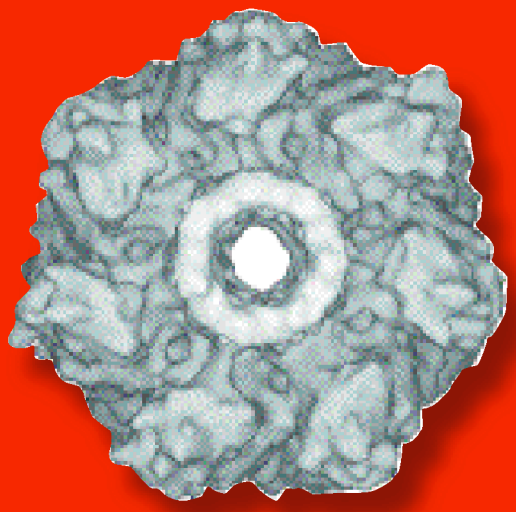
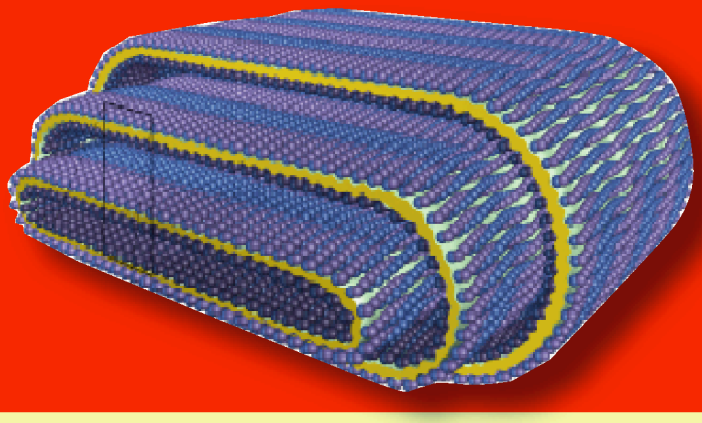
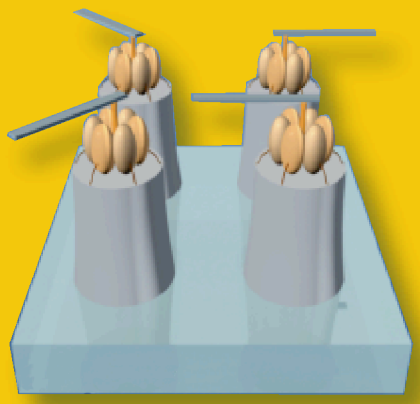


Biomolecular Materials





Report of the January 13-15, 2002 Workshop

Conducted by the Basic Energy Sciences Advisory Committee

to the Office of Science

U.S. Department of Energy

Cover Illustrations (from top):

Molecular graphics representation looking into the channel of the □ hemolysin pore. Song et al., 1996 (Figure 24).

Complexation of F-actin and cationic lipids leads to the hierarchical self-assembly of a network of tubules; shown here in cross-section. Wong 2000 (Figure 9).

Depiction of an array of hybrid nanodevices powered by F1-ATPase. Soong et al., 2000 (Figure 1).

Electronmicrograph, after fixation, of neuron from the A cluster of the pedal ganglia in *L. stagnalis* immobilized within a picket fence of polyimide after 3 days in culture on silicon chip. (Scale bar = 20□m.). Zeck and Fromherz 2001 (Figure 16).

Biomolecular Materials

Report of the January 13-15, 2002 Workshop
Supported by the Basic Energy Sciences
Advisory Committee
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This report is dedicated to the memory of Iran L. Thomas, Deputy Director of the Department of Energy Office of Basic Energy Sciences and Director of the Division of Materials Sciences and Engineering until his death in February, 2003. Iran was among the first to recognize the potential impact of the biological sciences on the physical sciences, organizing workshops and initiating programs in that area as early as 1988. More broadly, his leadership, vision, breadth of view, and dedication to science had an immeasurable impact on the science programs of the Department of Energy and the nation over the past 20 years. He will also be remembered as a lover of the arts and as a caring and generous friend.

Table of Contents

Executive Summary

The Office of Basic Energy Sciences in the Department of Energy Office of Science, and the Basic Energy Sciences Advisory Committee convened a workshop in January, 2002 to explore the potential impact of biology on the physical sciences, in particular the materials and chemical sciences.

Twenty-two scientists from around the nation and the world met to discuss the way that the molecules, structures, processes and concepts of the biological world could be used or mimicked in designing novel materials, processes or devices of potential practical significance. The emphasis was on basic research, although the long-term goal is, in addition to increased knowledge, the development of applications to further the mission of the Department of Energy.

The charge to the workshop was to identify the most important and potentially fruitful areas of research in the field of Biomolecular Materials and to identify challenges that must be overcome to achieve success. This report summarizes the response of the workshop participants to this charge, and provides, by way of example, a description of progress that has been made in selected areas of the field.

The participants agreed on several conclusions. First and foremost, they agreed that:

> ***The world of biology offers an extraordinary source of molecules and inspiration for the development of new materials, devices and processes. Progress in research in a number of areas in this field has been rapid and the panel foresees a revolutionary impact of the linkage of biology and materials science on science and technology in general, and the mission of the Department of Energy in particular.***

In particular the panelists agreed that:

> ***The interest of the Department of Energy in biomolecular materials and biological processes is very broad. There is a need for lighter and stronger materials to improve fuel economy. There is a need for functional materials to control transport across membranes, to make separations and purification processes more efficient. There is a need to increase energy efficiency by using low temperature processes to make materials. There is a need for energy producing processes that can convert light, carbon dioxide, and water to high-density fuels and thereby decrease, at least to some extent, our dependence on fossil fuels. Finally the high specificity of biological reactions, producing little or no side products, and the inherent biodegradability of biological systems strongly suggest that these systems need to be explored by DOE for their potential beneficial effects on the environment.***

Having agreed on these principles, the participants stepped back to explore potential research directions in the field. The world of biology is immense. As described in Section 2 of this report, living organisms perform an extraordinary number of functions, virtually all of which can be seen to have relevance to materials, processes or devices. Some of these impacts have already been explored, at least to some extent, most have not. At this stage an outline of productive directions in the field can be identified only through broad brush strokes.

Specifically, the participants felt that a DOE program in this area should focus on the development of a greater understanding of the underlying biology, and tools to manipulate biological systems both *in vitro* and *in vivo* rather than on the attempted identification of narrowly defined applications or devices. The

field is too immature to be subject to arbitrary limitations on research and the exclusion of areas that could have great impact.

Future Directions.

These limitations aside, the group did respond to the charge and develop a series of recommendations. Three major areas of research were identified as central to the exploitation of biology for the physical sciences. Sections 3, 4 and 5 in this report are devoted to those areas.

Self Assembled, Templated and Hierarchical Structures. Biology acts at the nanoscale, synthesizing and manipulating molecules with dimensions as small as tenths of nanometers. Through successive rounds of complexation and linkage of these molecules, it develops structures on the meter length scale. All of this is accomplished without conscious direction. Understanding and control of the processes involved in this self-fabrication are critical to the successful exploitation of biology. This is discussed in Section 3.

The Living Cell in Hybrid Materials Systems. Despite the extraordinary advances in the past decade in our understanding of biological systems, many remain far too complex for us to use, mimic or recreate. As a result, it must be expected that for well into the future, many of the cellular functions we wish to exploit will have to be performed by intact, living cells themselves. Thus, methods to incorporate living cells or tissues into non-living structures and devices, and to have them communicate with those structures and devices will be required. This area is discussed in Section 4.

Biomolecular Functional Systems. Living systems perform a wide variety of functions that could be controlled and used *in vitro*. Critical to this goal is the thorough understanding of the molecular components of these systems and how they interact, leading to our ability to manipulate those components and interactions. In some cases, intact cells (as found in nature or altered by design) will need to be used (see Section 4). However, in other cases, this will involve removal of the particular functional system from the organism. In still other cases it will involve the recreation or mimicking of it outside the organism. This area is discussed in Section 5.

Workshop participants also discussed the challenges and impediments that stand in the way of our attaining the goal of fully exploiting biology in the physical sciences. Some are cultural, others are scientific and technical.

Barriers.

Cultural Challenges. Those who know the biology, the biologists, are, more often than not, descriptive scientists, whose goal is to identify the molecular components of biological systems and understand how they work together to produce the observed function. They are, in general, not focused on synthesis or creation of these molecules or systems, or mimics of them, nor are they focused on their adaptation to functional systems working outside the organism. This culture has changed somewhat in recent years with the focus on the molecular basis of disease and the identification of targets and then lead compounds for pharmaceuticals. The number of biologists with an explicit interest in the non-biomedical application of their systems however, remains small.

1. On the other hand, until recently, chemists, physicists and materials scientists, who traditionally do have an interest in creating materials, processes and devices, have had little formal training in the biological sciences. A very sophisticated understanding of a field is required to exploit it, thus truly interdisciplinary training needs to be significantly enhanced. We are already seeing this, with the organization of departments and groups in "chemical biology" and the significant increase in the enrollment of chemistry, physics and materials science students in biological

science classes.

2. The application of biology to the physical sciences is by definition a multidisciplinary activity requiring extensive collaboration. There have, however, historically been few collaborations between biologists and materials scientists, although there have been some with physicists and more with chemists.

Scientific and Technical Challenges.

1. Biological systems are not generally robust. They function best at room temperature, although some have been found in freezing or boiling environments. They are subject to deterioration in non-sterile environments. They generally require an aqueous milieu. Thus issues of the adaptation of biological systems to the harsher environments of materials, processes and devices, and their strengthening for long-term viability must be addressed.

2. We do not, even after the revolutionary advances of the past few decades, understand biological systems well enough to control and manipulate them. Basic research into the molecules, structures and processes is required before adaptation and mimicry can be achieved. Processes such as molecular recognition, self-assembly, protein folding, energy transduction, nervous system function must be further elucidated.

3. Biological systems are, at their highest level of function, exceptionally complex, with large numbers of components interacting in very specific ways. Many systems are multifunctional and highly responsive to their environment. Issues of simplification or of precise assembly of multicomponent complex objects without sacrificing their function need to be addressed

4. Theory, simulation and modeling have not been applied to biological systems to the extent that they have become routine in the materials sciences, physics and chemistry. This field must be developed.

5. Characterization tools, especially at the single molecule level need to be developed. This is a particularly challenging issue because the National Institutes of Health, the primary federal agency for support of biological and bio-medical research has, in the past, not emphasized instrument development to the extent that the DOE programs have.

Recommendations.

Program Relevance. In view of what has recently developed into a generally recognized opinion that biology offers a rich source of structures, functions and inspiration for the development of novel materials, processes and devices support for this research should be a component of the broad Office of Basic Energy Sciences Program.

Broad Support. The field is in its early stages and is not as well defined as other areas. Thus, although it is recommended that support be focused in the three areas identified in this report, it should be broadly applied. Good ideas in other areas proposed by investigators with good track records should be supported as well. There should not be an emphasis on "picking winning applications" because it is simply too difficult to reliably identify them at this time.

Support of the Underlying Biology. Basic research focused on understanding the biological structures and processes in areas that show potential for applications supporting the DOE mission should be supported.

Multidisplinary Teams. Research undertaken by multidisciplinary teams across the spectrum of materials science, physics, chemistry and biology should be encouraged but not

artificially arranged.

Training. Research that involves the training of students and postdocs in multiple disciplines, preferably co-advised by two or more senior investigators representing different relevant disciplines, should be encouraged without sacrificing the students' thorough studies within the individual disciplines.

Long-Term Investment. Returns, in terms of functioning materials, processes or devices should not be expected in the very short term, although it can reasonably be assumed that applications will, as they have already, arise unexpectedly.

The workshop participants wish to thank and acknowledge Patricia Dehmer, Director of the Office of Basic Energy Sciences; Iran Thomas, Director of the Division of Materials Sciences; and the Basic Energy Sciences Advisory Committee for their vision in identifying biomolecular materials as an important new field and in sponsoring this workshop.

Foreword

In 1999, the Basic Energy Sciences Advisory Committee (BESAC) convened a workshop to design a roadmap for research in complex systems. The report of the workshop, *Complex Systems – Science for the 21st Century*, outlined an exciting science agenda that both integrated the disciplines of physics, materials sciences, chemistry, biology, and high-performance computing, and also could be built on the foundations that had been put in place a year before by the National Nanotechnology Initiative. In June 2001, Dr. James Decker, Acting Director of the Office of Science, U.S. Department of Energy, asked BESAC to help refine that research agenda. "In the world beyond nano," Dr. Decker wrote in his charge letter to the Chair of BESAC, "it will be necessary to use atoms, molecules, and nanoscale materials as the building blocks for larger supramolecules and hierarchical assemblies. As was described in *Complex Systems – Science for the 21st Century*, the promise is nanometer-scale (and larger) chemical factories, molecular pumps, and sensors. This has the potential to provide new routes to high-performance materials such as adhesives and composites, highly specific membrane and filtration systems, low-friction bearings, wear-resistant materials, high-strength lightweight materials, photosynthetic materials with built-in energy storage devices, and much more. The magnitude of the challenge is perhaps more daunting than any faced before by these disciplines. I would greatly appreciate BESAC's help in defining these challenges."

Table 1. Speakers

Mark Alper	Lawrence Berkeley National Laboratory / University of California at Berkeley
Samuel Stupp	Northwestern University
Lia Addadi	Weizmann Institute of Science
Paul Alivisatos	University of California at Berkeley / Lawrence Berkeley National Laboratory
Hagan Bayley	Texas A&M University
Angela Belcher	University of Texas at Austin
Carolyn Bertozzi	University of California at Berkeley / Lawrence Berkeley National Laboratory
Jean Fréchet	University of California at Berkeley / Lawrence Berkeley National Laboratory
Reza Ghadiri	Scripps Research Institute
Wolfgang Knoll	Max-Planck Institute for Polymer Research, Mainz
Chad Mirkin	Northwestern University
Carlo Montemagno	University of California at Los Angeles
Thomas Moore	Arizona State University
Daniel Morse	University of California at Santa Barbara
David Nelson	Harvard University
Cyrus Safinya	University of California at Santa Barbara
Peter Schultz	Scripps Research Institute
Nadrian Seeman	New York University
Douglas Smith	University of California at San Diego
Viola Vogel	University of Washington
Ulrich Wiesner	Cornell University
X. Sunney Xie	Harvard University

BESAC considered a number of workshop topics that were suggested by this charge. One involved the exploration of biomolecular materials, materials based on biological structures and principles but whose study and use encompasses research at the interfaces among the many disciplines enumerated in Dr. Decker's charge. As a result of the rapidly increasing interest in research applying the principles and structures of biological systems to the physical sciences, this BESAC workshop was held in San Diego, California, January 13-15, 2002. Mark D. Alper of the Lawrence Berkeley National Laboratory and the

University of California at Berkeley and Samuel I. Stupp of Northwestern University were co-chairs. Twenty-two leaders (Table 1) in a wide variety of areas linking biology, physics, materials sciences, and chemistry were invited to discuss progress in the field, define promising future directions and identify barriers to their pursuits. Thirty other participants attended. The agenda for the meeting is shown in Table 2. This report of the presentations and discussion at the workshop begins with an introduction followed by a discussion outlining the wide potential for research in the field, identifying molecules, structures and principles in biology that could reasonably be applied to solving problems important to the Department. This is followed by three sections discussing areas in this broad field the workshop attendees felt were of particular interest at this time, and also amenable for productive research, given our present knowledge of the underlying biology and the tools and techniques existing for their manipulation.

Table 2. Agenda.

Doubletree Golf Resort San Diego, 14455 Penasquitas Drive, San Diego, CA 92129		
Sunday, January 13, 2002		
7:30 pm	Speakers' Dinner	
Monday, January 14, 2002		
8:00 am	Welcome	Pat Dehmer, Iran Thomas DOE/BES
8:10 am	Introduction	Mark Alper
8:20 am	Workshop Organization	Samuel Stupp
Bio-Inorganic Systems — Angela Belcher , Chair		
8:30 am	Opportunities at the Biology/Materials Interface	Paul Alivisatos
8:50 am	Silicon Biotechnology: Proteins, Genes and Biomolecular Mechanisms	Daniel Morse
9:10 am	Control of Minerals by Organisms - Nanometers to Millimeters and More	Lia Addadi
9:50 am	Protein Control of Inorganic Materials	Angela Belcher
10:10 am	Towards a Tetravalent Chemistry of Colloids	David Nelson
10:30 am	Discussion	
Biomimetics and Biomolecular Self Assembly — Sam Stupp, Chair		
11:00 am	Self-Assembly of Cell Cytoskeletal Proteins	Samuel Stupp
11:20 am	Functional Materials Design, System Construction, and Computation. Adventures in Information Space & Complexity	Reza Ghadiri
11:40 am	Supramolecular Assembly of Cell Cytoskeletal Proteins	Cyrus Safinya
12:00 pm	Working Lunch	
1:00 pm	DNA Nanotechnology	Nadrian Seeman
1:20 pm	Functional 2-3 Dimensional Bio-inorganic Nanostructures	Chad Mirkin
1:40 pm	Discussion	

Biomolecular Functional Systems — Mark Alper, Chair		
2:20 pm	Signal Transduction and Active Transport at the Nanoscale	Viola Vogel
2:40 pm	Dendridic Macromolecules and Bioinspired Functional Nanoscale Assemblies	Jean Fréchet
3:00 pm	Engineered Protein Pores with Applications in Biotechnology	Hagan Bayley
3:20 pm	Providing Energy of Biomolecular Processes with an Artificial Photosynthetic Membrane	Thomas Moore
3:40 pm	Using Biology to Make New Materials	Peter Schultz
4:00 pm	Discussion	
6:30 pm	Working Dinner	
Tuesday, January 15, 2002		
8:30 am	Discussion	
Cell Engineering and Cells in Artificial Environments — C. Bertozzi, Chair		
9:00 am	NanoEngineering Biotextiles	Carlo Montemagno
9:20 am	Probing Biochemical Reactions: From Single Molecules to Single Cells	Sunney Xie
9:40 am	Supramolecular (Bio-) Functional Interfacial Architectures	Wolfgang Knoll
10:00 am	Artificial and Biological Machines	Carolyn Bertozzi
10:20 am	Structure and Shape Control in Hybrid Materials	Ulrich Wiesner
10:30 am	Manipulation and Visualization of Single Biomolecules: Applications in Materials Science and Biophysics	Doug Smith
10:40 am	Discussion	
12:00 pm	Working lunch	
1:30 pm	Individual Group Discussions	
4:00 pm	Group reports	Chairs
6:00 pm	Dinner	

1. Introduction

Mankind has made use of biological materials for millennia. Through most of this time, they were used as nature made them. Homes were built with wood, straw, leaves; ropes were fashioned from vines; tools were shaped from bone, antler, horn; living yeast was used to catalytically ferment alcohol or to leaven bread. More recently, mankind sought to extend his exploitation of nature by mimicking her principles, building, for example, bird-like wings to free him from the ground, and Velcro, reported to have been inspired by the mechanism by which burred seed shells stick to a dog's coat (Ball 1999).

For most of recorded time, however, nature's living systems were regarded as "special." It was not until the 19th century that the principle of the "vital force" was finally set aside and the concept of making biological molecules and employing biological processes outside the living cell was demonstrated. The extracellular synthesis of urea from cyanate by Friedrich Wöhler in 1828 demonstrated that "life" was not a requirement for the synthesis of molecules found naturally only in living organisms. [As Wöhler wrote to Berzelius, "I must tell you that I can make urea without the use of kidneys, either man or dog."] Years later, in 1897, Buchner demonstrated that the entire 12 step/12 enzyme pathway converting glucose to ethanol could proceed in extracts from yeast cells that had been killed and completely disrupted through grinding with sand.

