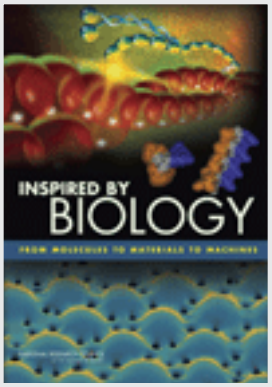

Inspired by Biology: From Molecules to Materials to Machines

DETAILS

170 pages | 7 x 10 | PAPERBACK
ISBN 978-0-309-11704-3 | DOI 10.17226/12159

AUTHORS

Committee on Biomolecular Materials and Processes, National Research Council

INSPIRED BY BIOLOGY

FROM MOLECULES TO MATERIALS TO MACHINES

Committee on Biomolecular Materials and Processes

Solid State Sciences Committee

Board on Physics and Astronomy

Division on Engineering and Physical Sciences

Board on Life Sciences

Division on Earth and Life Studies

NATIONAL RESEARCH COUNCIL
OF THE NATIONAL ACADEMIES

THE NATIONAL ACADEMIES PRESS
Washington, D.C.
www.nap.edu

THE NATIONAL ACADEMIES PRESS 500 Fifth Street, N.W. Washington, DC 20001

NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the committee responsible for the report were chosen for their special competences and with regard for appropriate balance.

This study is based on work supported by Contract No. DE-FG02-05ER46197 between the National Academy of Sciences and the Department of Energy and Grant No. DMR-0426181 between the National Academy of Sciences and the National Science Foundation. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the views of the agencies that provided support for the project.

Library of Congress Cataloging-in-Publication Data

Inspired by biology : from molecules to materials to machines / Committee on Biomolecular Materials and Processes, Board on Physics and Astronomy, Board on Life Sciences, Division on Engineering and Physical Sciences, Division on Earth and Life Studies.

p. cm.

Includes bibliographical references.

ISBN 978-0-309-11704-3 (pbk. book) — ISBN 978-0-309-11705-0 (pdf book) 1. Molecular biology. 2. Biomolecules—Analysis. 3. Materials—Biotechnology. 4. Biomedical materials. 5. Biomedical engineering. I. National Research Council (U.S.). Committee on Biomolecular Materials and Processes.

QH506.I4817 2008

570—dc22

2008016751

Copies of this report are available from the National Academies Press, 500 Fifth Street, N.W., Lockbox 285, Washington, DC 20055; (800) 624-6242 or (202) 334-3313 (in the Washington metropolitan area); Internet, http://www.nap.edu; and the Board on Physics and Astronomy, National Research Council, 500 Fifth Street, N.W., Washington, DC 20001; Internet, http://www.national-academies.org/bpa.

Cover: Three images are shown on the cover of this book, one each to represent a molecule (middle), a material (bottom), and a machine (top) in biomolecular materials research. (Top) Myosin V (blue-green), a molecular motor that moves cargo around the cell by walking on actin (red). Courtesy of Paul R. Selvin, University of Illinois at Urbana-Champaign; created by precisiongraphics.com. (Middle) Antimicrobial peptoids are designed to mimic the amphipathic structures of antimicrobial peptides; models of the folded structure of a synthetic peptoid are shown in views both parallel and perpendicular to the helical axis. Residues are color coded: cationic, purple; hydrophobic, orange; all others, gray. Published in N.P. Chongsiriwatana, J.A. Patch, A.M. Czyzewski, M.T. Dohm, A. Ivankin, D. Gidalevitz, R.N. Zuckermann, and A.E. Barron, "Peptoids that mimic the structure, function, and mechanism of helical antimicrobial peptides," *Proceedings of the National Academy of Sciences USA* 105(8):2794-2799 (2008). Copyright 2008 National Academy of Sciences, U.S.A. (Bottom) Array of microlenses on the skeletal plate of a brittlestar *Ophiocoma wendtii* that functions as a sophisticated optical element. The whole structure is composed of an intricately shaped single calcite crystal. The lens size is approximately 50 microns. Courtesy of J. Aizenberg, Harvard University.

Printed in the United States of America

THE NATIONAL ACADEMIES

Advisers to the Nation on Science, Engineering, and Medicine

The **National Academy of Sciences** is a private, nonprofit, self-perpetuating society of distinguished scholars engaged in scientific and engineering research, dedicated to the furtherance of science and technology and to their use for the general welfare. Upon the authority of the charter granted to it by the Congress in 1863, the Academy has a mandate that requires it to advise the federal government on scientific and technical matters. Dr. Ralph J. Cicerone is president of the National Academy of Sciences.

The **National Academy of Engineering** was established in 1964, under the charter of the National Academy of Sciences, as a parallel organization of outstanding engineers. It is autonomous in its administration and in the selection of its members, sharing with the National Academy of Sciences the responsibility for advising the federal government. The National Academy of Engineering also sponsors engineering programs aimed at meeting national needs, encourages education and research, and recognizes the superior achievements of engineers. Dr. Charles M. Vest is president of the National Academy of Engineering.

The **Institute of Medicine** was established in 1970 by the National Academy of Sciences to secure the services of eminent members of appropriate professions in the examination of policy matters pertaining to the health of the public. The Institute acts under the responsibility given to the National Academy of Sciences by its congressional charter to be an adviser to the federal government and, upon its own initiative, to identify issues of medical care, research, and education. Dr. Harvey V. Fineberg is president of the Institute of Medicine.

The **National Research Council** was organized by the National Academy of Sciences in 1916 to associate the broad community of science and technology with the Academy's purposes of furthering knowledge and advising the federal government. Functioning in accordance with general policies determined by the Academy, the Council has become the principal operating agency of both the National Academy of Sciences and the National Academy of Engineering in providing services to the government, the public, and the scientific and engineering communities. The Council is administered jointly by both Academies and the Institute of Medicine. Dr. Ralph J. Cicerone and Dr. Charles M. Vest are chair and vice chair, respectively, of the National Research Council.

www.national-academies.org

COMMITTEE ON BIOMOLECULAR MATERIALS AND PROCESSES

ARUP K. CHAKRABORTY, Massachusetts Institute of Technology, *Chair*
JOANNA AIZENBERG, Harvard University
ANNELISE E. BARRON, Stanford University
KEN A. DILL, University of California at San Francisco
SHARON C. GLOTZER, University of Michigan
YALE E. GOLDMAN, University of Pennsylvania
ELIAS GREENBAUM, Oak Ridge National Laboratory
W. JOHN KAO, University of Wisconsin at Madison
DAVID NEEDHAM, Duke University
V. ADRIAN PARSEGIAN, National Institutes of Health
ALAN RUDOLPH, Adlyfe Inc.
CYRUS R. SAFINYA, University of California at Santa Barbara
CHARLES F. STEVENS, Salk Institute for Biological Studies
DAVID A. WEITZ, Harvard University

Staff

DONALD C. SHAPERO, Director, Board on Physics and Astronomy
FRANCES E. SHARPLES, Director, Board on Life Sciences
ADAM P. FAGEN, Senior Program Officer, Board on Life Sciences
NATALIA J. MELCER, Senior Program Officer, Board on Physics and Astronomy
BETH MASIMORE, Christine Mirzayan Science and Technology Policy Graduate Fellow
CARYN J. KNUTSEN, Senior Program Assistant, Board on Physics and Astronomy
PHILLIP D. LONG, Senior Program Assistant, Board on Physics and Astronomy (until August 2006)
VAN AN, Financial Associate, Board on Physics and Astronomy

SOLID STATE SCIENCES COMMITTEE

PETER F. GREEN, University of Michigan, *Chair*
BARBARA JONES, IBM Almaden Research Center, *Vice-Chair*
DANIEL P. AROVAS, University of California at San Diego
COLLIN L. BROHOLM, Johns Hopkins University
PAUL M. CHAIKIN, New York University
GEORGE W. CRABTREE, Argonne National Laboratory
ELBIO DAGOTTO, University of Tennessee and Oak Ridge National Laboratory
DUANE DIMOS, Sandia National Laboratories
SIDNEY R. NAGEL, University of Chicago
MONICA OLVERA DE LA CRUZ, Northwestern University
ARTHUR P. RAMIREZ, Alcatel-Lucent
MARK D. STILES, National Institute of Standards and Technology
ANTOINETTE TAYLOR, Los Alamos National Laboratory
DALE J. VAN HARLINGEN, University of Illinois at Urbana-Champaign
FRED WUDL, University of California at Santa Barbara

Staff

DONALD C. SHAPERO, Director, Board on Physics and Astronomy
NATALIA J. MELCER, Senior Program Officer
MERCEDES M. ILAGAN, Administrative Assistant
VAN AN, Financial Associate

BOARD ON PHYSICS AND ASTRONOMY

Preface

The National Research Council of the National Academies convened the Committee on Biomolecular Materials and Processes (BMAP) to assess current work and future promise at the intersection of biology and materials science. The Solid State Sciences Committee of the Board on Physics and Astronomy developed the charge for this study in consultation with the Board on Life Sciences and the study's sponsors at the Department of Energy and the National Science Foundation. The Committee on BMAP was charged to identify the most compelling questions and the emerging scientific opportunities at the interface between biology and condensed matter and materials research, suggest strategies to best meet the identified opportunities, and consider connections to national priorities, including health care, security, the workforce, and economic and societal needs. The committee did not address tissue engineering in this report, because it has been reviewed elsewhere[1] and was considered outside the scope of the committee's charge. The complete charge is reproduced in Appendix A.

The Committee on BMAP is composed of experts from many different areas of biomolecular materials research (see Appendix B for biographical sketches of committee members). The full committee met in person three times (see Appendix C) to address its charge. The committee formed subgroups to study areas in further detail and to develop the text of the final report. At its meetings, the committee heard from experts in the field and from the federal agencies that support BMAP

[1] National Research Council, *Capturing the Full Power of Biomaterials for Military Medicine*, Washington, D.C.: The National Academies Press (2004).

research. Conference calls and e-mail were used to coordinate the work of the committee between meetings. This final report reflects the committee's enthusiasm and excitement for the research opportunities in BMAP.

The report is the product of input from many people. On behalf of the committee, I extend my thanks and appreciation to all who participated in this endeavor. I also thank the speakers who made formal presentations at the committee meetings (Appendix C); those presentations and the ensuing discussions strongly informed the committee's deliberations. In addition, the committee would like to thank the following people for their insights: Ian Anderson, James R. Baker, Jr., Sergey Bezrukov, Mark S. Humayun, Nicholas A. Kotov, Ronald G. Larson, John Miao, Dean A. Myles, Kevin Plaxco, Rudi Podgornik, Clinton Potter, Roger Pynn, Don Rau, David A. Tirrell, Gregory Voth, Karen Wooley, Wenbing Yun, and Joshua Zimmerberg. In particular, Theresa Reineke is thanked for her insight and contribution to the challenges in the area of synthesis.

Finally, I also thank the National Research Council staff (Natalia Melcer, Adam Fagen, Don Shapero, Frances Sharples, Phillip Long, and Caryn Knutsen) for their guidance and assistance throughout the development of the report.

As chair, I am grateful to the committee members for their wisdom, cooperation, and commitment to ensuring the development of a comprehensive report.

Arup Chakraborty, *Chair*
Committee on BMAP

Acknowledgment of Reviewers

This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the National Research Council's (NRC's) Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their review of this report:

Robert H. Austin, Princeton University,
William F. Carroll, Jr., Occidental Chemical Corporation,
Robert J. Full, University of California at Berkeley,
Laura L. Kiessling, University of Wisconsin at Madison,
Robert S. Langer, Massachusetts Institute of Technology,
Monica Olvera de la Cruz, Northwestern University,
Jose N. Onuchic, University of California at San Diego,
Joel M. Schnur, Naval Research Laboratory, and
David A. Tirrell, California Institute of Technology.

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations, nor did they see the final draft of the report before its release. The

review of this report was overseen by Peter B. Moore, Yale University. Appointed by the NRC, he was responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

Contents

Summary

The ability of biological systems to carry out extremely complex functions in a vast array of environments has long inspired scientists to create synthetic systems that work with similar precision and efficiency. While a lack of understanding of how biological systems function has hampered their ability to make such materials and devices, scientists are nonetheless using an expanding toolbox of new ways to measure, manipulate, and compute properties of matter, living and nonliving. These efforts are beginning to uncover the principles that govern how biological systems work. Application of the principles uncovered by these investigations will one day allow scientists to create synthetic materials, processes, and devices that can carry out tasks with the precision of biological systems. As demonstrated by the opportunities and examples presented in this report, now is a very exciting time for research at the intersection of the biological and materials sciences.

Practical design of biologically inspired materials has the potential to improve the well-being of people everywhere and our nation's economic competitiveness by addressing some of the most urgent national challenges. Biomolecular materials and processes may improve medical therapeutics, allow the creation of reliable sensors to detect biological and chemical threats, and facilitate the transition to energy independence. To realize these opportunities and fully harness the potential of biology to inform the development of materials and processes, further advances in fundamental physics, chemistry, and materials science will be required. Three closely related strategies for the creation of new materials and systems may help to realize the potential of biomolecular materials and processes: biomimicry, bioinspiration, and bioderivation.

- *Biomimicry.* This strategy relies on first learning the mechanistic principle used by a living system to achieve a particular function. One then attempts to adapt that principle to achieve similar function in a synthetic material. One example is the encoding of information into building blocks when they are synthesized. One can also try to create materials that mimic whole cells in their response to external stimuli. Such materials could be used in devices for detecting hazardous biological and chemical agents.
- *Bioinspiration.* Merely knowing that a task can be achieved by a living system can inspire scientists to develop a synthetic system that performs the same function, even if the synthetic system uses a scheme quite different from that employed by the biological system. Nature provides examples of systems whose exceptional properties and performance might be replicated for all sorts of applications. The adhesive gecko's foot, the self-cleaning lotus leaf, and the fracture-resistant mollusk shell have all fueled interest in smart biological materials. Yet attempts to create synthetic analogs have been largely unsuccessful, in part because our fundamental understanding of the biological systems is limited.
- *Bioderivation.* This strategy involves using an existing biomaterial in concert with an artificial material to create a hybrid. A prominent example is the incorporation of biologically derived proteins into polymeric assemblies for targeted drug delivery.

Progress will be facilitated by the efforts of research agencies, the scientific community, and other stakeholders. In particular, five recommended steps will help to overcome the scientific challenges associated with these strategies and to translate the resulting knowledge into achievements of social and economic value.

The synergistic application of approaches traditionally considered to belong to distinct disciplines will be called for. While such concerted efforts are already emerging in isolated cases, substantial interagency and interdepartmental cooperation in support of interdisciplinary research and development (R&D) efforts will be needed.

Recommendation 1: The Department of Energy (DOE), the National Institutes of Health (NIH), the National Science Foundation (NSF), and other relevant departments and agencies should jointly sponsor programs of innovative research at the intersection of different disciplines. Initiatives of this type will provide incentives for universities to work across traditional departmental boundaries. The Office of Science and Technology Policy (OSTP) should take the lead in coordinating such programs.

Physicists, chemists, biologists, and engineers need to work together to create new biomaterials and technologies. Educating scientists and engineers so they can work at the intersection of these fields is crucial.

Recommendation 2: University physics, chemistry, biology, materials science, mathematics, and engineering departments and medical schools should jointly examine their curricula, identifying ways to prepare scientists and engineers for research at the intersection of the physical sciences, engineering, and the life sciences. The educational programs being created should be evaluated from a wide range of viewpoints, including input from leaders in industry and at the national laboratories.

Communication between scientists and engineers from different disciplines is hampered by difficulties in understanding methods, concepts, and jargon. Mechanisms that facilitate communication across and between disciplines are essential.

Recommendation 3: DOE, NIH, NSF, and other relevant departments and agencies should support the development of 1- or 2-week summer courses to train physical scientists and engineers in the tools and concepts of biology and medicine and, conversely, biologists in the tools and concepts of the physical sciences. Special attention should be given to finding ways of communicating fundamental physicochemical concepts to biologists using the mathematical knowledge common to the biology community. Such summer courses would help bridge the physical and life sciences communities, allowing them to exploit research opportunities at the intersection of the fields.

Fundamental research is necessary to realize the applications envisaged in this report and could lead to yet-unimagined technological applications, but the translation of new discoveries into useful products is also crucial. Thus both fundamental and applied research should be carried out.

Recommendation 4: DOE, NIH, NSF, and other relevant departments and agencies should collaborate to link fundamental research with commercial applications. While it is imperative to recognize and exploit the connections between fundamental advances and opportunities to transition them into practice, curiosity-driven fundamental research on outstanding unsolved questions should be encouraged, because it could lead to unforeseen technological advances.

It is difficult for a single laboratory to house the diverse instrumentation and expertise required for interdisciplinary research in biomolecular materials and

processes. Standard equipment in biology laboratories, for example, is not usually found in engineering laboratories and vice versa. Further, many researchers do not in any case have access to facilities, shared or private, containing such equipment and instrumentation. National facilities that house clusters of moderately sophisticated instrumentation and individuals with the associated expertise are important for fostering interdisciplinary research in biomolecular materials and processes.

Recommendation 5: DOE should continue to evaluate the effectiveness of recently created facilities to provide access to midrange instrumentation and computational facilities for the advancement of interdisciplinary research in nanoscience and technology. Based on what is learned from this evaluation, analogous, but distinct, centers could be created to facilitate research in biomolecular materials and processes.

1

Introduction

Research in biomolecular materials and processes can contribute to the understanding of nature and can advance technology in areas of importance to the nation's health and security. The relevance of these materials and processes to national challenges means that vigorous pursuit of this research is likely to pay substantial dividends not only for U.S. economic competitiveness and well-being but also for its intellectual leadership. In this report, only a few of the many examples of such research were selected for elaboration. This chapter begins by describing some of the concepts that appear throughout the report and subsequently describes current research in four areas. This research is detailed in Chapters 2 and 3 of the report. Finally, a brief summary of enabling tools for research in biomolecular materials is presented. Enabling tools are described in more detail in Chapter 4 of the report.

UNIFYING CONCEPTS

There are a number of concepts whose detailed understanding would advance many of the research areas described in this report. The Committee on Biomolecular Materials and Processes refers to them here as unifying concepts. Scientific understanding of how systems behave far from equilibrium, how complex systems are controlled via feedback regulation, how they exploit or avoid stochastic effects, and the nature of intermolecular forces remains primitive. Their elucidation would greatly help in the development of biomolecular materials. For example, scientists have a good theoretical understanding of systems at or near equilibrium, yet liv-

ing biological systems function far from equilibrium; in fact, a biological system is at equilibrium only when it is dead! A theory of systems far from equilibrium is needed to understand biological systems properly. Another key concept is control via feedback regulation. The functional precision of biological systems often relies on feedback regulation. Further, many biological processes (and nanoscale devices) involve small numbers of molecules, and the behavior of such systems is influenced in important ways by stochastic fluctuations. The average response often does not mean much. Biological systems have developed mechanisms to take advantage of stochastic fluctuations as well as to quench their effects, in ways that scientists are just beginning to understand.

Even our understanding of electrostatics and solvation, the underlying forces that govern the action and interaction of charged molecules in polar media, still relies on decades-old approximations or on lessons from necessarily simplified computer simulations. Until all such forces can be accurately computed and combined, scientists will not have a detailed understanding of the action of water and simple ions on intricately constructed macromolecules, much less of the more complex interactions that occur in biological systems.

AREAS FOR RESEARCH

Alternative and Renewable Energy

Harvesting Light

The recently achieved molecular-level understanding of photosynthetic mechanisms has allowed scientists to create membranes that mimic the essential energy-harnessing properties of natural photosynthesis. A deeper understanding of the structure and function of the photoreaction center of biological systems is inspiring the design of synthetic materials and systems that are even more efficient than those found in nature.

Fuels

Cellulose is the world's most abundant biological polymer. Its use as a fuel feedstock to create ethanol is one way to reduce the release of carbon dioxide into the atmosphere, since the carbon dioxide formed during combustion is balanced by that absorbed as ethanol-producing plants grow. Cooperative research exploiting advances in plant genetics, process chemistry, biochemistry, chemical biology, and engineering will make it possible to convert renewable biomaterials like cellulose to useful fuels like ethanol.

Motors

From the subcellular level to the level of the whole body, movement is made possible by proteins that transform chemical to mechanical energy. For many years now there has been the hope of creating synthetic systems capable of similar efficiency and control. Now that we are able to measure force and motion at the molecular level and now that the structure of the component proteins has been resolved, it may soon be possible to mimic these living structure and motility systems to create robust artificial devices such as molecular sorters, filters, concentrators, switches, and power sources.

Health and Medicine

Clinical Diagnostics

The ability to diagnose major diseases has improved dramatically in the past two decades. Biomolecular materials have been key to these advances. It is now possible to design biomolecular materials that undergo large physical changes when they bind to a target molecule and to design systems that exploit the consequences of cooperative binding events.

Drug Delivery

There has been a longstanding interest in using biomolecular materials for the delivery of therapeutic agents. Nanometer-size particles show promise as vehicles for the targeted delivery of payloads such as siRNA and DNA and as labels to monitor such delivery.

Prosthetics

The design, fabrication, and integration of functional biomaterials into prosthetic devices present a number of challenges. Next-generation prostheses will likely incorporate feedback loops that involve sensing and actuating components.

National Security

Sensors

Cells can detect minute amounts of molecules with extraordinary sensitivity. However, because only very small physical changes occur when a sensor binds to its target, the problem has been translating such an event into a measurable

output signal. Current research on designer biological sensors whose physical states change significantly when they bind to their target may solve this problem. A specific challenge will be to create a sensor that detects engineered as opposed to natural threats.

Next-Generation Bioinspired Materials

Supermaterials

Biology presents many examples of materials able to work under extraordinary conditions. The mechanisms that allow geckos to walk on a ceiling and lotus leaves to be self-cleaning are being revealed, as are many of the mechanisms used by other smart biological materials. These revelations may allow scientists and engineers to synthesize improved materials for specific applications.

Materials with Information Content

Modern polymeric materials serve mainly structural purposes—as plastics, clothing, paints and surface coverings, for example. They are mainly composed of repeats of a single type of monomer unit. The future will see materials that mimic the more flexible sequence-structure-property relationships of biopolymers.

Self-evolving, Self-healing, and Self-replicating Materials

Populations of living organisms sometimes seem to reengineer themselves, evolving to meet new challenges. Likewise, individuals can adapt to environmental pressures. Current research is aimed at understanding these strategies. Scientists and engineers may someday be able to use these strategies to develop new materials that correspondingly mimic the ability to evolve and adapt.

Enabling Tools

The exciting current state of research in biomolecular processes and materials has been powered by new experimental and computational tools for interrogating complex systems at a high level of detail. Further advances in the development and application of these tools are crucial to the advancement of the field.

Experimental Probes

Advances in experimental technologies may soon allow the imaging of cells at the angstrom scale with subsecond resolution. The measurement of forces and motions of nanomachines and the study of the assembly of complex functional structures have also been dramatically advanced. Further development of single-molecule imaging technologies, electron microscopy, and X-ray and neutron scattering will be very important.

Theoretical and Computational Probes

Theory and computation have a rich and proud history in the physical and engineering sciences. A grand success of theory in the life sciences was the determination of the structure of DNA, which emerged from the confluence of theory, computation (wooden models), and diffraction experiments. Today, theory and computation are slowly emerging as an important complement to experimentation in many areas of the biological sciences. However, for theory and computation to become full partners with experiment, significant advances are needed. Such advances would include ways to sample configuration space and dynamic rare events, efficient algorithms for stochastic simulation of spatially resolved cooperative dynamic events, and the creation of a fundamental theory of systems far from equilibrium.

Chemical Synthesis

Researchers have achieved great mastery over small-molecule organic synthesis and characterization. However, at the macromolecular scale, researchers have not gained a corresponding level of synthetic control. Fully characterizing the structure of multifaceted three-dimensional architectures is also currently problematic, yet the solution is of the utmost importance for accurately mapping structure to function. At the most fundamental and essential level, materials researchers must acquire the ability to synthesize, modify, and manipulate novel macromolecules with atomic-level control.

2

Understanding Biomolecular Processes: Toward Principles That Govern Biomaterial Design

The application of new technologies such as DNA chips and fluorescent labeling of molecules has led to remarkable progress in the ability to collect detailed data about biological processes. Advances in genetics and proteomics are producing huge amounts of data. New knowledge of gene regulation and cell signaling is resulting in an ever more detailed understanding of these complex phenomena. With this increasing amount of data comes increased understanding of the mechanisms that underlie many of these processes. For example, the understanding of the role of restriction enzymes has led to completely new applications in gene manipulation. These enzymes selectively cut strands of DNA to protect cells against viral invasion, yet they do not cut the cell's own DNA. As another example, the discovery of small interfering ribonucleic acids (siRNAs) will have enormous impact on studies of gene regulation and cell signaling. The list of such discoveries is constantly growing.

This improved understanding of the principles that underlie biological dynamics and function also creates new opportunities in biomolecular materials. It is now possible to design materials that not only mimic the properties of biological materials but also mimic the function and underlying principles of biological systems. This represents a significant step forward for biomolecular materials. Current research could lead to qualitatively new materials and to qualitatively new functions and methods for use of these materials. Such possibilities become feasible when new knowledge of biological processes is combined with the ability to fabricate new materials.

While good progress has been made so far, the potential impact of new func-

tional materials through continued understanding and exploitation of biological processes is enormous. In this chapter, specific examples of research on biomolecular processes are discussed. The focus of the chapter is on learning the principles of biomolecular processes, which could then be used to design biomolecular materials.

Imagine that one could . . .

- Create sensors having the exquisite sensitivity and accuracy of the immune system, able to detect minute quantities of molecules with a very high precision.
- Create new biomolecular materials with highly adaptable and controllable properties based on the mechanical design principles of cells, where biomolecular motors can actively control the stiffness of the networks that give the cell its rigidity.
- Assemble new materials with the incredibly detailed precision made possible by interactions that result from the sequence of oligonucleotides.
- Engineer advanced materials that mimic evolution and adapt their properties to address new environmental pressures or to self-heal disruptions.
- Develop advanced materials that self-replicate, storing structure and function information in the materials themselves, just as is done in the genomes of all living species.

These revolutionary scientific goals represent the future impact of the new materials that will be possible through increased understanding and utilization of biomolecular processes. Following discussion of certain areas of research, specific challenges and opportunities are discussed at the end of this chapter.

