall process, seems to be the primary control mechanism; there are limited technological options that can provide the requisite external sensors and actuators to configure external loops with effective monitoring and regulation at the nanometers scale and milliseconds time-constants. The implications of self-regulation are important and will be discussed in a subsequent section.

Constructing Nanoscale Processing Systems

The concept of nanomechanical assemblers that enable the construction of nanomachines, including itself, in an atom-by-atom fashion, externally controlled,[21] has received solid criticism derived from chemical and physical reasoning.[22,23] However, if the nanomechanical fabrication of nanomachines is "replaced by their self-assembly from modular parts in the sense of a molecular self-instruction process, the creation of replicatable nanomachines seems to be feasible today"[24]. The technological ideas used to propose construction of replicatable nanomachines are also applicable in the construction of nanoscale processing systems and constitute the basis for the prototype proposed in the following paragraphs.

One may envision two possible types of nanoscale processes:

1. Processes with "semidefined" boundaries, where the supramolecular unit operations, arranged in a 2- or 3- D configuration and attached to a scaffold, are immersed in a fluid medium. A surface (on which the scaffold is attached) defines part of the process boundary, while the boundary defining the end of the fluid medium is far (relative to the size of the scaffold) removed from the scaffold-anchoring surface.

2. Processes with "defined" boundaries, akin to the plasma membrane of a eukaryotic cell, where the unit operations are closely packed in a 3-D confined space.

For either type, a nanoscale process will be composed of the following components: *Molecular scaffold; Supramolecular unit operations; Material transport elements; Monitoring and control signals.*

Molecular Scaffold. This is a critical component for the fabrication of a nanoscale process and provides a series of functions, including all or a subset of the following: (1) Structural support for the resulting process, (2) points in space where the supramolecular units will be anchored, (3) support for the movement of molecules from unit to unit, (4) points for potential measurement of operating states and/or control inputs, and (5) encoding of chemical information for the localized regulation of a unit or the replication of the process itself.

Two general categories of scaffolds have been developed: Scaffolds with biological molecules (DNA, viruses), and inorganic scaffolds (branched nanocrystals, crossed nanowire grids, high-density nanowire arrays).

1. *Biological scaffolds*: 1-D and 2-D DNA "tiles" with specific affinities at their edges can self-assemble (through the DNA's complementary base-pairing) to a variety of predetermined spatial arrangements (1-D "tracks," 2-D grids, stars, cubes) thus rendering desired geometries of scaffolds for nanoscale processes.[25] Functionalization of selected DNA tiles at regular intervals can be used to organize specific molecules in predetermined arrangements. Such scaffolds have the advantage of being relatively easy to design (it is commonplace to engineer DNA binding interactions), can be adapted to new and more complex geometries, and can take advantage of numerous DNA-conjugation strategies to attach new molecules to the scaffold. However, one can only use such grids in an environment friendly to the native form of DNA (i.e., aqueous, correct pH and salt concentrations, moderate temperatures). Scaffolds can also be constructed with viruses as the elementary building blocks, as follows: Functionalize the outer shell of viruses with strands of DNA, and allow them to assemble into clusters via DNA hybridization with viruses bearing the complementary sequence. Selecting appropriate virus mutants[26] to bind inorganic structures, or chemically modifying viral amino acids[27] so that they can attach to a wide variety of particle moieties, allows the construction of scaffolds that can be used in fluid media that are not DNA-friendly. Viruses allow for more complex chemistry on their surfaces, and are thus promising anchors to fairly complex supramolecular unit operations of a nanoscale process. Biological scaffolds are easy to construct (through self-assembly) in a purposeful and deliberate manner with fully predictable properties. However, they do not easily allow the use of external input signals, which may be needed to direct the operation of the nanoscale process. DNA-based scaffolds, though, may be designed to carry the chemical information of the desired preprogrammed operations for the nanoscale process. In addition, one can use a virus crystal, instead of

individual viruses, as a scaffold, a kind of "biological zeolite" with potentially functionalizable cavities.[28]

2. *Inorganic Scaffolds.* Crossed nanowire grids[29] or high-density arrays of nanowires[30] have been constructed and could be used as scaffolds for nanoscale processes. They are chemically durable and can be chemically functionalized, but are not as chemically flexible as the biological scaffolds. Their major advantage is their ability to interface with electrical influences, not only to monitor the process of reactions (taking place in the anchored unit operations), but also to affect them with highly precise control inputs, e.g., redox chemistry. Branched nanocrystals of varying composition have also been constructed in well-defined geometries.[31] Such components could, if integrated with other unit operations, organize structures of electrical and chemical signals in conditions not accessible with purely biological scaffolds.