The impact of these discoveries on materials science was immense – although not, to this day, fully exploited. Nature, through evolution – the extraordinary linkage of natural variation and selection – has, over billions of years, learned to develop thousands of extraordinarily sophisticated materials and chemical processes that can serve us well in our search for the advanced materials required to meet our demand for improvements in productivity, conservation, and safety. As in other fields, opportunities often lie untapped until the need and the tools to exploit them arise. In this area, biomedical applications came first driven by human health considerations. But the time for applications to the physical sciences is now here, and the past few years have seen a burgeoning of our interest in pursuing this exciting field of endeavor.

Despite the great interest over the past decade, successful and widespread use of biology in materials science remains a formidable challenge. The application of biological materials or of materials that mimic biological systems lags far behind our enthusiasm for them. Our ability to control chemical reactions with nature's exquisite sensitivity, to make polymers with precise molecular weight, or to assemble macromolecules into large-scale structures is at a very primitive, descriptive stage. We are even further from an understanding, much less the ability to imitate, the metabolic, catalytic, and regulatory processes that harness energy for vital processes and synthesize all vital substances.

There is however reason to be optimistic that we will, in the not too distant future, come to understand the very complex physics and chemistry of biological processes. A revolution has taken place in biology over the past few decades. We now have a vastly increased knowledge and understanding of the biochemistry and molecular biology of biological materials and how their unique properties arise from their structure. We now have a vastly increased arsenal of tools to

analyze, characterize and manipulate these systems and we now have theories and highly developed simulations to guide and interpret experiments. We are, as a result, developing a vastly increased ability to modify biological materials and processes for our needs and to synthesize, *de novo*, new materials that are based on biological principles.

As Wöhler and Buchner demonstrated, there are no mysterious vital forces governing the behavior of biological systems. They are, instead, governed by the coulomb and chemical potentials that govern everything in the universe. Quantum mechanics, Newton's laws, and thermodynamics determine the motions of particles, mass transport, and energy balance. Geometry influences how things can be packed. The difficulty is that we don't yet understand how these relatively simple forces can give rise to such complex phenomena. At the molecular level, we don't fully understand the relationships among structures, properties, and functions. We don't understand chemistry well enough to make these complicated molecules easily. At the level of molecular assemblies and sub-cellular components, we don't understand how they are organized and how they function collectively. The cellular level, with all of its interacting components, mass and energy flows, is beyond our ability to even describe completely.

Section 2 outlines the awe inspiring array of molecules, structures, and processes developed by living organisms and available for our use or modification. It is an impressive list, providing, in effect, an "existence proof" of what can be done and challenging us to exploit it. It must be remembered, however, that even this impressive catalogue does not describe the upper limits. Despite their many interesting and useful properties, biological materials and processes evolved under severe constraints that limited their development. For one, nature does not optimize structures and processes – evolution stops when it has made structures and processes that are "good enough" for their specific, or narrowly defined purpose and successfully adapt their host organism to its environment. Further, each structure or process is limited by the fact that it must "co-exist" and interact with the other structures and processes on that organism. On a more fundamental level, only a small number of the 92 naturally occurring elements have been used, and only small ranges of pH, temperature, and pressure have been explored. Often, the constraints do not prevent our use of these materials and processes. Clearly wood is a ubiquitous structural material, and fermentation is a well-developed industrial process. However, these processes are limited in their properties and applications of biomolecular materials. Wood cannot substitute for carbon fiber reinforced composites in airplanes, and fermentation by itself will not produce absolute alcohol. There is a real possibility that, once we understand the principles of nature's construction, we will be able to use these principles for our own design goals and "improve on nature."

The interest of the Department of Energy in biomolecular materials and biological processes is very broad. There is a need for lighter and stronger materials to improve fuel economy. There is a need for functional materials to control transport across membranes, to make separations and purification processes more efficient. There is a need to increase energy efficiency by using low temperature processes to make materials. There is a need for energy producing processes that can convert light, carbon dioxide, and water to high-density fuels and thereby decrease, at least to some extent, our dependence on fossil fuels. Finally the high specificity of biological reactions, producing little or no side products, and the inherent biodegradability of biological systems strongly suggest that these systems need to be explored by DOE for their potential beneficial effects on the environment.

Following the cataloging of some of nature's structures and processes in Section 2, we

discuss, in Sections 3, 4, and 5, three broad areas of research that emerged at the workshop as having significant discovery potential because of the breadth of knowledge that already exists in the underlying biology, because of the applicability of this knowledge to materials research in the physical sciences, and because of the promise seen in the preliminary research already begun. These three areas are:

- self-assembled, templated, and hierarchical structures, both bio-inorganic and bio-organic,
- the living cell in hybrid materials systems,
- biomolecular functional systems.

Finally, it should be noted that this report reflects the focus of the workshop on a discussion of materials and processes designed for nonmedical applications, consistent with the mission of the Energy Department as distinguished, for example, from the mission of National Institutes of Health (NIH).

2. What Does Biology Offer[1]

2.1 Introduction. Before addressing the specific question of a proposed research agenda for the Department of Energy, it is useful to view the full breadth of the potential impact of the field of biology on materials and chemical applications.

Therefore, we look here at the humbling catalogue of what organisms can do, and consider how our imaginations might allow us to harness, adapt, and mimic these capabilities for advanced materials or processes. ***This is, however, by no means a list of what can be accomplished. Some or many of these biological processes may not be reproducible outside the living organism. Some or many that are, may not be superior either in simplicity or effectiveness, to fully nonbiological solutions. Those that are, may take years or decades to develop.*** **On the other hand**, progress is surprisingly rapid. Speaking of his field of self-assembling electronic devices, Fraser Stoddart of UCLA has been quoted as saying ***"Something that I thought would …[for]ever be a dream in my lifetime now stands a good chance of becoming a technological reality before this decade is out"*** (Ball 2001).

2.1.1 Adaptation to the Environment. Organisms sense their environments and alter their properties to adapt to them. Shifting humans to high altitude results in the spontaneous increase in the production of the molecule 2,3 bis-phosphoglycerate. This metabolic product of glucose binds to the protein hemoglobin in the blood, causing a change in its shape to decrease its affinity for oxygen. This decreased affinity allows the hemoglobin to deliver more oxygen to the muscles and brain at each cycle through the blood stream, a critical adaptation to the lower oxygen levels at high altitude. This effect progresses over hours to days as we acclimatize to the altitude. Other responses to the environment, discussed below, can occur in less than a second (withdrawal from a hot surface) or more than years (evolving lungs for breathing air).

2.1.2 Amplification of Signals. Blood clotting, gene expression, and the activation of enzymes involved in the control of cellular energy production require the amplification, by many orders of magnitude, of signals carried by as few as one molecule, photon, electron, or ion. Amplification is a multi-step pathway. At each step one enzyme molecule activates a very large number of copies of the next enzyme in the pathway, which, in turn, activates many more copies of the enzyme catalyzing the next step. This sequential activation/amplification continues until the final product is produced at sufficient levels to achieve its macroscopic function.

2.1.3 Atomic Level Control of Structure. One of the most striking capabilities of living organisms is their ability to produce extraordinarily complex molecules with virtually error-free control of the selection and location of each individual atom. This is a critical ability because the structure, properties and function of molecules can depend sensitively on the type or position of a single atom. Mirror images of the same molecule, for example, can have drastically different properties. The converse also applies. Molecules can be designed at the atomic level for very specific structures and functions through atomic level control of design. This level of control has been a grand goal of synthetic chemists and materials scientists for years. In fact the challenge extends beyond the molecular to the systems level. Deer antlers, for example, do not need to be shaped after initial synthesis. Our control of structure at this level would allow "net shape

manufacturing," in which the product is produced in the shape required; thus, no expensive and wasteful machining is necessary.

2.1.4 Benign Processing. Biological processes are generally less hazardous and involve fewer toxic materials than their synthetic counterparts. For example, synthetic nanocrystals, which are of such great interest now, are often synthesized at very high temperatures with hazardous precursors. Organisms, on the other hand produce magnetic and semiconductor nanoparticles, often with great homogeneity, at room temperature and pressure. Teeth, shell and other ceramics are produced biologically under far more benign conditions than are synthetic ceramics.

2.1.5 Color. Certain birds (for example, peacocks) fish, snakes and butterflies appear colorful, but without synthesizing the "traditional" light absorbing pigments. Instead, they produce overlapping scales made of carbohydrate that impart iridescence by creating interference patterns. These give the appearance of different colors depending on the nanometer scale spacing, the thickness of the layers, the angle of viewing, the wavelength of the light illuminating them, the refractive index of the liquid between the layers. At near grazing angles, for example, only ultraviolet light is reflected, making the material virtually invisible. It should be possible to develop materials using similar properties that change their response to light in the presence of applied electrical or magnetic fields. Such materials could be used, for example, in "smart" windows that would reversibly reflect or transmit light, thereby controlling the heat load in buildings.

2.1.6 Combinatorial Synthesis. Vertebrates produce upwards of one hundred million different antibody molecules, each with a slightly different binding site. As a result, in virtually all cases, there are a few select antibodies with a shape that allows them to first bind to invading viruses or bacteria, and then, after a few days time, to arrange for the synthesis of many more copies of themselves to overwhelm the invader and ward off disease. In many cases, the current state of theory and modeling is too primitive to allow the prediction of the structure of a material from the desired mix of properties. The ability, through combinatorial synthesis to develop millions of extremely similar, but critically different structures, and the ability to rapidly scan them all for the desired properties, as is done in the immune system, can potentially save enormous amounts of time and expense in the development of materials. A variety of techniques can be employed. In phage display, variants of a given protein are presented on the outer surface of bacterial viruses. In inorganic "combi-chem", ink jet printers deposit nanoliter amounts of a variety of compounds in small wells that can be processed and analyzed in parallel. Battery manufacturers have recently announced that their next generation cells will contain materials discovered through combinatorial methods. Combinatorial synthesis has become a major focus in the search for better and cheaper catalysts for organic reactions.

2.1.7 Computation. The holy grail of computer designers remains the development of a device that mimics the human brain. Nothing comes close to its ability to store and retrieve information, often from apparently unrelated "data entry" events. Its ability to focus on a subset of the huge number of simultaneous stimuli and inputs it receives is also unmatched, as is its ability to "reason" by combining bits of information and weighting them appropriately as it sums their input into the solution of a problem. No device competes in use of spoken language. It is true that silicon computers are orders of magnitude faster than the millisecond biological processes of the brain but none match the brain's ability to process in parallel. It is interesting to note however, that the unit of biological information, the DNA or RNA nucleotide, occupies a volume of about one cubic

nanometer, and can be "accessed" for "read out" with exceptional specificity.

A number of biological materials are being studied for their ability to "compute", although clearly, nothing approaching a "biological computer" has been developed. The protein bacteriorhodopsin has been shown to have the capability for holographic data storage. Adleman's report in *Science* (Adleman 1994) sparked a great deal of interest for his use of DNA to solve complex mathematical problems. Shapiro and his colleagues at the Weizmann Institute have also reported in *Nature* (Benenson et al., 2001) on the use of DNA and its restriction and ligation enzymes in a system that can do computation. Clearly an understanding of the method of parallel processing and the mechanism of the self-assembly of the billions of neurons into a functioning brain will be of great benefit, with many, as yet undefinable, applications.

2.1.8 Conformational Change. Many cellular responses to external stimuli are based on the fact that proteins can alter their structure, and therefore their properties, in response to changes in their environment. This response is mediated through the binding of one or more molecules from that environment at a specific site on the protein. This binding event can cause one part of a ~10nm protein to move more than 1nm relative to another. Enzyme activity is regulated in this fashion, with the protein shifting from an active conformation to one that is less effective either in binding the substrate, performing the chemistry of the reaction, or both. The oxygen binding protein hemoglobin also exhibits conformational changes. On a shorter time scale than that for the increase in the concentration of 2,3 bis-phosphoglycerate (see Section 2.1.1), hemoglobin responds to a change in proton concentration. In low proton environments, as in the lungs, it assumes a conformation that enhances its ability to bind oxygen. In high proton environments, as in the muscles, however, these ions bind to hemoglobin and cause it to change shape, releasing oxygen for its use in the metabolic processes that create energy required for biosynthesis, muscle contraction and other functions.

2.1.9 Control of Interfaces. Organisms have learned to control and exploit a wide variety of interfaces with disparate materials. In biomineralized tissues such as bone, teeth, shell, inorganic materials of a variety of compositions are in direct contact with various organic materials. Proteins often direct the synthesis of the inorganic phases. These processes serve as models that could, for example, be applied to the controlled growth of mineralized phases for functional thin films or particulate applications (Klaus et al., 1999). Phage display and other such techniques have been used to identify proteins that bind specifically to semiconductor and other nanocrystals (Whaley et al., 2000; Whaley and Belcher 2000; Lee et al., 2002; Seeman and Belcher 2002). The crystalline ordering of viral particles could be used to direct the ordering of nanocrystals in arrays that might promote collective behavior. Biocompatibility, an inherent interfacial property of biomolecular materials is also becoming increasingly important, as we design hybrid devices that exploit the many cellular functions we cannot yet reproduce in the absence of the living cell (Section 4). Whole cells have been attached to surfaces in bioreactors for fermentations. Hybrid circuits with both semiconductor chips and synaptically connected neurons have been explored. Nerve cells from the snail *Lymnaea stagnalis* have been immobilized, through non-specific linkages, on silicon chips using polyimides such that a voltage pulse on the chip could excite the neuron (Zeck and Fromherz 2001). "Metabolic engineering" has been employed to alter the surfaces of cells to improve their binding to specific sites on non-living surfaces including metals, polymers, or ceramics, while the cells maintain their natural functions (Section 4.3).

2.1.10 Control of Polymer Properties. The properties of polymers depend on the type and sequence of their constituent monomers.

Increased numbers of types of monomers allow an increased variety of structures, properties and functions. Most synthetic polymers are made from a single type of monomeric unit – for example, styrene in polystyrene, ethylene in polyethylene. To achieve different properties, two or even three types of monomers can be incorporated into a single polymer. Alternatively, a number of different polymers can be blended. Even these systems are at best rudimentary analogues of natural polymers. Biologically produced nucleic acids, on the other hand, use four primary monomers (ATGC in DNA, AUGC in RNA), and a few others, in smaller amounts, that are methylated or acetylated. Proteins generally use 20 primary amino acids as monomers, some of which are modified after initial synthesis, for example to hydroxy-proline or gamma-carboxy-glutamate, to, in effect, create a far larger library. Polysaccharides are even more complex. Although some are made of a single monomer, others draw from many, varying in size, stereochemistry, charge, and attached functional groups, each affecting the properties of the polymer in a different way. Cyclic polysaccharides have been used for example to insulate photoluminescent polymer chains (Cacialli et al 2002). Recent research has expanded the number of monomers beyond those nature uses in both proteins and nucleic acids, allowing the incorporation of amino acids or nucleotides with an almost unlimited variety of sizes or with specific redox, optical, electrical, magnetic, and chemical properties. Equally important to the materials properties of polymers of biological origin is the fact that nucleic acids, proteins or many carbohydrates, are made to a precise, uniform length, giving greater control of properties alignment and crystallization. Naturally occurring biopolymers do have a limited number of different backbone structures, but research has progressed in broadening this range, further increasing the breadth of properties that can he achieved. Techniques are being developed to use these materials in very imaginative ways, for example, in a technology to "write" thin lines of proteins or nucleic acids on a variety of inorganic surfaces using the tip of an atomic force microscope as a quill, and a solution of the polymer as the ink (Demers et al., 2002).

2.1.10.1 Nucleic Acids. DNA and RNA are of course involved in the storage and use of cellular information, and, in the case of RNA, in catalysis and as scaffolding in supramolecular structures. These materials are found base-paired in double strands in the well-known "double helix" form of DNA or in single strands, which often, through the same mechanism, fold back on themselves and base-pair into precisely defined 3-dimensional structures. Their role in information storage and retrieval is an impressive one, with, as mentioned above, one bit of information occupying only one cubic nanometer, yet still allowing rigorous specific addressability (regulation of specific gene expression) and accuracy in reading. The DNA double helix can in fact serve not only as an information carrier but also as a structural material (Section 3.2.1), exhibiting the properties of a long, relatively rigid rod with a persistence length of approximately 50nm. Capitalizing on this, nanocrystals and complex organic groups with electronic or optical properties have been linked to individual single strands of DNA of defined sequence. These single strands then are base paired with other defined sequence oligonucleotides to align those functional groups in precise positions in space. Some of these structures have demonstrated energy transfer from donor to acceptor groups, presenting intriguing possibilities for electronic materials. DNA molecules with complex topologies have been formed into specific objects, nanomechanical devices and periodic arrays, with potential ultimate applications in nanoelectronics and nanorobotics. For example, DNA base pairing has been exploited in the fabrication of rudimentary structures, demonstrating and, nand, or and nor gates. Other devices, involving DNA single strands that compete

with each other for binding complementary strands demonstrate information manipulation (Seeman, 1999). DNA molecules with complex topologies have been formed into specific objects, nanomechanical devices and periodic arrays, with ultimate applications for nanoelectronics and nanorobotics. The precise structures of folded single strands of RNA, for example in transfer RNA, are critical to the role of these molecules in genetic information storage and transfer, but could also be exploited in using them in materials applications. Opportunities for the use of these materials abound, especially when the difficulties in producing large amounts of nucleic acids are overcome.

2.1.10.2 Proteins. Proteins fold into precise three-dimensional shapes, driven by their amino acid sequence. The range of properties that these polymers exhibit, or contribute to is enormous, including lubricity, adhesion, viscosity, stiffness, toughness, flexibility, optical clarity, and, in the case of, for example, aspartame, taste. A very large number of proteins and their functions remain undiscovered or uncharacterized. The emerging field of proteomics will, no doubt, result in the discovery and understanding of many of these, some performing novel, useful functions and others helping us understand how the structure of proteins determines their function.

2.1.11 Energy Conversion. Chemical energy powers organisms without the use of flammable fuels or high temperatures. Biological molecular motors involve the direct conversion of chemical energy into mechanical energy without the inefficient production of heat seen in conventional motors and engines. Much of this process, and also the use of energy for biosynthesis of cellular materials, involves the recycling of "energy carrying" molecules between their [incorrectly] so-called "high energy" and "low energy" states. In particular, the molecule adenosine diphosphate can be "activated" in the presence of metabolic energy through its binding a third phosphate group to make adenosine triphosphate (ATP). Removal of that group involves a significant negative free energy that can "drive" energy requiring processes. The photosynthetic sequence of light energy capture, production of "high energy" molecules, and the use of those molecules to produce metabolic and mechanical energy is one that, if duplicated, would revolutionize energy conversion. Chemical energy can also be stored as a molecular or ionic gradient across a membrane.

2.1.12 Enzymes. Enzymes accelerate reactions by up to 13 orders of magnitude at room temperature and atmospheric pressure. Most important is their exquisite specificity for both starting materials and products, and thus their synthesis of the desired molecules while producing no byproducts. The activity of enzymes can be controlled over several orders of magnitude through the binding of specific effector molecules. Selective activation of enzymes could allow control of complex, multi-component, specific chemical conversions such as the synthesis of fuels from carbon dioxide and water using sunlight or other energy sources.

2.1.13 Evolution. Materials ideally suited to perform in the environments for which they were originally designed are often poorly suited to new environments that have arisen since their design. Living organisms evolve constantly to allow them to survive and in fact thrive in new environments. One of the most frequently quoted (although recently challenged) cases involves the change in color of moths in England from white to black during the industrial revolution and back to white again after institution of pollution controls. The principle lies in what might be called error prone manufacture. Each unit (organism) manufactured (born) has a naturally occurring slight variation (mutation) from the norm in one or more of its thousands of characteristics. In some cases, this alteration leads to death or decreased fertility. In most

cases, these alterations are unnoticed in normal use (life), but when conditions change, those individuals with the particular mutation that improves their survival, and hence fertility in that new environment take over the population. In many cases, this process occurs over several generations, each one slightly different from its parents. Evolution has also been employed in the laboratory in the "maturation" and optimization of antibodies and other proteins where the continual production of variants and the selection of the "better" variants, has led to better functioning products (Yin 2001). Development of materials that themselves evolve is a very long term goal, perhaps beyond our grasp. However, the use of the principles of evolution to optimize materials, as in the antibody work, is an excellent example of biologically inspired materials science.