MULTIPLE COOPERATIVE INTERACTIONS

Nature has evolved impressive means to sense and respond to a wide variety of stimuli. For example, in response to many external stimuli, individual cells can modulate their mechanical properties, shape, growth rate, motility, secretory functions, and the biochemical and charge characteristics of their surfaces. The extraordinary precision with which they sense environmental cues and the exquisite control with which they modulate their properties to affect specific functions is not matched by any synthetic material. The ability to even crudely mimic this ability of cells is expected to find application in a myriad of technologies.

The key process that facilitates this precision, sensitivity, and selectivity, and the exquisite control in response, is recognition based on the many weak but highly cooperative interactions that are buttressed by positive and negative feedback modules spanning a spectrum of time and length scales. Scientific understanding of the

principles underlying such hierarchically arranged cooperative processes involving feedback is still rudimentary. With an understanding of these principles, scientists will be able to create materials with unprecedented functional capabilities.

The process of unraveling the principles will be expedited by bringing theoretical and computational approaches together with experimental approaches such as genetic, biochemical, and imaging experiments. Expertise in the statistical mechanics of complex systems from the study of soft materials will also be a great asset. In addition, the effects of stochastic fluctuations must be included and properly treated. Ideas from network theory may also help researchers to understand cooperative processes that take place on very different length and time scales. Research at the crossroads of statistical mechanics, materials science and engineering, and molecular and cell biology should pay dividends for both fundamental biological research and the understanding of these essential highly cooperative processes and interactions. Some examples of research focused on understanding the specificity of detection and the precision of response in biological systems follow in the next subsections.

Cells

Many living cells carry out specific functions when they sense certain external stimuli. One example of cells that function this way is the T lymphocytes, also known as T cells. They have evolved to deal with pathogens that are no longer in the blood or on mucosal surfaces but have penetrated other cells. Because they combat pathogens that have not been previously encountered, they are critical components of the adaptive immune system in higher organisms such as vertebrate animals. They can rapidly and sensitively detect the presence of biological and chemical hazards. Moreover, they can detect minute quantities of hazardous molecules without frequent false positive responses. Thus mimicking T cells would be one way of overcoming an important challenge facing society today—namely, the rapid and sensitive detection of biological and chemical hazards in the environment, including unknown pathogens that could be engineered, perhaps from existing agents.

Specialized cells, called antigen-presenting cells (APCs), display molecular signatures of the pathogen on their surface (Figure 2.1). Antigen-derived proteins are cut up into small peptide fragments by enzymes in APCs. These peptide fragments can then bind to major histocompatibility proteins, and this complex of peptide (p) and major histocompatibility (MHC) is the molecular flag of the pathogen displayed on the surface of APCs. Peptides derived from proteins of the host organism can also bind to MHC molecules, and these self pMHC molecules are also displayed on APC surfaces. T cells can detect as few as 10 antigen-derived pMHC molecules in a sea of tens of thousands of self pMHC molecules.

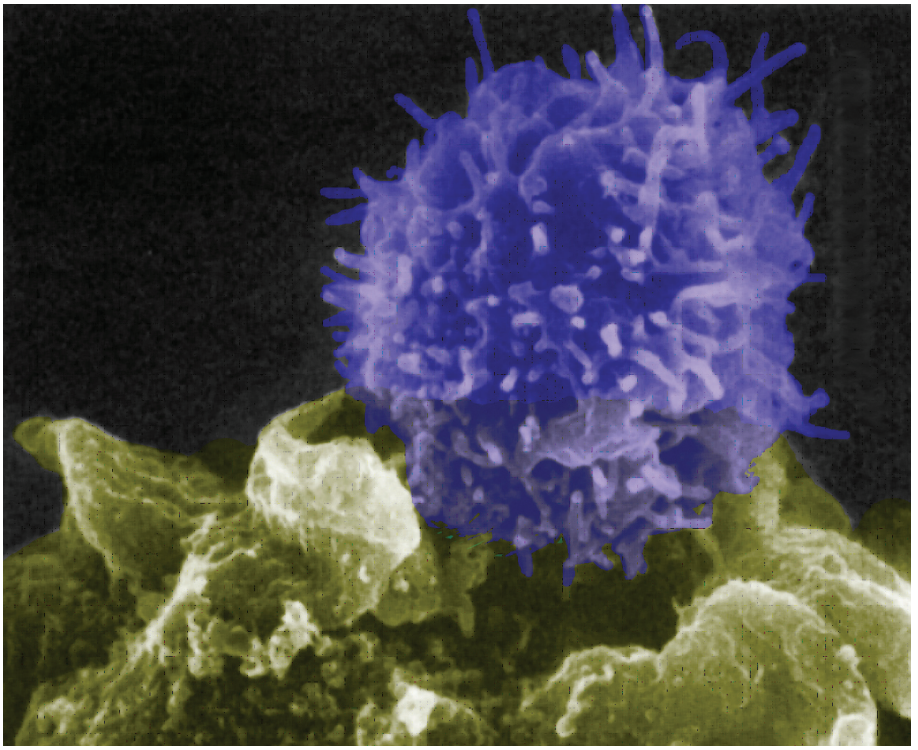

FIGURE 2.1 T cell (shown in blue) interacting with an antigen-presenting cell (in green). The latter display molecular signatures of unknown pathogens. T cells can detect fewer than 10 such molecules based on cooperative interactions between membrane-bound and cytoplasmic molecules. SOURCE: Michael L. Dustin, Kimmel Center for Biology and Medicine of the Skirball Institute of Biomolecular Medicine, New York University School of Medicine, Program in Molecular Pathogenesis.

An understanding of how T cells operate is still emerging. Recent results from studies that bring together the physical and biological sciences suggest that a deep understanding of how T cells use cooperative interactions and feedback regulation of signaling cascades for sensitive detection could develop in the coming years. Related studies of other cellular components of the immune system (e.g., natural killer (NK) cells and macrophages) that serve as sentinels have also been illuminating. Further work along these lines will result in an understanding of the biomolecular processes that could then be harnessed to design synthetic materials that can mimic the specificity of the cells that comprise the immune system.

Understanding how cells of the immune system function is greatly aided by advanced experimental technologies that provide vivid images of the spatiotemporal evolution of key cellular components. The activation of a T cell (and, indeed, other types of cells) is an emergent property in that it is the consequence of cooperative dynamic events that involve a myriad of membrane-associated and cytoplasmic components. An understanding of the mechanistic principles is essential for the future creation of cell-mimetic materials that will incorporate only those components necessary to affect a particular stimuli-dependent response.

More generally, the exquisite sensitivity of cells to their environment and their complex yet detailed response to stimuli can be harnessed in many other ways to create new materials. For example, cells can themselves be used as detectors in many different sensors, from specific assays to test for disease or individualized response to drugs, to highly selective and sensitive detectors of pathogens. This use requires an interface between the cell, its control and response circuitry, and the more traditional electronic circuitry of modern instrumentation. This interfacing of systems represents an important challenge in biomaterials research. Another potential use of cells themselves is in personalized medicine. For example, the response of cells from an individual could be tested against different drug combinations to optimize the choice for the individual. All these uses require research into the behavior of cells and into the new biomolecular materials required to create the interface between the cell and ancillary electronics or other readout mechanisms.

Cell-mimetic Materials

Future research opportunities also exist in the creation of bioinspired systems that mimic the behavior and properties of T cells. These will probably be based on synthetic vesicles that contain the key biochemical elements of the signaling machinery and secretory apparatus identified by studies of the biomolecular processes inherent in T cells. One specific class of candidate materials that may suit this purpose are polymersomes, capsules formed from bilayers of complex amphiphiles (for example, short peptide amphiphiles or co-assembled cationic-anionic amphiphiles). Polymersomes are stable structures into which molecular functionality can be incorporated. A major challenge is to determine how to make them interact with the environment in a more flexible manner as cells of the immune system do. For example, can they be made to open and close "pores" in response to signals? The challenge here is the development of structures that serve as the support surface and encapsulate the cell while remaining robust and flexible.

It is also important to understand the organization of components on living cell membranes and synthetic vesicles. The challenge here is to develop systems that can both sense the stimuli and simultaneously respond to them in some proactive way. This effort will benefit from a combination of spectroscopy, materials

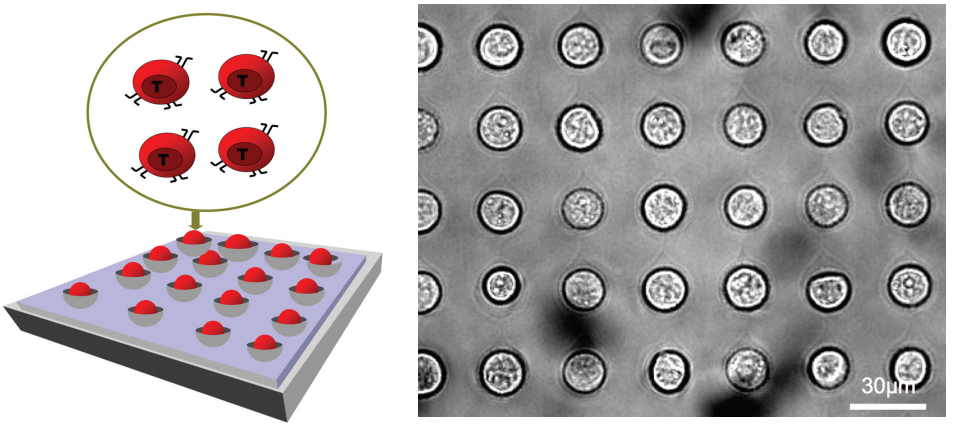

FIGURE 2.2 Hydrogel microwells used to create microarrays of single live lymphocytes. Micrograph at right shows array of live B cells. SOURCE: H. Kim, R.E. Cohen, P.T. Hammond, and D.J. Irvine, "Live lymphocyte arrays for biosensing," *Advanced Functional Materials* 16:1313 (2006). Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

science, and biology. In addition, platforms where materials such as polymersomes can sample the environment will also be essential for developing sensing devices that mimic the immune system. High-throughput experimental techniques may prove particularly valuable. One example is the patterned surfaces that can mimic lymphoid tissue in living systems (see Figure 2.2).

PROCESSES FAR FROM EQUILIBRIUM

One of the distinguishing features of virtually all biological systems is that the description of materials and kinetics using equilibrium statistical mechanics often no longer applies. This is mainly because the molecular motors and other molecules that are present convert chemical energy, usually in the form of adenosine triphosphate (ATP), into mechanical energy, increasing the level of mechanical activity in the cell. It also results in fluctuations within the cell that can appear remarkably similar to Brownian motion but that are not driven solely by thermal effects. Interestingly, when Brown first observed the motion that now bears his name, he attributed this motion to "vital" processes due to living objects, and it was only after he observed the same effect in clay and other inanimate objects that he realized that the motion is in fact ubiquitous. It was, in fact, Einstein who ultimately confirmed the purely thermal origin of what is now known as Brownian motion.

However, certainly within the cell, and probably in many other places, the result of constant but random motor activity is fluctuations that have many features in common with thermally induced Brownian motion, much like those first imagined by Brown during his seminal studies.

The nonequilibrium fluctuations that result from motor activity have a significant impact on the behavior of all the dynamics within the cell. They can affect the mechanical properties of the structures within the cell, and they can also affect the signaling of the cell and its response to stimuli. Thus a better understanding of these effects is essential. More generally, application of all the tools of statistical mechanics to the dynamics of biological processes is hampered by the fact that so many things are way out of equilibrium, so that it will be essential to develop new theoretical and conceptual tools that can directly address such nonequilibrium processes.

Clearly the nonequilibrium transport of molecules is critical for cell function and often accomplished by molecular motors. These are discussed further in Chapter 3. Another critical transport process is that of ions. The precise measurement, molecular description, and elaborate analysis of ion transport through ~1-Å-wide channels combine to give what is probably the best example of rigorous physical thinking on a biological material. The determination of the structure of a chemical- and voltage-dependent potassium channel, together with the electrical observation of single channels, allows researchers to speak quantitatively of the mechanisms that control the channel's opening and closing, to allow potassium ions to move out of a firing nerve cell. At the next level, the nanometer level, there has been systematic work not only on sizing channels to study the physics of transport through well-defined structures but also on determining how these channels might prove to be conduits for specifically designed antibiotics or nutrients to enter cells. Detailed understanding of transport processes will facilitate new applications. For example, can the understanding of ion transport be generalized to nonbiological systems, where control of charge can be a mechanism to control the structure or function of biomolecular materials?

While motor activity within the cell drives nonequilibrium fluctuations, the cell is, nevertheless, always near room temperature, and many biochemical processes can be described by traditional equilibrium statistical mechanics. This is particularly true of the enzymatic reactions that are so critical to the function of the cell. The interplay between the equilibrium and nonequilibrium phenomena will provide much insight into the nature of many essential cell functions, and this represents an important area for further investigation. Moreover, as these processes become better understood, new ways to harness the use of cells and the tissues that are constructed from them will surely emerge.

DESIGN PRINCIPLES FOR MECHANICS

The cell is a remarkable construction that combines both specific function and flexibility of performance with an array of mechanical properties. It is highly controllable and highly adaptable, and its properties can change significantly in response to external stimuli. The cell can change its shape and become motile; it can both sense and respond to forces in its environment. At the same time, the cell is sufficiently rigid to maintain its own shape. Understanding the design principles that control the mechanics of the cell and other living systems would facilitate the development of biomolecular materials that share these physical properties.

The elasticity of a cell comes from several different load-bearing structures: The cytoskeleton is an elastic network throughout the cell, made primarily of actin filaments and many cross-linking proteins. Microtubules are the stiffest rodlike element in the cell and provide both structural support and physical pathways for transport of material within the cell. Intermediate filaments form a structural component that provides elasticity to the cell and bears tension. The stiffness of the networks that make up the cell is highly controllable. The networks are under internal tension, which is balanced both by adhesion of the surface of the cell to the surrounding medium and by the compressional load-bearing capability of the microtubules. Internal tension is provided by activity of biomolecular motors within the cell. The elasticity is highly sensitive to the degree of internal prestress, providing a sensitive control mechanism through regulation of motor activity.

Cells exert forces in many ways: Molecular motors within the cell can provide forces of several piconewtons each and, operating in concert, can exert much larger forces. The elasticity of the networks that make up the cell can also provide a force when they are strained. The cell is also constantly remodeling its shape, and the polymerization of the network components during the course of this remodeling also provides a force. The cell adheres to its surroundings and can exert a tension on the matrix, coupled through focal adhesions, the points where the cell is adhered to the external environment. Motor activity within the cell is coupled to the matrix through these focal adhesions to exert the external force. Cells also respond to the elasticity of their surroundings; for example, the differentiation of stem cells is strongly influenced by the stiffness of their surroundings.

Molecular motors, in conjunction with the cytoskeleton, control cell shape, division, targeted intracellular transport, and many other cellular motions. These motors are briefly described in Chapter 3. Muscle contraction is an extreme example of cell motility that allows higher organisms to maintain posture, move, walk, and swim. At the microscopic level, the contractile organelle is termed the sarcomere. Micrometer-sized filaments of polymerized actin and myosin interdigitate and slide to produce force and shortening. The molecular mechanism of the sliding follows the general operational properties of protein motors described in Chapter 3.

Hundreds of sarcomeres arranged in series and in parallel within the muscle cell increase the force and motion to the macroscopic levels required to achieve locomotion. Muscle contraction is highly adaptable to the conditions of work, and individual muscle cells vary considerably in their speed, metabolic requirements, and resistance to fatigue. The transduction of metabolic energy into work is more than 50 percent efficient. Man-made actuators for animal and robotic locomotion have generally been based on different principles, such as electrical or magnetic forces. The energy density of these actuators is usually lower. Understanding the principles of muscle contraction and, even more particularly, how they self-assemble into the highly regular sarcomeric structure may lead to bioinspired actuators, which could be used in man-made devices as prosthetics or as actuators or drives in other devices.

Another important mechanical function within cells is the transport of materials. As the cell grows or remodels, material must be transported from one location to another. Given the size of a cell, one would expect reasonably rapid diffusion. In fact, the interior of a cell is a very crowded environment, slowing any diffusive transport. As a result, the cell typically relies on molecular motors to actively transport materials. Mimicking such active transport could qualitatively change the way transport is accomplished in biomolecular materials. This would make it possible to specifically target what and where things are transported, using active processes that overcome the limitations inherent in random diffusion.

The number of certain molecules in a cell can be very low, especially those involved in gene expression—for example, DNA, specific messenger RNAs (mRNAs), and transcriptional and translational regulators. The randomness of both the interactions and the conformational transitions of these molecules leads to fluctuations in the content of a given protein in cells that are otherwise identical. Theoretical and experimental studies have shown that such fluctuations are inevitable at the low copy numbers of regulators and the rates of cellular processes. So-called "gene noise" and its dynamic characteristics have been detected from variations of mRNA and protein expression between cells. These fluctuations limit the precision of expression in cellular regulatory networks, but they may also be advantageous for development, adaptation, and evolution by facilitating the sampling of nearby alternative states that may improve cell function. Signaling processes such as those that may be important to mimic in creating cell-mimetic materials can also involve molecules present in small copy numbers. The influence of stochastic fluctuations on such signaling processes has received less attention. It is possible that signaling processes have developed ways to quench the deleterious effects of fluctuations while exploiting them to generate phenomena such as discrete decisions. Biomimetic and nanoscale materials may also exhibit statistical fluctuations when very small copy numbers are involved; these need to be taken

into account in the design of the material, as they may be advantageous for adaptation to changing conditions.

The adaptability of the mechanical properties of the cell serves as a model for the properties that can be achieved by biomolecular networks. However, to fully exploit the remarkable properties of such materials, it is essential to determine the underlying design principles that determine their properties. What gives small amounts of these materials such very high strength? What is the underlying cause of the highly nonlinear behavior? What is the role of the specific cross-linker? How does the cross-linker determine the network architecture? What impact does the architecture have on the mechanical properties of the network? How is the nonlinearity controlled?

If these basic materials design principles are understood, they should provide the requisite guidance for fabrication of new materials with similar properties. For example, a consequence of malaria infection is that the mechanical properties of the red blood cells are altered, making it difficult for them to squeeze through blood vessels. If one could change the mechanical properties of cells, one might be able to address significant problems like malaria. Can materials be designed that can change their mechanical properties in response to environmental cues? Can an intrinsically hard material be designed that becomes soft when it senses certain stimuli? Such a capability might allow a material to enter compartments that otherwise exclude it and then carry out some function. Can the control circuitry of the cell, which depends on the highly regulated activity of biomolecular motors, be adapted to other materials? This would allow the stiffness of a material to be changed by several orders of magnitude by tuning motor activity.

Knowledge of the fundamental design principles of the cell will facilitate the building of bioinspired structures, formed either directly from biomolecular materials or, alternatively, from strictly synthetic nonbiological analogs. Such bioinspired materials can be designed to mimic the behavior and dynamics of the cell and to recreate its remarkably adaptive and highly controllable mechanical properties. Any material fabricated with these design principles would, ideally, be scalable: The same principles could apply, for example, to the construction of macroscopic networks, which could have similar strength-to-weight ratios and which would have similar controllable mechanics. These materials would represent a truly new class of material.

SELF-ASSEMBLY, DIRECTED ASSEMBLY, AND SPATIOTEMPORAL ASSEMBLY

Assembly of complex structures is ubiquitous in nature, from the one-of-a-kind patterns of individual snowflakes to the membrane that surrounds every cell. Assembly is a common paradigm in materials, biological and nonbiological

alike, but the mechanisms driving the assembly processes are profoundly different. Evolution has perfected assembly mechanisms that lead to remarkably complex structures in biology; examples include microtubules, cell membranes, viruses, and a myriad of other structures that far surpass traditional materials in both design and functionality.

Assembly processes can be divided into several categories. Self-assembly refers to the spontaneous organization of preexisting components under the influence of forces acting among the components. Self-assembly is generally considered to be a reversible process, tunable by varying a thermodynamic parameter such as temperature or density, and one that can be controlled through judicious design of the components. Typically, self-assembled structures form based on thermodynamic principles in which free energy is minimized subject to constraints. In polymers, liquid crystals, metal alloys, and other nonbiological materials, van der Waals and electrostatic interactions between atoms and molecules conspire to produce bulk materials whose elementary building blocks self-organize into stable equilibrium patterns. This same mechanism is also observed in colloidal and nanoparticle systems, where additional solvent-mediated interactions contribute to self-assembly.

Self-assembly is not always sufficient to ensure the organization of molecular or other building blocks into highly organized structures. Sometimes the system can get stuck in kinetic traps. To overcome these limitations, guided or directed assembly exploits the application of external fields, such as electric, magnetic, or shear, to help align the assembling particles, affording another means for structural organization. Alternatively, templated self-assembly involves the use of scaffolds or templates to provide a pattern on which building blocks order; such templates can be used to promote the formation of a desired structure in preference to competing structures with similar free energies.

Nature exploits all of these categories of assembly processes. However, nature also uses a much more sophisticated assembly process that combines many spatial and temporal scales. For example, precise specific interactions among proteins lead to the self-assembly of highly organized complex structures, often with no known analogue in nonbiological systems. These structures may be dynamic, stable far from equilibrium, and may reorganize based on dynamically changing interactions among the constituents, leading to complex, emergent behavior. Processes carried out in living cells, for example, depend on the spatial organization of many different chemical components. These assembly processes are often hierarchical: Self-assembly of elementary building blocks results in secondary building units, which assemble into tertiary building units, and so on. This hierarchical assembly can lead to powerful functionality. However, a fundamental understanding of these spatiotemporally correlated assembly processes remains elusive. Such understanding is essential if we are to exploit the mechanisms that control many biological

systems. It is also essential to be able to mimic these assembly processes to create new materials that possess new functionality.

One important example of directed assembly that has already been widely investigated is the use of DNA as a building block to program the self-assembly of larger structures. For materials, this approach holds the promise of fabricating more complex structures than are currently attainable. Both DNA and RNA are molecular materials for programmable assembly in which the selective affinity of base pairs on strands of these molecules is exploited for fabricating nanostructures with designed geometries. Through judicious design of the base pair sequence, oligonucleotide strands can be fabricated to bind reversibly with complementary strands. This unique property of DNA allows this "molecule of life" to be used as an architectural element, both as a building block itself and as a linker of building blocks; this has resulted in new areas of research, such as DNA computing, structural DNA nanotechnology, and programmable self-assembly (Figure 2.3).

Like RNA, DNA assembles into structures other than the double helix, including hairpins and multiway junctions. Cohesive or "sticky" ends on these motifs allow for their use as architectural elements for larger nanostructures, including cubes (as in Figure 2.3), truncated octahedral rings, knots, bricks, and three-dimensional crystalline arrays. These DNA structures and nanoarrays can further serve as templates for nonbiological materials and as scaffolds for nanoelectronic components and nanomechanical devices; examples include a bipedal walker and a translation device. The field of structural DNA nanotechnology is an emerging area of biomolecular materials research at the intersection of the physical and biological sciences. Another potential application is in computation; DNA nanostructures have been used as molecular building blocks for self-assembled tilings, which can, in turn, be used for molecular computation. The first two-dimensional example of this was an "exclusive or" (XOR) logic function.

Hierarchical Self-assembly

A classic example of precise hierarchical self-assembly is the virus. All viruses are made up of protein coats (called capsids) that protect the viral genome. Capsids are self-assembled from groups of proteins called capsomers, which interact noncovalently to create the capsid structure; in some viruses, chaperones help to direct their assembly. In about half of all viruses, the capsid is roughly spherical and takes the shape of a perfect, 20-sided polyhedron or icosahedron composed of integer multiples of 60 proteins. Influenza, herpes simplex (HSV-1), human rhinovirus (which causes the common cold), and hepatitis B are all examples of icosahedral viruses. Other capsid shapes include prolate spheroids or ovoids (aberrant flock house virus (FHV) and alfalfa mosaic virus (AMV)), cones (HIV), and rods (tobacco mosaic virus and H5N1, or avian flu, virus).

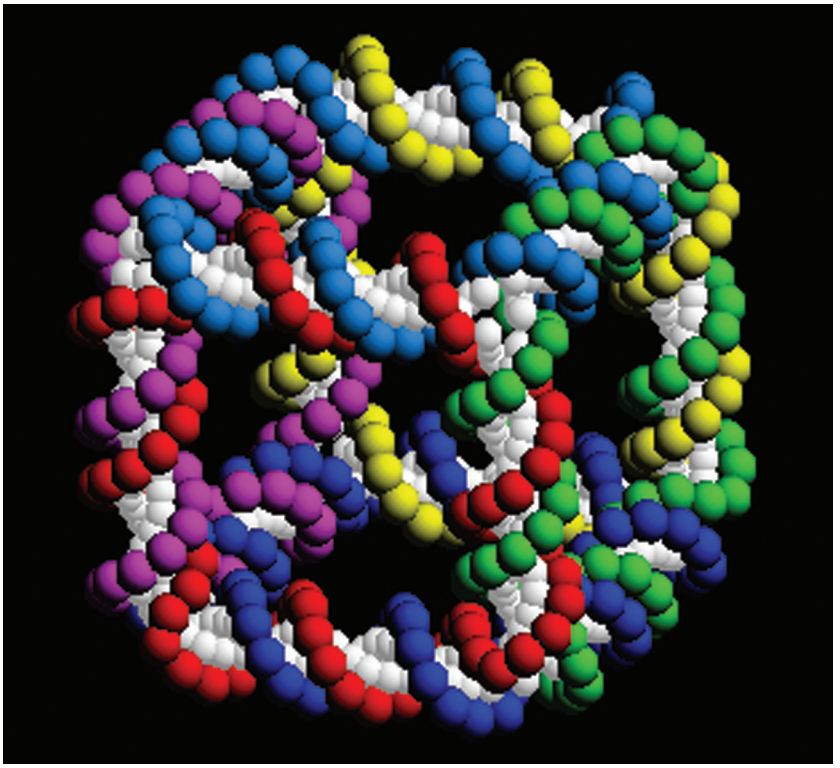

FIGURE 2.3 DNA cube with six different cyclic strands. Their backbones are shown in red, green, yellow, magenta, cyan and dark blue. Each nucleotide is represented by a single colored dot for the backbone and a single white dot for the base. Note that the helix axes of the molecule have the connectivity of a cube. However, the strands are linked to each other twice on every edge, making this molecule a hexacatenane. The red strand is linked twice to the green, cyan, magenta, and dark blue strands and only indirectly to the yellow strand. Each edge of the cube is a piece of double helical DNA, containing two turns of the double helix. SOURCE: Nadrian C. Seeman, New York University.