Supramolecular Unit Operations. Self-assembly seems to be the only path to the fabrication of supramolecular unit operations with the desired functionality and precision in geometrical shape and size. Depending on their size, we can distinguish two general classes of supramolecular unit operations: *mesoscale* units and *nanoscale* units. For a review of research on self-assembled nanoreactors, see Vriezema et al.[32].

Simple rules (such as hydrophobic interactions) can direct the self-assembly of *mesoscale unit operations*. Micelles, lipid bilayers and ensuing vesicles, meso-porous silica, are all created through judicious patterns of molecular hydrophobicity relative to the surrounding medium. They have been fabricated to function as nanoreactors,[33] nanoreservoirs with controlled chemical-release,[34,35] and nanoseparators. Dendrimers have also led to the creation of mesoscale units.[36,37] The geometry of dendrimers[38] can be designed so as to define relative positional hydrophobicity and, thus, control their self-assembly into supramolecular structures with different, and prespecified, interior and exterior molecular environments. They have led to nanoscale reactors and separators, and energy transforming (light harvesting) units.[39,40]

Hydrogen bonding, π-stacking, metal coordination, and electrostatic interactions direct the self-assembly of *nanoscale unit operations* with exquisite molecular precision, possessing a wide range of geometries (symmetric or not), and diverse functionalities.[39,40,41,42,43,44]

Electrostatic or orbital overlap forces can be very useful for assembling molecular complexes, e.g. catenanes or rotaxanes,[43,45,46] and are generally strong enough to localize two components together to react, creating interlocked components. Electrostatic forces are highly dependent on the redox state of the components, creating the possibility of "bistable" complexes (i.e., the existence of two distinct molecular states that can be accessed under different redox potential conditions) and the creation of single-molecule switches.[47] The use of interlocked molecules as nanoswitches, valves, circuits, elevators, etc. has been widely explored, especially in the context of molecular electronics. They have been formed on monolayers and extensively modeled,[48,49] and a whole host of possible interlocked structures has been created[50].

Viruses are particularly attractive for use in molecular unit operations for three reasons: (a) They effectively bridge the meso- and nanoscales (they can form relatively "large" structures, up to 300 nm in the tobacco mosaic virus (TMV) case), (b) they allow precise chemical control at the single-residue level, while forming predictable and robust structures, and (c) their chiral surfaces and the functional diversity offered by the proteins allow for the formation of unit operations with rich functional diversity. For example,[27] modifying the inside and outside of the TMV creates the possibility of a "molecular tubular reactor": the outside functionalization could be used to anchor it in a particular location on a scaffold, and the inside could contain catalytic moieties that promote a particular reaction as reagents flow through it. If a more batch-like behavior is desired, one could use the MS2 virus which forms a spherical vessel and presents modifiable residues on its interior.[51]

Mechanisms for Mass Transport. Most of the mass transport mechanisms that can be envisioned are derived from biology, in that (a) the synthetic structures perform similar functions as known biological molecules, and/or (b) the molecules themselves are biological molecules shown to operate *in vitro*. In the cell, mass transport is the outcome of Brownian diffusion, concentration gradients, and protein-directed activity. To move molecules against their concentration gradients, many mechanisms require energy from either ATP (or GTP) hydrolysis or coupling with an energetically favorable reaction (e.g., ion or molecule transport through *antiporters* and *symporters*). Current research has designed and produced the following synthetic mass transport molecules: (1) Nanotubes (made of carbon, DNA tiles, etc.); (2) DNA scaffolds, DNA walkers, DNA motors; (3) Synthetic molecular shuttles, motors, elevators, gears, which provide mechanical advancement of molecules from one unit operation to another.