2.1.14 Extreme Environments. In general, biological systems are regarded as impractical for many applications because of their sensitivity to extremes of environment. Organisms have, however, adapted to live in the below freezing waters of Antarctica or the ocean depths, and in the near boiling conditions of hot springs or deep ocean thermal vents. Those living at the ocean depths are protected against exceptionally high pressures, those living in salt flats are protected from high osmotic pressure, cells that line the stomach are adapted to extreme acidity (pH 1) and others have been shown to be exceptionally resistant to ionizing radiation. In each case, specific protective mechanisms have evolved. Transferring these capabilities to organisms or systems that are employed for specific functions could protect them from these "biologically extreme" environments, although it must be kept in mind that what is regarded as extreme biologically is not at all extreme in conventional materials synthesis.

2.1.15 Hierarchical Construction. Biological structures are extraordinarily complex, far more so than synthetic systems, and their sophisticated properties reflect that complexity. However, to a great degree, their synthesis is far less complex, relying on sequential hierarchical construction principles. For example, the synthesis of collagen fibers, whose thickness can be measured in millimeters, can be described as a sequence of relatively simple steps starting with the association of groups of atoms and gradually building in complexity to generate advanced properties. The groups of atoms in amino acids are assembled in a linear polypeptide polymer through the DNA-directed, protein synthesizing machinery. This single peptide chain then folds with two others of similar structure into a triple helix collagen molecule. Collagen molecules than associate in a spontaneous but highly controlled process to produce fibrils, which associate in a similar manner to, eventually, produce the final, exceptionally strong collagen fibers. Each step along the path is programmed into the structure of the material, allowing, through kinetically and thermodynamically driven self assembly, the development of great structural complexity with minimal complexity of design.

2.1.16 Lightweight Materials. Living systems are inherently light-weight. They use, almost exclusively, carbon, hydrogen, nitrogen, oxygen, with lesser amounts of phosphorus and sulfur and very small amounts of others. They also produce low density hydrogels and related structures. Our ability to mimic living systems and develop lightweight structural and functional materials would lead to enormous reductions in weight and fuel use in, for example, automobiles.

2.1.17 Lubricants. Enormous inefficiencies, loss of function and expense result from inadequate lubrication of the contact surfaces of moving parts. Living systems must solve the same problems and have evolved molecules to lubricate joints, portions of the eye, and internal organ surfaces. These usually highly charged molecules could serve as a model for biomimetic lubrication.

2.1.18 Mass Production. Large scale production of materials can often be expensive. Organisms can, and in fact already, serve as factories. Their regulatory mechanisms also allow for the control of the levels of each molecule made. Certain proteins can be present in as few as 10 copies per cell. Alternatively, activators and "strong promoters" can lead to very high levels of production of defined products, often at amounts approaching tens of percent of total cell volume. Genes for the naturally occurring plastic polyhydroxybutyrate have been transferred into plants. Acres of farmland devoted to these transgenic plants could be inexpensively harvested and the polymer extracted. Genes for proteins are now being inserted into goats or cows in a manner that leads to their secretion into the easily collected milk, which, of course, can be "grown" for the cost of animal feed.

2.1.19 Materials Recycling. Biosynthesis is accomplished almost exclusively by enzyme catalysis of chemical reactions that make bonds between small molecules to make larger ones. These enzymes increase the rate of reactions that would be otherwise far too slow to support life. Other enzymes, produced by, for example, soil bacteria, and widely present in the environment, break these bonds, "bio"-degrading the molecules, also at rates far exceeding those of the uncatalyzed reaction. Energy input is, of course, required in one direction, usually the synthetic direction, since bonds are being made and entropy is being decreased. As a result, the degradative reactions usually proceed exergonically, and with relatively low activation energies. Easily degraded structures are of increasing importance. Industries are being required to be responsible for the entire product cycle. The manufacturing process will have to include recycling: cradle-to-cradle (new product), rather than cradle-to-grave responsibility will become the norm. The biological model could serve well.

2.1.20 Membranes. Cellular membrane are extraordinary multi-functional structures. They define the boundaries of cells and of sub-cellular organelles. Cell surface membranes help, through the use of embedded proteins and carbohydrates, to identify the cell to the outside world and receive signals from that world. They house transport systems, motors, rotors, energy transduction devices and exquisitely sensitive and selective sensors. They create non-polar compartments in the midst of a fully aqueous environment. They are self-healing, self-assembling, can grow as the cell it surrounds grows, and can split into two as the cell divides. Membranes are highly flexible and can adapt their shape to a variety of structures and also to perturbations in those structures as the cells progress through the various stages of their lives or perform their myriad functions. They are also quite robust, despite the fact that the individual component molecules are not covalently linked to each other. A great deal of effort has gone into the mimicking of the cell membrane and much success has been achieved. Artificial, self-assembled monolayers serve in a wide variety of efforts to study self-assembly or other membrane associated properties. Membrane mimics are made with artificial molecules, mirroring the self-assembling amphiphilic properties of membrane lipids, but incorporating greater rigidity through cross linking, or functionality through light absorbing chromophores, inserted channels (Section 5.6) or molecular recognition groups. Use of other molecular components allows for multilayered membranes with their own sets of properties.

2.1.21 Model Materials for Studies of Basic Materials Physics. Basic physical laws such as those of Newtonian mechanics, statistical mechanics and quantum mechanics provide our only rigorous foundation for understanding the properties of materials. However, factors such as complexity, nonlinearity, and many-body interactions tend to frustrate our ability to make connection between the microscopic physical laws and macroscopic materials properties.

Fundamental experimental studies of materials science must be aimed at elucidating the basic connections between physical laws and materials properties; but, as a practical matter, an experimentalist has to pick example or "model" materials to study. Biological materials can act as model materials that revolutionize such studies since they have properties that can currently be controlled to a far greater extent than synthetic materials. An example of this is in the field of polymer materials and polymer rheology. Half a century of theoretical and experimental research had been dedicated to understanding the connection between microscopic molecular and macroscopic materials properties of polymeric fluids without having the experimental ability to directly control or visualize the molecular dynamics. The field was revolutionized in the early 1990s by the introduction of techniques for directly visualizing and manipulating single DNA molecules using optical tweezers and fluorescence microscopy (Perkins et al., 1994). Use of DNA allows the preparation of a polymer solution in which every polymer in the solution has exactly the same length, and structure. Optical tweezers allow individual molecules to be mechanically manipulated and the forces acting on the individual molecules to be directly measured. Fluorescence microscopy allows the molecular conformation of individual polymers to be directly visualized. For the first time this allowed rigorous testing and refinement of molecular theories by direct comparison with molecular measurements (Smith et al., 1999; Babcock et al., 2000).

2.1.22 Molecular Recognition. Many biological macromolecules have the extraordinary ability to recognize specific other molecules in an environment containing large numbers of very similar structures. This selectivity is the basis for the functioning of the cell membrane as a biosensor, the self-assembly of collagen and other structures, the structure and replication of DNA as a genetic or structural material, the specificity of enzymes for reactants and products. The mechanism of this recognition involves several factors. The first is a geometric fit similar to that between two pieces of a jigsaw puzzle. Individual atoms in a molecule are in such precisely defined positions that each molecule assumes a defined shape, which is complementary to the shape of the molecule to which it will bind. In addition, groups of opposite charge line the surfaces of two bound molecules. Other binding forces including the so-called hydrophobic interactions and polar interactions (hydrogen bonding, salt bridges) are also found along the interface. Complexity is added by the fact that proteins are not fixed in shape but rather are quite flexible, rapidly and spontaneously shifting among a set of possible conformations. Generally, only one conformation of a given protein binds a particular target molecule, and the binding of that molecule locks the protein into that particular configuration. This is a powerful tool in protein design and function but it does add complexity to the task of designing proteins for specific molecular recognition functions.

2.1.23 Motors, Rotors, Pumps, Transporters, Tractors, Springs, Switches, Ratchets. Cellular function depends to a great extent on a variety of motors, rotors and related devices. These transport systems use chemical energy to move ions, macromolecules, organelles, chromosomes, and even whole cells. A newly characterized motor packs DNA into the heads of viruses as they are produced in cells. Using a motor generating 60 piconewtons of force to counter an internal pressure 10 times that of a champagne bottle (60 atm), the DNA is packaged, perhaps to provide the "spring" to release it into the next cell that is attacked (Smith 2001). The enzyme ATP synthase, and the various components of the electron transport chain, which are responsible for the production of most of the cellular ATP from ADP and phosphate, are themselves molecular pumps and motors. Energy released by the oxidation of nutrients is used by the electron

transport chain to pump protons across the membrane. These protons "flow" back through the synthase, causing it to rotate and drive the synthesis of ATP. Thermodynamics requires that this process can be run in reverse. Thus, the release of energy accompanying the conversion of ATP to ADP and phosphate causes the rotation of the protein and the pumping of protons out across the membrane. The synthase converts chemical to mechanical energy (or the reverse) with nearly 100% efficiency (Yasuda et al., 1998).

Another rotational motor imbedded in the cell membrane drives the high-speed rotation of flagella, the tails of bacterial cells that enable them to "swim" towards nutrients and away from repellants. Linear motors such as myosin, kinesin, DNA polymerase also move within the cell using the direct conversion of chemical energy to mechanical energy. To a great extent, the nature and structure of the molecules involved in these functions are known. Some could replace micro-mechanical devices now made by lithographic techniques. For example, single steps in the motion of

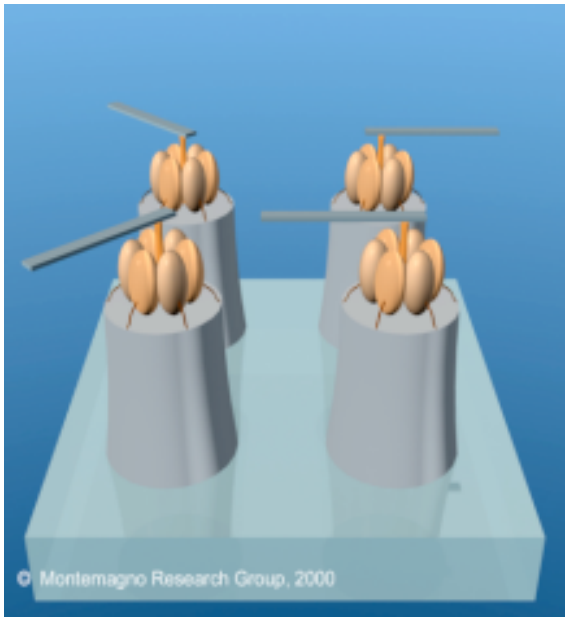

Figure 1. Depiction of an array of hybrid nanodevices powered by F1-ATPase. Soong et al., 2000.

these motors have been analyzed and resolved to be on the order of 10 nanometers. Some manipulation has also been achieved. Rigid rods have been attached to the synthase and observed in a microscope to rotate in a circle, driven by the rotation of the protein (Figure 1). Techniques, such as single molecule spectroscopy allow the study of individual motors and rotors.

Biomolecular ratchets and springs store or release energy and rectify motion. The energy for springs is provided by hydrolysis of a nucleotide or binding of a ligand. Ratchets are powered by Brownian motion in polymerizing filaments. For example, the spasmoneme of the vorticellid contracts upon exposure to calcium, by 40% of its length (2.3 mm) in milliseconds, at a velocity approaching 8 cm/sec. No energy source is required (Amos 1971, Moriyama et al., 1999, Mahadevan and Matsudaira 2000). Small changes in protein subunits, amplified by linear arrangements in the filaments can lead to structures that store energy and then release it on demand, creating movement. Other structures act as molecular switches or "dimmers". For example, enzyme activity can be controlled on a continuum from full activity to complete inactivity by a variety of effectors (Zhou et al., 2001). Ion channels can be controlled over the full spectrum of activity by an equally diverse group of ions and molecules. Some have speculated on the interfacing of millions of these efficient biological devices to produce "macro" levels of power. Nature again shows the way, bundling actin and myosin molecules to make muscles. Jiménez and colleagues (Jiménez 2000) have mimicked the system by designing a molecular assembly in which two synthetic "filaments" mimicking actin and myosin slide along each other to contract or stretch. Others have done pioneering work to achieve self-assembly of these motor systems from their components and, for example, to control the motion of kinesin using defined micro-butule tracks (Nêdêlec et al., 1997; Hiratsuka et al., 2001).

2.1.24 Multi-Functional Materials. Many biological materials perform several functions simultaneously. Skeletal plates of calcite

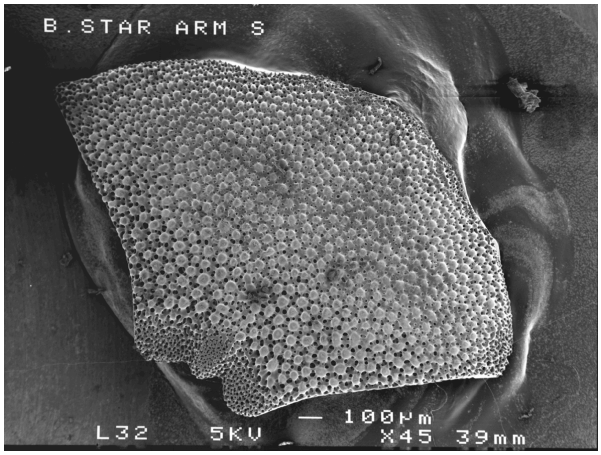

Figure 2. Scanning electron micrograph of a dorsal arm plate from a light-sensitive brittle star. The arm-plate, which is made up of single crystal calcite, is decorated by myriads of microlenses that concentrate light on the underlying nerve bundles. Courtesy of L. Addadi.

(calcium carbonate) on the arms of the brittlestar ***Ophiocoma wendtii*** provide not only structure and protection but also contain thousands of lenses, which serve to focus light on nerve receptors beneath, somewhat like a compound eye (Figure 2). The plates, which are clear, birefringent single crystals of calcite, allow the organism to detect changes in light intensity on its body surfaces and change color from night to day (Aizenberg et al., 2001). Multifunctional scales coat the wings of butterflies and simultaneously aid in the aerodynamics of the wing, assist in temperature control, and provide colors and patterns on the wing, serving as an avoidance defense mechanism against predators.

2.1.25 Organic Synthesis. Living systems are perhaps the ultimate factories. Extraordinarily complex and large materials are synthesized from an exceptionally small list of simple precursors. The key to this lies in the use of a complex of interlinked metabolic pathways, charting a sequence of comparatively simple organic chemical reactions that convert the starting materials, through as many as 20 reactions or more, to the product. Enzymatic control of the reactions insures that no byproducts are produced. A large number of common intermediates insures that a minimum amount of duplication is involved.

2.1.26 Optical Systems. Organisms have developed a variety of optical systems, not the least of which is the eye of higher organisms. As described above (2.1.24) ***O. wendtii*** produce calcite micro lens arrays. This concept has already been adapted for directional displays and in micro-optics. Opals and butterfly wings also manipulate light in a manner that could serve as a model for photonic systems (Sambles 2001).

2.1.27 Self-Assembly. Perhaps the most powerful of properties of biological systems is their ability to assemble individual molecules into large, complex, functional structures. The information for the assembly lies in the molecular structure of the components, their geometry and their precise alignment of hydrogen-, ionic-, polar- and "hydrophobic" bonding groups (Section 2.1.22). Membranes (Section 2.1.20) assemble themselves because the lowest energy state of their component amphipathic molecules is the membrane lipid bilayer. Proteins (Section 2.1.10.2), composed of multiple individual subunits, self-assemble, aligning the individual subunits precisely with respect to one another to perform a function as dependent on the relationship of the individual molecules as a watch is dependent on the meshing of its gears. The ribosome is self-assembled from 80 proteins and 4 pieces of RNA. Hierarchical construction (Section 2.1.15) is simply a process involving sequential, increasingly complex, self-assembly steps.

Viral self-assembly is another remarkable example. The bacteriophage phi29 has about 20 genes coding for proteins out of which the virus is constructed and a variety of other proteins and RNA which transiently aid that construction process. The phage's capsid, or shell, self-assembles about a molecular scaffold which later disassembles itself. The empty capsid is then filled with DNA by the action of a transiently formed molecular motor powered by ATP (Smith et al., 2001). Once the DNA is packaged, the motor falls apart. While this assembly normally occurs inside the cell, molecular biologists have identified and cloned all of the genes of the proteins needed

to assemble the virus from purified components ***in vitro*** (Guo et al., 1986). In a 20 microliter test tube reaction each of 100 billion DNA molecules (end to end, 400 miles of DNA) are packaged into 100 billion viral capsids in approximately 5 minutes. These viruses are all infectious and each one has the potential to make an infinite number of copies of itself by repeating the process. A variety of successful attempts at self-assembled structures mimicking these sorts of biological systems have been described (Ball 2001).

2.1.28 Self Healing, Repair, Damage and Fault Resistance or Tolerance. Living organisms are, of course, capable, to varying degrees, of self-repair and healing. Simple organisms, or very young organisms can replace entire sections of their bodies. More complex or older organisms are more limited in this, although the human liver, for example, can regenerate itself even after much of it has been removed. On a more molecular level, membranes have the capability to repair holes, and proteins can refold after being denatured. DNA polymerase, which copies DNA, reviews its own work, and excises errors, replacing them with the correct base. The application of this principle to non-living materials and devices is almost as difficult to imagine as it is to calculate the energy and cost savings that would result if it could be achieved.

2.1.29 Signal Transduction. Living organisms detect changes in their chemical and physical environment and rapidly respond to them. The process involves molecular recognition and a resulting conformational change in "receptor molecules." This change in shape can lead to enhanced or reduced enzyme catalysis, or transport of ions or molecules. Signal amplification (Section 2.1.2) is usually a critical component, as these systems often transduce a change in tens or hundreds of molecules into a physiologically significant response. Many groups have mimicked this stimulus-driven conformational change (Krauss 2000).

2.1.30 Smart Materials/Sensors. Smart materials are those that alter their structure and properties in an almost immediate response to a change in their environment, thus, on a much shorter time scale than adaptation and evolution. In many cases these are reversible changes, and in some cases, the extent of the change is controlled to reflect the degree of change in the environment. In some cases, individual molecules are 'smart' (Section 2.1.8). In other cases a system of molecules responds. An individual molecule of the enzyme glutamine synthetase, for example, monitors the level of nine independent factors in its environment simultaneously and adjusts its rate of catalysis on the basis of a summation of these positive and negative inputs. On a more complex level, entire metabolic pathways respond to single molecules, such as hormones, growth factors or pathway products or other metabolic intermediates. In some cases the response to the stimulus is a functional one. In other cases, it is simply a record of that stimulus. In cells, "analytes" are detected by "surface mounted" protein and carbohydrate receptors whose structure is defined at the level of each atom and the spatial relationship between those atoms, allowing molecular recognition, with high affinity and specificity. In most cases, these responses are triggered by alterations in the shape of the receptor resulting from the binding of its target.

The ability to change chemical activity, color, electrical conduction, mechanical properties in response to a change in the environment would be quite valuable in a variety of materials applications. Perhaps the most advanced smart materials at this time are sensors, which translate their detection of defined targets into measurable optical, electrical or mechanical signals. Biological systems provide a very high standard to attain, demonstrating discrimination, sensitivity and adaptability that can approach the detection of single molecules or photons. Dogs can distinguish individual humans by smell. Honeybees have been trained to detect

explosives at levels as low as tens of parts per trillion (Rodacy 2002). Other organisms have exceptional senses of taste, touch, hearing, sight. The Melanophila beetle senses infrared emission of a forest fire at a distance of 50 kilometers. A dish-like organ under its wings contains structures that are tuned to absorb the appropriate wavelengths and then increase their volume and apply pressure to structures that trigger nerve impulses. Vipers, pythons and other snakes identify warm blooded targets objects by detecting the minute differences in radiated temperature, with exceptional discrimination levels. As miniaturization progresses, and micro- and nano-scale devices are developed, sensors for extremely small forces will be required. Recent work (Liphardt et al., 2001) has shown that the unfolding of single strands of RNA, which involves only the breaking of hydrogen bonds, can be measured using optical tweezers, allowing speculation that these measurement tools could be adapted as (nano-) mechanical sensors. Cantilevers have been shown to allow the detection of the change in energy resulting from the binding of very small numbers of molecules, again allowing speculation about new, ultrasensitive mechanical sensors. Although investigators have had difficulty adapting these and other such systems to working devices, it cannot be assumed that these problems will not be resolved.