The controlling factors that govern the self-assembly of proteins into these highly ordered structures precisely, rapidly, and repeatedly to propagate an infection in living organisms are not yet understood. Many theoretical models exist, most involving the complex interplay of specific and directional noncovalent interactions among protein building blocks. Control of these interactions and the subsequent assembly process would allow the design of antiviral drugs to interrupt virus replication or the fabrication of empty viral capsids containing disease-fighting

drugs. Moreover, an understanding of the basic design and assembly principles of these structures would permit the use of complex, hierarchical self-assembly processes to construct many other completely different structures, either from proteins or from inorganic nanoparticles designed to assemble as proteins. New "patchy" particles with diameters between 1 and 1,000 nm are now being synthesized with unprecedented anisotropy as building blocks with directional and specific interactions for assembly into complex structures. Furthermore, viruses themselves, or bioinspired structures imitating viruses, could be used as building blocks for the construction of new materials on a larger scale. For example, highly precise and symmetric capsid and capsidlike structures can serve as templates for novel new materials such as oriented quantum-dot nanowires. The use of viruses or other biomolecular materials such as proteins, including engineered proteins, as structured building blocks to create larger structures represents an important opportunity in hierarchical self-assembly.

Another example of hierarchical self-assembly is implicated in amyloid diseases, in which peptides assemble into beta-sheet tapes, which assemble into ribbons (double tapes), fibrils (twisted stacks of ribbons), and ultimately fibers (entwined fibrils). Such fibrils are thought to play a critical role in diseases such as Alzheimer's and Pick's. Building block chirality is thought to be very important in the hierarchical assembly of many such biological structures, but the process underlying the order of various subprocesses in this type of assembly is poorly understood. By contrast, assembly in hard materials such as ceramics and metals is not typically hierarchical, and in soft materials and complex fluids such as surfactants and block copolymers the hierarchy is often limited to the self-organization of secondary aggregates into ordered liquid crystalline lattices. An understanding of how nature exerts spatiotemporal control over the assembly of groups of building blocks to create precise structure at successive scales would be of great value. For example, it could allow development of drugs to constructively disrupt the fibril formation responsible for Alzheimer's. In addition, it could open the way to the exploitation of similar principles to create hierarchically arranged structures with nonbiological materials.

Complex Spatiotemporal Assembly

In many biological systems, different spatial patterns form over time. One such example is provided by the formation of the immunological synapse. As described earlier in this chapter, the orchestrators of the adaptive immune response are a class of cells called T cells. When they interact with antigen-presenting cells (APC) and recognize the molecular markers of pathogens, different types of receptors and ligands organize themselves into specific spatial patterns that evolve with time (Figure 2.4).

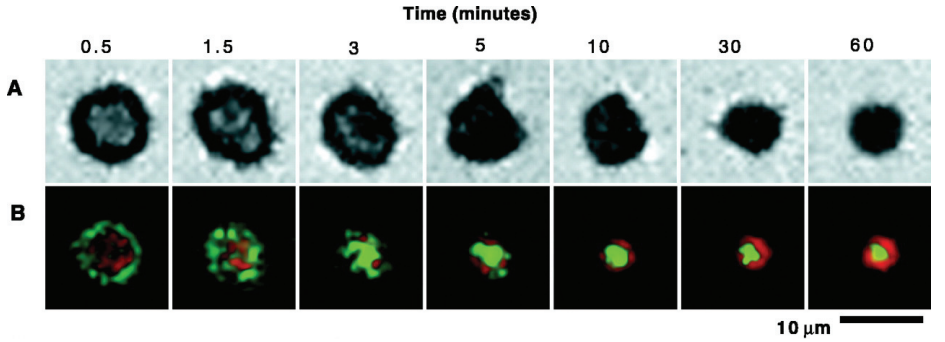

FIGURE 2.4 Formation of the immunological synapse: T cells in contact with a supported planar bilayer. (A) Images of contact formation as seen with interference reflectance microscopy and (B) as seen with fluorescing Oregon green. SOURCE: A. Grakoui, S.K. Bromley, C. Sumen, M.M. Davis, A.S. Shaw, P.M. Allen, M.L. Dustin, "The immunological synapse: A molecular machine controlling T cell activation," *Science* 285:221 (1999).

Panels A and B show how a T cell interacting with a supported bilayer mimics the APC that contains ICAM-1 and pMHC. These images are taken looking up. Panel A shows the time evolution of the shape of the T cell during synapse formation. The darker the color, the closer the apposition between the T cell membrane and the supported bilayer. Panel B is an overlay of peptide-MHC molecules (green) and adhesion molecules (red) concentrations in the intercellular junction. Movies that make these observations of the spatiotemporal evolution of protein patterns and cell shape vivid can be seen online.[1] Similar spatiotemporal patterns were first observed at the junctions between a T cell and an APC.

These spatial patterns are thought to form by a guided self-assembly processes. The intrinsic tendency of receptor-ligand pairs of different sizes to separate due to coupling between membrane elastic forces and topographic size differences is amplified by cytoskeletal motion triggered by T cell signaling. Although there is still no consensus on the exact function of these spatiotemporal patterns, the patterns are thought to mediate specific functions. A fundamental understanding of such guided self-assembly of spatiotemporal patterns of molecules could be exploited in the design of biomolecular materials that perform different functions over time.

Even more complex examples of a spatiotemporal, hierarchical assembly process are motility organelles such as the bacterial flagellum and membranous organelles such as the Golgi apparatus and the endoplasmic reticulum. These are

[1]Movies of these observations are available at www.sciencemag.org/feature/data/1040037.shl. Last accessed March 30, 2008.

examples of highly ordered, precise constructs, whose structure arises from self-assembly and is required for functionality. Bacterial flagella, for example, contain an intricate, 20-nm-diameter helical filament self-assembled from flagellin proteins. Depending on the filament's stress state, the proteins within can pack in either a left-handed or a right-handed configuration. This transition in configuration reorients the swimming direction of the cell. It arises from subnanometer conformational changes in the protein subunits that constitute the flagellum. A quantitative understanding of how applied stresses and torques lead to an overall polymorphic transition from one chirality to another has remained elusive. Such an understanding could provide the basis for nonbiological sensors, actuators, and other nanomechanical devices.

Assembly promises to remain an overarching theme of research at the intersection of biology and materials in the foreseeable future. Hierarchical organization, external fields, and biomimetic motifs for guiding self-assembly, and dynamic self-assembly in dissipative systems, are likely to grow as key themes. Assembly of dynamic structures that are responsive to external stimuli, such as the bacterial flagellum, holds great promise for nanotechnology. The exploitation of anisotropic, noncovalent interactions at nano and colloidal scales is an important biological approach that could be applied to the assembly of nonbiological materials. Breakthroughs in understanding biological assembly processes, and in mimicking it to create new materials and devices, will revolutionize materials fabrication and development.

SELF-REPLICATING, SELF-HEALING, AND EVOLVING MATERIALS

A tenet of all biology is that organisms have evolved to their current state and continue to evolve as they are subjected to environmental pressure. This has allowed finding effective solutions to problems. They are not necessarily the best solutions, but they are solutions that work. As a result, biological species are constantly changing, adapting, and evolving.

Can similar design principles be applied to materials? A key feature of evolution is the genetic encoding of information, which provides an essential means for modification and allows the modifications to become permanent. A living organism responds to environmental pressure by changing its behavior or structure. While this response may ultimately be specified in the genetic code, there is also strong evidence that many changes do not involve direct modification of the genetic code; instead, they involve modification of the complex response of the entire system, exploiting the multiple cooperative interactions and feedback regulation discussed earlier in this chapter. While evolution is highly complex and still poorly understood, the potential for mimicking it is enormous. This could allow the development of materials that actively adapt to their surrounding environment.

It would also make possible the development of materials that modify their behavior to perform new functions in response to changing conditions. These materials could be self-healing, repairing themselves upon being damaged.

Self-replicating Materials

An important feature of living systems is that all the information for the individual member of the species is encoded in the genome. Indeed, each cell has all the information required for reproduction encoded within its genome. This is the key to both producing and reproducing life. This is also the essential challenge: to create a biomaterial that mimics this information content. The challenge might be met by using the same material and sequence information as is used in nature.

Considerable effort is being focused on the use of DNA to assist directed self-assembly of colloidal particles. This methodology exploits the precision and specificity with which DNA can bind. Thus, for example, the formation of colloidal crystals and crystalline alloys by binding with DNA oligonucleotides has already been demonstrated, and similar structures can certainly now be fabricated.

Even if the basic principles are completely understood, and even if they can be incorporated in a material, significant challenges would remain to manufacture the material. Again it is likely that the optimal way to do this, at least initially, is to follow the lead of biology and to use biological materials directly. While this will limit the number and type of materials that can be produced, it will allow known principles to be exploited directly. As researchers learn more about the principles, it is conceivable that other materials will be made following those principles. These could lead to the fabrication of a whole new class of materials.

Self-replicating materials are now being made by another means, called synthetic biology. Organisms such as *E. coli* are being programmed to perform specific activities, usually mimicking specific computer gates such as functions of Boolean logic, making the bacteria work in ways that are analogous to simple computers based on binary logic. This represents an opportunity to harness some of the methodology of nature for other uses. Ultimately, the functions will reflect functions found in biology rather than in the physical sciences. This will call for knowing much more about the specific functions in biological systems. While synthetic biology can reproduce functions familiar to more traditional computation, possibilities well beyond these could be explored. For example, might not some of the complex calculations performed by living organisms be designed into synthetic biology systems, and might not such a capability be harnessed to perform computations?

Self-healing Materials

One distinguishing feature of living organisms is their ability to heal. This ability could take the form of preventing an invasion of foreign substances, as exemplified by the immune system discussed above. Alternatively, it could entail regrowing regions that die or regenerating regions that are injured. Because all the structural and functional information is encoded in the genome, each portion of the living system can, in principle, be regenerated. Stem cells could be one resource for constructing self-healing systems. Understanding the mechanisms by which stem cells differentiate into specific cells might allow similar adaptability in biomimetic systems.

There are numerous other examples where a deeper understanding of interactions at the molecular or cellular level could lead to other self-healing systems. For example, the adsorption of biomolecules at interfaces could serve as a geometric constraint that allows new function and interactions to occur, and these could be harnessed to create alternative forms of self-healing materials. Many biologically important interactions, such as those between membrane proteins, occur at interfaces, and they remain poorly understood. As our understanding of such interactions improves, they can be exploited to create new materials. The key to understanding many of them is a better comprehension of the effects of the inhomogeneity at the interface of the dielectric constant of the material.

Materials That Evolve

An essential feature of biology is the adaptability of living systems to their environment and to changing environmental pressures. Understanding the way living systems adapt will help us to design biomolecular materials with the same traits. Living organisms also adapt over time, through the process of evolution. Adaptation occurs at the molecular level, through the evolution of new proteins; at the cellular level, as cells evolve in their function and responses; and at the level of the organism as species evolve. Understanding the process of evolution, particularly at the molecular level, may be able to show a new way to the development of more sophisticated biomaterials, as described in the examples below.

One endeavor where the principles of evolution are already being exploited is "directed evolution," a way to create new enzymes. This approach adapts the Darwinian process of evolution to create new molecules by subjecting the original enzyme to externally applied pressure and then screening and selecting the improved species. It can be an effective method for creating new enzymes while simultaneously providing new insights into the nature of evolution itself. The concept is also being applied to develop new microbes and cells with improved performance. For example, a directed evolution approach is being used with yeast

cells to create cell lines that can produce ethanol more efficiently. Such an example suggests that some evolutionary traits could be exploited to create biomolecular materials. Improved understanding of existing evolutionary pathways would allow the more rational design of new pathways, but the biggest challenge would be to make the biomaterial evolve itself by subjecting it to external pressures.

Representations of the genomes for all living species and knowledge of the full expression of each gene, the functions of the proteins each gene produces, and, most important, the complex signaling and coupling between the expression levels of these proteins and their complex control circuits are still in their earliest stages. Current techniques to exploit this genetic information to create new materials use only the most rudimentary parts of this knowledge. As the amount of detail that is known increases, and as a more general understanding begins to emerge, increasingly sophisticated methods will be developed to more fully exploit the complex systems of control and signaling that are inherent in living systems.

OPPORTUNITIES AND CHALLENGES

In this chapter, the committee has identified many areas where research is elucidating the underlying processes of biology. This knowledge is the basis for developing new technologies and making new biomolecular materials and processes. The committee discussed the essential nonlinearity in biological processes, illustrating this in particular through the behavior of cells, and considered its implications for new materials, with the creation of materials with cell-mimetic capabilities a prime example. The committee discussed the conversion of chemical energy to mechanical energy by molecular motors, with the consequence that most biological materials cannot be described by equilibrium processes. The committee considered the design principles for mechanical properties of the cell. It also discussed the myriad forms of self-assembly in nature and the implications for new biomaterials and processes if they could be mimicked. The committee considered as well two remarkable properties of living organisms—namely, their ability to evolve in response to new conditions and their ability to regenerate or heal themselves in response to damage. These properties will open up opportunities for new biomolecular materials. Some of the challenges to scientific understanding where new understanding is beginning to emerge are listed below. They are followed by mention of some opportunities that might arise if researchers are able to address the challenges.

- A unique feature of many response and signaling systems in biology is their use of cooperation to create a response to stimuli. This allows them to achieve a very high degree of precision and sensitivity, while reducing spurious false positives.

—Opportunity: Biosensors that combine high sensitivity and high precision
—Opportunity: Biomimetic structures that mimic the specificity of T cells in identifying and selecting pathogens

- The large number of motors that convert chemical energy into mechanical energy ensures that the properties of living species cannot be described with an equilibrium description but must instead be described in the framework of nonequilibrium systems.
  —Opportunity: New description of biological function for materials development
- The essential design principles that describe the mechanical properties of a cell are not fully understood. Some cell components are under tension, while others balance this and are under compression. They are under a steady pre-stress, which apparently drives them into a nonlinear elastic state. The relationship between these properties and the mechanical behavior of the cell remains undetermined or only poorly determined as does the biological rationale for this behavior. However, the design principles for the elasticity of the cell must be understood if its behavior is to be mimicked.
  —Opportunity: Actively controlled biomolecular materials using molecular motors
  —Opportunity: Highly adaptable and controllable biomolecular materials
- Biological systems possess very high specificity in their ability to recognize molecules and to respond and control themselves when this recognition occurs. This ability to very precisely recognize specific targets can be exploited to create new functional materials.
  —Opportunity: Exploit specificity of DNA interactions to fabricate biomolecular materials
  —Opportunity: Use viruses as building blocks for the assembly of more complex materials
  —Opportunity: Mimic viral function in synthetic materials
- Biological systems are the ultimate example of the construction of highly complex structures and systems from simple and common building blocks. This is accomplished by very fine control of the spatiotemporal assembly. Mimicking this behavior would allow changing the paradigm of manufacturing by following the model of biological systems.
  —Opportunity: New manufacturing capability that relies on self-assembly
- Much of the diversity in all living species comes from their ability to evolve, changing both their structure and their function in response to external pressure. Understanding the details of how this is accomplished will allow the development of synthetic bioinspired materials that are able to evolve themselves when external pressures are applied.

—Opportunity: Design new enzymes or microbes with improved functionality
—Opportunity: Improved efficiency for biofuel production
—Opportunity: Materials that are self-healing to recover from disruption

- All living species carry the full information about their structure and function, as well as information about the nature of the complete system. This information is carried through sequence-specific structure, and understanding the details of this information storage and propagation will facilitate the design of bioinspired systems that incorporate this ability.
  —Opportunity: Materials that can self-replicate
  —Opportunity: Materials that can adapt by changing the stored information
  —Opportunity: Modification of materials properties with analogues of RNAi

SUGGESTED READING

Arnold, F.H. "Design by directed evolution," *Accounts of Chemical Research* 31:125 (1998).

Janmey, P.A., and D.A. Weitz, "Dealing with mechanics: Mechanisms of force transduction in cells," *Trends in Biochemical Science* 29:364 (2004).

Mizuno, D., C. Tardin, C.F. Schmidt, and F.C. MacKintosh, "Nonequilibrium mechanics of active cytoskeletal networks," *Science* 315:70 (2007).

Nicolis, G., and I. Prigogine, *Self-Organization in Nonequilibrium Systems,* New York, N.Y.: John Wiley & Sons, 1977.

Thorpe, M.F., and A.E. Carlsson, *The Role of Theory in Biological Physics and Materials: A Report to the National Science Foundation.* Available online at http://biophysics.asu.edu/workshop/report/pdf/bio_mat.pdf. Last accessed on March 24, 2008.

Whitesides, G.M., and B.A. Grzybowski, "Self-assembly at all scales," *Science* 295:2418-2421 (2002).

3

Advanced Functional Materials

A key objective of scientists is to incorporate functional properties of biological materials into new materials and devices. The properties sought include molecular recognition, sensitivity and specificity of response, energy storage and conversion, force dynamics of elasticity, adhesive and other mechanical properties, and optical filtering and detection. In this chapter, key advances, opportunities, and challenges are reviewed in creating biomolecular materials with these specific functional attributes. To organize this chapter, important socioeconomic areas are described where applied biomolecular functional materials are having a strong impact: alternative and renewable energy, health and medicine, and national security. In each of these areas, exemplars of fundamental discoveries and challenges are explored. Finally, the efforts under way to exploit the functional properties of new bioderived, bioinspired, and biomimetic materials are discussed.

Advances in functionalizing biomolecular materials will have enormous impact on U.S. society. To date, there has been demonstrable progress harnessing the functional power of biomolecular materials, especially in alternative and renewable energy, health and medicine, and national security, as described in this chapter. The interest in fuels derived from plants is largely based on a greater understanding of energy conversion processes in these materials as well as a greater understanding of how to manipulate the plant genome. The significant role medical diagnostics play in everyday life (for example, the new generation of glucose testing for diabetics) is a direct result of the understanding of biomolecular recognition events and the ability to manipulate them in useful, easy-to-use devices. Approaches based on bioinformatics and synthetic biology are currently being vigorously pursued, with

the goal of making cells produce products of societal and economic value. Most of the focus today is on products of interest to the pharmaceutical industry. Related approaches may also be of value for the synthesis of new biomolecular materials.

While significant progress has been made, the potential future impact of understanding and utilizing functional biomolecular materials is enormous. Imagine that one could . . .

- Engineer biological enzymes to convert organic matter to usable fuels with very high efficiency in order to substantially reduce dependence on foreign sources of fuel.
- Manipulate biomolecular recognition events to create a biosensor with no false alarms that responds with sensitivity and specificity, mitigating threats before people or other key assets are exposed.
- Create new arrays of medical diagnostic assays that can predict susceptibility to and progression of disease.
- Deploy new materials that will protect people and material assets from chemical and biological contamination.
- Design and fabricate new materials that capture the superlative properties of adhesion in a gecko foot or the elegant strength in design of a diatom or mollusk shell.

These seemingly futuristic touchstones represent some of the future impact that could be realized through the understanding and exploitation of functional properties of biomolecular materials. Following discussion of certain areas of research, specific challenges and opportunities are outlined at the end of this chapter.

ALTERNATIVE AND RENEWABLE ENERGY FROM BIOMOLECULAR MATERIALS AND PROCESSES

Life requires energy and the continual conversion of energy from one form to another. Biological systems have adopted diverse means by which to convert between different forms of energy in order to compete and survive. These unique, adaptive, energy-converting properties of biomolecules have inspired biomaterial scientists. There have been great advances in the understanding of functional biomolecular processes that efficiently convert energy in biological systems. These include the chemical conversion of high-energy-containing materials such as polysaccharides (one of which is cellulose) into fuels, the production of electrical energy with enzymes for fuel cell or battery applications, the conversion of light energy into chemical energy in photosynthesis, and the conversion of chemical to mechanical energy by biological motor proteins.

Biofuels and Processes

Rudolph Diesel contemplated that the engine named in his honor would be powered by vegetable oils. However, the widespread availability of inexpensive petroleum during the twentieth century altered that vision. Twenty-first century realities have rekindled interest in Diesel's original thinking.

Cellulose is the world's most abundant biological polymer. Studies demonstrate that with technological advances, biofuels, such as ethanol from cellulose, could supply a significant fraction of the world's demand for transportation fuels in a way that is carbon dioxide neutral and does not compete with land for food production. The key to achieving these advances is research in plant genetics, biotechnology, process chemistry, and engineering that will lead to new manufacturing concepts for converting renewable biomass to valuable fuels and products.

For certain practical applications, biologically based feedstocks are already having an impact. These include solvents, plastics, lubricants, and fragrances. For example, poly(lactic acid) is a hydrolytically degradable plastic that is currently manufactured on a million-kilogram scale in the United States and on a smaller scale in Europe and Japan. The route to the final product ferments corn dextrose for the production of lactic acid, which is then dimerized and polymerized. The final product is used in food packaging and the clothing industry.

Substrate utilization remains a key challenge in the use of biological feedstocks for meeting energy needs. The abundant products of photosynthesis are primarily renewable cellulose, hemicellulose, and lignin. Although energy-rich, these abundant products are not easily transformed into usable fuels. This challenge, along with process methods that treat these substrates as chemical engineering feedstocks, will be the focus of much of the effort in this area of research. Figure 3.1 summarizes the key global biomass resources from agricultural residues, wood, and herbaceous energy crops.

Polysaccharides and lignin are separated and processed to make feedstocks for materials and fuels. Current methods for separating the constituent biomolecular components rely on thermochemical processing; they are relatively harsh and energy-intensive. The development of more benign and economical processing steps is clearly a challenge. In principle, enzymes could be brought to bear, but doing so in a cost-effective manner remains problematic. The science of genomics, supported by new tools such as proteomics, metabolomics, and imaging in conjunction with the genomic databases, could give us insight into how microorganisms utilize untreated biomass to produce metabolic energy.

The logical companion to bioinspired renewable energy production is the utilization of that energy. This is the focus of research in microbial fuel cells. Microbial fuel cells are electrochemical cells that convert chemical energy into electrical energy by using bacteria as a catalyst to convert substrate materials into

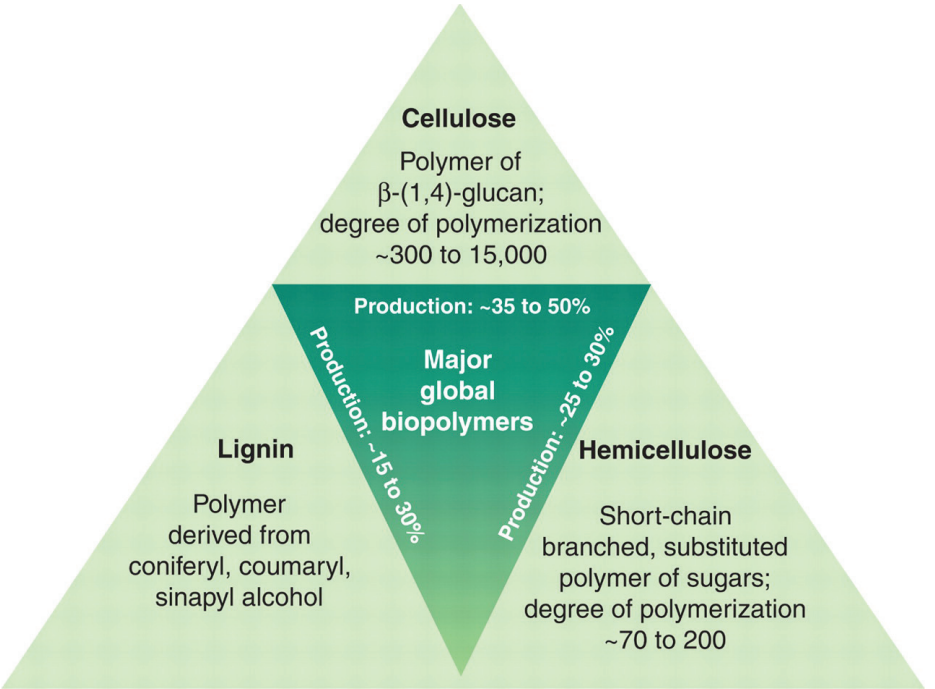

FIGURE 3.1 Key biomass resources: agricultural residues, wood, and herbaceous energy crops. SOURCE: A.J. Ragauskas, C.K. Williams, B.H. Davison, G. Britovsek, J. Cairney, C.A. Eckert, W.J. Frederick, Jr., J.P. Hallett, D.J. Leak, C.L. Liotta, J.R. Mielenz, R. Murphy, R. Templer, and T. Tschaplinski, "The path forward for biofuels and biomaterials," *Science* 311:484-489 (2006).

available electrons. The principle of operation of a microbial fuel cell is illustrated in Figure 3.2. Organic substrates (e.g., glucose) are oxidized to carbon dioxide in the anode compartment. Electrons from the substrates are transferred to the anode. The pathway by which this transfer is achieved is another topic of current research. Several options include soluble electron relays that shuttle between microbe and electrode, direct membrane contact, or nanowires that are produced by the bacteria themselves. In principle, a broad spectrum of substrates can be utilized as fuel. The geometry of the cell is such that electrons are forced to flow through an external circuit and load resistor to the cathode compartment. Simultaneously, a proton-conducting membrane that separates the anode and cathode compartments is needed to maintain electroneutrality. Among the science and engineering challenges associated with this research are the molecular mechanisms for transporting

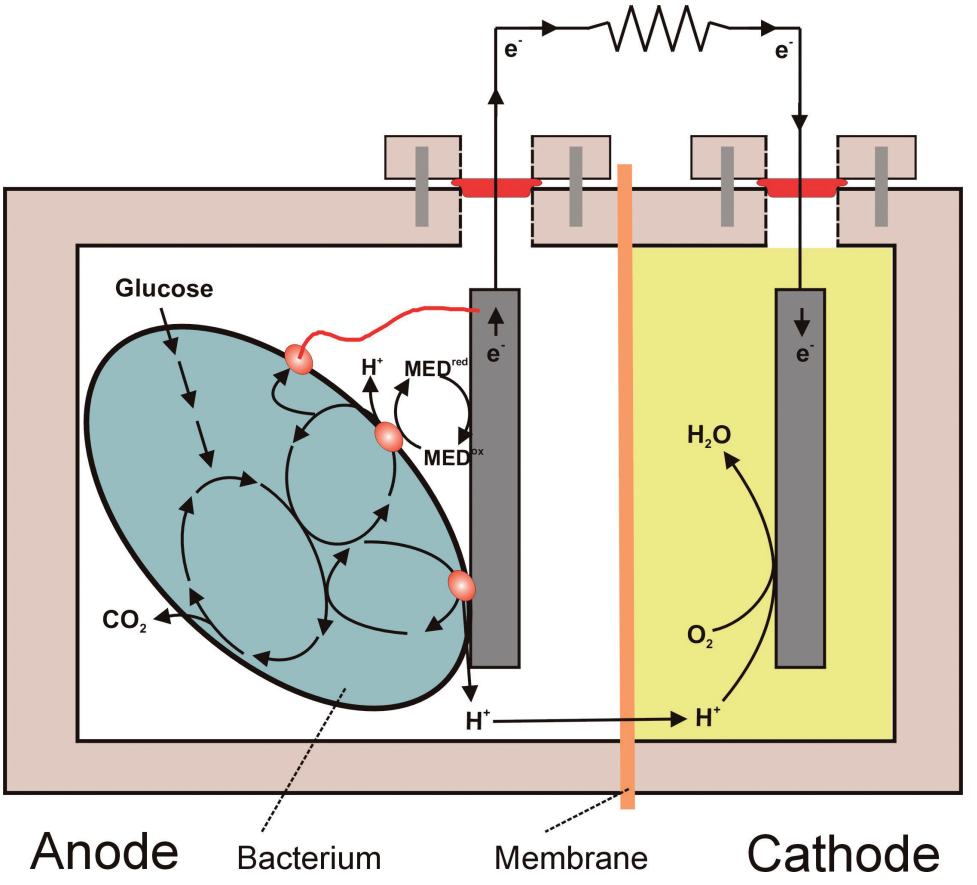

FIGURE 3.2 The microbial fuel cell and its operating principles. SOURCE: B.E. Logan, B. Hamelers, R. Rozendal, U. Schröder, J. Keller, S. Freguia, P. Aelterman, W. Verstraete, and K. Rabaey, "Microbial fuel cells: Methodology and technology," *Environmental Science and Technology* 40:5181-5192 (2006). Copyright 2006 American Chemical Society.

reducing equivalents to the anode, internal resistance of the device, efficiency, the range of organic substrates, and the selection of microorganisms. These problems lie at the intersection of physics, chemistry, biology, and bioengineering. Figure 3.3 illustrates the overall concept of the fully integrated agro biofuel, material, power cycle for sustainable technologies.