Nanotubes can be used to construct 2- or 3-D molecular scaffold structures for nanoscale processes. As such, they can (1) anchor the "unit operations" at specific points by directing the nucleation of "unit operation" self-assembly, and (2) be used as tracks for molecular transport between specific units. Molecular transport along the nanotube scaffold from one unit operation to another can take place through the interior of the tubes or the exterior surface of the tubes with cargo-carrying molecular motors. Many potential molecular motors can be found in literature. In fact, experiments have shown that biological molecular motors can operate *in vitro*, e.g., in the presence of ATP, kinesin can move along microtubule structures.[52] Other molecular motors have been made from other materials, such as DNA.[53,54] The use of DNA-based motors is a particularly interesting avenue, especially in conjunction with a DNA-nanotubes scaffold, which can potentially use DNA hybridization as a means of directed movement along the nanoscale process. Other novel supramolecular transport structures include: (a) *Molecular shuttles and elevators* based on rotaxanes,[45,55,56] and (b) *Molecular gears.*[57]

The above discussion focused on inter-unit mass transport, but it is not complete without discussing the critical step of mass transport across the boundary of a unit operation. Molecular structures across the boundary form high molecular-specificity channels, pumps, or pores to induce transport of molecules that cannot permeate the boundary. Many synthetic channels and pores have been designed to mimic biological functions for the transport of multiple substrates, such as: ions;[58,59] water;[60,61,62] light gases;[63] larger molecules.[64] Certain molecule-specific channels can also couple substrate translocation with a reactive conversion.[64]

Energy Sources to Power Nanoscale Processes. A network of energy sources is essential for a nanoscale process in

order to power (1) the physicochemical transformations in the various unit operations, and (2) the movement of molecules among unit operations (since molecules constantly buffeted by solvent cannot move in any directed or purposeful way without a constant input of energy to overcome the randomizing effects of the environment). Such a network must be designed in a way that satisfies the following requirements: (a) It can power the targeted active components of the process (unit operations and mass transport vehicles) with molecular precision. (b) It is fully integrated with the molecular monitoring and control system of the process, in order to affirm full observability and controllability of the system's operation. The following sections examine these two requirements and the design options that are available.

(1) *Forms of Energy.* The first designs toward powering simple mesoscale factories will be predominantly based on (a) thermal energy (the basis of traditional chemical synthesis, even for transformations in mesoscale reactors, and the majority of current supramolecular reactors), or (b) chemical energy (the basis of powering biological processes, e.g., ATP hydrolysis). The former is not fully controllable and cannot be targeted with molecular precision. Thus, it is acceptable for processes with mesoscale unit operations, where the localization accuracy and resolution of spatial control is of order 0.5 μm or higher, but inadequate for adaptive and complex processes with units at the nanoscale. Chemical energy released during the hydrolysis of ATP can be both precisely targeted to the active components of the process (e.g., enzyme activation by phosphorylation allows the enzyme to use ATP only when it is needed) and fully controllable (e.g., a control system can remove the phosphate when the enzyme is no longer needed). Hess et al.[52], for example, showed that it is possible to create self-assembled structures by molecular motors powered by ATP. Of course, the use of ATP restricts the processes to using ATP-dependent enzymes and biological reaction conditions, which severely limits the synthetic possibilities of such systems.

Light energy is an alternate option. Balzani et al.[65] have suggested using light as the principle energy components in Stoddart-like molecular machines, arguing that light allows temporal resolution at the scale of the operating molecular processes, and can simultaneously be used as a monitoring mechanism. However, such approaches would require localizing and regulating the activity of photons to a single molecular unit at a time (i.e., if we want to go beyond "bulk actuation" to control specific units). Thus, the effective use of light in such systems will require precise control of energy use through light-harvesting mechanisms as in photosynthesis. Baskaran et al.[66] are trying to utilize and harvest light at the single-molecule level by creating mimics of photosynthetic "collector antennae". The possibility of manipulating such light-collecting systems at the single molecule level (by functionalizing them to affect their self-assembly properties, for example) would provide a promising route toward photon-powered systems.

Magnetic nanoparticles, if attached properly to supramolecular structures, could serve as tiny "locomotives" to pull them along the structural elements of the system scaffold, e.g. nanotubes, viral scaffold, or DNA tracks. However, the issue of spatial control resolution remains a major challenge.

(2) *Interfacing Energy and Control Systems.* The simplest way to interface the energy production and dissipation system with the monitoring and control mechanisms of a nanoscale process is to make them one and the same. For example, catenane or rotaxane molecular switches use a variety of stimuli (acid/base reactions,[68] electrochemistry,[47] light[56]) to create motion, but the chemical modification simultaneously serves as the driving force for the transformation, which is usually charge- or sterically-based. The difficulty with such systems, however, is again the lack of resolution, that is, it is impossible to localize (at the level of a single unit operation) the control of factors like pH without affecting neighboring unit operations. Thus, while single-molecule switches exhibit a variety of attractive functions, these nanosystems will require spatially precise fuel systems, analogous to enzymes and ATP. The same problem arises for a variety of molecular machines that rely on physicochemical properties like ion binding or chemical modification for activity. Thus, it would be of great utility to develop a small-molecule fuel that can be used controllably and reliably by a large variety of molecular unit operations and machines.