2.1.31 Structural Materials. Nature's materials have exceptional strength and toughness (Smith, B.L. et al., 1999; Hinman et al., 1993; Waite et al., 1998; Curry 1977; Jackson1988). Spider dragline silk, synthesized primarily from carbon, hydrogen, oxygen and nitrogen, has long been envied for its fracture energy which is two orders of magnitude greater than that of high tensile steel (Hinman et al., 1993; Heslot 1998). Its use has been hampered by the availability of the material, (which is more difficult to obtain than the weaker material from easily grown silk worms.) Recently, however, researchers have been able to splice portions of the genes for spider silk into cells from a variety of other organisms that can be grown in tissue culture (Lazaris et al., 2002). Systems also exist which would allow the transfer of the genes to goats that have been bred to produce the silk in their milk. The proteins produced are not yet identical to natural silk; synthesis of only one of the two proteins has been achieved, and even that one is produced in a form that is shorter and weaker than the natural product. Unfortunately, we do not yet fully understand the structure of the silk proteins, the mechanism by which the spider processes the proteins into fibers, or the techniques required to manipulate the extremely long pieces of DNA that code for the large silk proteins.

Other natural structural fibers such as collagen and keratin, each with their own exceptional properties could find applications if their synthesis were possible. Research is also progressing in the use of synthetic materials to mimic the biological models, for example hydrogels that can reversibly bind water to mimic the ability of collagen to absorb shock.

Extensive work has been proceeding for decades to improve our understanding and ability to mimic the "hard" biological structural materials such as bone, teeth, shell, which have exceptional combinations of mechanical properties and light weight. Abalone shell, a composite of $CaCO_3$ and organic polymers is 3000 times more fracture resistant than a single crystal of $CaCO_3$ (Curry 1977; Jackson 1998) and artificial composites of ceramics and adhesives are also inferior to the natural material (Smith B.L. et al., 1999; Almquist et al., 1999). Mineralized components are, as are the organic components, usually made of a small group of simple compounds, in this case hydroxy apatite or calcium carbonate or phosphate. They do, however, form a wide variety of structures with these building blocks, thus achieving a wide variety of properties which can perform a wide variety of functions. This variability in crystal structure arises because the organic protein phase controls the crystal structure of the mineral and a variety of such

structures are possible. The synthesis of the silica needles in a marine sponge, for example, is achieved by the enzymatic condensation of silicon alkoxides. Enzyme molecules aligned in linear repeated assemblies in the form of a rod, allow the deposition of the mineralized spines of defined shape around them (Morse 2001).

2.1.32 Systems. On a larger scale, the performance of biological systems often exceeds that which is attainable with current technology. Shark skin, for example, exhibits better hydrodynamic behavior than polished surfaces, an effect attributed to the organization and structure of the surface. It has served as a model for drag reducing coatings (Bechert, D., in Ball 1999) (Figure 3). Lotus leaves are remarkable in their ability to reject dirt. Their fine surface roughness prevents tight binding of dirt particles (and even glues), which are then easily washed away by water which is itself repelled by the waxy coating. Structural surfaces on insect legs allow for reversible adhesion to surfaces as do the microscopic setae on the feet of geckos, which can hang upside down and on vertical walls depending on van der Waals bonds (Autumn et al. 2002). Valves in veins are designed to allow blood flow in only one direction; flow in the opposite direction forces the two flaps of the valve together, closing the channel. Yu and coworkers (Yu 2001) designed a hydrogel valve that mimics these check valves and could find use as an actuated control in microfluidic systems.

2.1.33 Template Directed Synthesis. Much of biological synthesis occurs through enzyme catalyzed reactions, with the great specificity of these catalysts providing the high level of efficiency and minimization of byproducts. Equally high fidelity is achieved through templated synthesis, with the product produced through its specific match to a preexisting model. DNA and RNA synthesis uses the sequences of bases on an existing single strand to determine the sequence of bases to be organized in the daughter strand.

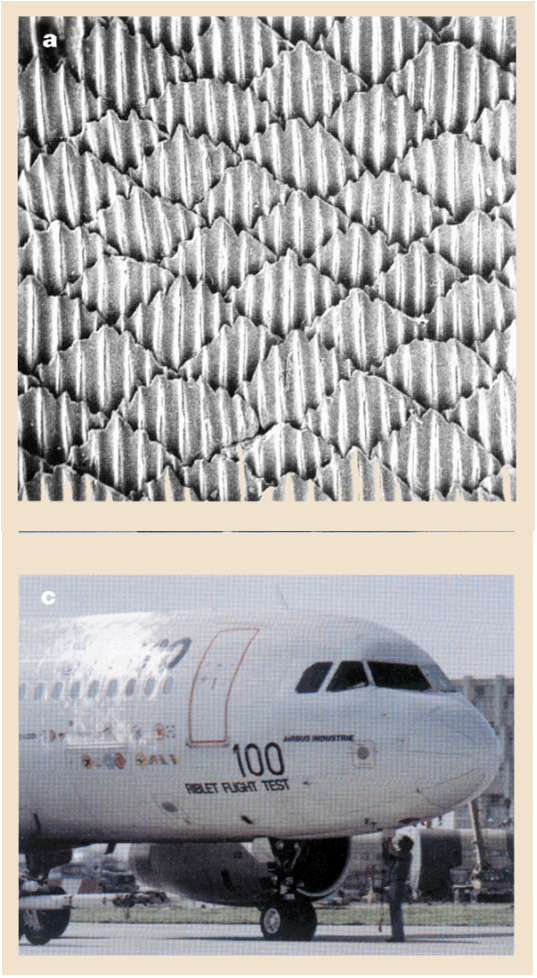

Figure 3. The riblets on shark skin (top) provided the inspiration for modeling studies of the drag reduction they confer, and eventually led to trials on an aircraft coated with a plastic film with this same microscopic texture (bottom), where an up to 8% reduction in drag was observed. Ball 1999.

Deposition of mineral phases is directed by the shape of the underlying proteins. This synthetic strategy could be valuable in the

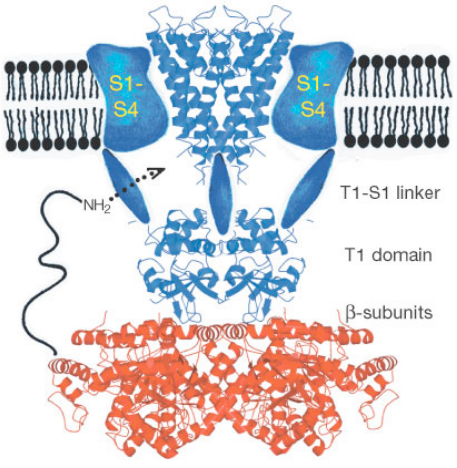

Figure 4. Model of a voltage-dependent K^+ channel. Zhou et al., 2001.

synthesis of many other types of materials provided that the design of the appropriate template can be achieved.

2.1.34 Transport Systems. Living organisms must transport a wide array of molecules and structures both in and out of the organism or its constituent cells, or to various specific locations within these organisms, cells or organelles. Membranes are extraordinarily selective in allowing materials to penetrate. In some cases, even the hydrogen ion is prevented from passing. On the other hand, membranes can develop systems to specifically allow the passage of molecules of choice (Figure 4). In many cases these "channels" open or close in response to either voltage changes or the presence of other molecules. In other cases, chemical or voltage gradients are used as energy sources to drive transport against a concentration gradient (e.g., Gulbis et al., 2000). Separations technologies would benefit greatly from these capabilities.

3. Self-Assembled, Templated and Hierarchical Structures[2]

3.1 Introduction. Nature, through the course of evolution, has developed techniques to construct increasingly complex systems, reaching her most glorious achievement in the living organism and, in particular, the human brain. Perhaps as impressive as the product itself is the means employed to achieve it. Nature begins with a surprising small set of building blocks, modifies some, produces variants of others, and then organizes tens, hundreds, thousands or millions of them, in most cases in precisely defined three-dimensional functional structures. To accomplish this, it uses the processes of self-, templated-, and hierarchical assembly, manufacturing techniques that are driven only by the laws of kinetics and thermodynamics acting on the structural and electronic properties of the assembled building blocks. It is as if all the parts of an automobile were thrown in a box and, within minutes, the fully functioning car drove off – on its own.

The long term goal of the study of biomolecular materials is the development of systems employing or based on biological processes that function independent of the living organism. Construction is a critical issue. At the nanoscale we cannot manipulate building blocks one by one, placing each in its required location relative to the others. (Techniques have been developed to use scanning probe microscopes to manipulate and arrange individual atoms one-by-one (e.g., Eigler and Schweizer 1990; Avouris et al.,1996) but these cannot, at least now, push molecules or structures into place, or do more than one at a time. Thus, the concept of using nature's self-assembly principles outside the living organism to construct materials, structures and devices in non-living systems has captivated imaginations for centuries. Now, however, with our newly achieved understanding of these biological processes, and the structure/property/function relationships of the building blocks we wish to use, we can reasonably hope to achieve this goal.

The building blocks in this process can be biological molecules or abiological mimics of them. In the first case, we take advantage of the inherent self-assembly "guides" that nature has built into these molecules. In the latter case we take advantage of the synthetic skills of the organic chemist, allowing us an almost infinite variety of structures and therefore potential properties and functions. Taken together a linkage of chemistry and biology can provide multiple avenues and opportunities to build assembled systems that can be used to advance energy production and storage, photonics, electronics, composite design, catalysis, diagnostics, and computation. In some cases the properties and eventual functions can be predicted from the choice of building blocks and the nature of the self-assembly process. In other cases, we see only a set of unique new structures whose functions remain to be discovered, as have so many before, by serendipity playing before the prepared mind.

The key to self-assembly lies in the structure of the building blocks. They must accomplish two tasks – their desired functionality and the ability to link to other components in a predetermined fashion. The breadth of possibilities is breathtaking. First, nature provides us with a wide choice of length scales. Amino acids (for proteins), nucleotides (for nucleic acids), sugars (for carbohydrates) and simple lipids (for lipid assemblies) have dimensions of the order of nanometers. Proteins, carbohydrates and complex lipids are one to two orders of magnitude larger.

DNA, viruses, liposomes, cellular machines, while larger still, are orders of magnitude smaller than micrometer size cells and organelles. The power of variability at each of these length scales is also easily seen. The properties of polymers depend on their length and precise sequence. 4^{20} distinct 20 base oligonucleotides (DNA or RNA) can be made using the four major nucleotide monomers. 20^{20} peptides of that length can be constructed from the constituent amino acids. Orders of magnitude more oligosaccharides can be made from their many sugar monomers, many arising from the fact that these polymers can be branched at any of a large number of sites. Moreover, as mentioned above, one can easily modify nucleotides, amino acids and sugars through chemical synthesis to make even these numbers appear small. An oligomer with virtually any desired chemical functionality, surface binding group, chromophore, redox-active moiety, catalytic agent, and spectroscopic probe is thus accessible. Finally, new backbones can be employed, while maintaining the uniquely biological properties of sequence specificity, defined length, and defined three-dimensional structure. Using these monomers or polymers as building blocks coupled with the ability to do automated synthesis, we have a "toolkit" of unmatched variability, allowing us to develop structures with an almost limitless range of functions. In all cases however, the structural and electronic needs of self-assembly must also be achieved.

Self-assembly can involve several types of materials.

- **Organic Materials.** Use of proteins or nucleic acids with or without synthetic polymers in novel structures based on their inherent H-bonding, polar, ionic, and hydrophobic self-assembly properties.

- **Organic-Inorganic Hybrid Structures.** Bone teeth, shell are highly-evolved organic/inorganic structures with exceptional mechanical properties. A great deal of work involves attempts to mimic these structures to achieve these properties in artificial systems. Other work involves the use of bio/organic structures to bind functional inorganic crystals in novel photonic or electronic devices.

- **Abiological Molecules Mimicking Biological Structures.** The principles of biological structure can be used as the basis for self-assembly of fully nonbiological materials. The sp3 geometry of carbon bonding can, for example serve as the conceptual basis for tetravalent colloid crystals. Similarly the hydrophilic/hydrophobic forces that drive water, protein, and membrane structure could be the basis for the supramolecular assembly of complex functional assemblies of a wide variety of nonbiological materials.

3.2 Organic Materials

3.2.1 DNA-Based Materials. The specificity of the intra- and intermolecular interactions of nucleic acids underlies the basis of genetic information. Self-assembly, in nature, of structures such as duplex DNA, DNA-RNA hybrid double helices, L-shaped tRNA's, and single stranded RNA hairpin and looped structures allows us to exploit these molecules as the basis for rationally programmed synthesis of complex three dimensional structures. One aspect of this "DNA nanotechnology" is predicated on the construction of DNA motifs that contain branched molecules or their generalizations. These motifs can then be combined into more complex structures exploiting "sticky ends" which allow the base pairing of two independent pieces of nucleic acid which have complementary, unpaired ends (Figure 5). These linkages involve the same self-assembly specificity as DNA or RNA duplexes and can thus be equally easily programmed into the primary structure of these molecules. The local

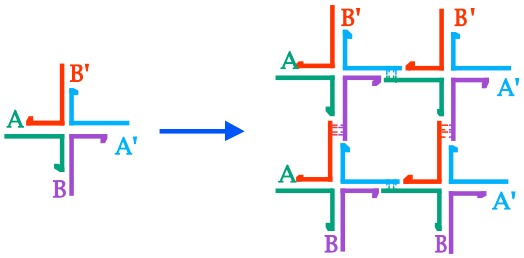

Figure 5. Linkage of nucleic acid oligomers using sticky ends. The unpaired regions of A' and B' are complementary to those at A and B, allowing spontaneous linkage as shown on the right. Adapted from Seeman 1982.

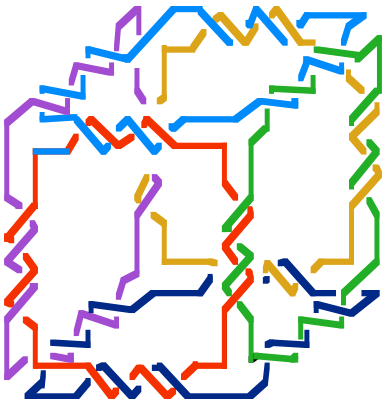

Figure 6. Schematic of a DNA cube. Adapted from Chen and Seeman 1991.

product structures that result when the two components cohere are B-DNA, the conventional double helical structure. This assembly has been shown to be capable of producing complex objects (Chen et al., 1991) such as, for example. a DNA cube, (Figure 6), periodic arrays (Winfree et al., 1998), and nanomechanical devices, such as, for example, the structures shown in Figure 7: one, (a) a device based on the B→Z right-handed to left-handed DNA transition, and the other, (b) a device based on the switching of base pairing between oligomers of identical sequences (Mao et al., 1999; Yan et al., 2002).

The first of these examples, the B-Z device (Figure 7a) operates by switching between the conventional right-handed B-DNA structures and the unusual Z-DNA structure. Z-DNA has two requirements, an appropriate sequence (usually something like $[CG]_n$) and proper solution conditions. The appropriate sequence is at the center of the device, and consists of 20 nucleotide pairs whose ends rotate 3.5 turns relative to each other when they switch to Z-DNA from B-DNA. The Z-state is achieved by addition of $Co(NH_3)_6\ Cl_3$, which facilitates its formation. Removal of this activator returns the system to the B-state. The dangling filled circles in the figure represent a pair of FRET dyes to monitor the transition.

The second example, the sequence-specific (Figure 7b) device is based on the differential hybridization topology of two pairs of strands, shown as green strands on the left (the PX state) and as purple strands on the right (the JX_2 state). The green strand pairs and the purple strand pairs associate with 1.5 turns

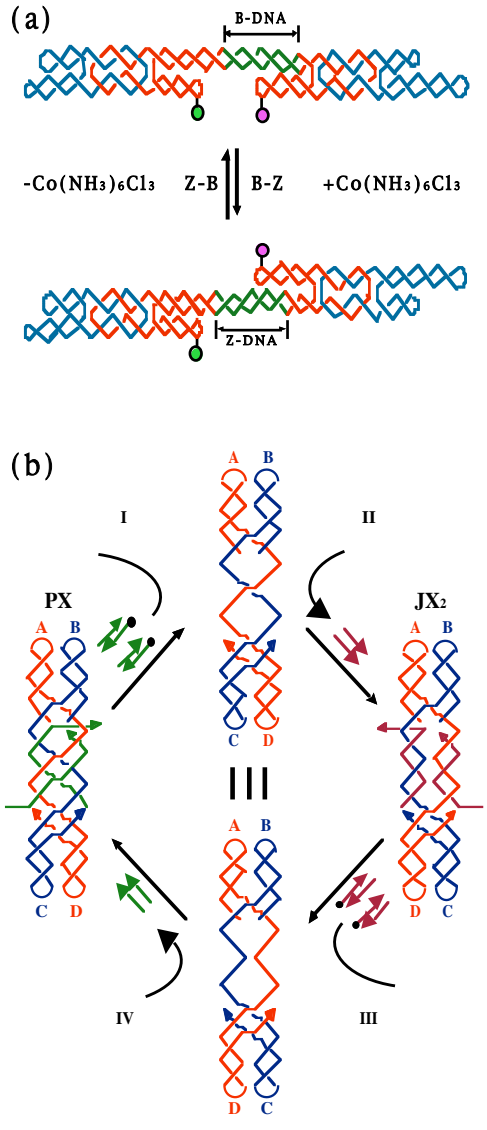

Figure 7. (a) A DNA device that flips between conformation as conditions convert the double helix from the B, (right-handed) to Z, (left-handed) DNA forms. Mao et al., 1999. (b) A sequence-dependent device. Yan et al., 2002.

each of their devices, but that they have extensions drawn horizontally in the figures. Starting with the PX state, if one adds the full-length Watson-Crick complements to the green strands, including the extensions, the green strands will be removed from the rest of the device. If the complements contain biotin groups (black dots) they can be removed from solution by streptavidin-coated magnetic beads. The unstructured intermediate at the top of the figure can be converted to the JX2 structure by the addition of the purple strands. The tops of the PX and JX2 structures are the same, but the bottoms are rotated a half-turn relative to each other. The JX2 structure can be converted back to the PX structure by the same procedure, as shown at the bottom of the diagram. The green and purple strands and the segments to which they are complementary can be varied, leading to a variety of devices that can be addressed individually.

DNA base/pairing specificity is the basis of the growing field of DNA-based computation (Adleman 1994). Methods to achieve this involve parallel methodologies that include DNA-based logic gates and computation by self-assembly (Winfree 1995; Mao et al., 2000). The importance of computation by self-assembly is that this area can lead to algorithmic self-assembly: whereas the incorporation of a large number of distinct molecular tiles, N, in an array would naively be seen to require N distinct tile types and the costs associated with their individual preparation, algorithmic assembly, in which the pattern of each surface leads to the appropriate linkage of the component pieces, can result in the production of similar arrays from far fewer tiles, thereby saving expense. Likewise, algorithmic assembly can ultimately lead to highly precise dimensions for constructs in one or more dimensions, in emulation of the ways in which biological systems make polymers of specific lengths (Winfree 2000).