There is growing interest in finding alternative biological materials from which to make fuel. While biofuels still represent a small fraction (less than 15 percent) of the world's overall energy, the yearly growth rate of 15-20 percent contrasts significantly with the rate of 1-2 percent for fossil fuels. Today, established biofuel

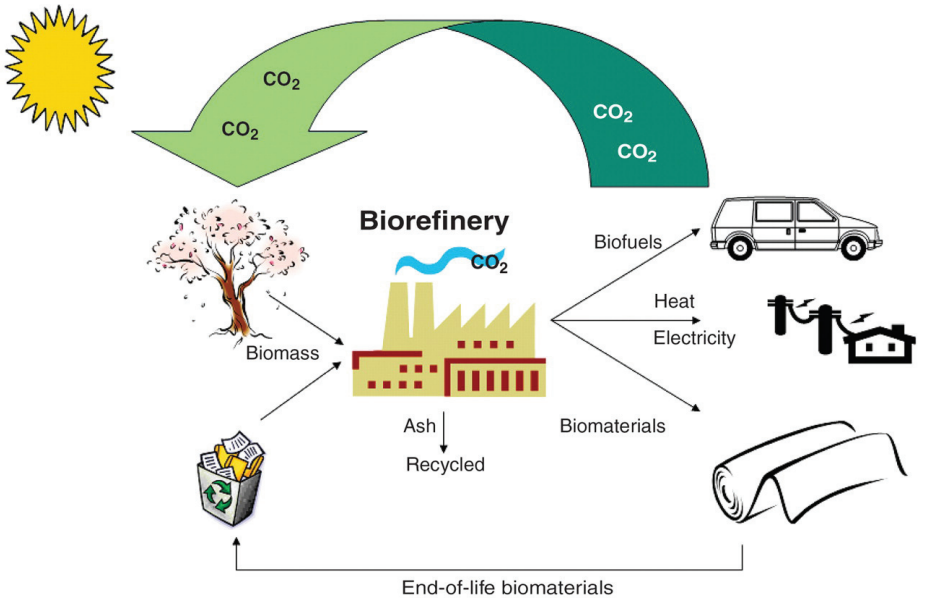

FIGURE 3.3 The fully integrated agro-biofuel-biomaterial-biopower cycle for sustainable technologies. SOURCE: A.J. Ragauskas, C.K. Williams, B.H. Davison, G. Britovsek, J. Cairney, C.A. Eckert, W.J. Frederick, Jr., J.P. Hallett, D.J. Leak, C.L. Liotta, J.R. Mielenz, R. Murphy, R. Templer, and T. Tschaplinski, "The path forward for biofuels and biomaterials," *Science* 311:484-489 (2006).

industries are a reality in countries like Brazil and Germany. The production and processes to efficiently produce fuels are being pursued for palm oil, cottonseed, peanut oil, castor oil, sunflower, and cattle fat. Success in this industry will require focused research to understand and utilize plant genetics, chemical processing methods to recover useful materials from the crops, and means to efficiently produce carbon-rich fuels from these biomaterials.

Biomimetic Photosynthesis

Photosynthesis, the conversion of solar energy into stored chemical energy, is a key biological process that sustains life on Earth. The energy-rich molecules produced by photosynthesis are primarily reduced carbon compounds such as cellulose, hemicellulose, and lignin, which are difficult to convert into chemical fuels. Other photosynthetically produced carbon compounds such as glucose are directly fermentable to ethanol.

One of the great scientific challenges of the twenty-first century for researchers inspired by naturally occurring photosynthesis is extracting and mimicking the essential solar-energy-conserving reactions. The general equation of photosynthesis guides the way: water + carbon dioxide + sunlight → oxygen + stored energy. Biomolecular and bioinspired energy transducers need not replicate all of photosynthesis. Nonbiological photoreactions could in principle mimic the reactions of photosynthesis by producing small fuel molecules such as hydrogen, methane, or methanol. In all these reactions, molecular oxygen would be produced, since water is the source of electrons for the fuel molecules. For example, in the case of renewable hydrogen production by light-activated water oxidation, the reaction is $2H_2O$ + sunlight → $O_2 + 2H_2$. Current research is focused on synthesizing efficient catalysts for molecular oxygen and fuel formation. Cutting-edge photosynthesis research is aimed at understanding the molecular mechanism of oxygen evolution, whereas cutting-edge solar fuel research is aimed at understanding the catalytic pathway for the production of the fuel molecules.

Understanding light-activated oxygen evolution by plants and cyanobacteria is one of the great challenges in photosynthesis. Progress has been made in elucidating the structure of the photocatalytic oxygen-evolving center in natural photosynthesis. The oxygen-forming steps are mediated by an Mn_4Ca-tyrosine catalytic center illustrated in Figure 3.4. The photocatalytic oxygen-evolving complex (OEC) is the starting point for bioinspired energy transduction research for biomimetic photosynthetic fuel formation. Within the next 5 years the synthesis of this structure with proven oxygen-evolving capabilities should be complete. This work includes linkage to a photochemical reaction that drives the oxygen evolution reaction. Advanced computation, simulation, and modeling techniques—in addition to chemical synthesis— should be brought to bear on this problem, with the goal of understanding how this high-potential multielectron transfer reaction is achieved. Even with oxygen evolution fully understood, less than half the scientific challenge of bioinspired energy transducers for renewable energy production will have been overcome. The other part of the challenge focuses on the photocatalytic centers for fuel formation: the chemical fate of the electrons and protons in the reaction $2H_2O$ + solar energy → $O_2 + 4H^+ + 4e^-$.

Biomolecular energy transducers are a rich area for basic research, with potentially important applications in the field of renewable fuels and chemicals production. The key challenge, following the example of natural photosynthesis, is the conversion of solar energy into stored chemical energy using electromagnetic energy contained in the visible portion of solar emission spectrum. A molecular-level understanding of the mechanisms of photosynthetic oxygen evolution must be achieved. This includes the de novo synthesis of photocatalytic oxygen-evolving complexes that are able to drive the light-activated oxidation of water. Understanding oxygen evolution at the molecular level, which is well under way, will permit the

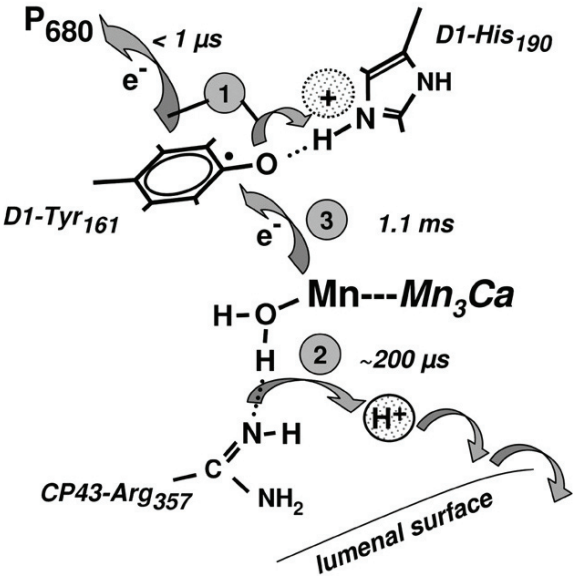

FIGURE 3.4 Mechanistic model for the formation of photosynthetic oxygen. SOURCE: M. Haumann, P. Liebisch, C. Muller, M. Barra, M. Grabolle, and H. Dau, "Photosynthetic O_2 formation tracked by time-resolved X-ray experiments," *Science* 310:1019 (2005).

synthesis of artificial oxygen-evolving structures without the need to synthesize or recreate an entire biological organism. The desired fuels will determine the catalysts and chemistry to be studied. For example, simple fuel molecules like hydrogen can be produced with well-known metallic catalysts or mimetic analogues of the enzyme hydrogenase. Drawing further inspiration from natural photosynthesis, light-induced charge separations of photosynthetic reaction centers occur across a membrane that separates oxidizing and reducing equivalents. Bioinspired photosynthesis will build on this example by constructing artificial photosynthetic membranes that can photoproduce oxygen and fuel molecules on opposite sides of the membrane. A priority research goal should be the synthesis of artificial photosynthetic membranes that mimic the essential energy-conserving properties of natural photosynthesis with equal or greater energy conversion efficiency. Theoretically, synthetic systems could be more efficient than natural systems for three reasons: (1) photosynthesis has evolved to maximize survivability, not efficiency; (2) in principle, a synthetic system, combined with suitable catalysts, should be able to produce small fuel molecules such as hydrogen, methane, and methanol, which are immediately useful, unlike cellulosic biomass; (3) synthetic chromophores

might be able to harvest a larger fraction of the solar emission spectrum than the chlorophyll molecules of green plants. Of course, development of a real-world system, including long-term operational stability, will be a key challenge after the basic science problems have been solved.

Bacteriorhodopsin (BR) is a stable transmembrane protein of the bacterium *Halobacterium halobium* and thrives in salt marshes at high salt concentrations. Like photosynthesizing green plants, BR converts light energy into chemical energy. Whereas green plants convert light energy into chemical energy by photon-induced charge separation, BR is a photon-driven proton pump. In its native state, BR is a backup source of metabolic energy that is activated when available oxygen becomes too low for normal respiration, a transition that is accelerated by the poor solubility of oxygen in concentrated salt solutions. Following photon absorption, protons are pumped out of the cell through the cell membrane at the rate of one proton per photon. The Gibbs energy of the hydrogen ion gradient that is formed by this process is converted into chemical energy in the form of adenosine triphosphate (ATP) molecules by ATP synthase enzymes.

As illustrated in Figure 3.5, BR molecules are composed of seven α-helical subdomains that form a hexagonally close-packed, oriented structure that assembles into large aggregated patches in the cell's membrane. The structure of a single BR molecule is illustrated in Figure 3.6a, along with the movement of protons from the inside to the outside of the cell. Photon absorption triggers a series of spectroscopic changes that include a trans-1,3-cis transformation, rearrangement of electronic charge within the protein, protonation, deprotonation, and conformational changes. As illustrated in Figure 3.6b, the spectroscopic states have characteristic lifetimes and absorption spectra that collectively are known as the BR photocycle.

The creation of gradients is a recurring theme in the harvesting of light and its conversion to chemical energy in biomolecular systems. The physical and chemical principles of the action of BR have inspired research in the field of light-activated proton gradient production. For example, BR processes involving light-driven proton pumping and production of ATP catalyzed by F_0F_1-ATP synthase have been demonstrated in artificial liposome photosynthetic membrane and other systems. In all cases energy is conserved by the creation of proton gradients following photon absorption that utilizes a suitably designed chromophore.

The biomolecular principles for converting light energy into chemical energy are now understood to follow a conceptually simple plan that provides guidance for bioinspired research. Design principles drawn from naturally occurring biological systems can be used to form the basis of near-term (5 years) research. Nanoscale energy sources based on light-induced formation of gradients should be mastered in a range of synthetic systems to illustrate the themes and variations of this mode of energy transduction. In addition to light-energy harvesting, research in

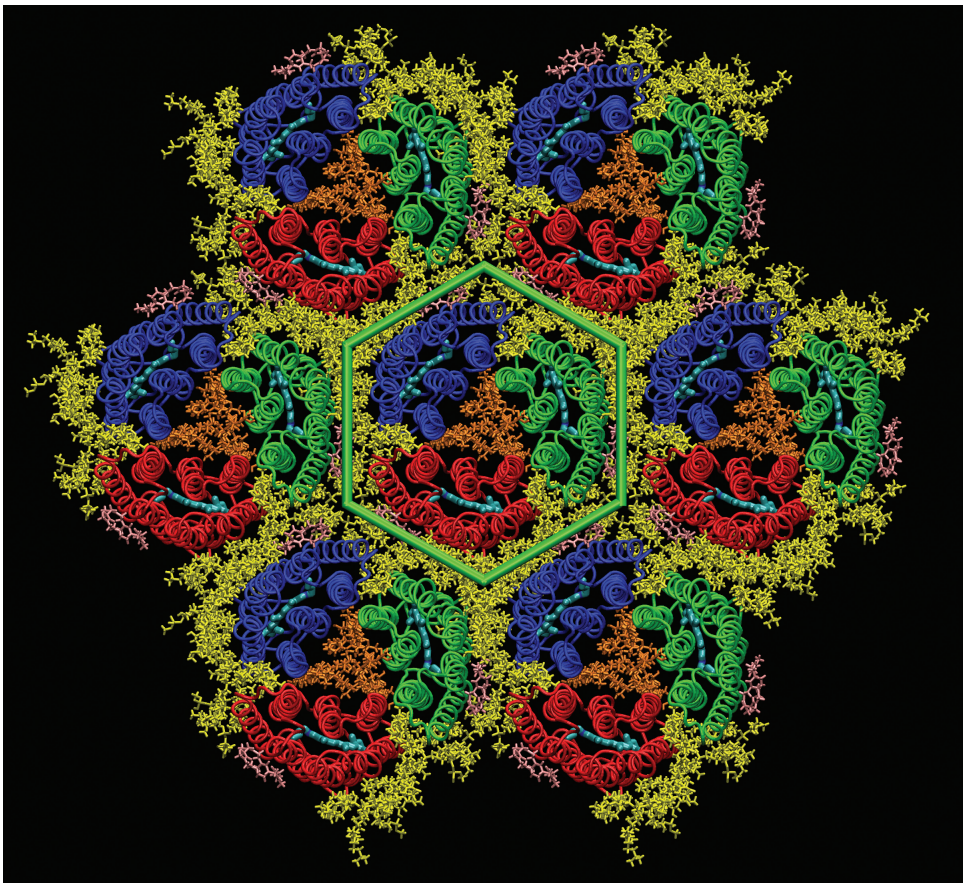

FIGURE 3.5 Illustration of the seven α-helical domains that constitute BR molecules. SOURCE: J. Baudry, E. Tajkhorshid, F. Molnar, J. Phillips, and K. Schulten, "Molecular dynamics study of bacteriorhodopsin and the purple membrane," *Journal of Physical Chemistry B* 105:905 (2001). Copyright 2001 American Chemical Society. Reprinted with permission.

BR has taken on a life of its own in fields removed from the biological origin and energy-transducing function of the protein, including protein-based field-effect transistors, artificial retinas, spatial light modulators, three-dimensional volumetric memories, and optical holographic processors. These applications will require standardization of materials, components, and manufacturing methods in order to be successfully developed.

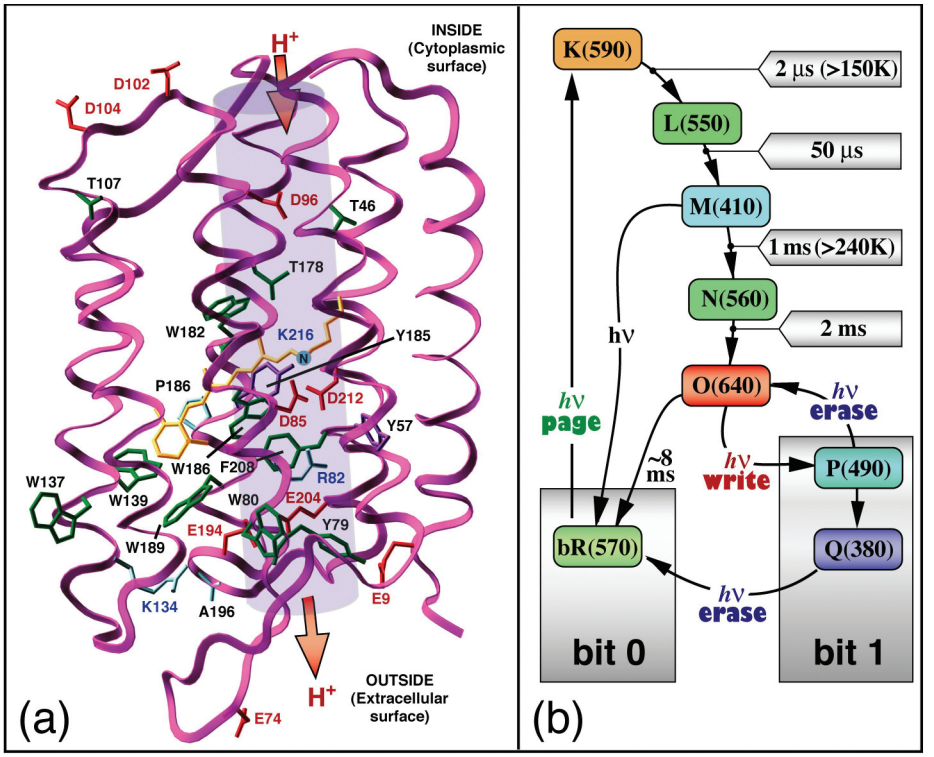

FIGURE 3.6 BR structure and photocycle. (a) BR is an ion pump that results in the net transfer of a proton from the intracellular to the extracellular surface. Key residues are highlighted, and the proton transfer channel is shown. (b) The main and branched photocycle in BR used for three-dimensional memory device applications. The branching reaction involves the O → P transition that is optimized using directed evolution. M and O state lifetimes and yields can also be optimized using directed evolution. SOURCE: Adapted from J.R. Hillebrecht, J.F. Koscielecki, K.J. Wise, D.L. Marcy, W. Tetley, R. Rangarajan, J. Sullivan, M. Brideau, M.P. Krebs, J.A. Stuart, and R.R. Birge, "Optimization of protein-based volumetric optical memories and associative processors using directed evolution," *Nanobiotechnology* 1:141-152 (2005).

Biomolecular Motors

All active cellular movements, such as separation of the chromosomes and the two daughter cells during cell division, determination and modulation of cell shape, locomotion, and targeted transport of intracellular cargos, such as membrane bound vesicles containing neurotransmitters or waste products, involve the

transduction of chemical metabolic energy into mechanical work. Production of the cell's main energy source, ATP, is accomplished by chemical metabolic reactions and also by mechanical-to-chemical energy transduction. The mechanoenzymes that carry out these essential roles are termed molecular motors, and they sort into several families with related structural and mechanistic features. Understanding and controlling these functional properties in detail could facilitate harnessing them or their operating mechanisms for actuators in nanoscale devices, such as molecular sorters, filters, concentrators, switches, and power sources. However, the principles of their operation are very different from those of macroscopic chemical-to-mechanical energy transducers, partly because their shapes and positions undergo thermal vibrations that are comparable to the functionally relevant motions. A major challenge in biomotor research is to understand how to integrate these functional biomolecular materials into useful devices that operate efficiently. High-resolution structural biology, rapid-reaction biochemical kinetics, and single-molecule biophysical studies of these machines are improving understanding dramatically. Other challenges to using them in practical fabricated devices are to make them robust enough to retain activity for the intended life span of the device and to program them to assemble appropriately for their designed role. The combination of materials science with protein and nucleic acid molecular biology and biochemistry holds promise for achieving these advances.

Linear Molecular Motors

Biological cells are highly crowded environments in which molecular machines transport cargos to specific locations and in which metabolic enzymes interconvert energetic compounds to perform useful work. Series of molecular motors and mechanoenzymes have evolved to perform these tasks. They are remarkably efficient macromolecular machines used in nature to determine cellular shape and to accomplish intracellular transport, cell locomotion, muscular contraction, and cell division. The main high-energy compound that serves as the fuel for energy-requiring processes is ATP, which these machines cleave to the diphosphate (ADP) and orthosphospate (P_i). Splitting each ATP molecule liberates approximately 10^{-19} J, which can be transduced into various types of chemical, electrical, or mechanical energy to perform work. Of the three cytoskeletal filament systems that shape eukaryotic cells (actin, microtubules, and intermediate filaments), the first two are tracks for molecular motors. The three families of motors are called myosin, kinesin, and dynein, and all three are found in numerous intracellular locations. Bacteria also contain actin filaments and tubulin homologs, but no molecular motors have yet been discovered that actively translocate on the prokaryotic cytoskeleton.

Considerable progress has been made in understanding the fundamental properties of biomotors, and various attempts are under way to fabricate useful devices

that incorporate these new materials. Figure 3.7 shows a typical scheme molecular motors use to generate force or move a cargo. An internal structural change, often rotation of a lever, stretches a compliant (springy) element, transferring the chemical energy from the ATP into potential energy of extension of the spring module. Whether this produces force between two cellular components or causes them to move relative to each other is dependent on their mechanical properties and how they are tethered to other structures. Typical forces produced by individual molecular motors are 2-10 pN, and the distances they move per ATPase cycle are 10-40 nm. These values imply remarkable thermodynamic efficiency of energy transduction. For instance, a single actomyosin event in muscle can produce 10 pN of force with 10 nm of displacement. The energy-storing spring is nearly linear, so the mechanical work output is ½ 10 pN × 10 nm = 50×10^{-21} J, about half of the energy liberated from the ATP. Whole muscle contraction is also approximately 50 percent efficient, which should be compared to man-made chemical-to-mechanical energy transducers (e.g., an automobile engine) that can operate at about 20 percent efficiency. Muscles regulate their biochemical energetic utilization (the ATPase rate) according to the requirements of the mechanical load, fast shortening, or heavy lifting. This mechanical feedback makes the myosin motor a smart machine.

Flagellar Motors

A flagellar motor is a biological rotary engine. Found in bacterial membranes, it spins a flagellum around its axis to cause the bacterium to swim (Figure 3.8). The flagellar motor assembly contains about 25 different proteins that form the motor-switch-shaft-propeller complex. The motor itself has about 12 proteins. The energy for rotation is derived from a proton gradient across the membrane in some flagellar motors such as that in *E. coli* or from sodium ions in others such as marine vibrio. The flagellar motor is one component in a larger biochemical system in the bacterium that governs the swimming of the cell along nutrient and chemical gradients in a process called chemotaxis. The presence or absence of "food" triggers a biochemical cascade that can turn the motor on and off (that is, begin the rotation) or can turn the rotation from clockwise to counterclockwise.

Only 35-40 nm in size, a flagellar motor powers the motion of a cell that is nearly 100-fold larger (about 1-2 microns). *E. coli* has 6-8 flagella-motor complexes per cell. Each motor consumes about 10^{-16} W, running on ion flows of tens of femtoamps, and takes about 26 steps per revolution. While the motor can run at nearly 100 percent efficiency at low speeds, it is only a few percent efficient at high speeds. As with other biological systems, it self-assembles, requiring no external assembly machinery.

The final step in mitochondrial phosphorylation of ADP to form ATP is

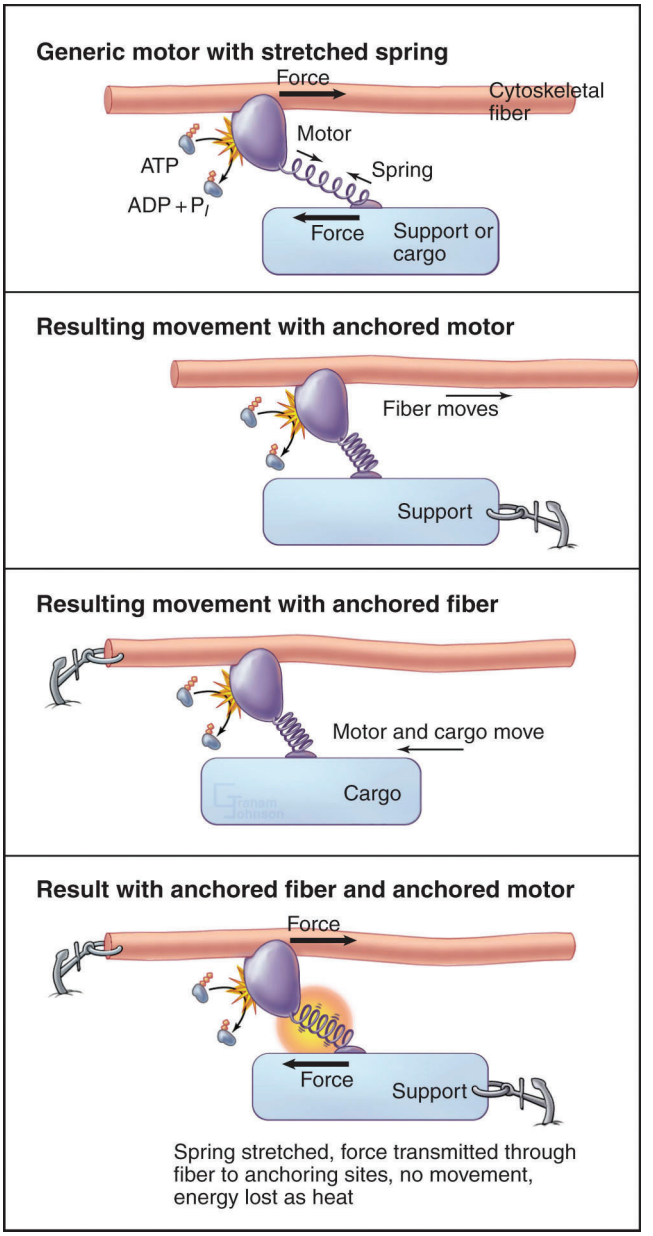

FIGURE 3.7 Typical scheme molecular motors use to generate force or move a cargo. SOURCE: T.D. Pollard, W.C. Earnshaw, and J. Lippincott-Schwartz, *Cell Biology, 2nd Edition,* Philadelphia, Pa.: Saunders, 2007. Copyright Elsevier 2007.