Network of Monitoring and Control Signals. There exist three broad classes of signals, which are used for local monitoring and control of biological processes, and could be deployed for the control of artificial nanoscale processes: (a) Signaling molecules (e.g., ions or ligands), which can transfer a signal through molecular reactions (e.g., charge transfer in mitochondria membrane proteins). (b) Signaling molecules, which can cause molecular conformational changes (e.g., allosteric enzyme activation/deactivation). (c) Concentration gradients and/or spatially directed abundance of electrons, ions, or molecules (e.g., cationic concentration control of the propagation of action potentials in nerve cells).

Synthetic supramolecular structures for use in control mechanisms of nanoscale systems are growing in number. Chemists have devised structures which change conformation or activity based on the local environment, acting as signaling molecules for monitoring or control. Examples include signaling molecules undergoing molecular modifications, induced by the following factors:

1. pH (e.g., dendrimer destabilization[38]; vesicle-to-micelle transition[35]). pH can be effectively monitored and controlled by ion pumps/channels or local reactions.

2. Temperature (e.g., transition temperature of vesicles changes permeability to specific molecules[34]; thermally-controlled self-complexing molecular switches[69]).

3. Chemistry (e.g., allosteric catalyst activation in the presence of Cl- ions and CO[70]; dendrimer self-destruction through molecular trigger signal, releasing attached molecular cargo[36]).

4. Electrochemistry (e.g., molecular switch with differing cation- and anion-interaction behavior[71]; ion-controlled molecular tweezers[72]; electrochemically-induced rotaxane switch[46]).

5. Photochemistry (e.g., light-induced rotaxane switch[56]; light-operated molecular scissors[73]).

Local concentration gradients and/or spatially-directed molecular abundance have inspired multiple synthetic mechanisms, which allow control of local molecular concentrations. Examples include the following: guest-induced assembly of cavitand cages[74] and chemically-controlled nanovalves to capture and release specific molecules[75].

Systems Engineering for Nanoscale Processes

A nanoscale process, like its macro- and microscale counterparts, involves the following systems engineering design and implementation aspects:

(a) Conceptual synthesis of the integrated nanoscale process.

(b) Design and fabrication of the supporting scaffold and of the unit operations in the process flowsheet.

(c) Synthesis of the molecular network that supplies the energy sources and sinks for energy dissipation.

(d) Synthesis of the molecular network that implements the signaling paths for monitoring controlling of the process.

In the following paragraphs we will offer a series of research propositions for tackling each of the above four engineering problems.

Conceptual design of nanoscale processes: Synthesis of interacting molecular networks

The conceptual design of a nanoscale process is founded on three interacting molecular networks. The basic network provides the chemical and physical transformations defining the process. The second network represents the molecules and reactions, which supply the energy to the basic process, and the third network establishes the molecular signals for the operational monitoring and control of the previous two networks.

Research Proposition 1. Research should be directed toward the systematic synthesis of the three interacting molecular networks, which define a nanoscale process.

Literature on the synthesis and analysis of chemical and biochemical networks is rich and extensive. In this case, though, we are confronted with the synthesis of multiple interacting networks with differing objectives. This is a new problem, which has not been tackled in its entirety.

Groundup synthesis of molecular networks for given functions, e.g. networks of catalytic peptides,[76] which are designed to create particular network architectures and constructed through self-assembly, is a characteristic example of the research direction that we need to pursue further. The significance of these networks is that they are designed to be both auto- and cross-catalytic, so a particular peptide can catalyze its own formation as well as the formation of other peptides in the network (Figure 4). Such "molecular ecosystems" can be modeled by simple or colored graphs and, in principle, could lead to any type of molecular networks required by the nanoscale process. For example, variants of the network of catalytic peptides can be used to establish molecular networks that carry out computations, e.g. Boolean logic,[77] which in turn could partake in the monitoring and control of a nanoscale process.