3.2.2 Polymer-Organic Hybrid Structures.

While DNA and, for that matter, proteins, are remarkably valuable for their ability to provide encoded as well as directed self-assembly, synthetic polymers built on their model possess a great deal of versatility of function. For example, random copolymers obtained from a generic vinyl monomer and a dendritic macromonomer might provide a unique template along the polymer backbone capable of spatial assembly and organization of functional structures that have been linked to them. The preparation of a random styrenic copolymer with dendritic pendant groups has been demonstrated (Hawker 1992) as has the preparation of similar copolymers with pendant dendrons functionalized with easily removable protecting groups (Tully et al., 1999) for further synthesis.

Another approach to the precise assembly and organization of functional structures into a supramolecular entity involves their ligation to the periphery of shape persistent dendrimers. The preparation of several types of complex dendritic assemblies (Malenfant et al., 1998; Andronov and Fréchet 2000a,b) has been demonstrated. A rigid rod-like core in a dumbbell-type structure (Malenfant et al., 1998) with dendrons at both ends effectively acts as a fixed spacer between these dendrons, thereby preventing van der Waals contact between them. The shape-persistent properties of these and other dendritic assemblies can be exploited in order to install a well-defined number of structures on the same molecule, while preserving a minimum separation. This type of ensemble would provide unique insight into the properties of complex but well-defined nanostructured assemblies.

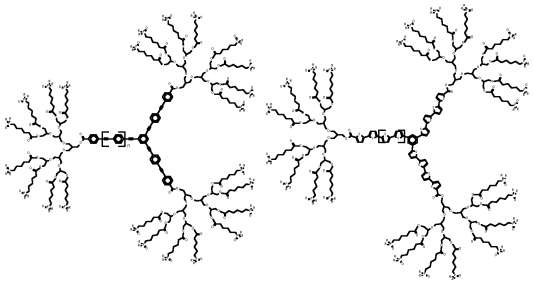

Figure 8. Dendrons with strong ligands linked via rigid rod-like oligomers. Malenfant et al., 1998.

Building blocks possessing dendrons with strong ligands at their periphery (Figure 8) can also be used. The focal point of the dendrimer can connect to a well-defined rigid rod-like oligomer (Martin and Diederich 1999) of the desired length. Two specific examples include an oligothiophene similar to those already prepared (Andronov and Fréchet 2000b) and a variety of phenylacetylene oligomers (Moore 1997; Tour 1996).

3.2.3 Lipid-Protein-Nucleic Acid Hybrid Structures. Studies of the biological membrane, a lipid-protein complex that performs a myriad of functions, (Section 2.1.20) have led to the development of supramolecular assemblies in reconstituted biological polymers which exhibit hierarchical self-assembly on length-scales spanning subnanometer to microns. Use of filamentous actin (F-actin) (a component of muscle protein) and membrane lipids has led to the assembly of a network of connected tubules on the mesoscale (>1 micron) (Wong et al., 2000). On the nanometer scale, these tubules consist of stacked (F-actin-lipid-F-actin) multilayers (Figure 9). Since the physical and chemical concepts leading to the self-assembly are general, by replacing the biological components with synthetic analogs (e.g., replacing lipids with diblock co-polymers, or replacing F-actin with synthetic polymers)

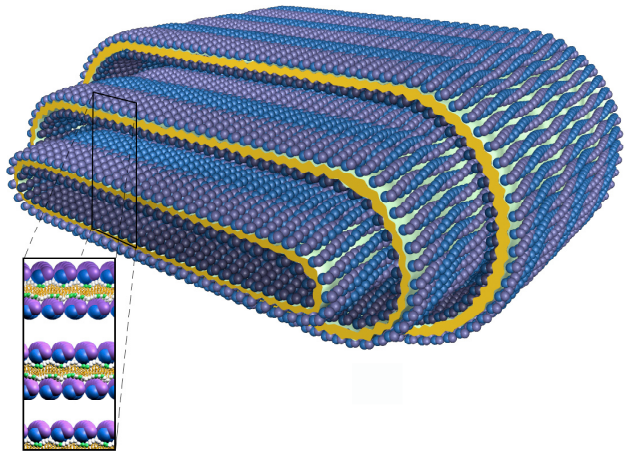

Figure 9. Complexation of F-actin and cationic lipids leads to the hierarchical self-assembly of a network of tubules; shown here in cross-section. Wong 2000.

"plastic" mesoscale tubules and tubule networks could be realized for possible applications in chemical delivery, nanoscale templating, and nanoscale mask development.

Cationic lipids (CLs) are currently used in clinical applications as vectors to deliver genes. For example, a significant fraction of worldwide clinical trials on developing cancer vaccines use CLs (Henry 2001). Recent work has found that CL-DNA complexes exhibit an unexpectedly large degree of order on the sub-to-many nanometer length scales. The two key CL-DNA structures are inverted hexagonal (H_{II}^{C}),where DNA is coated by inverted structured lipids arranged on a hexagonal lattice, and lamellar ($L_{\square}^{C}$), with alternating lipid-bilayer and DNA monolayers (Raedler et al., 1997; Koltover et al., 1998) (Figure 10).

The lamellar lipid-DNA complexes have novel materials applications. It was found that certain electrolytes (e.g. Mg^{2+}, Mn^{2+}, Co^{2+}...) can mediate unusual attractions between DNA

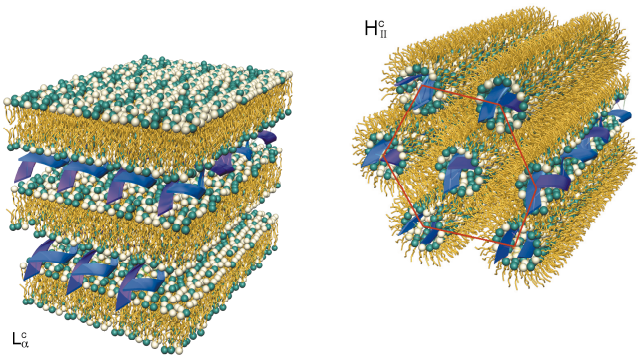

Figure 10. Structures of the lamellar (L) and hexagonal (R) complexes of DNA and cationic lipids derived from synchrotron radiation data. Raedler et al., 1997; Koltover et al., 1998; Koltover et al., 2000.

chains bound to lipid membranes, forcing the chains to form the most compact state of DNA on a surface, with the electrolytes trapped between the DNA chains (a precursor of nano-wires) (Koltover 2000). Potential applications of the DNA-lipid-electrolyte hybrid material are in high-density storage and retrieval of genetic information, and in parallel processing of nanometer scale wires. In many of these hybrid structures, the mechanical properties of the biological are also of great interest. The

protein components of viral capsids self-assemble as containers for the viral DNA or RNA. These structures are able to resist strong forces, even when loaded with tightly packed (and highly charged) strands of DNA and RNA. A theoretical understanding of the ability of capsids to resist being blown apart by these forces might suggest new ideas for making strong materials.

3.2.4 Biomolecular Materials in Microchannels. Self-assembly of biomolecules within confined geometries has the potential to control lengths on scales comparable to the physical confinement. Micro-channels are able to control ordering from the sub-micrometer to the many-micrometer scales. Several applications of microchannel-based confinement and alignment of macromolecules can be envisioned. First, highly oriented biopolymer samples can be used as templates for making inorganic materials with desired microporosity. Second, patterned surfaces consisting of ordered monodisperse defects organized in two dimensions may be developed with applications in templating and chemical delivery (Pfohl et al., 2001).

3.3 Organic-Inorganic Hybrid Structures. Nature has provided a truly inspiring set of examples of the use of self-assembly and templated synthesis in organic/inorganic composites.

These materials serve a wide variety of functions. Each has a complex shape, structure and organization that has been tuned for function over millions of years of evolution. In the course of this evolution, a number of fundamental problems in basic materials chemistry have been solved. The most striking is the ability to control self-assembly at several successive hierarchical levels, building structures step-by-step from the Ångstrom to the macroscopic length scale.

At the molecular level, these composites involve biological macromolecules that serve as templates for the synthesis of mineral surfaces, controlling their nucleation, growth, orientation, microenvironment and structure (Belcher et al., 1996; Falini et al., 1996; Cha 1999; Meldrum et al., 1993). The heart of the problem thus lies in the rules of molecular recognition between proteins and crystal surfaces, a subject that has been studied in some depth. Despite this work, however, detailed mapping of the active sites of interaction at organic-inorganic interfaces, in terms of protein sequences, structure and assembly is critical information that is just now starting to emerge and a complete understanding remains one of the primary challenges. Achievement here will then lead us to the subsequent challenge: the need to translate this information from natural systems to artificial materials with the same level of control.

In some cases, imitations will be very closely related to the structures of the natural products such as bone, teeth, shell. In other cases, artificial systems will be developed based on the two underlying principles: one, the rules of organization learned by studying these systems and two, the concept that the desirable properties of these two disparate types of materials can be combined in a material whose properties are inaccessible to structures containing only organic or only inorganic materials. Orthogonal to this approach is the line of research that separates the study of mineralized biological structures into those that are structural and those that are functional. Successful work has already been reported in all these areas (Colvin et al., 1992; Brust et al., 1995; Li et al., 1999; Mann Alivisatos et al., 1996; Mirkin et al., 1996; Brown 1992).

3.3.1 Natural Mineralized Tissues. Organisms use three conceptually different strategies to build their skeletal parts. Each could serve as the basis for biomimetic organic/inorganic synthesis. The easiest approach to the filling of a given shape with solid material applies when there is no

internal order or structure, i.e., when the material is amorphous. The most striking examples are found in silicaceous sponge spicules and diatoms. The recently determined sequence of a family of proteins that catalyze the polymerization of silica is providing information on the mechanism of assembly of this natural biological material. It is hoped that this understanding will be applied to the development of new synthetic pathways. For example, these findings, coupled with genetic engineering to identify the structural determinants responsible for the catalysts, have led to the predictive synthesis of a 'biomimetic' self-assembling peptide that simultaneously catalyzes and directs the structures of silicas and silicones from the corresponding precursors (Morse 2001). However, not all amorphous phases used by organisms, for example calcium carbonate, are stable under non-biogenic conditions. Here, specific macromolecules impose a structured microenvironment on the forming amorphous phase, continuously inhibiting the formation of crystallization nuclei. Thus amorphous materials must have some structure that, it is suspected, encodes their fate.

A second solution involves building a material as a single crystal, deposited from an amorphous phase that very slowly transforms in a controlled manner. The regulation of the transformation is crucial to the shape, structure and microtexture of the final material. Examples of single crystal skeletal elements are the calcitic sea-urchin larval spicules Figure 11 and adult spines. The striking calcitic microlenses found in the dorsal arm plates of light-sensitive brittle stars also belong to this group (Aizenberg et al., 2001).

A third solution involves building skeletal materials as polycrystalline assemblies formed inside self-assembled matrixes composed of biological macromolecules such as proteins and polysaccharides. Control of the microenvironment of crystal formation in these systems, which include bone, teeth and

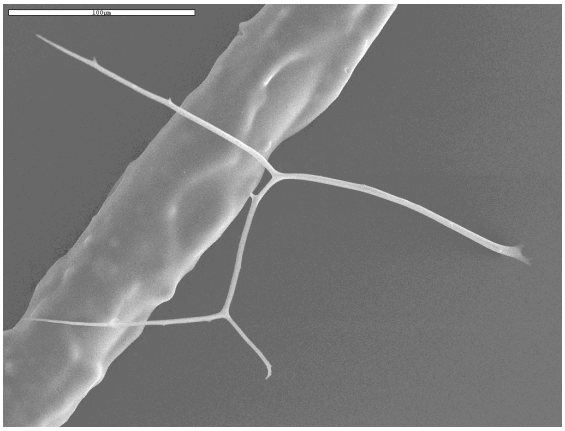

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Figure 11. Scanning electron micrograph of a larval spicule from *L. pictus* (age 72 hours) mounted on a glass fiber. The whole spicule is a single crystal of calcite. Of note is the elegance, sculpted shape and smoothness of the spicule, relative to the man-made glass fiber. Courtesy of L. Addadi.

mollusk shells, is crucial.

Each of these approaches provides inspiration for the design and construction of technologically important materials based on totally new concepts. Thus, they open opportunities beyond those arising by simply mimicking specific materials.

3.3.2 Polymer-Directed Mineralized Composites. Inspired by the biomineralization phenomena discussed in the previous section, various synthetic approaches toward organic-inorganic hybrid materials have recently been pursued. Among these, the study of amphiphilic polymer based polymer-ceramic hybrid materials is a particularly exciting emerging research area offering enormous scientific and technological promise. Through the choice of the appropriate synthetic block copolymer systems (e.g., poly(isoprene-*block*-ethylene oxide, PI-b-PEO) as well as ceramic precursors (e.g., organically modified ceramic precursors, ORMOCER[$), unprecedented morphologic control on the nanoscale is obtained in the block copolymer directed synthesis of silica-type materials (Figure 12) (Simon et al., 2001). As in natural systems, control lies in the unique polymer–ceramic interface; the hydrophilic portions of the block copolymers can be completely integrated into the ceramic phase. The resulting composites are then described as 'quasi two-phase systems'

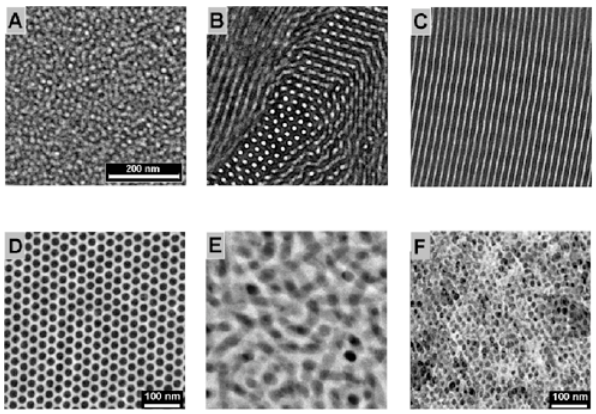


Figure 12. Transmission electron micrographs of block copolymer directed polymer-silica hybrids with different proportions of inorganic phase, each resulting in a unique morphology. Scale bars in B., C., and E., as in A. Simon et al., 2001.

allowing for a more rational hybrid morphology design based on the current understanding of the phase behavior of diblock copolymers and copolymer-homopolymer mixtures. The structures generated on the nanoscale are a result of a fine balance of competing interactions, another feature of complex biological systems. In addition to morphologies known from conventional block copolymer studies new phases may be discovered. For examples, the existence of a bicontinuous, cubic 'Plumber's Nightmare' phase was recently suggested for these block copolymer-ceramic hybrids (Finnefrock et al., 2001). This indicates subtle, not yet understood differences to conventional block copolymer systems and emphasizes the need for future interactions with theory and simulations. Nano-engineering of such hybrids towards applications has been demonstrated in the area of nano-objects of predetermined sizes, shapes and compositions for nanobiotechnology as well as mesoporous materials for separation technology and catalysis (Simon et al., 2001).

The potential of this approach for new materials lies in the versatility of the polymer chemistry as well as that of the sol-gel chemistry that can be exploited in the materials synthesis. Focusing on the polymer side, Figure 13 shows a complexity diagram for blocked (compartmented) macromolecules (Simon et al., 2001). It illustrates that when the number of building blocks along the chain is increased, the complexity of the resulting structures is elevated significantly. For the case of passing from AB diblock copolymers to ABC triblock copolymers this has already been demonstrated (Stadler et al., 1995; Breiner et al., 1997). A whole range of new morphologies has been found for ABC triblocks. The understanding of their detailed phase behavior is a current area of intensive research. It will be an interesting challenge to try to use those polymer systems as structure directing agents for the generation of polymer-inorganic hybrid materials. In this way, for example controlled access to inorganic nano-objects in the form of rings or helices should become accessible. This is only one possible research pathway, however, since the variety

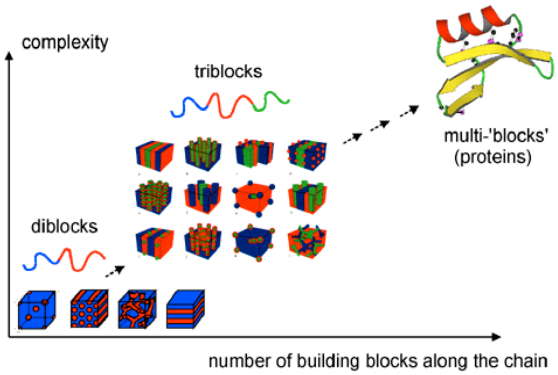


Figure 13. Complexity diagram of blocked macromolecular systems. Simon et al., 2001.

of the polymer chemistry as well as the inorganic sol-gel chemistry is only limited by one's imagination (Figure 13). Clearly, simple theoretical models of how proteins control the kinetics of biomineralization and affect the strength of resulting material would be very valuable.

3.3.3 DNA-Inorganic Hybrids. The use of DNA as a template for nanometer scale molecular organization was first proposed in 1982 (Seeman 1982). The self-assembly and organization of nanocrystals and nanoparticles on the molecular level using DNA followed (Figure 14) (Loweth et al., 1999; Mahtab 1996; Alivisatos 1996; Mirkin 1996; Elghanian 1997;

Storhoff 1999; Robinson and Seeman 1987).

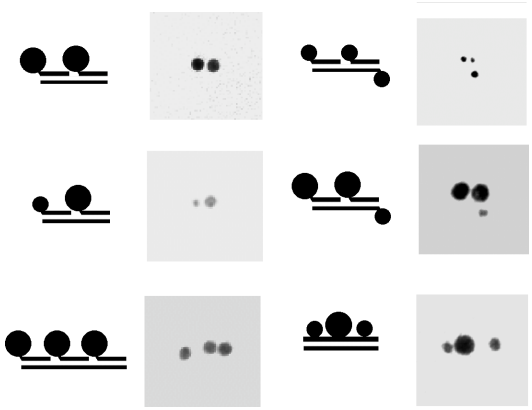


Figure 14. Nanocrystals aligned through their attachment to DNA strands. Alivisatos et al., 1996; Loweth et al., 1999.

These methods employed rely directly on hybridization of the two complementary DNA strands (Section 3.2.1). Because the DNA duplex acts as a rigid rod over comparatively long distances, the approach offers the potential for control over particle composition, particle periodicity, interparticle distance, and composite stability which can be achieved through careful selection, and often design, of nanoparticle and biological building blocks. This in turn would allow the development of a wide variety of devices such as sensors and spectroscopic enhancers and advances in microimaging methods (Mirkin et al., 1996; Alivisatos et al., 1996; Robinson and Seeman 1987). Although the strategy has been extensively developed for gold nanoparticles, it has been explored with several other nanoparticle compositions as well, including, Ag, CdS, core-shell inorganics, liposomes, and polymer particles. In fact, individual linkages are controlled to hybridize (join) or melt (release) at specific temperatures, through control of the relative proportions of A-T and G-C pairs.

The incorporation of other molecular electronic components into 3D DNA arrays could lead to new computation capabilities, both in terms of density and speed (Robinson and Seeman 1987). The incorporation of nanomechanical devices within such arrays could lead to the multiple states necessary for nanorobotics based on arrays of molecular scale devices with controlled mechanical movement (Yan et al., 2002). Algorithmic assembly in 2 and 3 dimensions, particularly when combined with nanodevices could lead to extremely smart materials. Similarly, the addition of functional protein systems to these complexes could lead to novel combinations of enzymatic or binding activities. It seems likely that extension of individual constructs from micron to larger scales will require hierarchical techniques, similar to Reif frames (Reif 1999). Ultimately, DNA also offers the attraction of self-replicating systems, although at this time self-replication of branched DNA molecules is likely to be somewhat oblique (Seeman 1991). It is also of some interest to understand theoretically the strength of these materials, and quantities such as the critical force for DNA pull-out which causes these structures to tear apart. The optimal overlap length for complementary base pair sequences and the effect of sequence heterogeneity also need to be understood.