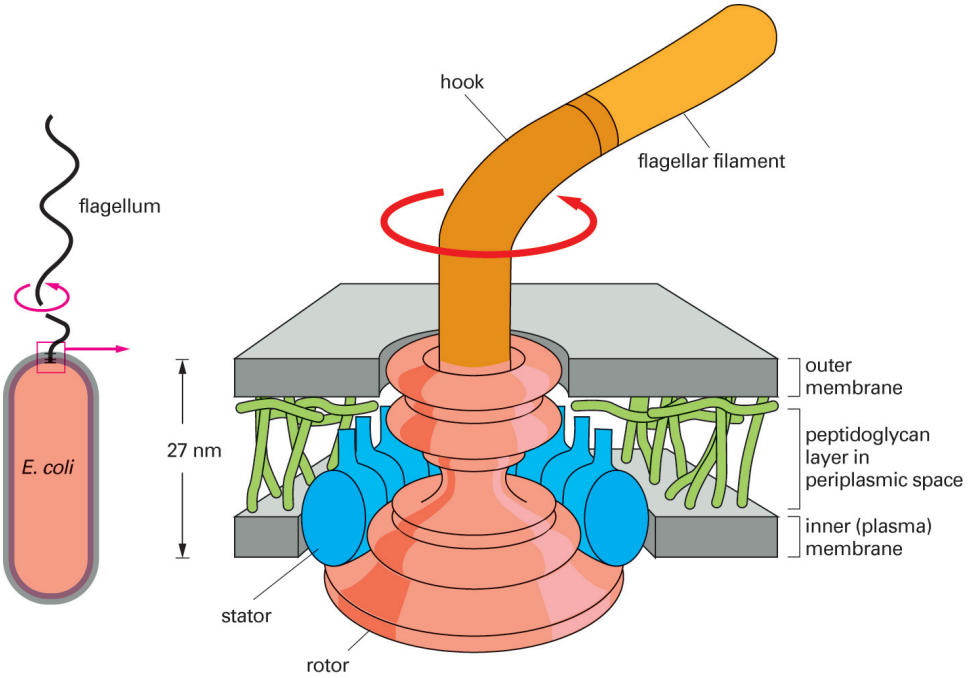

FIGURE 3.8 Schematic illustration of a flagellar motor. SOURCE: B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, and P. Walter, *Molecular Biology of the Cell, 4th Edition*, New York, N.Y.: Garland Science, 2002. Based on data from T. Kubori, N. Shimamoto, S. Yamaguchi, K. Namba, and S. Aizawa, "Morphological pathway of flagellar assembly in *Salmonella typhimurium*," *Journal of Molecular Biology* 226:433-446 (1992), and N.R. Francis, V.M. Irikura, S. Yamaguchi, D.J. DeRosier, and R.M. Macnab, "Localization of the *Salmonella typhimurium* flagellar switch protein FliG to the cytoplasmic M-ring face of the basal body," *Proceedings of the National Academy of Sciences USA* 89:6304-6308 (1992). Copyright 2002. Reproduced by permission of Garland Science/Taylor & Francis LLC.

accomplished by another remarkable mechanoenzyme, the F_1 ATP synthase (Figure 3.9). This is a rotary motor-generator that is cranked around by another motor, F_0, which in turn derives its energy from a proton gradient across the outer mitochondrial membrane. F_0 has many similarities to the bacterial flagellar rotary motor, described in the preceding section. F_1 is constructed like a citrus fruit whose segments are six protein subunits (α and β in Figure 3.9), with a central rotating shaft (γ, δ, and ε) in its core. The direction of the motor is readily reversible. Torque generated is approximately 50 pN·nm, and considering that the force is applied between the stator and rotor at a radius of about 1 nm, the effective linear force

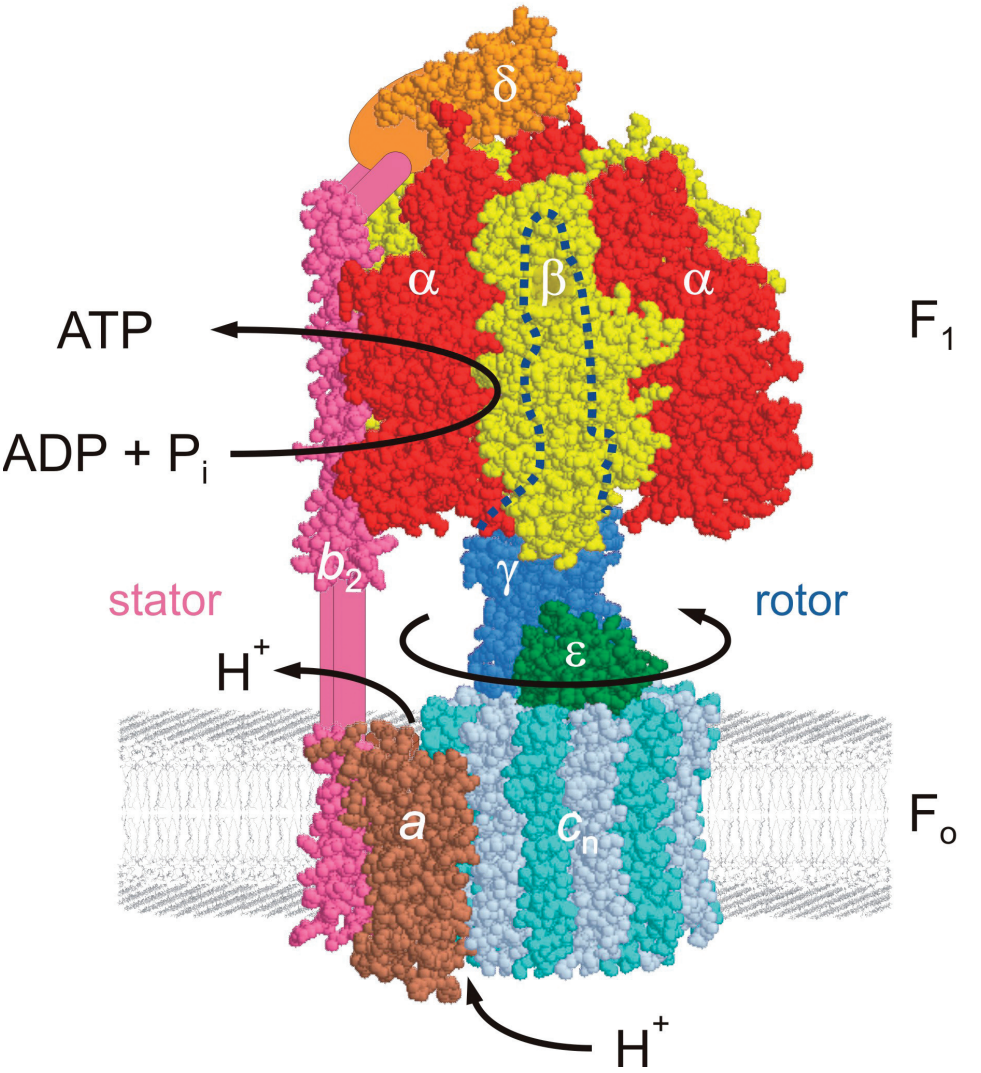

FIGURE 3.9 F1 ATP synthase enzyme. SOURCE: A.E Senior and J. Weber, "Happy motoring with ATP synthase," *Nature Structural & Molecular Biology* 11:110-112 (2004).

produced is approximately 50 pN. The efficiency of energy transduction, either synthesizing or splitting ATP, can approach 100 percent!

From the perspective of new materials and new nanotechnologies, these and other biological motors exemplify nanomachines that (1) convert cellular chemi-

cal or ionic gradient energy sources into work, motion, and propulsion, (2) are controllable by switching systems involving cooperative transitions within the protein subcomponents, and (3) assemble themselves from individual components, mitigating the need for expensive and complex assembly processes. In 5 years, more details of the structures and mechanisms are likely to emerge. It is likely to take 20 years before researchers can construct stable and robust molecular-scale devices that utilize biomotors or their propulsion principles.

Many exciting scientific and technical challenges remain in understanding these mechano-chemical energy transducers, which offer the promise of power and propulsion systems for nanotechnology. First, while the structures of many of the individual proteins are known—such as the flagellar filaments and the motor domain of myosin—the structures of other component proteins and the full atomic structures of the assemblies have not yet been determined. How they are functionally modulated in activity by cellular signals, how the cargos and target destinations are specified, and how the motors are integrated with other cellular activities are not known. The molecular motors appear and assemble into functionally competent supramolecular complexes when needed for various roles during the cell cycle and then disassemble to be used elsewhere for different jobs. How they cooperate in this manner is almost a complete mystery. A central problem in rotary motors is that researchers do not yet understand how torque is generated by the transfer of ions. Researchers also do not understand how motor proteins get proper anchoring in the fluid bilayer membrane to generate torque or linear force. Biophysical and structural research under way will likely resolve these questions in 5 to 10 years and reveal the design trade-offs made during evolution to tune the individual molecular motors for their specific tasks in the cell.

To use molecular motors in human-designed materials and devices, the assembly and control mechanisms described above will have to be understood at a very sophisticated level. Proteins are perishable because they are sensitive to temperature and solvent conditions and they are food substrates for microorganisms such as bacteria. There are new efforts to exploit biomotor properties and evaluate them in new device applications. These efforts largely revolve around combining sensing and actuation and evaluating the ability to detect and/or amplify a signal in a biosensor or diagnostic system. Among the concepts being pursued is the use of linear motors (kinesin and microtubules, actin-myosin) to shuttle cargo consisting of antibody-coated beads through a microfluidic channel that contains an antigen or analyte of interest. In the proposed device, the cargo picks up the analyte of interest on the surface of the bead and shuttles it to a depot, resulting in a concentrated antigen that can be detected with a secondary antibody conjugate.

The ability to create robust systems around biomotor-based devices will depend on a number of important factors, including stabilizing the motors for long-term function in an in vitro environment. Biological systems have adopted unique mech-

anisms to stabilize components for long-term function, and the environment in which this is accomplished in the natural system is complex and dynamic. Creating a controlled in vitro environment in which the motor can function optimally for a useful period of time in a device application is a challenge. The long-term storage of proteins has been accomplished using sugars for (1) conformational stability in the dry state and (2) storage of biomolecular components that could be used in devices. The use of sugars should extend the storage lifetime of components in devices once many of the other challenges in building them are overcome. Realizing the value of biomolecular motor devices will also require characterization tools at the scale of the device that is being engineered. We will also need to better understand how these devices work and can be used in technology or product applications.

An early application of a new device using molecular motors was recently reported. The device is a molecular sorter with kinesins that direct microtubules labeled with different fluorescents into separate paths using hydrodynamic and electrical steering forces. Another group of researchers placed F_1 motors on microfabricated inorganic pedestals and attached nickel nanopropellers to form a hybrid biotic/abiotic device. Such bioinspired actuators might serve as concentrators or nanopumps in nanoscale devices. In the future, the flagellar motor or the F_0-F_1 ATP synthase might serve as the model for new energy sources that convert chemical concentration gradients into chemical energy that can be stored and transported. Molecular-scale propulsion might be useful in devices that enter the bloodstream or nervous system to remove clots, to counteract toxins, to attack plaques, or to deliver pharmaceuticals or therapeutic genes.

ADVANCED FUNCTIONAL MATERIALS IN HEALTH AND MEDICINE

One obvious area for the application of advanced functional materials that incorporate or mimic biomolecular components is health and medicine. In this section, three specific examples of the application of such materials in health and medicine are explored: medical diagnostics, drug delivery, and prosthetics. These application areas take advantage of different functional capabilities of biomolecules, including molecular recognition, signaling, adhesion and binding, and mechanical properties. There are challenges in creating integrated systems in which working functional components are maintained. Design and fabrication of new devices or products is proceeding based on advancing knowledge of useful functional biomolecular properties. Many devices have been developed into robust systems that can be used to look at clinical variability in human response. Thus a medical diagnostic or therapeutic device exploiting functional capabilities will reach its ultimate utility when the breadth of biological response variability is known. One need only look at the variability generated in a drug clinical trial or diagnostic profile of disease in a human population to appreciate this challenge.

The presence of regulatory review to ensure the safety and efficacy of devices that incorporate functionalized biomolecular materials is an important gatekeeper in deciding which research advances may proceed to widespread human use. Such review can account for the long lead time between a research advance and its impact on health and medicine.

Medical Diagnostics

The health of the U.S. population has significant implications for the economy and society as a whole. As modern medicine progresses, the ability to diagnose and ameliorate diseases has improved markedly. The cost of health care has also risen dramatically owing to the costs associated with hospitals, physicians, and diagnostic and treatment modalities. For decades now, advances in medicine and health care have been tightly linked to the development of novel technologies. Understanding biological mechanisms has improved diagnosis and treatment, so that longevity and the quality of life after diagnosis of the major illnesses have improved dramatically.

One area in which the unique molecular recognition and binding properties of biomolecules have been exploited to advantage is medical diagnostics. This $6 billion industry currently has a tremendous impact on health and medicine. A number of biomolecular components are of great use in the design and fabrication of diagnostics. Nucleic acids, proteins, carbohydrates, and lipids are potential sources of information insofar as they are cellular or molecular biomarkers of interest. The use of antibodies is widespread, with enzyme-linked immunosorbent assay (ELISA) testing alone being a good example of the power of high-impact products based on biomolecular functional recognition and binding properties. The application of nucleic acid testing in infectious disease blood testing as well as polymerase chain reaction (PCR)-based and other genomics-based testing is now a common practice that saves lives. Genomic and proteomic profiling is becoming more widely practiced as a means to assess a patient's condition.

Biomolecular materials have a number of advantageous capabilities that warrant continued research and have already been proven valuable as detection elements in diagnostics. These capabilities include exquisite specificity, sensitivity for binding or affinity (down to picomolar concentrations, 10^{-12}, for antibodies and attomolar concentrations, 10^{-18}, for DNA hybridization), and generalizability of biomolecular recognition (one can make an antibody or peptide via phage or bacterial displays or DNA/RNA via systematic evolution of ligands by exponential enrichment [SELEX] that will bind almost any water-soluble target). Amplification schemes such as those used in PCR and more recently with conformational peptides enhance the signal over the background. These capabilities are also sometimes augmented by the cooperativity of biomolecules and aggregation events, exploiting

both kinetic and thermodynamic properties of biomolecules. They can also be utilized in matrices or materials. For example, the antigen-antibody capping reaction on the B cell surface is dominated by cooperative biomolecular events organized in an immobilized matrix of the membrane, resulting in very high sensitivity and specificity to antigen protein. These properties have proven fruitful in biosensor research and development.

Researchers have started the formidable task of designing biomolecules that undergo large physical changes in response to binding any arbitrary target molecule. Two approaches have been reported to date. The first is to take one of the rare, naturally occurring biomolecules that undergoes a large change in its physical state and reengineer its normal binding site such that it binds the target of choice. The alternative has been to take a biological sensor molecule that binds a target of choice and reengineer it so that it undergoes a large-scale change in its conformation upon binding. Because the scientific community's ability to rationally modify and develop designer biomolecules has improved recently, both approaches have seen notable successes.

Another promising approach is the use of molecular beacons (MBs), single-stranded stem-loop DNA molecules that adopt a linear conformation when a target oligonucleotide hybridizes to the loop region, breaking the stem. By placing optical reporter groups (e.g., a quencher/fluorophore pair) on the stem, binding is coupled to a large, readily measurable change in MB emission. More recently this approach has been expanded to the detection of nonoligonucleotide targets via the use of "aptamers" (DNA or RNA molecules selected in vitro for their ability to bind such targets) that have been reengineered to undergo binding-induced folding via any of a number of rational or semirational redesign approaches. Finally, this approach has been expanded to the use of polypeptide recognition units by (1) demonstrating that it is similarly possible to engineer binding-induced folding into small proteins and (2) by developing optical reporter groups that couple folding-induced changes in biopolymer dynamics into easily measurable optical outputs.

A significant challenge in creating useful diagnostics has been in the translation of a binding event into a measurable output signal. These binding events often require known conformational changes, which are often hard to predict or control. Fluorescence measurements require energy transfer; this requires a conformational change and transduction into energy transfer events from fluorophores attached to biomolecular materials such as antibodies. Controlling these conformational states is challenging, because they can be affected by solvent conditions, immobilization, and background absorption, resulting in interference. The development of biosurfaces that retain specificity and sensitivity remains a challenge and is expected to be a very active area for R&D of future biosensor assays. Additional approaches have been to attach biomolecules to a surface and then monitor their binding by studying mass (quartz crystal microbalances, microcantilevers), charge (field-effect

transistors), or index of refraction (surface plasmon resonance) changes at the interface. Many of these approaches are not yet robust enough to work in real-world conditions.

Cell-based biosensors offer the advantage of exploiting more complex responses and deriving signals from collaborative and adaptive cellular reactions. For example, the use of B cells to report on antigen presence relies on the sensitivity and specificity of the antibody-antigen capping reaction and associated calcium flux upon binding. The advantage of this system is the ability to engineer B cells to present different antibodies on their surface and thus engineer desired responses to known antigens. This system is being exploited for diagnostic applications because it provides the cellular machinery to amplify the signal through cellular processes. In principle, cellular response offers another advantage: the ability to respond to unknown or new threats to human health.

Challenges remain in the design and implementation of detection elements for diagnostics. For example, clinically relevant HIV detection (currently achieved by PCR amplification) requires about 1,000 copies of the genome per milliliter—that is, the attomolar (10^{-18}) level. Current detection limits are about 10 to 100 femtomolar (10^{-15}) with electronic and optical-based biosensors. The long-term goal of relevant DNA detection is thus attomolar to zeptomolar (10^{-18} to 10^{-21}) levels. PCR-free detection at these levels in a convenient electronic device is also a big challenge. The picture for detection of proteins and small molecules is more favorable. Protein diagnostics of cancer markers requires about picomolar (10^{-12}) levels. Such biosensors should become available within the next 10 years, and they will revolutionize point-of-care diagnostics and the real-time monitoring of, for example, pollutants and industrial intermediates.

A number of challenges also remain in implementing detection elements in diagnostic devices. One of these is controlling functionality at an interface, because most of these applications immobilize functional biomolecular components. Other challenges include (1) the design and synthesis of new tags for fluorescence, electronic, radiologic, or other contrast techniques to enhance the information gained from biomolecular components and (2) sample preparation techniques to increase signal to noise in clinical samples. The transport of sample following processing also can affect the performance of these systems. Significant progress has been made in microfluidic separation and preparation technology, while challenges in controlling optimal conditions remain. Finally, computational challenges arise from the complex and large datasets from multiplexed signal generation in these applications.

Targeted Drug Delivery, Targeted Imaging Systems, Targeted Radiation

There has been long-standing interest in understanding and utilizing different biomolecular materials for the specific delivery of therapeutic compounds. The use

of biopolymers for this purpose is widespread, with notable efforts in liposomes, hydrogels, and, more recently, nanoparticles. Various aspects of charge, elasticity, size, and functionalized surface biochemistry have been explored as methods by which to manipulate the pharmacodynamics of therapeutic compounds and to selectively deliver these agents to specific sites in the body. Transdermal delivery is also widespread, and it is recognized that delivery through the skin can be an effective way to use biomolecular materials in this application.

New advances have been made that take advantage of nanoparticles for tagging, labeling, and targeted drug delivery. The coincidence of their size with that of proteins makes this possible, and the potential for nanomedicine is enormous. Many groups are developing nanoparticle targeting of anticancer drugs and testing them on animal models of human cancers. This form of drug delivery improves the therapeutic response to anticancer drugs and allows the simultaneous monitoring of drug uptake by tumors.

Modified polyamidoamine (PAMAM) polymer nanoparticles (in the class of molecules known as dendrimers) smaller than 5 nm in diameter are used as carriers. One of the targets chosen for delivery is the high-affinity folate receptor for the vitamin folic acid, also known as the folate-binding protein. A therapeutic nanoparticle consists of acetylated dendrimer conjugated to folic acid as a targeting agent, later coupled to methotrexate as a drug and a fluorophore as an imaging agent. The conjugates are injected intravascularly into mice bearing human tumors that overexpress the folic acid receptor. In contrast to nontargeted dendrimers, a folate-conjugated nanoparticle concentrates in tumor tissue for over 4 days after administration. The tumor tissue localization of the targeted nanoparticle can be attenuated by prior intravascular injection of free folic acid. Internalization of the drug nanoparticle into tumor cells can be confirmed by confocal microscopy, which detects the fluorophore that is delivered with the same dendrimer platform. Targeting methotrexate increases its antitumor activity and markedly decreases its toxicity, allowing therapeutic responses not possible with a free drug.

Several other targeting ligands that can be placed on the surface of the dendrimer are currently being developed for in vivo applications, such as aptamers, peptides, antibodies, and antibody fragments that interact with specific target molecules on tumor cells. Conjugation of different, clinically approved drugs such as taxol or doxorubicine is being developed for alternative nanoparticle conjugates. The lack of ligands with sufficient affinity to achieve targeted delivery in vivo could be improved by attaching multiple copies of each molecule to a dendrimer. Prior work with sialic acid-conjugated dendrimers documents the cooperative binding of dendrimers functionalized with multiple ligands to the influenza virus, while molecular modeling and in vitro experiments suggest that folate-targeted dendrimers have cooperative polyvalent binding to cells.

Other potential uses of nanoparticles in medicine include the use of gold

nanoparticles as tags in the treatment of arthritis and various cancers. Unlike many semiconductor particles, gold rods are also nontoxic and can be efficiently delivered to the cells. Importantly, gold nanorods possess exceptionally strong optical absorptivity in the infrared range, where human tissues have relatively high transparency. The rods are targeted with antibodies specific to particular cell markers expressed in cancer. The presence of several affinity sites of different kinds on the nanorods ensures their efficient and selective binding to the cancerous tissues. Photoacoustic imaging makes it possible to localize small clusters of cancerous cells at much greater depths than regular optical imaging can afford, which is one of its most valuable features. This technique is being developed now to apply to other diseases, such as arthritis.

Targeting of drugs is also becoming increasingly sophisticated. Antibodies are being developed and identified in increasing numbers that can be used to help target either drugs or nanoparticle carriers of drugs to specific locations. In addition, as genetic information about disease becomes increasingly sophisticated, additional biomarkers are being identified that can also be used to target drug delivery. This increasingly sophisticated understanding of disease is also leading to new understanding of the genetic and proteomic pathways of disease, which is providing new classes of drugs and new strategies for attacking disease. This will lead to new opportunities to create the materials that deliver these drugs and the drug molecules themselves.

The increasing sophistication of the biomolecules themselves will also lead to new uses and functions. For example, in addition to using the biomolecular materials as the drug delivery vector, the biomolecules will ultimately themselves have a dual role, becoming both the delivery vehicle and the drug itself. Indeed, a major focus of much development effort in the biotech industry is the discovery of drug molecules that are much larger than the small molecules favored by the traditional pharmaceuticals industry. This dual role of biomolecules is likely to increase significantly.

As knowledge of both the human genome and the function of the genes increases, specific traits of individuals will start to be identified. This will bring with it a much more personalized form of medicine, where the genetic profile of individuals will be determined, at least for specific portions of the gene, and drugs and treatments will be designed that are specific to the individual. Both the identification of the specific traits and the new treatments and their delivery will again present enormous challenge and opportunity for new materials.

New materials are also advancing new biomedical imaging modalities. New magnetic resonance imaging (MRI) techniques take advantage of specific spins on molecules or possibly on nanoparticles to significantly enhance sensitivity. Nanoparticles are also being developed to act as fluorophores in the use of optical techniques to probe much deeper into the body. New nonlinear optical methods

are also being developed to overcome the strong scattering of light typical of tissues. These also enhance the use of optical methods for imaging. Even ultrasonic imaging is increasingly benefiting from the development of contrast agents to enhance its sensitivity.

Neural Prosthetics

Medical prosthetics are a good example of integrating a number of fundamental challenges in the design, fabrication, and integration of functional biomaterials and will require interdisciplinary advances in the physical, chemical, biological, and engineering sciences. Next-generation devices will need to integrate microelectrodes, power, and communications in a biocompatible material. Artificial materials that will replace limbs, for example, will have to be impedance-matched to the signals that drive them. For some applications, like artificial limbs, force dynamics and elastic materials that can be seamlessly integrated into a neural-controlled device will be required. Signals generated from neural sources will advance by incorporating closed feedback loops in which sensing and actuating components are integrated to control a limb. One example is in the area of devices for neuroprosthetics. There has been considerable research activity and early application in health and medicine of neuroprosthetics based on advances in the R&D for these systems. A good example is the more than 35,000 cochlear implants for the hearing impaired that stimulate the cochlea and make auditory processing possible for them. Another more recent example is the deep brain stimulators that have considerable therapeutic utility in Parkinson's disease and are being more widely used as a therapeutic option for mitigating symptoms of the disease. Many new areas are being explored for these next-generation devices, including their application as replacement limbs and sensory organs.