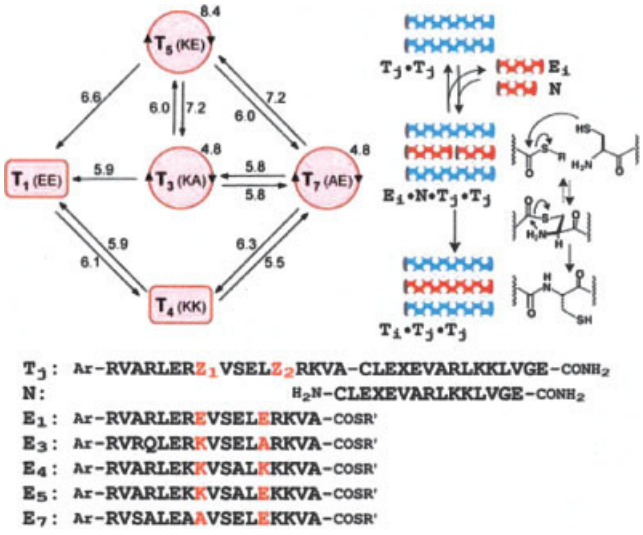

Figure 4. Synthetic network of template-directed peptide ligation.[77]

The graph at the top left represents the network of auto- and cross-catalytic reactions. The nodes represent the sequence similar peptides. The directed edges represent a cross-catalytic formation, while the circular nodes represent auto-catalytic formation. E_i: electrophilic peptide fragment, N: nucleophilic peptide, T_i: peptide template. Image reprinted with permission. Copyright 2004 American Chemical Society.

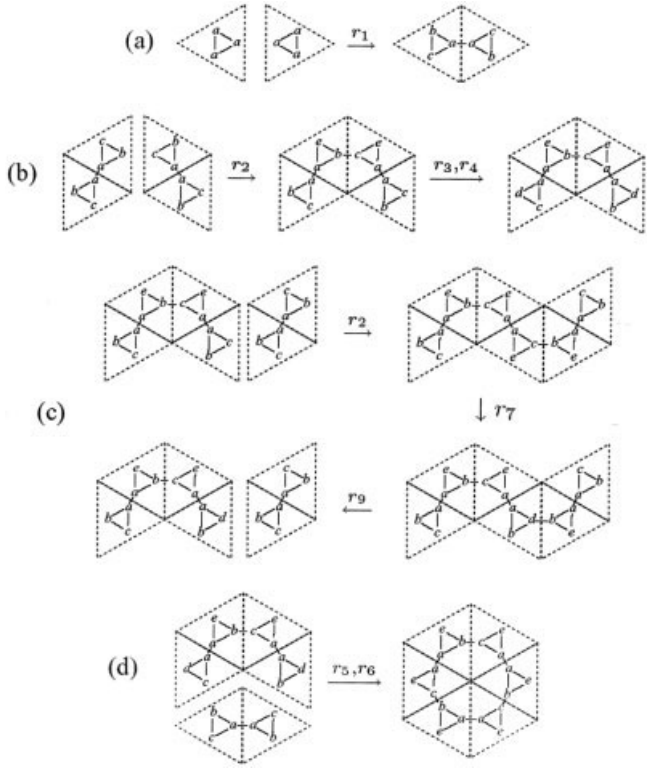

Figure 5. Graph grammars approach to self-organization.[86]

r_i represents the rules that lead from one conformation to another, and {a, b, c, d, e} represent the labeled vertices of the graph. Image reprinted with author's permission.

As the complexity of interacting molecular networks increases, we are confronted with questions of *computational irreducibility*[12] that is, our inability to predict the properties of such networks, without actually constructing them and seeing how they behave. The upside to this engineering roadblock, however, is that a network with even a small number of interacting components could produce arbitrarily complex (and potentially adaptive) behavior.

In designing the requisite molecular networks, the following questions arise:

- How does one select the molecular components of the three networks, so that they achieve the requisite integration, while satisfying their roles within the functional scope of each network? Biology offers excellent prototypes of molecules with such integrative roles, e.g. enzymes, RNA, DNA.
- What is the "chemical information" of the starting molecules that drives the auto- and cross-catalytic self-assembly of molecular networks, and how can it be modeled?