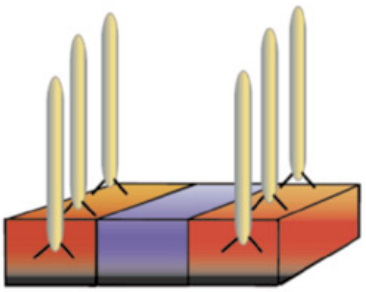
DNA scaffolds are also used to induce the dimerization of proteins. More recently, as mentioned, an AFM tip has been used to "write" DNA with attached nano-particles on surfaces of metals and oxides with precisely controlled feature size. The tip is coated with charged molecules to which the DNA sticks until transferred to the substrate. Feature size is controlled by varying the humidity and surface-tip contact time. Again, base pairing can allow specific binding of complementary DNA strands carrying desired active structures, to create circuits, photonic crystals, or catalysis (Demers et al., 2002).

DNA could itself be an electronic component. Its base pairing properties have also been used in studies of its potential role as a conducting material that can be used in targeted attachment of functional wires (Braun et al., 1998). Oligomers are attached to two electrodes and a DNA strand that can base

pair with both oligomers is hybridized to connect the electrodes. The DNA is then metalized with silver through ion exchange.

There also are many opportunities to use these materials to prepare high surface area "designer" catalysts, nanoelectronic devices, power structures (solar energy conversion and batteries), plasmon wires, and colloidal crystals for photonic applications (e.g. photonic band-gap structures, LEDs, lasers). However, to realize the full potential of these structures, we first must develop a firm fundamental understanding of the chemistry required to biofunctionalize the hundreds of different types of nanoscale building blocks currently available; the properties (electrical, mechanical, chemical, optical, and structural) of the almost infinite number of possible composite structures that can be prepared via the strategy; and the ways to modify anisotropic particles (e.g. rods, arrowheads, and prisms), in spatially well-defined ways.

3.3.4 Protein-Inorganic Hybrids. An alternative approach to study the rules of molecular recognition between biological (macro)molecules and organized inorganic surfaces has been illustrated in the use of combinatorial selection either through antibodies or proteins exhibited in techniques known as phage and bacterial display. These techniques are particularly useful in the search for organic molecules that interact with inorganics with important electronic or optical properties, because these appear infrequently in nature and thus have no evolved models. Large libraries of peptides with random amino acid sequences are produced and screened for binding to selected inorganic crystals. Proof of principle has been provided for both methods, identifying peptides that bind, with high specificity, a variety of semiconductors (Brown 1992; Brown 1997; Brown 2001; Naik et al., 2002; Lee et al., 2002) (Figure 15). An expansion of the use of these techniques, both in breadth (to many more types of materials) and in depth (to reach understanding of the interaction involved at the molecular level) is



(a)

(b)

Figure 15. Phage recognition of semiconductor heterostructures. In (a), phage (yellow), selected to recognize GaAs were placed on a substrate patterned with concentric rings of 1-□m GaAs lines (red) separated by 4□m SiO_2 spaces (blue). When viewed from above (b) the fluorescently labeled phage demonstrate their selective binding to the GaAs rings. Whaley et al., 2000.

expected. Use of peptides with multiple binding sites mimicking multidomain proteins such as fibronectin or, motor proteins such as kinesin could be exploited (Figure 15) to transport inorganic components of electronic structures to their positions and orientation in complex structures (Ball 2000). In fact, Schmidt and colleagues (Winter 2001) have linked CdS nanocrystals to neurons using custom designed, nanometer length octa-peptides that, at one end have sulfur containing cysteines to bind the semiconductor, and at the other end, designed RGDS sequences to bind the cell surface integrin a_V subunit on the neurons. It should also be noted that synthetic polymers can be used to organize inorganic functional nanostructures (Andronov and Fréchet 2000b).

3.4 Abiological Molecules Mimicking Biological Structures.

3.4.1 Colloidal Particles with a Valence. The richness of variety found in biological molecules is derived in large part from the fact that the central atom, carbon, is able to bind to four other atoms, in tetrahedral, sp3 geometries. Self-assembly and manipulation of micron or sub-micron colloidal particles have many potential uses, including particle based assays. and photonic band gap materials. Dense glassy and crystalline particle arrays (possibly involving DNA linker elements) usually display the high coordination numbers (Z = 12-14) characteristic of an isotropic pair potential. It is therefore of some interest to devise a means by which micron scale colloids could link with, for example, a four-fold valence, similar to the sp^3 hybridized chemical bonds on an Ångstrom scale associated with, for example, carbon, silicon and germanium. In contrast to close packed fcc and bcc colloid arrays, a tetravalent colloid crystal with a diamond lattice structure and appropriate dielectric constant is predicted to have a very large photonic band gap. Efficient creation of colloidal particles with a 1-, 2-, 3- or 4-fold valence would also find useful applications ranging from anchoring small catalytic particles to surfaces to linking a precise number of biomolecules such as kinesin, RNA or DNA to particles which could then be manipulated magnetically or with optical tweezers. Theoretical analysis of proposals to produce the equivalent of directional bonding on a micron scale (perhaps involving ordered states of surface active molecules on spheres) would be very useful here (Nelson 2002).

3.4.2 Hydrophilic/Hydrophobic Coatings on Inorganic Materials. Whitesides and his colleagues have reported a number of pioneering studies in which selected surfaces of inorganic materials such as gold plates (Clark et al., 2001) plastic "logs" (Oliver et al., 2001) and plastic "chips" with wires and imprinted devices (Gracias et al., 2000). are coated with hydrophobic groups and immersed in water to drive directed self-assembly into multicomponent structures.

We know the rules governing molecular recognition and assembly: geometry and the weak bonds, hydrogen, electrostatic, polar and "hydrophobic". What is far more difficult is the task of constructing molecules that not only perform their required function but also have structures that will self-assemble in precisely defined complexes. It is easier to make "sticky" structures that will link in random or multiple ways. Specificity is the problem but is fundamentally the same problem faced more broadly: how does one arrange the appropriate atoms in a structure that will perform in a totally predictable manner, both in self assembly and in function. Organic/inorganic self assembly presents a slightly different challenge. Here it is not the tailoring of two surfaces for interaction but rather of one, to control the organization of the other, again an area whose rules are yet to be written.

4. The Living Cell in Hybrid Materials Systems

4.1 Introduction. Materials transformations are a central function of living organisms. Bacteria, for example, can convert the simple six carbon sugar glucose, in the presence of appropriate ions such as sodium, chloride, phosphate, ammonium, into the literally thousands of diverse materials required for their growth: carbohydrates, proteins, lipids, nucleic acids, vitamins. They can fabricate structures such as membranes, and control elements such as receptors, repressors and inhibitors. These metabolic conversions depend on two cellular capabilities. The first is the ability to create "pathways"– long sequences of relatively simple reactions, each of which converts the product of the previous reaction to the starting material for the next. The net effect of a pathway can be a radical change in the structure and therefore function of the material. Metabolic conversions also depend the ability to control those pathways so that other chemical reactions that are kinetically and thermodynamically possible, but would divert pathway intermediates to undesirable, wasteful or, toxic products, are not allowed to proceed.

Cells also perform functions: sensing; energy capture, storage and transduction; chemotaxis; transport; for example. These are performed by complexes of molecules which self-assemble into functional units. Through a billion years of natural variation and selection these pathways and processes have evolved to an extraordinary level of efficiency and selectivity so that now they make highly efficient use of energy, perform functions, and produce valuable resources that are extremely difficult or impossible to recreate via traditional synthetic methods. In addition, they can be engineered to perform functions they do not perform naturally. Thus, their adaptation to DOE mission goals, for example, environmental bioremediation, energy harvesting, industrial fermentation, biosensing is both possible and potentially valuable. As a result, the workshop participants placed, as a high priority, the goal of developing a greater understanding of the biology underlying this variety of processes and the development of technologies that employ the cells to capitalize on them. One might, in principle, expect that in the future, individual processes of interest could be isolated from cells and the rest of the cellular machinery and used in an abiological environment. This was judged, in many areas, to be sufficiently beyond current reach to require that we need, in the short run, to develop techniques that achieve these ends through the use and maintenance of the entire living cell. (See, for example, Zeck 2001, Figure 16.) It is also important to note that this would involve the use of a wide variety of cells, from organisms ranging from viruses through plants, bacteria, insects and humans, since each type offers its own advantages and limitations. In each case, the optimal cell for the goal at hand would have to be identified, adapted to the need, and then used. The panel

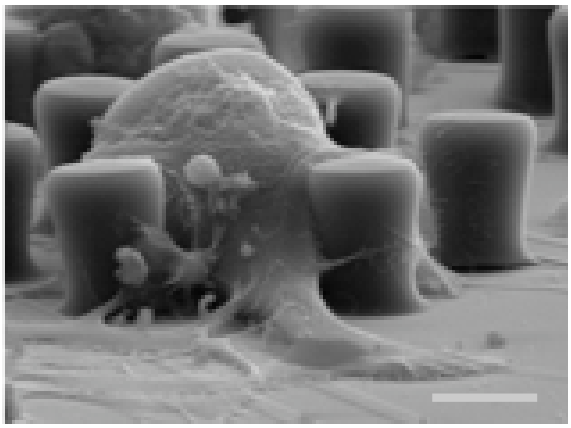


Figure 16. Electronmicrograph, after fixation, of neuron from the A cluster of the pedal ganglia in *L. stagnalis* immobilized within a picket fence of polyimide after 3 days in culture on silicon chip. (Scale bar = 20 □m.). Zeck and Fromherz 2001.

identified three important research areas in this field:

- **Metabolic Engineering.** Living organisms evolved molecules and processes to allow them to survive and reproduce in their particular niche in the environment. Thus, not infrequently, naturally occurring molecules or processes will not be ideally suited to a DOE mission need. One solution this problem would be to "engineer" cells so that they do produce the molecule or process required.

- **Engineering the Cell-Materials Interface.** Living cells have evolved very complex outer surfaces to allow them to survive in their environment, making specific contacts with other cells and extracellular proteins and communicating with them. Interfaces with inorganic surfaces are generally not natural; research is required to design the cell surface and the substrate to insure binding, maintenance of function and communication.

- **Artificial Cells.** Ideally individual cellular molecules or processes would be incorporated into biological/nonbiological devices and structures. As noted, in some cases it will be difficult to purify the specific structures from cells while maintaining their activity. Use of whole cells could serve many purposes, but it is likely that the complexity of cells and the myriad of molecules and structures in them could interfere with the single function required. Development of artificial cells that maintain function in a cellular environment but include only those functions required could be a solution to this problem.

- **Model System-Cell-Based Biosensors.** The discussion of the cell/materials interface led directly to a timely application of research in this area: The development of biosensors that use whole cells as the detection element and inorganic structures as the signal transduction and display elements.

4.2 Metabolic Engineering. The ability to engineer cellular processes for improvements in efficiency, or to establish entirely new metabolic systems, is of considerable and very broad interest for energy sciences. The availability, as a result of the recent major DOE and NIH genome programs, of complete genome sequences for humans, other eukaryotes and numerous microbes now provides the blueprint for establishing the genetic basis of metabolism. Accordingly, in this post-genomic era, metabolic engineering via genetic approaches will be greatly facilitated.

Two methods of metabolic engineering have been established: rational design and evolutionary selection. "Rational" approaches seek to define the specific genes, enzymes (primary gene products) and metabolites (secondary gene products) involved in a specific pathway and to alter these in predetermined ways to cause predictable changes in a cellular process. New pathways can be created in a cell by introducing both the genes encoding the enzymes in these pathways, and also the appropriate metabolic substrates. Applications to the production of biopolymers, both natural and unnatural, are particularly interesting. For example, bacterial cells can be modified to produce polysaccharides on a large scale by first transfecting them with the genes encoding the appropriate glycosyltransferases and then feeding those cells simple sugars as building blocks (Mahal et al., 1997). The chemical synthesis of such polymers outside the cellular context would be very costly and energy consumptive. Proteins are already being produced by cells through recombinant overexpression, but the full exploitation of these systems is limited by the fact that most cells can use only the 20 naturally occurring

amino acids in protein synthesis. Recent breakthroughs(Section 5.4) have enabled the incorporation of unnatural amino acids into proteins during large-scale overexpression in cells, allowing the introduction of a wide range of new optical, electronic, chemical and structural capabilities (Kiick et al., 2002). This has been accomplished by rational design of new enzymes for amino acid activation and the design of new genetic codes for translation of modified genes into modified proteins. A four-base codon allows one to expand the genetic code beyond the 20 natural amino acids to include almost any synthetic amino acid of interest. It may also be possible to engineer the cellular protein biosynthetic machinery to generate novel polymer backbones. Polyester synthesis, for example, generally unknown in biology, would allow the biosynthetic generation of novel functionalized polymers that fold into discrete conformations, enact specific functions, yet ubiquitously biodegrade via ester hydrolysis in the environment.

Given the vast information content of an organism's genome and the complexity of most metabolic systems, rational metabolic engineering can be very difficult. Identifying the elements to alter and engineering them while leaving other systems intact requires great knowledge and technical skill. In some cases there may be several genotypes that produce the target phenotype and altering only one might not have any effect. To access new metabolic properties in rapid fashion, "evolutionary" approaches to cell engineering are taking a prominent position. These approaches in effect allow the organism to select the method by which it is altered. They proceed by iterative cycles of mutagenesis to create genetic diversity followed by selection for a given property of interest. The selection criteria must be carefully designed so that only cells with the desired novel metabolic property have the required reproductive advantage. After multiple rounds of such selection, cells with optimized properties can be characterized at the genetic and biochemical level to determine the molecular basis of the improvement or novel property.

This evolutionary approach has been used to select cells that overproduce certain carbohydrate structures on cellular glycoproteins in order to extend their lifetimes for biotechnology applications.
Diversity/selection methods have also been applied to develop novel protein biosynthetic enzymes that prefer unnatural amino acids. Combined with the genetic coding strategies described above, this technique can be used to engineer new organisms that may acquire a competitive advantage in the presence of unnatural amino acids. Applications to the production of novel protein-like materials and environmental remediation can be envisioned. Moreover, new catalysts that exploit unnatural functional groups can be evolved for specific applications.

Despite recent advances, there is considerable room for the development of new techniques for metabolic engineering. A major stumbling block at present is the lack of knowledge regarding the integration and regulation of metabolic flux within cells. Genomics and proteomics programs are now well-established around the world, but, by contrast, "metabolomics" (the complete inventory of all metabolic pathways and intermediates) is still in its infancy. In order to accelerate metabolic engineering efforts, metabolic profiling efforts must be undertaken so that the fundamental circuitry of cells is established. High-throughput mass spectrometry methods are predicted to play a major role in these efforts.

Computational modeling and theoretical predictions will play a major role in future metabolic engineering efforts. Experimental systems need to be designed so that the relationships between environmental stimuli and changes in metabolic flux and output can be elucidated. These experimental parameters can be used to generate kinetic and thermodynamic models of metabolic networks. Ideally, metabolic modeling would

provide algorithms for rational engineering of new systems. Mutagenesis techniques can be applied to the generation of vast arrays of cellular variants with myriad metabolic properties. High-throughput screening of these can identify a range of properties, and subsequent metabolic and genetic profiling of the variants will provide a basis set from which models can be generated. In addition, there may be a role for theory in the design of libraries for evolution-based experiments so that sufficient "diversity space" is covered.

Methods for probing cellular processes *in vivo* and in real time are still quite limited despite their paramount importance for cell engineering. New techniques for tagging specific proteins or metabolites with fluorescent probes will be particularly useful in this regard. The above methods for expressing proteins with unnatural amino acids are predicted to contribute significantly, as they will allow the delivery of fluorescent amino acids to a specific site in a protein of interest. The development of novel chemistries that permit highly selective protein modification within a cellular context is another important goal.

Non-invasive magnetic resonance and infrared spectroscopy methods have enormous potential for tracking metabolic pathways within cells. Probes for these techniques, including fluorine atoms and azido groups respectively, have minimal structural impact on their host biomolecules and can be detected based on their orthogonality to native cellular components. Already, the coherent anti-Stokes Raman spectroscopy (CARS) technique has been used to image endogenous biomolecules such as lipids and DNA within living cells (Figure 17) (Cheng et a., 2001; Volkmer et al., 2001; Zumbusch 1999). Further application of this and related methods should elucidate the distribution of metabolites within cells in a temporal manner. Since biological work is accomplished a single molecule at a time, spectroscopic methods that probe single molecules are of significant interest. The

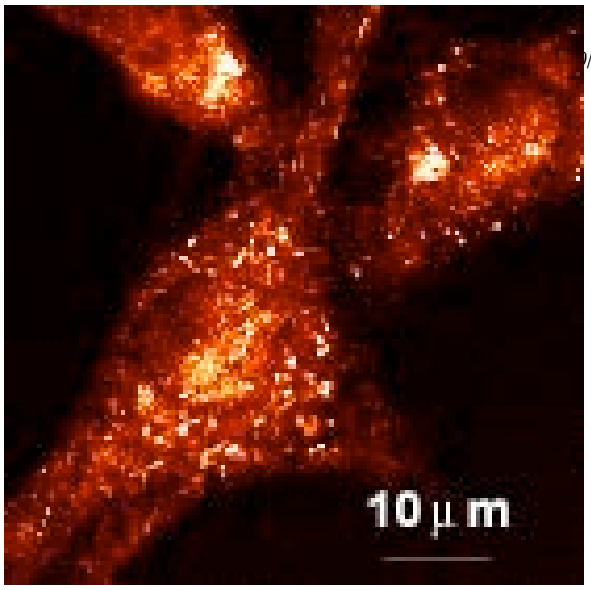


Figure 17. Coherent Anti-Stokes Raman Microscopy (CARS) allows sensitive 3D imaging of live and unstained cells based on vibrational spectroscopy. It provides a point-by point chemical map of cellular constituents, such as protein, DNA and lipid. This CARS image is of NIH3T3 cells using the Raman shift of 2870 cm^{-1}, the frequency of the aliphatic C-H stretching vibration. The bright spots are due to mitochondria and other organelles that are rich in C-H bonds. Cheng, et al., 2001.

ability to probe the action of a biomolecule within its native cellular context may transform our understanding of processes such as vesicle transport, gene transcription, and protein translocation. In general, new methods for probing cellular processes with spatial and temporal resolution, and in real time, are urgently needed.

4.3 Engineering the Cell/Materials Interface. In their native environments, animal cells are found in tissues where they attach to other cells and to the extracellular matrix. Cell adhesion is mediated by multiple, reversible receptor-ligand interactions that provide high avidity and specificity when engaged in concert. Several groups have sought to engineer the adhesion of cells to artificial substrates by mimicking natural cell adhesion processes. These efforts have largely focused on decorating the material surface with extracellular matrix-like structures that serve as specific recognition determinants for cell surface receptors. The integrin family of adhesion molecules plays a primary role in attachment of cells to extracellular matrix

components such as fibronectin, vitronectin and laminin. A minimal epitope for integrin binding that is shared by these matrix components is the Arg-Gly-Asp (RGD) tripeptide. The RGD motif has been incorporated into polymer matrices which can then support the adhesion of cells in a biospecific manner. Alternatively, wheat germ agglutinin, a lectin that binds the cell surface sugars N-acetyl glucosamine and N-acetyl neuraminic acid can be linked to polystyrene microspheres to mediate the attachment of living cells to the microspheres. Optical tweezers can be used to bring the cells and microspheres into contact in controlled geometries in a process known as "light-driven microfabrication."