For neuroprosthetics, the use of recording and stimulating electrodes in neural devices presents the challenge of creating materials that function well at the interface of the device and the tissue. This interface needs to be biocompatible and stable over many years of use. There is a growing trend toward measuring signals over ensembles of neurons to capture more signals over wider arrays of electrodes. These ensembles are thought to contain patterns of information encoded by neurons. They have also been used to explore dynamics and plasticity events in the brain that will be important for using these devices in controlling neural prosthetics. Dramatic new demonstrations of the power of recording or stimulating neuronal ensembles (10-1,000 neurons) include the recent demonstration by nonhuman primates of utilizing an implanted neural interface and "thought" to control a prosthetic arm in a controlled cursor computer game. This has led to a surge of new activity funded by many agencies in the area of neural prosthetics, given the severe social impact of losing a limb in an accident or in

combat. These activities seek to integrate a working neural prosthetic interface in both the central nervous system (brain) as well as peripheral sites (Figure 3.10). Another example is the not-so-recent effort to create an artificial visual system by stimulating populations of retinal ganglia through an implanted neural device in the retina (Figure 3.11).

The development of these new devices presents a host of challenges in research on and application of advanced functional materials. In addition to the molecular and neural physics of cell stimulation, materials science and biocompatibility are important issues in these prosthetics. Fundamental research in biocompatibility, biofouling, and loss of electrical connectivity in these implanted devices is leading to a greater understanding of the dynamic interface between an electrode and neural tissue. There have been considerable efforts to identify biocompatible materials for many of these applications, including both coatings (polyethylene glycol, nerve

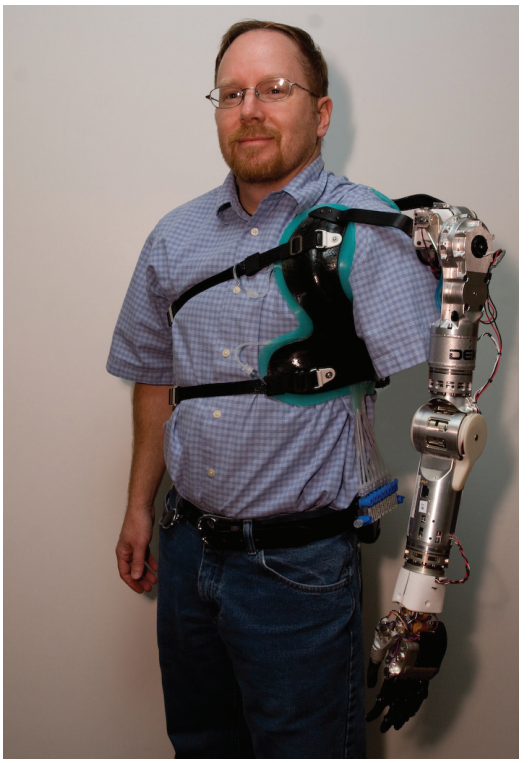

FIGURE 3.10 Conceptual model of DARPA's artificial arm that would be wired to the peripheral nervous system. SOURCE: DEKA Research and Development Corporation.

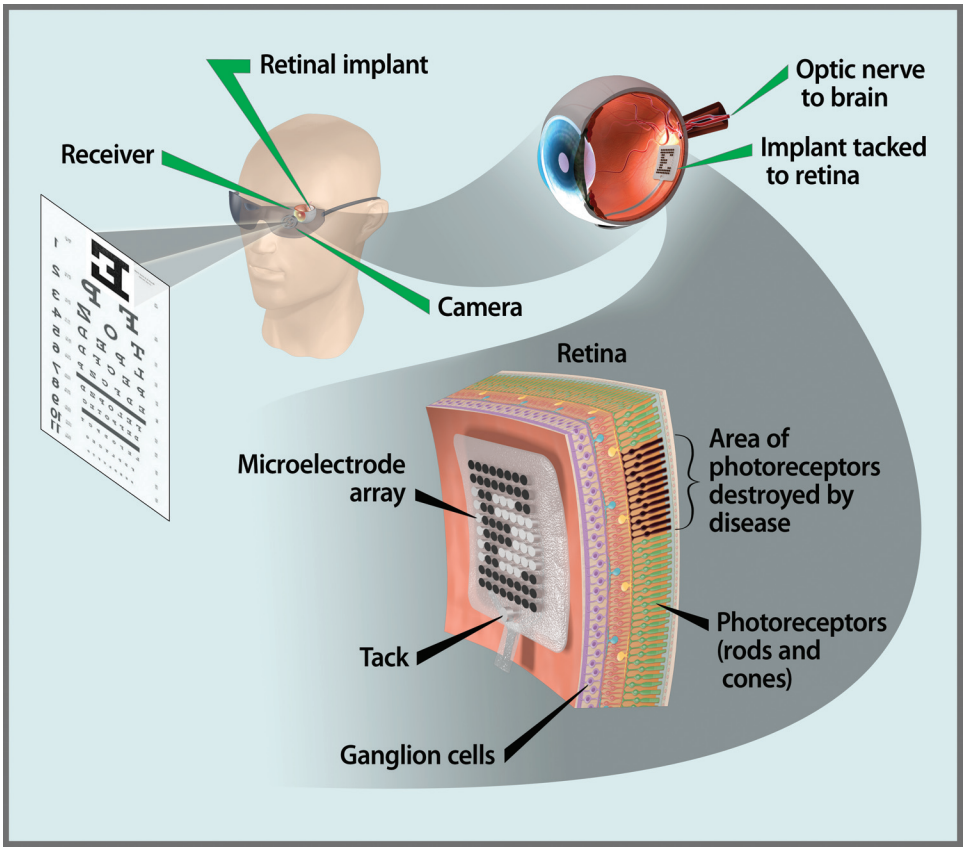

FIGURE 3.11 Retinal prosthesis for artificial sight. The microelectrode array stimulates surviving retinal neurons based on patterned signals that are received from the video camera. SOURCE: U.S. Department of Energy Artificial Retina Project.

growth factor) and the incorporation of biomolecular components that can reduce inflammation and stimulate growth into the surrounding tissue. These challenges, even if overcome, will also require significant advances in the understanding of neural code. There are also efforts to explore alternative "electrodes" for these applications, such as radiofrequency, magnetic, and optical energy to record and stimulate neurons. Many of these efforts are still in the early stages, and the focus is on understanding the signals harvested or induced by these methods.

The field of neuroprosthetics is enormously challenging. It comprises neuroscience, materials science, tissue engineering physics, chemistry, robotics, and more. In the next 5 years, research should focus on decoding the signals from neuronal

ensembles, fabrication and stability of electrodes, the physics and chemistry of electrode interactions with neural cells, and the opportunities for molecular structures and other materials to influence electrode design. Research in the 20-year time frame will build on these advances to construct integrated systems that restore neural function. A few examples of the focus areas of this research are artificial sight, weak electric field nanosystems for the study and control of cells and neurons, virtual-reality-based rehabilitation, rehabilitation robotics, deep brain stimulation, and next-generation neural interfaces, including molecular prosthetics.

ADVANCED FUNCTIONAL MATERIALS AND NATIONAL SECURITY

The unique functional properties of biological molecules offer new opportunities to create useful devices and systems for national security. Materials that have properties similar to those of biological materials may be used in applications such as environmental surveillance, protective clothing, and decontamination. These are discussed in the following sections.

Environmental Surveillance and Biosensing

Understanding changes in the environment has taken on ever-increasing importance for the United States and has implications for both global and national security. One focus of this report is the long-standing R&D in biosensors for the detection of harmful agents in the environment (pollutants and chemical or biological agents). The design and fabrication of useful biosensor devices for this purpose require the understanding and exploitation of a number of biomolecular functional properties. The unique ability of biomolecules to be sensitive, specific, and sometimes adaptive makes them very attractive for use in biosensor devices. Many of these same properties and challenges in the efforts to create working devices are similar to those faced by efforts in medical diagnostics, given that both applications exploit similar biomolecular functional properties.

There has been tremendous progress in engineering and using new engineered biomolecules in biosensors, many of which share the same advantages as the biosensors used in medical diagnostics. These biomolecules include antibodies, nucleic acids, proteins, and cells. They offer exquisite specificity to a target, and in some cases signal amplification can make them very sensitive detecting elements. One important consideration in the functional properties of these components in biosensors is that the signal transduction event is often based on key biomolecular interactions on immobilized surfaces. Most biomolecular interactions in biology take place at two-dimensional constructs (e.g., a lipid membrane), but the ability to mimic this environment in an engineered system is limited. In spite of these

limitations, the ability to detect the presence of pathogens in the environment is improving dramatically.

However, significant challenges remain for the real-world deployment of environmental biosensors, including both fundamental research challenges and the interpretation of responses in order to make effective decisions. For example, most of the biosensors deployed to detect the release of a biological agent in the environment have too slow a response time to limit public exposure to an agent (detect to warn). They are, however, informative about what exposure has already taken place and are useful in determining who should receive palliative care (detect to treat). While there has been progress in moving biosensors to the field, their success in the field is so far somewhat limited. In part, this is due to the very low tolerance for false alarms from these devices. Biosensors can raise false alarms for a number of reasons, including fouling and loss of functional activity. In order to optimize the utility of biosensors, a large community is addressing other reasons for false alarms, including manufacturability, sample processing, data collection, analysis, and information processing. The advent of multiplexing techniques to dramatically increase the quantity of signals generated by biosensors also contributes to solving the bioinformatic challenge, which is to derive useful knowledge and make decisions based on signals from these devices.

The lifetime of biomolecular components (and thus the shelf life of biosensors) is also a limiting feature of these systems. When they are external to an organism, functionalized biomolecular materials have a limited lifetime during which they maintain the biological conformational integrity of working components. There are tight tolerances for such system conditions as temperature and ionic strength, to name just a few. Some of the interfering components in real-world samples (e.g., proteases and heavy metals) can actually degrade properties and performance and limit the application of these systems. Some progress has been made in increasing the shelf stability of these systems through the use of protective agents and controlled storage, but this is an area that has not been carefully explored. Efforts to address stability should be based on clear demonstrations of utility in the field.

Functional Biomaterials for Decontamination and Protection

The use of biomaterials in the design and fabrication of materials that can neutralize environmental pollutants or chemical or biological threats has been an area of active research and development. These technologies have progressed to field testing with various polymers that contain a number of biomolecular components. Enzymes that degrade environmental contaminants have been engineered into polymeric materials with moderate success. One success is the incorporation of organic phosphatases into polyurethane materials for the decontamination of media exposed to nerve agents. Other nanoparticular detergent-like materials have

also been modified to degrade biological contaminants. These efforts continue to show promise. More recent efforts have integrated these advances into protective clothing, masks, and wipes, air filters, protective wear, paint and coatings.

The question often posed in the development of these materials is, How clean is clean? with the answer often requiring some follow-up detection method. Recent cleanup activities to address the anthrax contamination of congressional office buildings and a nearby post office reveal the challenges of developing and deploying these materials and using them to determine a safe end point in decontamination. Fundamental challenges remain in designing materials that maintain functionality for the desired properties and in ensuring that the media that are to be decontaminated (soil, water, material assets) are directly exposed to the functional agents.

NEXT-GENERATION BIOINSPIRED MATERIALS

The diversity of materials that nature offers as inspiration for the design of new materials is enormous. The flora and fauna on our planet exhibit a variety of sophisticated structures that are perfected to perform multiple biological functions. Understanding the underlying design principles of these unique biological materials drives much of the research aimed at developing new bioinspired materials. Societal interests in areas such as energy, nutrition, and health will also motivate the exploration and development of new bioinspired materials. Functionality can be defined in many ways for these materials, one of them being the biological ability to create supermaterials with exceptional physical and chemical properties.

Supermaterials from Biology

In the last decade, there has been an explosion of information about unusual natural structures that are superstrong, superadhesive, superhydrophobic, superhydrophilic, superefficient, self-cleaning, self-healing, and self-replicating, with superior designs and intricate shapes. Biological materials are also often multifunctional, a characteristic highly desirable in artificial materials and processes.

The various "super" properties of biological materials come from a sophisticated structural design exerted by self-assembled biomolecules, but the details of how this is achieved are still largely unknown, so that now more than ever is the time to study the underlying biological control mechanisms using advances in the physical sciences and applying this knowledge to bioinspired engineering. Scientists are beginning to answer important questions on how to use biological strategies to make materials that build themselves, repair themselves, and evolve.

Nature keeps surprising researchers by revealing new and ever-more-interesting biomaterials with unexpected properties. The gecko's foot, the lotus leaf, mussel byssus threads, mollusk shells, bones, spider silk, Venus's flower-basket, butterfly

wings, and brittle stars, described below, are just several inspirational examples that have escalated interest in smart biological materials on the part of physical scientists and engineers. These attributes lie beyond conventional engineering. If these biological systems can be reformulated in a synthetic context, it might one day be possible to design nano- and microscale materials and composites for various applications. Multidisciplinary research in this exciting area will become a critical theme of the biomolecular and bioinspired physical sciences.

Superhydrophobic

The lotus flower grows out of the mud, repels dirt when it unfolds, and then remains pristine. In 1975, two botanists discovered that its self-cleaning ability comes from the presence of microstructures coated with a nanoparticulate hydrophobic layer on the leaf surface (Figure 3.12). These structures render the surfaces superhydrophobic, so that water droplets are unstable and pick up dirt, insects, and contaminants as they roll off. Interestingly, a general theory that accounts for the lotus effect had been developed by physicists Wentzel, Cassie, and Baxter 50 years earlier, but its importance was appreciated only once the biological effect was described. Analogous rough surfaces with water-repelling properties were then reported to exist in a variety of organisms. The carapace of the desert beetle, for example, has another ingenious feature—it controls the direction in which the droplets roll, thus capturing and using the sparse water in the extremely arid environment. Biological discoveries have opened up for human use a new field of self-cleaning surfaces, and numerous technical inventions in the spirit of bioinspiration empowered by advances in nanotechnology keep appearing.

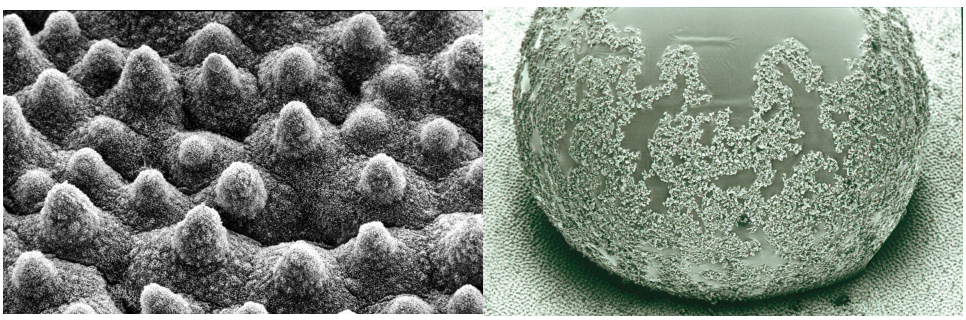

FIGURE 3.12 *Left:* Microstructures on the surface of a lotus leaf are covered by a nanocoating of wax. *Right:* A water droplet on the leaf adsorbs particles of dirt as it rolls. In this picture, one can also see the microstructured papillae on the cuticula. SOURCE: Copyright Wilhelm Barthlott, University of Bonn.

These initial successes are gratifying, but a real scientific triumph would be to go beyond the simple imitation of the biological structures and use nature's principles to create materials with properties beyond those found in nature. Can a surface nanostructure or surface chemistry (or a combination thereof) be designed that will repel any liquid, including organic solvents? Can a strategy be invented for the fabrication of reversible nanostructured surfaces that repel water in wet conditions and collect moisture in dry conditions in response to the environmental changes?

Superstrong

Nature fascinates scientists and engineers with numerous examples of exceptionally strong building materials. It has been shown that whether the structures are fully organic (for example, wood), hybrid organic/inorganic (for example, bone), or nearly fully inorganic (for example, mollusk shells and Venus's flower-basket), their supermechanics usually arises from the successive hierarchical assembly of the constituent structural units from the nanometer to the macroscopic scale (Figure 3.13). Nature's ability to improve inherently poor and brittle building materials, such as glass, calcium phosphate, or calcium carbonate, by introducing a molecular-level control of the structure implemented by biomolecules (proteins, polysaccharides) is unmatched in technology. The biomolecular components and cells improve the mechanical performance by dissipating the energy of advancing cracks and by creating a responsive living interface that provides a flux of ions and macromolecules for the localized material deposition or dissolution. As a result, the final products often confer a remarkable capacity for recovery, self-repair, and fault resistance or tolerance.

The fracture energy of spider silk, for example, is two orders of magnitude greater than that of high-tensile-strength steel, and shells are three orders of magnitude more fracture-resistant than a single crystal of the $CaCO_3$ that constitutes their structure. Spider silk and mollusk shells are regarded by many as the holy grail of materials science: Despite a concerted effort over the decades to explain their underlying mechanical principles, to determine the role and the structure of the proteins that make up the biomaterial, and, ultimately, to replicate it, there has not been much success yet. The same is unfortunately true for the extensive studies of the biomolecular mechanisms controlling the formation and structure of bone and teeth. Artificial bioinspired composites and elastic polymers are significantly inferior to their natural analogues.

While this challenge is not new, it remains critical to study the structural complexity of unique biological materials and the underlying biomolecular mechanisms of their synthesis and organization. Will it be possible to then disengage from nature and, by only using the concept of hierarchy, suggest new materials

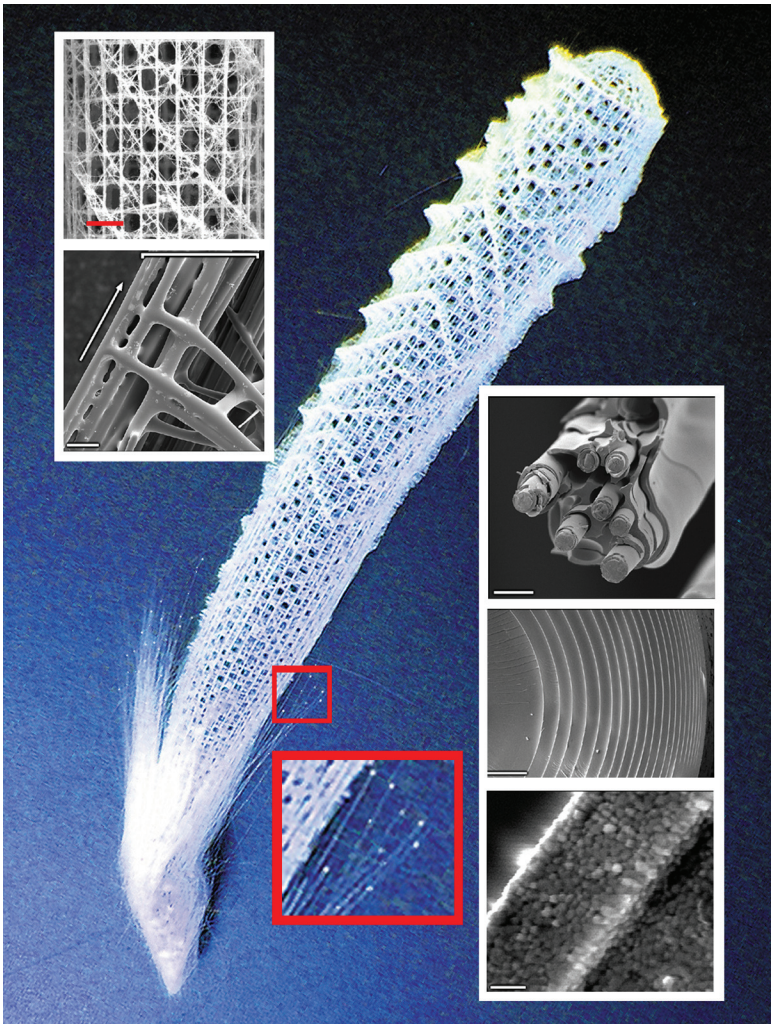

FIGURE 3.13 Skeleton of Venus's flower-basket, a glass sponge. This complex, tough glass architecture hierarchically designed from the nanometer to the centimeter scale houses a pair of mating shrimp fully protected from the environment. Scale bars from top down: 5 mm, 100 µm, 25 µm, 5 µm, 500 nm. Glass fibers forming a crown at the base of the sponge house possess wave-guiding properties similar to those of commercial fibers. The tips of the optical fibers are outlined in red. SOURCE: J. Aizenberg, Harvard University.

and design solutions not necessarily seen in nature, in which the properties of each structural level are not perfect but the successive levels compensate for the defects and together contribute to the mechanical stability and toughness of the resulting design?

Superadhesive

Mussels are adapted to survive in the wave-swept environments of tidal zones. Their competitive dominance and success in these extreme conditions are in part due to a unique anchorage system that reliably attaches them to any substratum (rocks, wooden piers, metal bridges, and so forth). The attachment of a mussel to a substratum is achieved by the mussel byssus, which is composed of a bundle of extracellularly secreted collagenous threads that are glued to the substratum by an adhesive plaque at their distal ends. The threads show unusual mechanical properties: a stiff tether at one end and a shock absorber with 160 percent extensibility at the other end (compared with a typical ~10 percent extensibility of other collagenous materials). The mussel byssus thus has two supercharacteristics that scientists dream of understanding and mimicking—high elasticity and superadhesion in a wet environment. The latter makes them very attractive for medical and dental applications in particular, as a wet, biocompatible glue that has no analogues so far. A previously unknown natural block copolymer was recently identified as being responsible for the elastic properties of the threads. In earlier work, the byssal's superadhesion was attributed to a new protein rich in the amino acid dihydroxyphenylalanine (DOPA). This protein is extremely sticky from the moment it is formed, and getting it to the place where you want it is nearly impossible. Nature solves this problem by using an ingenious just-in-time technique, whereby the protein is first formed without its sticky domain and the adhesive moieties are synthesized at the later attachment stage.

A gecko can climb a perfectly smooth wall or cling to the ceiling, supporting its entire body weight with only a single toe! Their supergripping has been shown to arise from highly developed hydrophobic nanomicrostructures: densely packed keratin microcolumns (setae) further split into bundles of nanospatulas cover a gecko's toes, providing an extraordinarily high adhesion to different surfaces (Figure 3.14). The gecko adhesive works in a vacuum and under water, leaves no residue, and is self-cleaning. Most importantly, the adhesion is reversible, and geckos alternatively stick and unstick themselves 15 times per second as they run up walls. In an attempt to mimic the properties of webbed gecko's feet, dense arrays of high-aspect-ratio polymeric microcolumns have been fabricated and shown to provide a significant adhesive capability. Their flexibility, however, which is necessary for conformal surface contacts, leads to an undesired outcome: The polymer fibers tend to stick to each other, entangle, and irreversibly collapse.

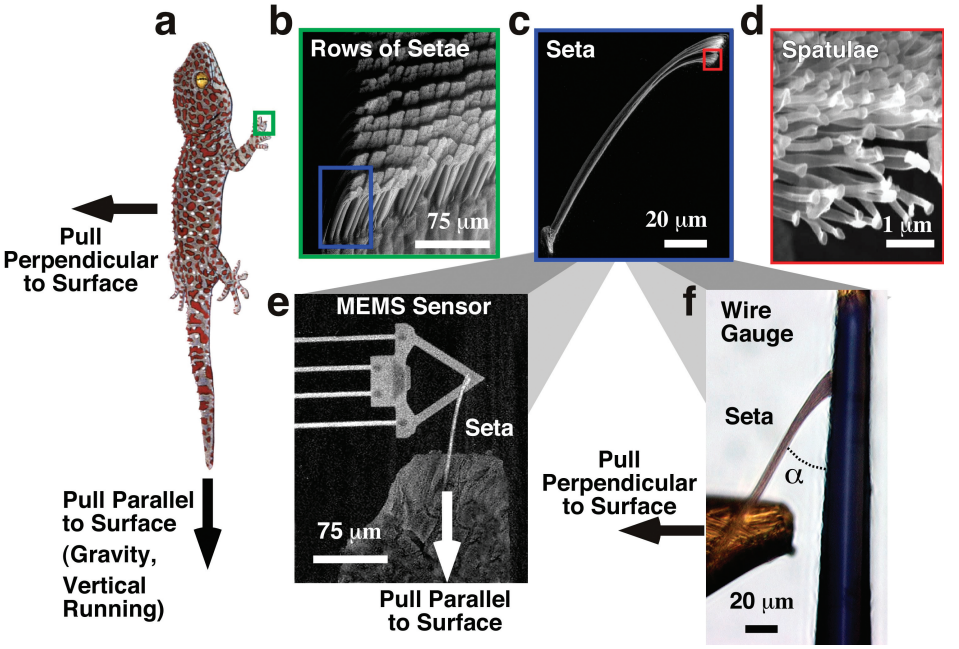

FIGURE 3.14 A Tokay gecko with its toe outlined (a). Scanning electron micrographs of rows of setae from a toe (b), a single seta (c), and the finest terminal branches of a seta, called spatulae (d). A single seta attached to a MEMS cantilever capable of measuring force production during attachment parallel and perpendicular to the surface (e). A single seta attached to an aluminum bonding wire capable of measuring force production during detachment perpendicular to the surface (f). Angle between setal stalk and wire represented by α. SOURCE: K. Autumn, Y.A. Liang, S.T. Hsieh, W. Zesch, W.P. Chan, T.W. Kenny, R. Fearing, and R.J. Full, "Adhesive force of a single gecko foot-hair," *Nature* 405:681-685 (2000).

Despite long-standing admiration for the superior properties of mussel threads and gecko toes, all attempts to mimic their design or to synthesize artificial polymers that are analogous to the bioadhesives in structure or function have been largely unsuccessful, and the mystery of a mussel's unique elastic, highly adhesive fibers and the magic of a gecko's "dry" glue with its reversible attachments remain unsolved, unmatched, and more challenging than ever.

Biooptics

Manipulation of light is a basic feature of many living organisms. Well-known examples of biological optical structures and processes are the eyes of higher organisms and the photosynthesis mechanism in plants. Over millions of years,

biological optical structures and processes have evolved that can compete with or even outperform the cutting-edge optical technology being developed by today's scientists: multilayer reflectors, diffraction gratings, optical fibers, liquid crystals, and structures that scatter light—all of them devices that can be described using optical theory—are found in animals as well, based on a diversity of designs. Iridescent sparkle and the blue color of a Morpho butterfly, which can be seen for hundreds of meters, are caused by a periodic photonic structure in scales covering the wings (Figure 3.15). No dye is involved. Structural color appears to be a common strategy in nature: "Living opals" are reported to be present in peacocks, beetles, and marine organisms.