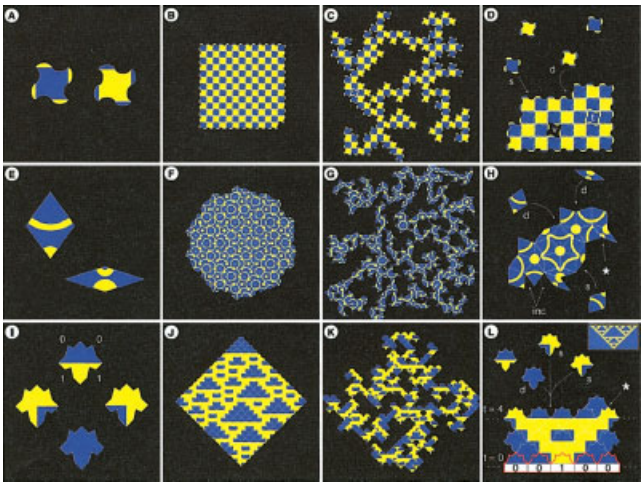

Figure 6. Tile self-organization.[91]

(A),(E),(I) are C, P and XOR tiles, respectively. (B),(F),(J) represent the periodic ideal structures of the self-assembly of these tiles. (C),(G),(K) show simulation results. (D) shows a schematic of tile-binding by a single (s) or a double (b) bond. Errors are formed by trappings (t) and vacancies (v). (H) shows binding by single and double bonds. The matching rules allow for incompletable sites (inc) that cannot bind anything without error (*). (L) shows tiles binding with an input nucleation site (outlined in red). Single and double bonds can form, and the three converging arrows represent three tiles competing for a site, two of which (s) would form an error (*). Image reprinted with permission. Copyright 2000 National Academy of Sciences, U.S.A.

- Given the starting molecules and their associated "chemical information", what is the structure of the resulting molecular network and how do the conditions of the environment in which the self-assembly takes place (e.g., initial and boundary conditions) affect the network structure?
- Given the desired structure and properties of a molecular network, how can we design the requisite "chemical information" of the starting molecules and the initial and boundary conditions of the environment, so that the resulting molecular network has the desired properties?

Fabrication and operation of nanoscale processes: Engineering of self-organizing processes

The design, fabrication, and operation of a nanoscale process is in essence the *engineering of self-organizing processes.* This is a daunting problem, because it requires the manipulation and coordination of a vast number of objects (molecules, supramolecular structures) into a coherent whole that performs a series of predefined global tasks. Addressing and manipulating each object is impossible. However, local interactions among the objects can lead to *self-assembled local objects* (e.g., formation of the supramolecular unit operations, material transport complexes, and segments of the molecular scaffold), which in turn can form *self-organized larger-scale structures* (e.g., unit operations positioned on the scaffold and connected with mass transport molecular structures).

Research Proposition 2. Nanoscale processes will be designed and fabricated through multiscale, self-organization of molecular entities. Research must be directed toward the understanding of the relationships between the local interaction rules, which lead to the self-assembly of local objects, and the rules that result in the self-organization of global processes (i.e., the nanoscale process). Position in space is important, and orientation of objects is an essential consideration in the formulation and deployment of the rules. Characteristic examples of the problems to be solved are the following:

- Self-organization of process scaffolds and precise positioning of nanoscale unit operations on them.
- Self-organization of nanoscale unit operations and associated material transport vehicles (e.g., nanotubes, molecular transporters of various types).
- Self-organization of molecular gates and channels at the boundaries of nanoscale unit operations.

All the molecular entities participating in the self-organizing processes behave as "programmed systems". Such chemical programming requires the incorporation of suitable instructions into molecular components for the generation of well-defined supramolecular entities, making use of recognition algorithms based on specific interaction patterns.[11] Thus, understanding self-assembly requires understanding the rules that govern the supramolecular association of individual building blocks. The procedure can be viewed as an algorithm that performs simple calculations via the rules that are "programmed into" the pieces (molecules and/or supramolecular structures). These rules are manifest through the physical-chemical interactions between the molecules, and a number of different chemical techniques have been used to engineer these interactions into the molecules.

Research Proposition 3. The molecular entities undergoing self-organization will be modeled as self-standing elementary computational processes. The components of a nanoscale process, as well as its final structure, will be the output of a complex computational process, resulting from the interactions of the elementary computational processes. To achieve computer-aided simulation and design of nanoscale processes, research must be directed toward the formulation of these elementary computational processes, their formal representation in a convenient language, and their deployment through the rules of the grammar of the associated language. The following are among the basic questions that must be answered:

- What is an efficient language to encode the computational processes modeling the behavior of molecular entities undergoing self-organization? Does this language lead to computationally reducible processes?
- How does one design the computational processes encoding the behavior of self-organized molecular entities, in order to achieve the desired structure of the self-organized system?