The defined molecular architecture of self-assembled organic monolayers (SAMs) has attracted significant attention to their use in mediating the adhesion of living cells. SAMs can be introduced on a variety of substrates (i.e., glass, gold or silicon). Those that specifically adsorb or repel extracellular matrix proteins have been patterned in spatially defined arrays using microlithography techniques to afford microdomains capable of supporting cell adhesion. The adhesive domains were composed of hydrophobic hydrocarbon chains or positively charged amino-terminated hydrocarbons, both of which promote the deposition of fibronectin and other proteins. Protein-repellent domains were composed of polyethylene glycol groups or perfluorinated alkanes. The lack of matrix protein deposition on these domains rendered them incapable of supporting adhesion. Using these tools, defined micrometer-scale arrays of adherent cells segregated by non-adherent domains could be engineered on artificial surfaces (Groves 2001). One of the limitations to this approach is the poorly defined nature of adhesion at the molecular level. The extracellular matrix proteins that supported cell adhesion were secreted by the cultured cells and heterogeneously deposited on the SAMs, precluding their precise definition. Furthermore, the interface between surface and cell may have been composed of many protein layers. In a biosensor device, for example, the transmission of information from the cell surface to the material surface might be impeded by such an intervening layer.

The current 'biomimetic' strategies for engineering cell adhesion focus on the

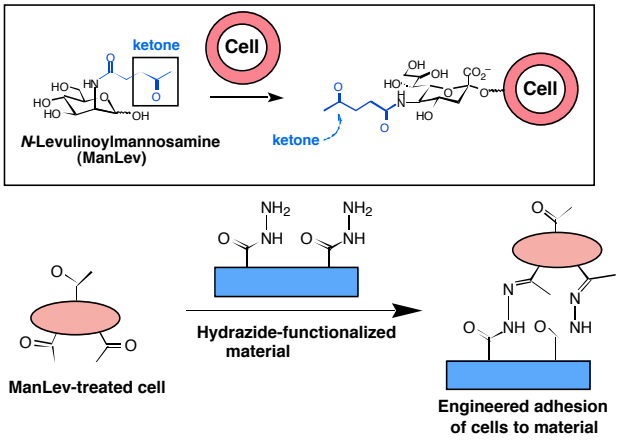


Figure 18. Chemical attachment of cells to synthetic materials. Cells are coated with ketone groups by metabolism of unnatural ketosugars such as ManLev. Ketones react selectively with hydrazide groups installed on material surface coatings. Mahal et al., 1997.

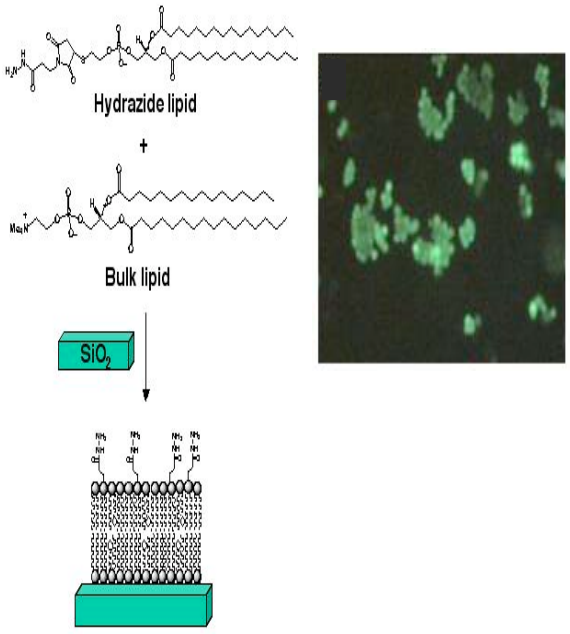


Figure 19. Generation of hydrazide-functionalized supported bilayers and adhesion of cells expressing ketone groups. Hydrazide-functionalized lipids and phosphatidylethanolamine bulk lipids generated a supported bilayer on glass to which HeLa cells adhered. Bruehl et al., 2002.

modification of materials with antibodies that bind naturally occurring epitopes on cell surfaces. This narrow focus limits both the types of materials and the types of cells that can be interfaced with each other. An ideal strategy for engineering cell adhesion would enable one to control the chemical composition of both material and cell surfaces and rationally define the nature of the interface. Toward this end, metabolic engineering approaches have been applied to introduce novel chemical reactivity onto cell surfaces. Reactive electrophiles such as ketones and azides can be incorporated into cell surface polysaccharides by simply feeding cells the corresponding keto- or azido-monosaccharide substrates (Saxon 2000). These functional groups are uniquely reactive with hydrazides and phosphines, respectively, but are essentially unreactive with any native cellular components (Figure 18). Cells engineered to express these electrophiles are primed to react with material surfaces functionalized with the complementary nucleophiles, thereby anchoring cell to material with a chemically-defined linkage. Such molecular-level control over the cell-material interface may facilitate integration into a device environment (Figure 19).

It should be noted, however that while the adhesion proteins that nature uses to mediate cell-cell interactions are typically a few hundred nanometers long, with many functional groups, most current technologies focus on the control of cell-surface interactions by using small fragments of these molecules. While these may be valid approaches to optimize one or another cellular response, they are far too simplistic to truly mimic the complexity of the natural environment of cells. In their native environment, cells are anchored to complex extracellular matrices (ECM) of multifunctional proteins. One such ECM protein, fibronectin is a large (>100 kDa), molecule composed of repeating, structurally-defined modules which are often less than 100 amino acids longs. Some modules carry cell adhesion sites, while others carry sites required for binding other ECM proteins and for matrix assembly. Structural models have been derived to describe how cells can stretch fibronectin and thereby switch the function (see also Section 5.3) of its recognition and binding sites (Vogel et al., 2001). Such protein conformation changes which can occur during normal cellular activities also play a key role in cell signaling. Systems have been designed to display these protein unfolding events as visible color changes using fluorescence resonance energy transfer (FRET). Since this phenomenon is dependent on the distance between a donor and acceptor, the degree to which the protein unfolds, increasing the distance between component parts labeled with donors and acceptors results in a color change (Figure 20; Baneyx et al., 2001). Similar alterations in molecular binding events as a result of protein conformational changes have been shown in the FimH protein of bacteria (Thomas et al., 2002, Section 5.3).

The strict environmental demands of mammalian cells in order to maintain viability have limited their utility in artificial devices. Thus, methods that increase the robustness of cells are of paramount importance. Selection-based cell engineering methods might be applied to this problem. Encapsulation strategies might also be explored. There are heartier cells from other organisms such as plants, which have cell walls, that might be

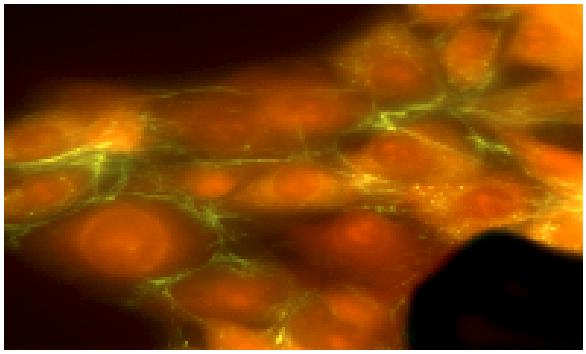


Figure 20. Imaging different protein conformations of the fibroblast cell adhesion protein fibronectin. The fibronectin was labeled with donor and acceptor fluorophores. In the compact state on the cell surface it appears red (FRET energy transfer). In the extended state in matrix fibers, the donors and acceptors are too far apart for FRET and they appear green. Baneyx et al., 2001.

used as hosts for metabolic and signaling machinery from other species. It will be of interest to reconstitute target metabolic pathways from mammalian or bacterial cells into plant cells for large scale production. For example, analyte detection and signal transduction mechanisms might be recapitulated in plant cell hosts for biosensing. Advances in plant cell genetics and metabolic mapping will be important components of such a program.

Finally, expanding the scale of cell-based devices from the single cell to organized cellular arrays or tissues is a major frontier. This will require that the impact of a cell's environment on its physiology be understood in some detail. Also, new methods for organizing multicellular systems into 2-D and 3-D architectures will be required. The invention of new biocompatible polymers and surfaces will be a major part of efforts in this direction.

4.4 Artificial Cells. Given the limitations of living cells with respect to robustness, the need for continuous nourishment, and other environmental demands, the notion of creating synthetic assemblies that perform specific complex functions is particularly attractive. Already, vesicle-based systems have been designed that harvest light to generate ATP (Section 5.2). The essential features of the system include a synthetic chromophore that, upon light absorption, creates a proton gradient across the membrane. When embedded in the vesicle membrane the ATP synthase, isolated from chloroplasts, uses the proton motive force to synthesize ATP from ADP and inorganic phosphate. This self-contained energy transducing system might be incorporated into a higher-order assembly to drive ATP-dependent chemical or physical processes.

In addition to vesicles, supported/tethered bilayers can be used to organize functional assemblies. Membrane proteins can be embedded in these structures, where they maintain their normal receptor or transporter properties. For example, translocation of ions across these bilayers can be accomplished by the action of an ATP-dependent ion pump embedded in the membrane. In principle, the resulting ion gradient could be used to drive cell-like processes.

4.5 Model System – Cell-Based Biosensors. Cells have shown great utility as "factories" for important products, both naturally occurring and designed. They also perform specific functions, many of which are of interest in the physical sciences. One such function allows their use as biosensors. Successful defense against chemical and biological warfare hinges on the ability to detect toxic substances (noxious gases, biological toxins and pathogenic organisms) rapidly at distant sites. The ability to analyze food and beverage samples prior to popular consumption is a major component of quality control and can minimize the occurrence of public health crises. The potential of cell-based biosensors is enormous when one considers that cells are naturally endowed with multiple, complex analyte recognition and signal amplification mechanisms that could be exploited for this purpose. Even at this early stage of development, several cell-based biosensors have been described. Many of the earliest examples utilized relatively robust bacterial cells as producers of enzymes capable of converting the analyte of interest to a detectable species.

More recently, cultured animal cells have been recruited for advanced sensor devices. Their diverse repertoire of recognition systems make them uniquely capable of detecting myriad substances, including bacteria and viruses. Their signal amplification mechanisms include electrochemical, enzymatic and morphological cascades, offering the opportunity to interface animal cells with a range of transducing devices. As an example, mast cells have been incorporated into an antigen-detecting device. The mast cells degranulate in response to antigen binding at the cell surface, releasing

heat into the environment that is detected in a calorimeter. Single neurons have been used to detect the presence of the neurotransmitter acetylcholine that, upon receptor binding, triggers a detectable change in membrane conductance and intracellular calcium levels.

Despite these achievements, the development of cell-based biosensors still presents significant design challenges. These requirements are reflected in all three areas of research identified by the panel in this section. One involves the engineering of the cellular metabolic machinery, for example modifying existing cellular amplification, signal transduction or receptor systems. Although cells have numerous endogenous analyte detection/signal transduction pathways that can be exploited in a biosensing device, there are many circumstances where the cell's natural machinery does not suffice. One must also engineer the cell surface to adapt the cell to bind the particular target of interest. Existing cellular receptors may need to be modified or new ones incorporated in the membrane to allow the cell to serve as a sensor for organisms or molecules it does not naturally respond to. A detailed understanding of cellular signaling pathways and receptor-ligand interactions and specificity is crucial for this.

Second, these systems also require the engineering of the interface between the cells and the microelectronics of the device. This is of paramount importance, in that the sensor response time and sensitivity are often dependent on the intimacy of cell-transducer interactions. In addition, animal cells often have stringent adhesion requirements for optimal viability. Bacterial cell-based biosensors have been constructed by immobilization of cells through polymer encapsulation, non-specific adsorption at a surface and physical containment within a semi-permeable membrane. These simple mechanisms are not readily imported to animal cell-based biosensors since animal cells are orders of magnitude more sensitive than microbial cells to their local environment. Consequently, significant effort has been directed to new strategies for controlling the attachment of animal cells to synthetic matrices with molecular level definition. (This same issue arises during attempts to integrate cells into other artificial environments, such as bioreactors and bioremediation filters.) Finally, there is the challenge of isolating the sensing/signal transduction machinery of the cell from non-essential components in an "artificial cell" to develop a far simpler, and therefore more easily managed and supported system.

5. Biomolecular Functional Systems

5.1 Introduction. Discussions of nanotechnology most often focus on miniaturized circuits for advanced computers. Discussions of biotechnology most often focus on metabolism, regulation, biochemical reactions and the production of small molecules, proteins, nucleic acids, reagents or pharmaceuticals. Discussions of living cells most often focus on systems producing metabolic products, degrading materials, growing and duplicating. Less thought has, until recently, been given to the fact that within the nanometer-size biological living cell lie complex structures that perform very sophisticated mechanical or energy transduction functions. Some capture light and convert it to chemical energy. Others transport substances across otherwise impermeable membranes. Others act as molecular trucks, moving structures and molecules from place to place within the cell. Others act as motors, rotors, ratchets, amplifiers, switches and other devices that perform the sorts of functions we normally associate with meter-size machines run by gasoline, oil or electric engines. There is increasing interest in these biological devices and increasing awareness that they could serve or be mimicked to meet needs for function either at the nanoscale level, or, in large-scale arrays, at the macro-scale level.

Four broadly drawn biological functions were singled for fruitful exploration of their potential impact on the physical sciences.

- **Energy Transducing Membranes and Processes.** Perhaps the most well known and best studied of these are the light harvesting systems in photosynthetic and related organisms. Photons are captured and focused to provide energy for cellular function. Another energy transduction system, oxidative phosphorylation, generates cellular energy through the reoxidation of electron carriers that are reduced in cellular metabolism. In most cases the energy is captured and used in its chemical form, ATP.

- **Motors, Rotors, Ratchets, and Switches.** Molecules such as kinesin and myosin are able to convert chemical energy into motion, as they transport other structures from place to place within the cell along "rails" of microtubules and actin, respectively. Bacterial flagella rotate and propel the organism through fluids, either directly towards an identified goal or randomly in search of a target if none is in "sight". Other biological molecules, such as enzymes, respond to signals as switches, activating or inactivating themselves or other molecules. Ratchets allow controlled motion in a single direction.

- **Enzymes.** These proteins (along with catalytic RNAs) accelerate reactions by up to 16 orders of magnitude, converting one specifically defined molecule into another equally well-defined product. They function through selective binding to their substrates, and then, by creating their own chemistry, lowering the activation energy along the path to products.

- **Pores, Gates, and Channels.** These membrane structures allow the controlled specific passage of ions or molecules into or out of cells or organelles. Often these proteins are highly regulated, responding to the presence of other molecules (ligand-gated) or to a potential (voltage-gated). Often, they can use energy to concentrate species against a gradient.

5.2 Energy Transducing Membranes and Processes.

> "On the arid lands there will spring up industrial colonies without smoke and without smokestacks; forests of glass tubes will extend over the plains and glass buildings will rise everywhere; inside of these will take place the photochemical processes that hitherto have been the guarded secret of the plants, but that will have been mastered by human industry which will know how to make them bear even more abundant fruit than nature, for nature is not in a hurry and mankind is."
>
> – Giacomo Ciamician 1912

Aerobic cells contain mitochondria or active membranes which pump protons across a lipid bilayer and establish the proton motive force (PMF). To accomplish this, they use the free energy made available by the spontaneous reoxidation by oxygen of the reduced redox carriers generated by the metabolic oxidation of nutrients. Anaerobic cells use ATP to pump protons for the PMF. Photosynthetic membranes in chloroplasts exploit the capture and concentration of light energy as the energy source to produce the PMF. Halobacteria, living in salt ponds, use light energy to establish a PMF independent of photosynthesis. However created, the PMF itself serves as an energy source, driving, through a variety of mechanisms, the synthesis of carbohydrates and other molecules; the production of chemical energy; the transport of sugars and other nutrients; the rotation of the flagellum, the cellular propeller; and other energy requiring cellular processes. Most often, this is achieved through the production of ATP, produced when the photons flow back across the membrane through the ATP synthase, a 12 nm diameter enzyme, a motor which rotates at 8000 rpm generating 80-1000 pN•nm of rotary torque (Noji et al., 1997) and catalyzes the formation of the phospho-anhydride bond linking ADP to Pi.

The mimicking of the capture and use of solar energy through systems like the photosynthetic centers or the halobacterial "purple membranes" has been a target of research for years. Similar efforts have focused on energy capture in metabolic oxidations that occur in aerobic cells or PMF formation in anaerobes. The primary challenge is to link the energy capturing system, whether it be optical or chemical, with the energy requiring function.

The two forms of cellular energy, the chemical and voltage potential in the PMF and the "activated" molecule ATP are in effect interchangeable, in that either can be harnessed to produce the other.

The PMF acts across membranes, ATP acts in solution, thus their applicability to artificial systems will depend on the particular needs of the system. One research direction involves the production of a "synthetic" PMF in artificial membranes that can be used as an energy source to power a variety of energy requiring processes of interest that have themselves been inserted into those membranes. These processes could include, for example, molecular motors, transporters, separation systems, pumps, biochemical reactions. In some cases the PMF could be linked directly to the process, in other cases it could be linked via the production and temporary storage of ATP. A considerable challenge lies in the isolation and use of the natural photosynthetic antennae or in the synthesis of artificial mimics of that system. In one example, highly branched phenylacetylene dendrimers have been synthesized and shown to act, with very high efficiency as photon energy traps (Shortreed et al., 1997). In another example, an artificial photosynthetic reaction center of light harvesting pigments was used. Captured photons were used to pump protons across the membranes of synthesized liposomes (hollow spherical lipid vesicles of predetermined size).

A PMF equivalent to that found in natural photosynthetic membranes was produced (Steinberg-Yfrach et al., 1997; Steinberg-Yfrach et al., 1998; Gust et al., 2001). and successfully linked to ATP synthase molecules embedded in the membrane. The 150nm diameter liposome, was manufactured through self-assembly from the known membrane components, phosphatidyl choline, phosphatidic acid, cholesterol and the ATP synthase was isolated from spinach chloroplasts (Figure 21).

These systems represent major advances, but are still quite rudimentary. Many barriers remain. Of primary importance is the need to increase the rate of proton transport and improve the robustness of the system. The artificial reaction center would also perform better if it could be inserted into planar membrane systems rather than the spherical liposomes so that a wider range of transducers could be powered and explored. Artificial pumps for ions other than protons should be developed to broaden the applicability of the system, since the difference in chemical potential of any ion across a membrane represents an energized membrane that can be coupled to a transducer to perform work. Nature for example, also uses sodium to drive ATP synthase, and sodium and potassium

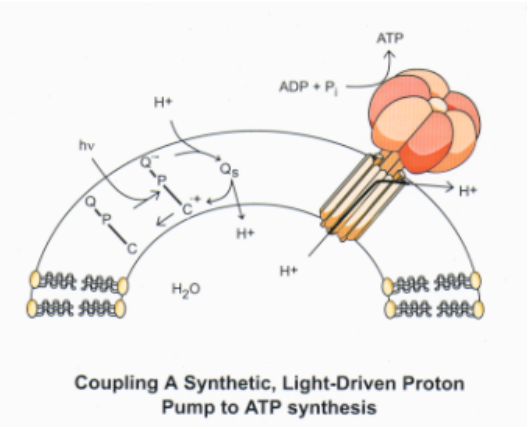


Figure 21. Liposome-based artificial photosynthetic membrane. Protons pumped into the liposome by a light-driven electron transfer system (C-P-Q) drive the production of ATP by the CF_oF_1-ATP synthase. Steinberg-Yfrach, et al., 1998.

pumps are critical for a variety of other biological functions. Success will lead to energy capture systems that are nonpolluting and do not rely on fossil energy.

5.3 Motors, Rotors, Ratchets, Switches.

Design of structures for controlling transport processes is tightly linked to the size scale involved. While sophisticated machines and devices have been developed for the macroscopic world, new principles have to be employed to control movement of objects at the nanoscale. Nature again provides a model, having evolved specialized molecules to actively transport molecules and structures over long distances (on the cellular scale), to specific destinations and against concentration gradients, all with very high (sometimes approaching 100%) efficiency. The goal for biomolecular research is to use these biological principles to create novel hybrid materials that are capable of dynamic spatial self-organization.