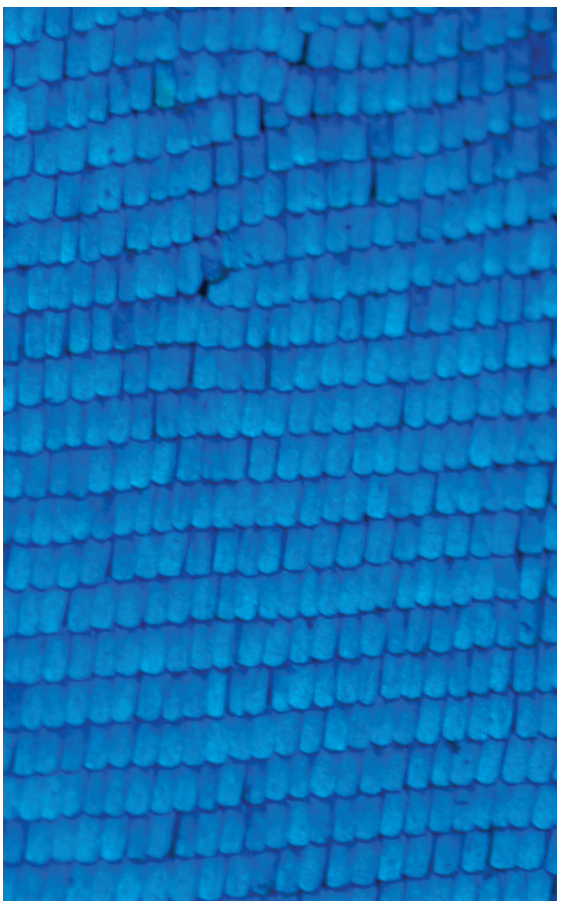

FIGURE 3.15 Optical microscope photograph of scales on a Morpho wing. SOURCE: P. Vukusic, University of Exeter.

In addition to the beauty and structural complexity of Venus's flower-basket, there is now a scientific understanding of the efficient fiberoptical lamp in the base of its latticework (Figure 3.13). The optical features of these biological fibers are remarkably similar to those of their commercial counterparts, and their mechanical properties are better still. Another example of a cutting-edge technology found in nature is the array of optically optimized, single-crystalline calcitic microlenses formed by a brittle star. In this amazing, tunable optical structure, the lenses are compensated for birefringence and spherical aberration and are combined with a microfluidic system that transports pigment to regulate the intensity of light reaching the receptors.

Recent studies mark the beginning of our appreciation for the complex optical structures found in biological specimens and open this field for future discoveries. Only now, when the physical and biological sciences are beginning to come together and new methods of nanoscale fabrication and characterization are developing, do these studies have a good chance of reaching fruition. It is noteworthy that only one optical device in animals has been taken through the manufacture stage: the fly-eye antireflector. Currently there are attempts to fabricate a functional microlens array combined with a microfluidic system, which mimics the features of brittle star optics. However, natural photonic and optical structures are often highly complex at the nanoscale, and mimicking their design may be beyond the ability of today's engineers. This might justify the study of cellular and biomolecular mechanisms and processes. Can researchers also exploit the flawless processes of optical manufacture employed by animals?

Biological Inorganic Supermaterials

Although polymers and organic materials have found their way into modern technology, inorganic materials will remain the basic elements in engineering. There is, however, a growing realization that the traditional methods of "heat and beat" will not be able to fulfill the requirements for future advanced materials, in particular the construction of higher-order architectures from the nanometer to the macroscale. New approaches that use molecular design and assembly should be developed. The ability to take inorganic building blocks and organize them into nanoscale, microscopic, or bulk materials is of importance in electronics, catalysis, magnetism, optics, sensors, and mechanical design.

Again, the best guidance in the search for new inorganic materials and fabrication strategies should come from the study of biological processes. The formation of inorganic materials in organisms, so-called biomineralization, results in exquisite, finely tuned hybrid superstructures that possess exceptional mechanical, optical, and magnetic properties. In addition to the well-known structural biominerals, such as calcium carbonates, calcium phosphates, and silica, a variety of unexpected

inorganic materials (Fe, Sr, Ba, Cu, Ag, Au, sulfides, oxides, sulfates, and hydroxides) have recently been found in organisms. The organisms control the inorganic polymorph, the location of nucleation, the size of the inorganic particles, their crystallographic orientation, and their intricate shapes and assembly, from the nanoscale to the macroscale. The importance of the study of the (bio)molecular processes that lead to these supermaterials and the relevance of biomineralization processes to modern technology were wonderfully depicted by Stephen Mann, one of the pioneers of biomimetic inorganic chemistry. He generalized that three biological principles are critical: (1) preorganization/assembly of (bio)organic molecules into structural and chemical scaffolds at the nanometer scale prior to mineral deposition; (2) templating and molecular recognition at organic-inorganic interfaces that result in controlled, site-directed nucleation of oriented inorganic nanoclusters within preformed supramolecular assemblies; and (3) larger-scale cellular processing of the nanostructures into higher-order, functional architectures.

The molecular details of these processes are largely unknown and require thorough investigation by cell biologists, structural chemists, materials scientists, molecular biologists, physicists, and engineers working in tandem. Recently it has been shown that even the most primitive use of these principles, when complex biological matrices were replaced by synthetic self-assembled molecules that mimicked biological supramolecular architectures and surface micropatterning was used to template site-specific oriented nucleation, could lead to significant successes in controlling the orientation, polymorph, shapes, positioning, and nano/micropatterns of synthetic inorganic crystals. "Currently we are only at the beginning of a biomimetic approach to inorganic materials, and there is a long way to go, but it is feasible that one day, the dusty, dirty world of minerals could be transformed by biological insights. A quiet revolution is underway."[1]

Materials That Mimic Proteins and Membranes

There are considerable efforts to mimic many of the functional properties of biomolecules and their assemblies. Most man-made polymeric materials used today serve structural purposes in plastics, fibers, paints, and rubbers. These polymers lack precise sequence specificity, and do not approach the functional sophistication of biomolecular materials. For example, proteins can catalyze chemical reactions, transport ions, convert energy to motion, repair other biopolymer molecules, and transduce signals and energy. Biomolecular functions can be linked together into complex, interacting and responsive systems that exhibit emergent, higher-level

[1] S. Mann, D.D. Archibald, J.M. Didymus, T. Douglas, B.R. Heywood, F.C. Meldrum, and N.J. Reeves, "Crystallization at inorganic-organic interfaces: Biominerals and biomimetic synthesis," *Science* 261:1286-1292 (1993).

functionalities. These powerful functionalities are possible because, unlike current man-made polymers, biopolymers are informational as well as structural in nature: Their functions are encoded within distinct sequences of diverse monomer sets. An exciting research area that has emerged in the past decade involves the mimicry of these functions in new man-made polymeric materials that are informational and designed to have a wide range of sequence-structure-function relationships.

For example, microbes are transfected with artificial genes with de novo-designed monomer sequences, allowing these biological cells to be harnessed for the production of nonnatural proteins. Researchers are synthesizing nonnatural amino acids that can still be polymerized by a microbe's protein synthesis machinery, leading to proteins with novel properties. In other cases, simple oligopeptide sequences are patterned after Alzheimer's disease peptides, both to study the aggregation process that underpins the disease and also to create new self-assembling materials. Other researchers are creating minimalist proteins—molecules that are simple enough that the sequence patterns can be designed without computers, often to test folding principles and create simple proteinlike functionalities. Folded peptides are being designed as cages, with gates that can be triggered to synchronize the release of dyes that allow complex biological and chemical events to be followed in real time.

An important recent development in creating useful and functional new proteins that can mimic particular functions is directed evolution. In this approach, a gene is first randomly mutated to create a library of variants. The library is then screened to select mutant proteins having the desired properties. This method is now widely practiced in academia and being industrialized to produce new proteins that are more stable or enzymatically more active or that have different substrate specificities.

Efforts are also under way to mimic the conformal activity of proteins in constructs termed foldamers, which are chain molecules composed of nonbiological monomers that, in principle, can fold like proteins and possibly function like proteins. One example is a class of polymers called peptoids, which are sequence-specific heteropolymers based on chains of *N*-substituted glycines. Peptoid-based helical bundles have been made that fold cooperatively, have hydrophobic cores, and can bind zinc tightly and cooperatively, serving as a proof of principle that such polymers are proteinlike in important ways. A family of peptoid 9-mers with hydrophobic side chains was discovered to form a uniquely folded, highly stable threaded-loop structure in acetonitrile, held together by hydrogen bonding, which surprisingly has a polar core and a hydrophobic outer surface. Like mirror-image proteins, peptoids are not degraded by protease enzymes and so may be useful as therapeutics and biomimetics. Peptoids have been used to create novel lung surfactant protein mimics, as well as bioavailable mimics of antimicrobial peptides. Excellent progress is being made as well in the design of protein-mimetic structures

based on β-peptides and chimeric α/β-peptides, including peptidomimetics that kill bacteria in the same manner as antimicrobial peptides and inhibit protein-protein binding interactions involved in viral fusion and the cell cycle.

Mimicry and patterning of lipid bilayer membranes is an active area of research to create spatial domains at the micro- and/or nanoscale, potentially leading to nanowires, nanonetworks, and to the formation of nanoscale "corrals" in which different membrane proteins may be localized. These efforts have obvious applications because many of the functional activities of biomolecules (for example, drugs) are manifest in lipid membranelike environments. Since 40 percent of the blockbuster drugs currently on the market act on membrane proteins, any new method that can successfully isolate, characterize, or utilize membrane proteins is an important advance.

To advance the field of mimicry of functional materials, there are several challenges that remain to be addressed: in synthetic chemistry, in the creation of new monomer types and new ligation strategies for linking them together with high efficiency, and to improve the yields of functional polymers. There are challenges in characterizing structural and physical properties of bioinspired or mimicked polymers that are created. And, there are substantial challenges in computational chemistry, to be able to design the sequences having targeted properties.

Integration of Functional Biomolecular Materials

Exciting progress is now being made, but a great deal of work remains, toward seamlessly integrating functional, nonbiological materials and devices with living, mobile biological systems, including cells and tissues. Such nonbiological materials must be stable for a tunable period of time and remain uninfected and ideally non-encapsulated when implanted in vivo. While certainly functional, today's metallic replacements for missing bones and joints remain at a primitive state of integration with the recipients' living tissue. An ideal compatibility of medical implants such as pacemakers and artificial hearts with the host site is clearly very important since these devices remain in place for 5-10 years.

Outside the medical realm but in the area of biotechnology, a host of biomolecules and their assemblies (cells, enzymes, antibodies, light-harvesting complexes) could provide useful functional system elements if they could be stably interfaced with man-made systems, many of which tend to be metallic or plastic (electrodes, batteries, computers, etc.). Many of nature's most useful proteins are embedded in lipid membranes, and learning to work with these types of proteins in artificial membranes will allow many important advances in biotechnology. Finally, a variety of machines could be designed to interface more intimately and effectively with their operators (for example, fighter jets, passenger vehicles, cranes and other heavy

equipment, surgical robots and lasers) if effective interfacing from the human mind and nerves to the machine could be created.

A variety of polymeric materials have been used in the human body, but so far, none perform as fully required or desired. Most of the presently used materials were "found" or "discovered" rather than "designed" materials. For example, polymers and plastics not originally created for use as biomaterials were found to be serviceable in artificial hearts, valves, and so on. Clearly, these found materials have very limited biocompatibilities. All of the presently used biomaterials eventually fail in vivo, in part because of problems with uncontrolled nonspecific cell/protein binding or fouling and a propensity for infections to occur at the site. Virtually all of today's biomaterials cause undesired clotting of blood; all patients implanted with cardiovascular materials require systemic anticoagulant therapy. All endotracheal tubes become infected after 5 days; 99 percent of systemically delivered nanoparticles or nanogels end up uselessly in the liver or spleen; 100 percent of small-diameter vascular grafts fail within 21 days in vivo; and virtually all implanted biosensors fail in vivo within 20 days. This highlights the tremendous work that remains to be done by engineers and scientists in understanding and controlling the interactions between abiotic and biotic interfaces.

Today's approach to integrating elastomeric or inorganic materials with living cells or tissue is to functionalize the surfaces with biocompatible and/or bioactive materials. Such tailored interfaces are now starting to make their way into clinical trials but still represent technology that is primarily academic and not yet commercialized. A significant amount of fundamental research has been done to discover the best ways of forming strong bonds between inorganic surfaces (such as gold, copper, silicon, silica, titania, and carbon surfaces) and tethered or bound polymers or biomolecules. Thiol moieties bind strongly to gold or copper, while reactive organosilanes can be used to modify silica or oxidized silicon. Some of the strongest bonding moieties yet discovered are inspired by natural systems, such as the modified L-DOPA amino acid that evolved to serve in the tethering elements of shellfish to allow them to adhere to shoreline rocks. When chemical approaches are taken, this is typically done using hydrophilic, uncharged oligomers or polymers, most commonly based on ethylene oxide. Polyethyleneglycol seems, at this time, to be the best polymer to reduce nonspecific binding of proteins and cellular material. On the other hand, some very physical approaches to modifying surfaces, including laser treatments, glow discharge plasma treatments, and supercritical fluid processing are showing significant promise as well in decreasing the nonspecific binding or fouling of abiotic interfaces with biomolecules. Second, for the best biological compatibility, the appropriate molecular signals, typically but not always proteins or their assemblies, must be presented on the engineered surfaces. Micropatterning of these surfaces appears to be a powerful way to control cellular behavior, as

well as the integration of microscale technologies with the use of biocompatible polymers and hydrogels.

Understanding the mechanisms of cell adhesion at the molecular level will be very important. Reengineered enzymes, more stable than natural proteins, may be critical for the tasks of interface engineering. Strategies for the synthesis and derivatization of nanoparticles such as cross-linked copolymer micelles and dendrimers are maturing rapidly, and these types of particles may soon find their way into clinical use. In general, the primary challenges faced today involve fooling biological molecules, which are very potent sensors of their environment, into believing that abiotic, human-made functional elements are not there. Using engineered biomaterials, artfully applied, researchers may succeed in putting realistic sheep's clothing on inorganic materials that are normally seen by living systems as the wolf.

OPPORTUNITIES AND CHALLENGES

The wide range of biological functions and advances in the understanding of their properties present enormous opportunities for further science and technological advances. In this chapter, selected topics were reviewed in the discovery and application of these functional properties, including alternative and renewable energy, health and medicine, and biomaterials for national security needs. Particular functional properties of biological systems were also reviewed, and superior functional properties that have not yet been fully appreciated or exploited were described. Finally, issues in mimicry, synthesis, and integration of functional biomolecular materials were discussed as important underpinnings to the science and technology of functional biomolecular materials. Some challenges to the scientific understanding of advanced functional materials are enumerated below where new understanding is beginning to emerge (first-level bullets). They are followed by opportunities that might arise if researchers develop enough of an understanding to address the challenges.

Alternative and Renewable Energy

There have been considerable advances in the understanding of energy storage and conversion properties of biomolecular materials and greater interest in engineering new properties that extend these important functional attributes. Efforts to create new functional biomaterials in plants, based on engineering the plant genome, that can be efficiently converted to usable, high-energy-containing materials are largely motivated by the promise of new sources of biofuel. Biomimetic photosynthesis is gaining momentum as researchers unlock the mechanisms involved when light is used to fix carbon.

- Challenges to scientific understanding: Expanded use of biofuels from plant and animal biomaterials
  - —Opportunity: Improved conversion efficiency and utilization of substrates
  - —Opportunity: Robust and controlled genetic engineering of new synthesis in plants
- Challenges to scientific understanding: Research and development of artificial photosynthetic and other proton gradient systems
  - —Opportunity: Efficient conversion of light to useful products
  - —Opportunity: Expanded selection of new biomolecules for energy conversion and storage
  - —Opportunity: Integration of photosynthetic complexes into artificial matrices
- Challenges to scientific understanding: Discovery of the structural and functional dynamics of biological motors in situ
  - —Opportunity: Assembly and integration of linear and rotary biological motors into useful nanomachines or devices that realize biomotor performance
  - —Opportunity: Stability and robustness of biological motor complexes

Health and Medicine

The use of biomolecular materials in health and medical devices is widespread and has already had a significant socioeconomic impact on society. The engineering of new biomolecules for increased functions such as sensitivity or specificity is an active area of research and has shown early payoff in the design of new tools such as diagnostic assays. Nanotechnology efforts have found new ways to manipulate the delivery of drugs to specific sites in the body and to enhance the delivery of new therapeutic compounds. Finally, the area of neuroprosthetics, which combines innovation across a number of disciplines, including neuroscience, materials science, microelectronics, and engineering, is moving rapidly.

- Challenges to scientific understanding: New functional biomolecular materials for diagnostic array detection with desired sensitivity and specificity
  - —Opportunity: Sample preparative and other methods to enhance signal detection and reduce background noise in a diagnostic platform
  - —Opportunity: Computational analysis of multiplexed, large diagnostic and profiling datasets for prognostic medicine
- Challenges to scientific understanding: Improved delivery of drugs using functionalized and controlled-size biomolecular materials

  - —Opportunity: Prediction and control of in vivo response to implanted materials
  - —Opportunity: Synthesis and characterization of controlled nanoparticles, polymers, and dendrimers with functionalized properties
  - —Opportunity: Targeted delivery to specific sites
- Challenges to scientific understanding: New neuroprosthetics with dynamic function for artificial limbs and sensors
  - —Opportunity: Development of reliable interfaces with long-term function
  - —Opportunity: Integration of recorded signals with response in closed-loop function
  - —Opportunity: Understanding information coding from neuronal ensembles

National Security

Global threats from the environment and in the context of military and homeland defense present unique opportunities for the application of functional materials. The most mature application may be biosensors, where the hope of developing detect-to-warn systems continues to drive this field across a variety of interested government customers (Department of Energy, Department of Defense, Department of Homeland Security, and Environmental Protection Agency). Biosensor performance has improved, but needed advances in reducing false alarm rates and integrating samples from different sources are still a challenge. Decontamination materials that can detect and protect, degrade, and perhaps regenerate are under investigation by a number of agencies and provide good application platforms to integrate advanced functional materials.

- Challenges to scientific understanding: Biosensors that reliably detect threats in time to prevent exposure and consequences of environmental threats (detect to warn)
  - —Opportunity: Reduction of false-alarm rates
  - —Opportunity: Sample processing and handling for optimal performance
- Challenges to scientific understanding: New decontamination and protective materials that incorporate functional biomolecules
  - —Opportunity: Methods of incorporation that optimize biomolecular function
  - —Opportunity: Cooperative functions that derive from multifunctional materials
  - —Opportunity: Demonstration in real-world conditions with reliable measures of determining safety after cleanup

Next-Generation Bioinspired Materials

- Challenges to scientific understanding: Wide selection of biological multifunctional systems to drive inspiration of new designs with advanced materials properties (e.g., strength, adhesion, optics, hierarchy, assembly)
  - —Opportunity: Enriching and harvesting underlying principles and processes to drive engineering activities in a productive way
  - —Opportunity: Fabrication and assembly of inspired materials
- Challenges to scientific understanding: Biomimicry of biomolecules with directed conformation and information content
  - —Opportunity: Defining desired properties and designing controlled synthesis
  - —Opportunity: Characterization and implementation of new materials
- Challenges to scientific understanding: Seamless integration of functionalized biomolecular materials in the body and in devices
  - —Opportunity: Preventing fouling and foreign-body reaction from functionalized biomaterials
  - —Opportunity: Controlling reactions at the surface to optimize desired integration of material
  - —Opportunity: Longevity and robustness

SUGGESTED READING

Department of Energy, *Basic Research Needs for Solar Energy Utilization*, Washington, D.C., 2005. Available online at http://www.sc.doe.gov/bes/reports/files/SEU_rpt.pdf. Last accessed March 26, 2008.

Gellman, S.H. "Foldamers: A manifesto," *Accounts of* ***Chemical Research*** 31:173-180 (1998).

Humayun, M.S., J.D. Weiland, G. Chader, and E. Greenbaum (editors), *Artificial Sight: Basic Research, Biomedical Engineering, and Clinical Advances*, New York, N.Y., Springer, 2007.

Jung, C.C., E.W. Saaski, D.A. McCrae, B.M. Lingerfelt, and G.P. Anderson, "RAPTOR: A fluoroimmunoassay-based fiber optic sensor for detection of biological threats," *Sensors Journal, IEEE* 3(4):352-360 (2003).

Langer, R. "Drug delivery: Drugs on target," *Science* 293:58-59 (2001).

Langer, R., and N.A. Peppas, "Advances in biomaterials, drug delivery, and bionanotechnology," *American Institute of Chemical Engineers Journal* 49(12):2990-3006 (2003).

Mann, S., *Biomineralization: Principles and Concepts*, Oxford University Press, 2001.

National Research Council, *The New Science of Metagenomics: Revealing the Secrets of Our Microbial Planet*, Washington, D.C., The National Academies Press, 2007.

National Research Council, *Polymer Science and Engineering: The Shifting Research Frontiers*, Washington, D.C., National Academy Press, 1994.

Pollard, T.D., W.C. Earnshaw, and J. Lippincott-Schwartz, *Cell Biology,* 2nd Ed., Saunders, 2007, Chapters 36-39.

Raber, E., A. Jin, K. Noonan, R. McGuire, and R.D. Kirvel, "Decontamination issues for chemical and biological warfare agents: How clean is clean enough?" *International Journal of Environmental Health Research* 11(2):128-148 (2001).

Ragauskas, A.J., C.K. Williams, B.H. Davison, G. Britovsek, J. Cairney, C.A. Eckert, W.J. Frederick, Jr., J.P. Hallett, D.J. Leak, C.L. Liotta, J.R. Mielenz, R. Murphy, R. Templer, and T. Tschaplinski, "The path forward for biofuels and biomaterials," *Science* 311:484-489 (2006).

Russell, A.J., J.A. Berberich, G.F. Drevon, and R.R. Koepsel, "Biomaterials for mediation of chemical and biological warfare agents," *Annual Review of Biomedical Engineering* 5:1-27 (2003).

Vogel, P.D., "Nature's design of nanomotors," *European Journal of Pharmaceuticals and Biopharmaceuticals* 60(2):267-277 (2005).

Vukusic, P., and J.R. Sambles, "Photonic structures in biology," *Nature* 424:852-855 (2003).

Wainwright, S.A., W.D. Biggs, J.D. Currey, and J.M. Gosline, *Mechanical Design in Organisms*, New York, N.Y., John Wiley and Sons, 1976.

Wang, L., J. Xie, and P.G. Schultz, "Expanding the genetic code," *Annual Review of Biophysics and Biomolecular Structure* 35:225-249 (2006).

Weiland, J.D., W. Liu, and M.S. Humayun, "Retinal prosthesis," *Annual Review of Biomedical Engineering* 7:361-401 (2005).

4

Probes and Tools for Biomolecular Materials Research

This chapter describes experimental and computational tools that promise to extend knowledge and understanding of biology and biomacromolecular materials in the next decade and beyond. Structural, biochemical, and physiological studies relate the motions and dynamics of these materials to particular cellular functions. A further motivation for these studies is the expectation that the process of learning how work is performed at the very small subcellular scales should pave the way for the development of functional biomolecular materials in future nanotechnological applications. Biological systems consist of collections of interacting molecules (proteins, carbohydrates, lipids, and nucleic acids) that give rise to a variety of supramolecular complexes with hierarchical structures spanning sizes from angstroms to micrometers. For example, the mechanical and structural properties of filamentous (F)-actin, one of the three components of the cytoskeleton in eukaryotic cells, is an area of intense research. F-actin further assembles to form either bundles, or loosely packed, two-dimensional and three-dimensional network structures in cells. The distinct functions resulting from these highly regulated structures interacting with other biomolecules and motors include cell shape and mechanical stability, cell adhesion and motility, and cell cytokinesis, the splitting of the cell body into two daughter cells during division. Special techniques are required to span spatial and temporal ranges appropriate to the functions of these networks.

While macromolecular crystallography has revolutionized our understanding of protein structure and function, the technique requires high-quality crystal samples. However, many proteins (notably membrane proteins) are difficult to crystallize, and the molecular basis of cellular function usually involves interactions

among more complex and highly disordered biological complexes (for example, the immunological and neuronal synapses that mediate cell-cell communication). Elucidation of the molecular details of the structure and dynamics of such noncrystalline supramolecular complexes requires continual advancement in the development of techniques to solve these biological problems. The techniques should also apply equally well to the broader class of man-made biomolecular materials.

The new experimental probes of biomolecular materials are expected to have a major impact on future science and technology, including imaging methods based on novel optical and electron microscopic techniques, new synchrotrons, and X-ray free electron lasers, which provide ultrashort and extremely intense pulses. There are significant synergistic advances in the development of instrumentation to probe the physical, chemical, structural, and dynamical behavior of individual molecules, so-called "single-molecule biophysics."

It must be emphasized that biomolecular materials are naturally complex, often containing many components, inhomogeneous characteristics, and fluctuations. These features make it difficult to intuit principles from experimental observations alone. Thus it is important to combine experimental techniques that provide crucial observations with powerful numerical simulations and insightful analytical modeling to elucidate the mechanisms underlying biomolecular processes. Theory and computation can be critical to the discovery and design process because they can be used to predict the consequences of different mechanistic hypotheses, interpret experimental results, and examine alternative design motifs for biomaterials.

Some of the potential advances to be achieved from next-generation experimental tools and computational methods inspire research efforts. Imagine that one could . . .

- View cells in atomic to molecular detail and at a time resolution of milliseconds, appropriate to observe the events during neuronal synaptic transmission or chromosome replication.
- Measure the forces and motions of nanobiomachines directly and on the millisecond to microsecond timescale.
- Determine the sequence of an individual DNA molecule, providing the ultimate sensitivity for forensic purposes or diagnosing inherited disease.
- Understand how biological machines conquer the chaotic and crowded conditions of the cell.
- Use principles of biological recognition to design new molecular interactions ab initio.
- Predict how molecules with a specific sequence of monomers will adopt a specific conformation and self-assemble into precise supramolecular structures.

- Use cell-mimetic cooperativity to design biosensors that can detect minute amounts of hazardous substances without noise-induced false positives.
- Describe systems far from equilibrium with a rigorous theoretical framework.

THREE-DIMENSIONAL ELECTRON MICROSCOPY

Electron microscopy imaging is an indispensable research tool of modern materials science, biology, and biomolecular materials. As compared to nuclear magnetic resonance (NMR) spectroscopy or macromolecular crystallography, which are used primarily for elucidating the structure of single protein molecules at angstrom resolution, three-dimensional cryo-electron microscopy (cryo-EM) is emerging as a powerful tool for capturing images, albeit at lower resolution, of directed- or self-assembled collections of biological molecules, complexes, and machines that function in concert.