Rothemund et al.[15] have observed "the importance of universal computation for autonomous fabrication tasks. . .recognized in von Neumann's seminal work on self-reproducing automata, where he postulated a *universal constructor* that, by reading an input tape specifying an algorithm for what to build, could carry out the commands necessary to construct an arbitrary object[78]". Research work already under way has proposed various ideas.[79] Klavins[80] has introduced *graph grammars* (Figure 5) to model self-assembly processes. *Tiles* and *Tiling Processes*[81] (Figure 6) have been favored for a long time as convenient means to construct computational elements and associated grammars for the simulation of self-organizing systems. However, it should be clear that if algorithmic programming concepts are to have an impact in modeling and designing molecular self-assemblies, the rules of the programming language must capture the impact that energetic and entropic effects have on the creation of self-assembled processes. Therefore, the following question needs to be resolved as early as

possible: "Do we use approaches, such as Monte-Carlo (MC), molecular dynamics (MD), or Brownian dynamics (BD), for the simulation of self-organizing processes, or do we rely on rule-based systems, which encode the essential physics of interaction among the interacting entities?". The former may be richer for simulation purposes, but inadequate for design purposes, which require the inversion of the modeling relationships; the vast number of potential alternatives would preclude their repeated application.

Cellular Automata (CA)[82] could be seen as the natural candidates for rule-based modeling of the interacting entities and of the self-organizing processes. The rich literature of CA has already established the link between rule-based computational processes and the underlying physics of a system. *Chemical Tinkertoys*[7] have been used to assemble chemical substructures, and, from them, larger supramolecular structures hierarchical synthesis. However, recent work on *Agent-Based* approaches promises to expand the capabilities of CA-based methods. Agent-Based[83] modeling allows the self-organizing "agents" to:

- interact with their environment and with other agents,
- change their actions (rules) in time as a result of these interactions, and
- show a goal-directed behavior, that is, they take the initiative to satisfy the proposed goals.

Such flexibility in autonomous actions could lead to nonlinear behavior, unpredictable designs, and learning over time.

Taming the complexity emanating from self-organizing processes is an essential aspect of the required research. The engineering of complex self-organizing processes could result in *computational irreducibility,*[84] implying that it would be impossible to predict the structure of the resulting nanoscale process without carrying out the computations, that is, constructing the complex process to completion. Although multiscale modeling approaches have had some success in taming the computational irreducibility of specific systems,[84] the issue remains open.

Research Proposition 4. Taming the complexity of self-organizing processes, and producing computationally reducible nanoscale processes of desired structures requires the judicious (a) control of initial and boundary conditions in the domain, where the self-organizing process takes place, and/or (b) multiscale representation of the self-organizing entities. Research should be directed toward (1) the understanding of how boundary conditions restrict the potential structures of self-organizing systems, and (2) how multiscale models ensure computational reducibility of the associated simulation and design processes.

For example, the existence of a rectangular, 2-D grid as the basic scaffold for a nanoscale process, with alternating hydrophilic and hydrophobic segments, imposes a pattern of boundary conditions that limits significantly the number of potential structures resulting from the self-organization of supramolecular unit operations. Also, coarse-graining of self-assembled systems is being broadly used to study the patterns of "complex" physical systems.[84]

Monitoring and control of nanoscale processes: Preprogramming and self-regulation

Judicious monitoring and control is needed in two phases: (a) During the self-organization of molecular entities in constructing the nanoscale process and (b) during the operation of a fully developed nanoscale process. In both phases, none of the components of a nanoscale process knows the global state of the process. The state space across the process is not continuous, and the interacting molecular networks (see paragraph 1, earlier) introduce fast logical switching, which requires hybrid (continuous and integer) representations. Performance must be met, be fault tolerant and robust. Monitoring and controlling a nanoscale process is a formidable challenge.

To control the structure of a system during the phase of its self-organization we must primarily rely on the design of the elemental pieces (e.g., molecules, supramolecular subsystems), since limited external manipulations are technologically possible to effect control at the requisite nanoscales. Therefore, controlling the self-organization process is a problem in the design and fabrication of the nanoscale system itself,[85] as suggested earlier by Research Propositions 2, 3 and 4.