Specific inspiration for this work comes from cells that use motor proteins such as kinesin (that walk along microtubules- longer-polymerized protein filaments), to actively transport cellular components to defined locations. The structure of two functional states of kinesin, one bound to ADP and the other bound to a non-hydrolyzable analogue of ATP, has been determined at the Advanced Light Source and the Stanford Synchrotron Radiation Laboratory, two BES user facilities (Kikkawa 2001) greatly assisting in the elucidation of the mechanism of action of this transporter. Challenges for the construction of molecular shuttles using biological motors include (a) guiding the direction of the motion on manufactured tracks, (b) controlling the speed, and (c) directing the loading and unloading of specific cargo. Guiding the movement of molecular shuttles on surfaces has been accomplished by various techniques relying on surface topography and chemistry as well as flow fields and electric fields (Hess and Vogel 2001). The high stiffness and persistence length of microtubules, for

example, enables the control of their placement by topographic surface features (Hess et al., 2002a) and makes possible the fabrication of microscopic "rectifiers" which sort microtubules according to their direction of motion (Hiratsuka et al., 2000). Approaches to loading incorporate biotinylated tubulin into microtubules which then binds specifically to streptavidin-coated cargo (Hess et al., 2001). The cargo itself could be molecules or micro- or nanofabricated devices. The sliding of myosin along actin fibers represents another type of motor, allowing the movement of structures attached to these proteins. Rates of motion vary considerably, from tenths to tens of micrometers/second. Mimicks have been made (Jiménez 2000).

The ATP synthase, discussed above in the context of converting solar energy to chemical energy can also be considered a molecular motor. Consistent with the law of "microscopic reversibility", addition of ATP will drive the rotation of the system, as it hydrolyzes ATP to produce ADP and Pi (Noji et al., 1997). This chemically driven rotating motor has great potential and has also been the focus of research. Through a combination of intra-biomolecular and bio-inorganic bonding strategies, these motor proteins have been self-assembled with 15 nm precision at specific locations on inorganic substrates patterned using electron beam lithography (Montemagno and Bachand 1999). Peptide linkers having an affinity for nickel were attached to the synthase molecules which were then placed on 200nm high nickel posts. 750-1400nm-long nickel "propellers" were also attached (Figure 1, page 12) to the rotors. Using conventional optical microscopy, rotation at speeds up to 7 Hz was observed following the addition of ATP (Soong et al., 2000). Alternatively, fluorescently labeled actin filaments have been attached and their rotation followed in fluorescence microscopy (Noji 1998).

The molecular motors based on the ATP synthase discussed above are also switches, with the capability of being turned on or off. Since they require input energy in the form of ATP to function, control and switching has been achieved through the control of the concentration of ATP available to the motor. Other artificial systems with motors have been constructed and turned on and off by light through photo-induced release of caged ATP (Hess et al., 2001). Switching has also been accomplished in the artificial system in which actin rods were attached to the rotating subunits of the ATP synthase and ATP added or removed. This type of control has its drawbacks, however. Any control mechanisms should be independent of regulation by ATP if used in a cellular context so that any other cellular ATP-dependent processes will be unaffected.

Some recent work with the synthase illustrates the power of existing biochemical techniques to solve problems such as this. Genetic modification of the ATP synthase has allowed the engineering of pockets in the enzyme with high affinity for binding zinc ions. When the zinc binds the protein, it becomes incapable of performing the conformational changes necessary for ATP hydrolysis and the accompanying rotation of its central shaft. It thus stops turning. Operation of this mutant motor protein demonstrated switching as zinc was supplied or removed (Figure 22). With a fluorescent actin filament attached to the rotor allowing single-molecule observation, rotation proceeded following the addition of ATP but

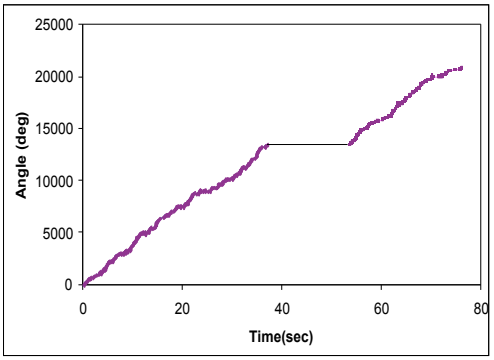


Figure 22. Controllable activity of F1-ATPase. The motor ceases rotation upon addition of Zn, but restarts when it is removed. Liu et al., 2002.

stopped in the presence of Zn. The motion restarted following addition of a Zn chelator and ATP.

Adhesion proteins found in the extracellular matrix of mammalian cells or on the surfaces of bacteria are also of interest. Some are able to switch their function when mechanically stretched (Vogel et al., 2001). The bacterial

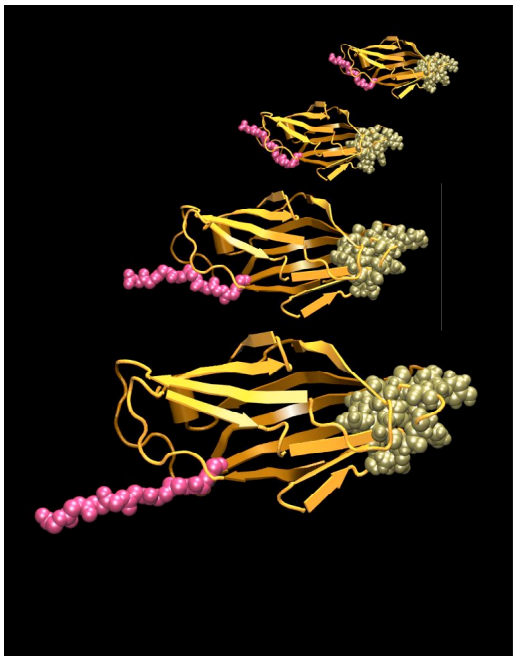


Figure 23. Sheer forces cause a conformational change in the bacterial adhesin (the lectin domain of FimH) of *E. coli*. Mechanical force separates the terminal strand purple balls) connecting the adhesin to the bacterium. This in turn causes a structural perturbation that propagates to the binding site (gold balls) switching it from low to high affinity. Thomas et al., 2002.

adhesion protein fibronectin, for example, switches its ligand affinity from low to high if stretched under shear flow (Thomas et al., 2002; Figure 23).

The fibronectin system (see also Section 4.3) also provides a glimpse of the value of computational tools to the study of protein function. Models have been constructed to understand how mechanical unfolding of these proteins may affect their structure and thus function (Craig et al., 2001; Krammer et al., 1999). It was found, for example, that the fibronectin module containing the cell binding site is among the first to unravel if stretched. Further, the mechanical stability of these modules can be altered several fold by single amino acid substitutions suggesting that "engineering" of these structures for particular functions is possible.

Enzymes, discussed in more detail below as catalysts, also act effectively as switches, responding to the environment, reversibly converting from active to inactive shapes. Binding to activator molecules alters their conformation to a structure that is capable of binding substrate and converting it to product. Binding to inhibitors alters their conformation to a structure that is incapable of binding substrate or incapable of converting it to product. In fact, regulation is not all or nothing, but can be graded from no activity to full activity. Further, these switches can respond simultaneously to many activators and inhibitors, summing the strength of each of these signals to arrive at an appropriate level of activity. Enzymes also play a role in switching other functions by controlling the production of actuators of those functions.

Finally, switching is also seen in the control of the use of genetic information. Transcription factors can bind to DNA to regulate the degree to which the information in the base sequence is used to produce protein. Again, a continuous spectrum of control is exercised, between completely repressed to fully active.

These are all processes that can be envisioned as components of nanoscale machines providing energy transduction, motion, catalysis or transport, in much the same fashion seen in the various components of the macroscale machines of today. Of particular note is that most biological systems achieve their exceptional level of efficiency by incorporating multiple functions into a single structure. This scheme could serve well as a model for artificial systems. Again, however, we encounter the same barriers as before: our incomplete knowledge of the structure and

function of the biological systems, difficulties inherent in isolating and then combining their components in a self-assembling, controlled and functional manner, the need to make them more robust.

Many challenges remain before these motor systems can be incorporated into hybrid systems. As before, they must be made more robust. Their complexity suggests difficulty in large scale manufacture, but they do self-assemble and, in fact, can easily be produced in large amounts through large-scale growth of the appropriate cells followed by efficient isolation and purification.

From an engineering perspective, motor proteins are an attractive alternative to MEMS actuated devices because of their small sizes (length scales hundreds of times smaller than those of equivalent MEMS devices), high speeds, high efficiencies, long lifetimes, and ease of mass-production. Hybrid combinations of biological and inorganic components in fabricated micro- and nanodevices promise unique advantage. In fact a motor-protein based device, using motor proteins as picoNewton force meters has been reported (Hess et al., 2002b).

5.4 Enzymes. Enzymes have long been regarded as the highest form of biological functional achievement, although perhaps only because the roles of other, more complex systems have yet to be elucidated. Regardless, these proteins are able to select a single specific substrate from a mix of thousands, interact with it chemically, or just structurally, and convert it to a specific product even if the laws of chemistry allow the production of several. Enzymes do not alter the thermodynamics of the process: the free energy change and equilibrium constant are unaltered, but these nanoscale objects can accelerate reactions by up to 16 orders of magnitude, equivalent to taking a reaction that takes 300 years and performing it in a microsecond. Some enzymes are "perfect, they accelerate reactions to a rate that is limited only by diffusion.

Further, enzymes can be regulated (see switches, Section 5.3). Binding of specific inhibitors or activators can increase or decrease their catalytic effectiveness by orders of magnitude. In fact, in many cases a given enzyme responds to multiple regulatory effectors. Glutamine synthetase, for example, responds to nine, in effect summing the positive and negative effects to determine a level of activity consistent with the balance of those effectors. This regulatory feature can also be used as a switch, as described above.

The difficulty inherent in the use of enzymes in devices or in materials synthesis is that nature designed only those enzymes required to catalyze the reactions necessary for life. Their specificity for substrate and product thus limits their usefulness in non-living applications.

The revolution in biology and biotechnology over the past few decades including the development of cloning, genetic and protein engineering, the deciphering of complete genomes, has opened the door to our overcoming that barrier. With help from other fields, for example, new tools such as the DOE/BES-managed synchrotron light sources and advanced nuclear magnetic resonance spectroscopy, the effort and time required to determine the structure of an enzyme has been greatly reduced, allowing us to understand its function, a basic element in our ability to tailor it to our needs. We can now use techniques such as cloning and mutagenesis to alter enzymes so that they catalyze reactions of interest. Active sites are being redesigned, although progress is slow and this is by no means a routine procedure. Progress is hampered by the fact that enzymes change their conformation, both before and during catalysis, so there is no "one" structure, with easily definable effects of a single change at point A on structure or function at point B. In addition recent results of "single-molecules" studies show that the activity of individual

enzyme molecules is quite different from what we see in ensemble studies (Lu et al., 1998).

Of great interest is the work in introducing non-natural amino acids into proteins at desired positions (Wang et al., 2001; Van Hest et al., 2000). This allows the incorporation into the protein of a far broader set of optical, electronic and chemical capabilities, with fluorescent, photoactivatable groups, or groups that introduce new chemistries. It also allows efforts to increase the stability of enzymes, a critical need if they are to be used outside the cell. [Of interest here is the fact that this technique, originally thought to be a solely human redesign of the natural system, using a "stop" codon to introduce the non-natural amino acid, has recently been observed in a rare methane producing bacterium organism (Srinivasan et al., 2002; Hao et al., 2002; Böck et al., 1991). This synthesis work has progressed to the point where the normal three-letter nucleic acid code has been substituted for by a four base code, allowing a much broader range of enzymes to be produced with a broader range of non-natural amino acids, with multiple substitutions in the same protein (Maglieri et al., 2001). Other efforts involve the development of artificial enzymes, generated through the selection of antibodies raised against carefully designed antigens, and then fine tuned through "molecular evolution." Non-protein enzymes, designed to attract and bind the substrate, subject them to chemical change, and then repel and release the product are being explored (Fréchet 2002).

5.5 Pores, Gates, Channels. Proteins also function as constituents of biological membranes in roles such as receptors, transporters and channels. While a great deal of work has been done on the engineering of soluble proteins, notably enzymes and antibodies, relatively little work has been done on the engineering of membrane proteins, in part because of the complexity of manipulating systems that are not soluble in water.

The membrane pore-forming protein α-hemolysin from *Staphylococcus aureus* has however been explored with great success in attempts both to push the frontiers of protein engineering and to develop new components for use in biotechnology, an excellent example of the tight link between basic science and target directed science (Bayley and Cremer 2001; Figure 24) and of the manipulation of biomolecular materials for ***in vitro*** applications.

α-Hemolysin is a 293 amino acid polypeptide that is secreted by the bacterium and then assembled into heptameric pores in the lipid bilayers. The crystal structure of the heptamer is known and has provided an extremely useful template for designing engineered forms of the protein. For example, work has progressed on the re-engineering of this pore-forming protein as a sensor for a wide variety of analytes: divalent metal cations, various anions, organic molecules, proteins and nucleic acids (Bayley et al., 2001).

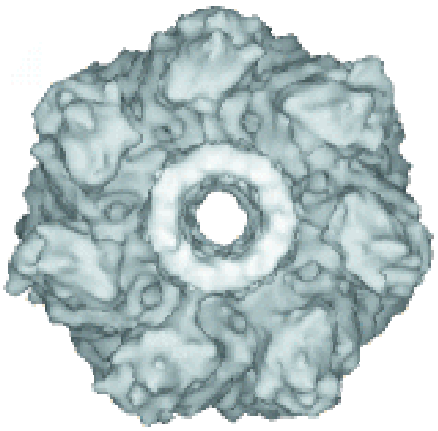


Figure 24. Molecular graphics representation looking into the channel of the α hemolysin pore. Song et al., 1996.

Analyte molecules modulate the ionic current driven through α-hemolysin pores by the transmembrane potential. Stochastic sensing, which uses currents from single engineered pores, is an especially attractive prospect. This approach yields both the concentration and identity of an analyte, the latter from its distinctive current signature. Further, several

analytes can be detected simultaneously with a single sensor element. The protein modification work has exploited recent advances in protein engineering that allow the alteration of entire protein domains by genetic engineering, and specific but drastic localized sites targeted chemical modification. The latter includes chemical modifications of the internal surface of the pore: for example, the attachment of poly(ethylene glycol) and its derivatives. It also involved a new twist in protein modification: non-covalent modification with adapters, including cyclodextrins, which continue to exhibit host-guest chemistry when lodged inside the pore. In an extension of the adapter concept, the pore has been genetically engineered to accommodate two or three adapters. Further, small molecules can be trapped in the "nanocavities" between the adapters.

As in other areas discussed, a major remaining challenge is to place the engineered pores in a robust environment to form components that can be used in practicable sensing devices. Possibilities include supported bilayers, bilayers across nanofabricated apertures and polymerized bilayers. A second challenge is to make arrays containing the protein for use in sensors or in high-throughput screening. Stochastic sensing devices based on alternative single molecule detection techniques can be envisioned, requiring physicists to join the groups or teams of chemists and biologists working on these proteins. These techniques include approaches based on fluorescence and force measurements. Because the sensor elements are of nanometer dimensions, it will be possible to build them into complex devices incorporating other components discussed in this report.

As was the case with the use of enzymes and pores, protein engineering will continue to contribute to numerous areas of biomolecular materials. Advances in our ability to make "designer" protein-based materials such as crystals, fibers, elastomers and adhesives can be expected.

An alternative approach to the construction of pores and channels is reflected in the work of Ghadiri and co-workers (Ghadiri et al., 1993). Hollow organic nanotubes hundreds of nanometers in length, with internal diameters of 0.7-0.8 nm, were self-assembled from rationally designed cyclic octa-peptides of alternating D- and L-amino acids. These can insert in natural or artificial membranes in a sequence-dependent fashion, allowing selective transport across those membranes (Fernandez-Lopez et al., 2001). Potential applications in catalysis, molecular electronics, molecular separation technology can be envisioned.

6. Promise and Challenges

There is a great deal of excitement around the world about the application of biology to the physical sciences, in particular, the materials and chemical sciences. First, as outlined in Section 2, the world of biology holds much promise for applications outside the organism. The list of molecules, structures, properties, concepts of biology that could have an impact is a long one. Second, this excitement stems from the fact that the dimensions accessible to the physical sciences have progressively shortened and now extend to the nanometer range, while those accessible to the biological and organic chemical sciences have progressively lengthened to that same range. Finally, there is excitement because the tools of molecular biology and biochemistry now allow a fuller understanding and a start at manipulation of biological systems.

There are, as has been discussed above, a number of obstacles that stand between us and the ready application of biology to the materials and chemical sciences. The primary barrier remains our incomplete understanding of biological systems themselves. Until a system is fully understood, with a clear definition of its component molecules and how they interact to create function, it is very difficult to manipulate. Despite the great advances in biology over the past few decades, an enormous amount remains beyond our grasp, not only in the description of specific systems but, and perhaps more importantly, in the use of those systems to develop the general underlying principles. Exploration of these areas must be a high priority.

Tool development is another area of challenge. It has been argued that the major advances in research depend on the development of tools. Clearly the impact of synchrotron radiation on the field of structural biology has been enormous and will continue to be so for quite some time. The tools developed as part of the DOE and NIH led Human Genome Project enhanced our ability to explore genes and proteins beyond what might have been imagined. The tools that will be developed in this new age of proteomics will be equally valuable. This too is an area that must be given high priority.

Unlike physics and chemistry, biology has not, until relatively recently, had as much support from theory, computation and modeling as it should. However, it is clear that the complexity of metabolic processes and the organization of the cell is such that this approach could bring great new insights. Theory does support efforts to understand protein folding; it needs to be extended to the studies of the structure of other molecules. Theory has also, for example, been applied to model simplified systems of microtubules and motor complexes (Lipowsky 2001). The ability to design structures for self assembly or templated or hierarchical assembly would also benefit greatly. The support of theory for biology must also be a priority.

Cultural issues were also seen to impede progress in the field. As with most interdisciplinary fields, differences in terminology and jargon must be overcome. Biology has traditionally been a field of description, answering the question “what molecular components are involved and how do they interact to create the function being studied.” The interest and skills of the synthetic chemists and physicists must be brought to bear, so that biologists focus more attention on synthesis and manipulation. At the same time, physics and chemistry students must receive more training in biology. As with any field, extensive study is a prerequisite for

grasping the required intuitive sense of an area of study. Whether this is done through a change in the course and research requirements for doctoral degrees or through the fostering of more interdisciplinary collaborations will depend on the specific research in question. Clearly, however, the training of the next generation of scientists must be broadened, perhaps through joint supervision by mentors in different fields.

Finally, there are properties inherent in biological systems that appear to limit their application outside the living organisms. Perhaps most important is the fact that they are not as robust as most inorganic structures. Compared to metals, ceramics, polymers, biological systems cannot withstand extremes of temperature, pH, ionic strength, pressure. In fact, in many cases it is extremely difficult to create systems in which they can function outside the living organism, under any conditions. Of further concern is the maintenance of activity when biological structures or cells are linked to inorganics in devices. On the other hand, systems could be developed to shield and protect biological structures, and they themselves can probably, at least to some extent, be made sturdier. Nature, after all did not design them for extremes – just for the environment the living things found themselves in. The ability to substitute new monomers in polymers, for example could aid in this effort. On a more basic level it is likely that our ultimate understanding of the fundamental physics and chemistry of biological processes might lead to the extension of these principles to different doses of molecules and materials that would be more stable and robust and by incorporating more of the predictable, more suited to nonbiological needs.

These are not insuperable obstacles. In fact they are already being overcome in select research groups around the world and in universities establishing departments, groups, or programs in “Chemical Biology” or Chemisty and Biology” and new areas of biophysics and bioengineering. Once they are more broadly overcome, and the rich world of biology becomes accessible to the physical sciences, we will have nothing short of another revolution in science.

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[1] Portions of Section 2 appear in a similar form in a report of another Federal agency workshop, as yet unpublished, in which an author (MDA) of this report was invited to participate.

[2] Text similar to portions of Section 3 first appeared in "The Molecular Foundry" published by the Materials Sciences Division of the Lawrence Berkeley National Laboratory, March 2001, written in part by MDA and APA who were also participants in this workshop.