During operation, control of nanoscale processes will rely primarily on *self-regulation* and secondarily on external control signals, such as those offered by inorganic (e.g., nanowires, nanotubes) or organic (conductive, semiconductive polymers) process scaffolds, allowing external control (e.g., analog electrical signals, digital logic) with appreciable computational scope.

The need for self-regulation has two very important ramifications:

1. The molecular structures of the supramolecular unit operations must be designed in such a way that they (1) provide specific functionalities (required by their role in the overall flowsheet), and (2) induce operational self-regulation. Examples from biology illustrate that self-regulation of molecular processes can be achieved implicitly (i.e., with molecularly integrated feedback loops), or explicitly (without feedback loops).

2. Self-regulation at a process-wide scale can only be achieved through judicious interactions among the supramolecular units acting as *independent agents*. Again, evidence from biology suggests that the following two mechanisms are possible: (1) A molecular network, distinct from the supramolecular unit operations, which implements a process-wide coordination of the independent agents, or (2) judicious molecular sub-structures in the interacting supramolecular unit operations, which regulate the interactions among these units.

Research Proposition 5. The design of the monitoring and control structure for a fully developed and operational nanoscale process must be solved simultaneously with the design of the process itself (see Research Proposition 1). The operation and control must be based on a combination of self-regulation (primary) and external manipulations (secondary; if possible), both at the local and global scales. Research is needed to extend existing approaches in order to ensure fault-tolerance and robustness for interacting molecular networks with auto- and cross-catalytic features. The desired result is a molecular network, which emulates the intended control structures and can be incorporated into the basic processing network.

The literature on the design of monitoring and control structures is vast and rich. Existing methods would be appropriate as starting points. Local and global self-regulation of dynamic systems has also attracted extensive attention. Fault-tolerance and robustness are essential for a

nanoscale process, and both have to be implemented through judicious design of molecular networks. This poses a great challenge. Nevertheless, these are not new technical issues for systems and control communities. The focus on molecular systems poses some discomfort to systems theorists, and this must initially be overcome.

Research Proposition 6. The elements of a nanoscale process are rule-based computational processes. Specific algorithms need to be developed for designing the self-regulating behavior of the process at large. Issues of computational irreducibility arise, again from the complexity of the underlying molecular networks, and need to be addressed.

The last proposition implies a certain affinity between the proposed research and that currently under way in computer science on the design of self-assembled robotic systems.[86] Self-organization of molecular entities can learn the value of procedural formalisms in taming complexity, while research in robotics becomes inspired by and learns to value the power of pre-programming in self-organizing molecular entities. This is a very fertile area of interdisciplinary discourse.

The operation of cells is directed by a preprogrammed procedure (the DNA code) and feedforward control processes (e.g., signal transduction pathways) driven by external stimuli. Analogous, although possibly different in implementation, molecularly preprogrammed operational procedures and feedforward control, driven by external stimuli (e.g., electrical current at position-specific points of the process scaffold), will be necessary to carry out the intended operational goals of nanoscale processes. However, as the effort focuses on *in vitro* artificial cells or completely synthetic nanoscale processes, alternatives to DNA are needed to encode preprogrammed operations. By defining the scope of the necessary information and the rules of specific programming languages for its computational deployment, one may delineate the character of the molecules that chemists could try to synthesize.

Research Proposition 7. The programmed operation of a nanoscale process can only be partially supplied by a computational algorithm external to the nanoscale process itself. Molecular processes, as integral parts of the nanoscale process design, could supply the desired computational procedures. Research is needed to establish the theoretical scope of such chemically-encoded programmed operations, which in turn could lead to the development of specific molecular networks deploying preprogrammed operations.

Epilogue

Engineering nanoscale processes is the engineering of complex systems. The Research Propositions, described in the previous section, aim at two targets: First, expand the methodological framework of classical PSE (i.e., design everything about a process at the outset) to include the features which are essential for taming the complexity of nanoscale processes (i.e., guide the system so it designs itself). Second, define the directions for the development of needed new PSE tools in synthesizing, fabricating and operating nanoscale processes. Creative formulation of new and reformulation of old problems will be required. Biology will serve as the quintessential paradigm in formulating the new problems, and the convergence of chemistry with computer science will provide the scope for developing new tools for Nanoscale PSE. The academic challenge is daunting and sets a threshold of achievements, which can propel research in exciting directions for many years to come.

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