The most widely used method of three-dimensional electron imaging is cryo-electron tomography (cryo-ET), which consists of three-dimensional reconstruction of an object from a series of tilt projections. In an alternative method, referred to as single-particle cryo-EM, a three-dimensional reconstructed image is obtained from the superposition of a very large number (20,000 to nearly 300,000) of particle images. The latter method is facilitated if the particle (e.g., an assembled molecular machine or a viral capsid) is monodisperse, so that all of the two-dimensional projected images used in the reconstruction can be assumed to be images of a single particle viewed from different orientations. On the other hand, to image the interior architecture of cells, where no two cells are ever identical, requires cryo-ET.

Figure 4.1 shows an example of a three-dimensional reconstruction of an in vitro assembled infectious P22 bacteriophage virion, a large asymmetric complex at 17 Å resolution, from the superposition of 26,442 particle images. The biology of bacteriophage P22, which infects *Salmonella enterica*, and its molecular-level mechanisms of action have been studied extensively by scientists worldwide as a model for virus assembly. In particular, studies are beginning to elucidate the non-covalent virus assembly pathway and the mechanism by which the double-stranded (ds) DNA genome is actively packaged followed by reinfection pathways and release of viral genome. The elucidation of the P22 structure at this resolution has revealed a likely mechanism for the late stages of dsDNA packaging and the sequence-independent switch, which senses that the virion has reached the correct physical packing density and signals termination of the genome packaging process.

Cryo-ET appears poised to become a key tool for creating three-dimensional images of directed assembly within inner cell structures in the next decade and beyond. In particular, cryo-ET is expected to be important for elucidating the spatial and temporal (via imaging of vitrified cells as a function of time during a

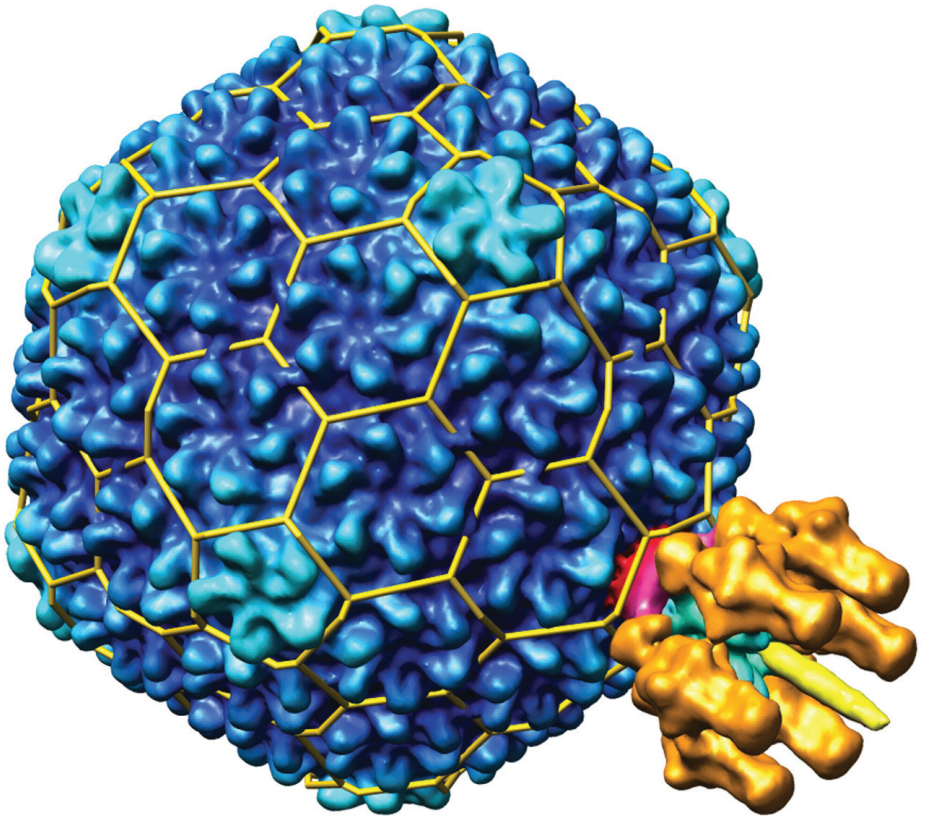

FIGURE 4.1 The structure of an assembled infectious P22 bacteriophage virion as revealed by three-dimensional cryo-EM at 17 Å resolution. The coat protein is shown in blue. The tail machinery complex, composed of multiple copies of four gene products (mustard, green, yellow, pink) exhibits 6-fold and 12-fold symmetry and is located at a single 5-fold vertex of the capsid. SOURCE: G.C. Lander, L. Tang, S.R. Casjens, E.B. Gilcrease, P. Prevelige, A. Poliakov, C.S. Potter, B. Carragher, and J.E. Johnson, "The structure of an infectious P22 virion shows the signal for headful DNA packaging," *Science* 312:1791 (2006).

particular cell function) distribution of supramolecular assemblies of biological complexes, molecular machines, and motors. Figure 4.2 shows images of the actin cytoskeleton in the slime mold *Dictyostelium discoideum* using state-of-the-art cryo-ET.

In order to appreciate the profound benefits that may be expected from cryo-ET as it becomes more readily available, one has only to think about the medical era before and after the availability of computed tomography (CT) scanners and

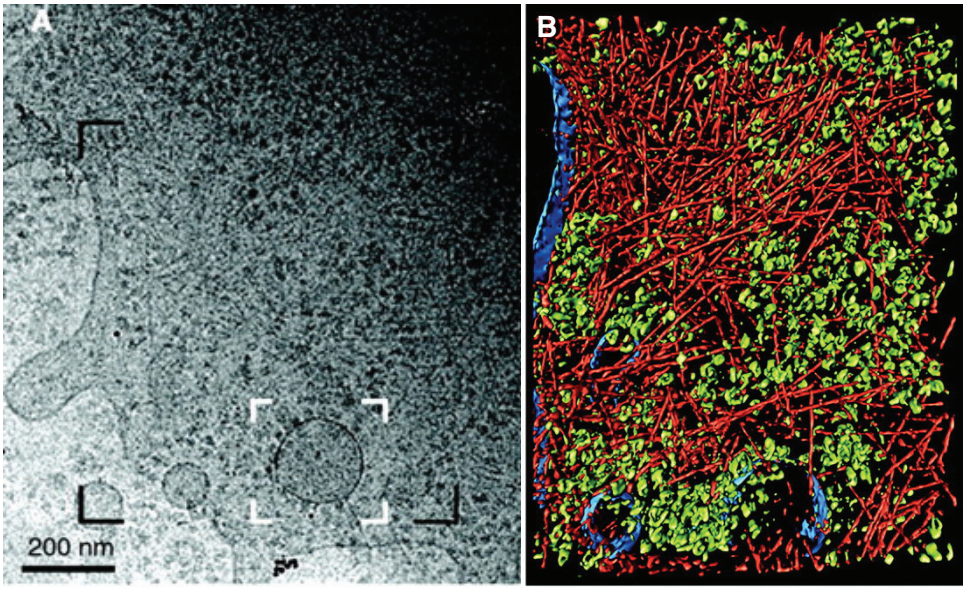

FIGURE 4.2 (A) Transmission electron micrograph of a peripheral region of the vitrified slime mold *Dictyostelium discoideum*. The thickness of the cell in this region was between 200 nm and 350 nm. (B) The cell-cytoskeletal architecture in a section of the slime mold visualized by three-dimensional reconstructions from cryo-ETs. The three-dimensional reconstruction resulted from a series of tilt projections of the region marked by the square vertices in (A). A partial cell volume 97 nm thick was used to produce this image at 5-nm resolution. The dense network of actin filaments is shown in red, ribosomes in green, and membrane in blue. A portion of the plasma membrane is seen in the top left corner and circular membrane vesicles are also seen in the lower part (center and to the left). The membrane vesicle in the lower middle part (corresponding to the white square region in (A)) appears to be decorated by ribosomes, thereby corresponding to a section of the rough endoplasmic reticulum. SOURCE: O. Medalia, I. Weber, A.S. Frangakis, D. Nicastro, G. Gerisch, and W. Baumeister, "Macromolecular architecture in eukaryotic cells visualized by cryoelectron tomography," *Science* 298:1209-1213 (2002).

magnetic resonance imaging (MRI) machines in clinical imaging. CT and MRI machines have allowed physicians to peer noninvasively into the human body, permitting precise three-dimensional visualization of organs and tissue and allowing, for example, rapid diagnoses for stroke and accident patients. The majority of CTs and MRIs currently in use are able to visualize objects about 1 cubic millimeter in size, a useful scale for clinical diagnosis. By the same token, cryo-ET and single-particle cryo-EM should allow researchers to visualize cells, their organelles, and the crucial biomolecular assemblies at a near molecular resolution, the appropriate spatial dimension to uncover their functional behavior.

HYPERRESOLUTION OPTICAL MICROSCOPY

Light microscopy, especially fluorescence microscopy of specifically tagged macromolecular components, has become the mainstay technique of cell biology. Its advantages are relative ease of implementation, tremendous adaptability and facility for labeling specific proteins and nucleic acids, and real-time and three-dimensional imaging. A big disadvantage with which optical microscopy normally contends is its limited spatial resolution relative to molecular dimensions. The spatial resolution for discriminating two objects in a conventional optical microscope is limited to about 250 nm owing to diffraction of the light by the imaging objective. Of course, many cellular components are much smaller than that. Several new techniques have been described that overcome this diffraction barrier in optical microscopy.

The first general method is termed "structured light." It is possible to form a spot of light or a grid with greater resolution by various nonlinear techniques. One such approach, called stimulated emission depletion (STED) microscopy, uses pairs of precisely timed and shaped laser pulses to generate an excitation area—point spread function (PSF)—much narrower than the standard 250 nm wide diffraction-limited spot. Figure 4.3 shows a schematic diagram of the optical arrangement and the mechanism for narrowing the PSF. For the fluorescent probe Atto532, a blue laser (470 nm) excites fluorophores with the usual diffraction-limited spatial distribution. A few nanoseconds later, an orange (670 nm) laser pulse triggers stimulated emission to quench the excited state of most of the fluorophores. This quenching is a reversible process, and the individual fluorophores can be excited and deexcited hundreds of times. The quenching beam is made donut-shaped by phase modulation, allowing probes only at the center of the original spot to remain excited. The reason the remaining PSF is so narrow is that de-excitation saturates abruptly at zero excitation (negative excitation does not occur) and a very intense STED pulse switches all of the probes except those within a few nanometers of the center. In Figure 4.3, the resolution achieved is 66 nm. There is no intrinsic limit to this narrowing, and 20 nm resolution has been demonstrated. Gains in axial resolution are also achieved by using two converging objective lenses on opposite sides of the specimen, and irreversible photobleaching is minimized by allowing triplet states to relax between excitation-quench pulses.

Images at high resolution are obtained by raster-scanning the sharp PSF excitation spot over the sample and sequentially collecting the fluorescence emission from each spot to reconstruct the distribution of fluorescent probes in the specimen. In one experiment, synaptotagmin-labeled synaptic vesicles were resolved much more clearly in STED images (Figure 4.3c) than by standard confocal microscopy (Figure 4.3b).

Fluorophores can be switched on and off by photophysical means other than

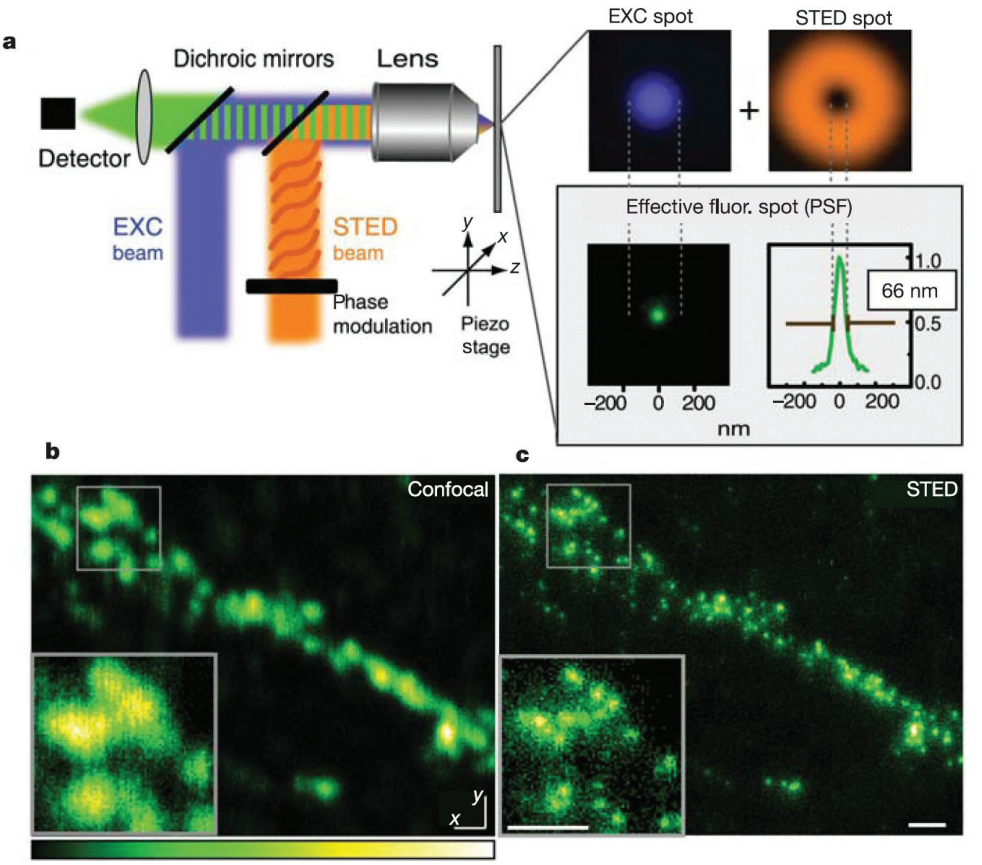

FIGURE 4.3 Schematic diagram showing (a) operation of stimulated emission depletion (STED) microscopy, (b) images of fluorescent-labeled synaptic vesicles using standard confocal microscopy, and (c) same vesicles imaged with STED microscopy. Scale bars are 500 nm for (b) and (c). SOURCE: K.I. Willig, S.O. Rizzoli, V. Westphal, R. Jahn, and S.W. Hell, "STED microscopy reveals that synaptotagmin remains clustered after synaptic vesicle exocytosis," *Nature* 440:935 (2006).

stimulated emission. Certain fluorescent proteins, organic dyes, and pairs of closely spaced cyanine dyes are photoswitchable between fluorescent and nonfluorescent metastable states using two different wavelengths. Again, switching these probes off is saturable, allowing the equivalent of STED narrowing of the PSF but at much lower laser intensities. The disadvantage of fluorochrome switching is that the image collection times are much longer.

Individual fluorescent molecules can be localized to within a few nanometers by collection of sufficient photons and fitting an appropriate kernel function to find the center of their PSF. Repeatedly switching a few single fluorophores on and off so that they are spatially separated allows determination of the position of each one at nanometer precision. After many cycles of excitation and reversible quenching, the overall spatial distribution emerges. Various versions of this scheme have been termed photoactivated localization microscopy (PALM) and stochastic optical reconstruction microscopy (STORM). This group of special fluorescence microscopic techniques and further developments are highly likely to accelerate understanding of spatial distributions, dynamics, and signal transduction in a broad range of molecular and cell biological problems.

X-RAY METHODS

The discovery of X-ray diffraction from crystals by von Laue and Bragg nearly 100 years ago marked the beginning of developments for visualizing the three-dimensional atomic structures inside crystals. Indeed, X-ray crystallography has since made a tremendous impact in materials sciences, physical sciences, and biology. It has now reached a point where, as long as appropriate high-quality crystals are obtained, it can determine any structure. However, many biological samples such as whole cells, organelles, viruses, and many important protein molecules are difficult or impossible to crystallize and are hence not accessible to crystallography.

Currently, there are two successful approaches to high-resolution, full-field X-ray imaging of noncrystalline samples: one that uses a high-resolution lens and another that does not use a lens but requires coherent illumination. Both of these imaging techniques have demonstrated rapidly improving resolution: 15-30 nm. While the first approach requires a high-resolution X-ray lens similar to that of a standard optical microscope, it does not require a source with high degree of coherence. In fact, using a laboratory X-ray source, sub-50-nm-resolution, three-dimensional imaging has been achieved. The second approach does not require an X-ray lens but requires a source with a high brilliance, such as a third-generation synchrotron source or the upcoming fourth-generation free-electron X-ray laser source.

Newly developed X-ray imaging techniques are expected to have a major impact on biomolecular materials research by facilitating fundamentally new ways of characterizing events at the nanometer scale and also as a function of time. In addition to bridging the resolution gap between optical and electron microscopy, they offer many unique capabilities resulting from the high penetration power (at short wavelengths) of X-rays for nondestructive and time-lapse imaging. Using nanoparticles as markers, X-ray three-dimensional cryotomography with nano-

meter resolution will allow the study of many important biomolecular processes. Nondestructive three-dimensional tomography will play an important role in the development of future-generation nanostructured biomolecular materials with the desired chemical, mechanical, and functional properties.

X-ray Tomography

A schematic illustration of a lens-based X-ray full-field tomographic imaging microscope is shown in Figure 4.4 (left). It consists of an X-ray source, a high-efficiency condenser lens focusing X-rays onto the sample, a high-precision rotation stage, an objective zone plate lens, and a high resolution charge-coupled device detector. Spatial resolution better than 15 nm has been demonstrated with 8 keV synchrotron X-rays. Using a laboratory X-ray source, a full field X-ray microscope with 50 nm resolution has recently been developed. High-resolution X-ray tomography opens up new avenues to nondestructively explore the internal structure of optically opaque solids with nanometer-scale resolution, previously not possible with other analytical techniques. As a proof of concept, Figure 4.4 (middle) shows a rendered three-dimensional image of an advanced copper-interconnect-based integrated circuit (IC) chip with 120-nm feature size.

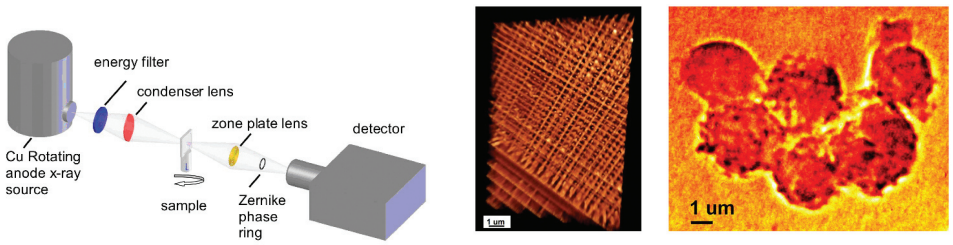

FIGURE 4.4 (Left) Schematic of a zone-plate-based full-field three-dimensional X-ray microscope operating in phase contrast mode. The principle of operation is very similar to that of a visible light microscope, where the visible light source is replaced by the commercially available X-ray source and the glass lenses are replaced with equivalent X-ray imaging optics. The Zernike phase ring alters optical path length for some scattered X-rays to obtain contrast by constructive/destructive interference with nonscattered rays. (Middle) An example of three-dimensional X-ray tomography with 120-nm feature size: volume rendering of a modern IC chip with sub-50-nm spatial resolution obtained with a laboratory-based X-ray microscope. (Right) Malaria-infected red blood cells imaged with a laboratory hard X-ray microscope (8 keV) in phase contrast mode. Dark protrusions from the cell boundaries are indicative of infection by malaria parasite. SOURCE: Wenbing Yun, Xradia, Inc.

To fully utilize the high penetration power and to image weakly absorbing objects, such as biological cells, the phase contrast technique has been applied to full-field imaging X-ray microscopy. For structures containing mostly low atomic number elements, such as biological specimens, phase variations provide much more contrast than absorption, especially for X-ray energies greater than a few keV. Figure 4.4 (right) shows an image of malaria-infected red blood cells obtained from a laboratory X-ray microscope operating at 8 keV in the phase contrast mode.

In the next decade and beyond, the spectral tunability and high brilliance of third-generation synchrotron X-ray sources will allow three-dimensional X-ray microscopy techniques to be employed to answer many key questions in biomolecular materials and processes: What are the elements? How are they arranged? What is their chemical nature? and What is the nature of the defects and how do they alter function?

X-ray Diffraction

A second X-ray based approach making rapid progress is the use of coherent X-ray diffraction to provide three-dimensional images of noncrystalline systems without requiring a focusing lens. When a coherent wave of X-rays illuminates a noncrystalline specimen, the far-field diffraction intensities are continuous and weak. This continuous diffraction pattern can be sampled at a frequency finer than the inverse of the specimen size (that is, oversampled), which corresponds to surrounding the electron density of the specimen with a no-density region: the higher the sampling frequency, the larger the no-density region. It has been shown that when the no-density region is larger than the electron density region, the phase information is uniquely encoded in the diffraction pattern and can be recovered directly by an iterative process that takes advantage of the knowledge that the electron density outside the object is zero and within the object is positive. The first successful experimental demonstration of coherent diffraction imaging was carried out in 1999. Since then, it has been successfully applied by several groups for imaging a variety of samples ranging from nanocrystals and biomaterials to double-walled carbon nanotubes.

In a recent proof-of-principle experiment, coherent X-ray diffraction imaging was used to study *E. coli*, a eubacterium of typical size 0.5×2 μm, transformed with a recombinant yellow fluorescent protein (YFP) that could be marked by potassium permanganate precipitates. The detected densities bear a strong resemblance to the pattern of fluorescence seen from comparable bacteria examined with the confocal microscope (Figure 4.5).

Since no X-ray lenses are needed in this technique, the resolution of coherent X-ray diffraction imaging is only limited by radiation damage to the samples. X-ray experiments have indicated that radiation damage can be greatly reduced by

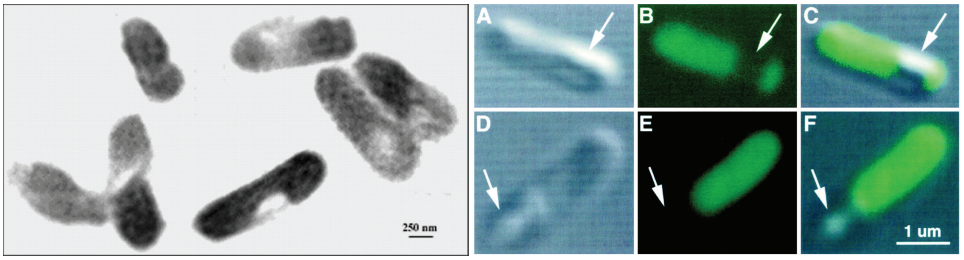

FIGURE 4.5 Coherent X-ray imaging of *E. coli* bacteria. *Left:* An image directly reconstructed from a coherent X-ray diffraction pattern. The dense regions inside the bacteria indicate the distribution of His-tagged YFP labeled with $KMnO_4$ precipitates. The semitransparent regions are devoid of YFP. *Right:* Individual bacteria are seen using transmitted light (A, D) and fluorescence (B, E), where the YFP (green) is seen throughout most of the bacteria except for one small region in each bacterium that is free of fluorescence (arrows). C and F show the fluorescent image superimposed on the transmitted light image. SOURCE: J. Miao, K.O. Hodgson, T. Ishikawa, C.A. Larabell, M.A. LeGros, and Y. Nishino, "Imaging whole *Escherichia coli* bacteria by using single-particle X-ray diffraction," *Proceedings of the National Academy of Sciences USA* 100:110 (2003).

freezing the samples to liquid nitrogen temperatures and sub-10-nm resolution should be achievable. X-ray free electron lasers also offer promising prospects with their ultrashort and extremely intense pulses. In this case, radiation damage could be circumvented by recording the diffraction pattern from single macromolecules before they are destroyed. By using many identical copies of the molecules, a three-dimensional diffraction pattern could be assembled, which could then be directly converted to an image by using phases recovered by oversampling.

Small-Angle X-ray Scattering

Synchrotron techniques of small-angle X-ray scattering (SAXS) are important structural probes of disordered or partially ordered nanostructured complex substances, including biomolecular materials. Owing to its relative ease of use and availability at several synchrotron X-ray sources worldwide, SAXS is a primary method for in situ studies of phase behavior, intermacromolecular interactions, structures, and the kinetics of formation of bioassemblies on multiple length scales, from nanometers to micrometers.

Among the many systems on which SAXS has had a significant impact is the elucidation of the basic structural units of the β-amyloid fibers implicated in Alzheimer's disease. In this condition, the deposited misfolded β-strands spontaneously assemble into stacked β-sheets as a result of hydrophobic interactions

and twist around a fiber axis. It is expected that SAXS studies will elucidate the structure and formation kinetics of many biomolecular material systems, including the misfolded proteins that cause amyloidosis diseases such age-related macular degeneration, type II non-insulin-dependent diabetes, and bovine spongiform encephalopathy ("mad cow" disease).

The combined application of SAXS and cryo-EM, a growing focus of research in the next decade, complements electron and X-ray tomography as powerful probes of noncrystalline biological structures. The X-ray methods quantitatively measure statistically averaged structures in reciprocal space, whereas cryo-EM provides a direct space model (although at lower resolution) of its subjects. Several recent studies of higher-order assembly of cytoskeletal microtubules and filamentous actin have in fact demonstrated the importance of the combined techniques in elucidating such partially ordered hierarchical structures.

NEUTRON SCATTERING

Neutron scattering and diffraction provide detailed information on the structure and dynamics of biomaterials and systems across time and length scales that range from pico- to nanosecond of time resolution and from 1 to 10,000 Å of spatial resolution. Neutrons are scattered from atomic nuclei (as opposed to X-ray scattering by electrons) and are thus exquisitely sensitive to hydrogen atom position, content, and dynamics in biological materials. This feature allows the structure and composition of complex or composite biomaterials to be determined and distinguished according to the bulk hydrogen content of the individual components. Moreover, neutrons scatter differently from hydrogen and its deuterium isotope, so that in mixed systems, individual components can be selectively labeled with deuterium in order to highlight them. The power of the technique is thus most fully realized when combined with synthetic capabilities that allow the design and production of specific, random, or uniformly deuterium-labeled macromolecules to permit selected components of structure, dynamics, and interactions of macromolecular structures to be analyzed in situ in multicomponent systems in solution, at surfaces, or in single crystals. Neutron scattering is thus a powerful tool for characterization and analysis of complex structure-function and interfacial relationships between membrane, polymer, and macromolecular systems at the intersection of biology and materials science.

Because neutrons interact with nuclei rather than electrons, neutron-scattering lengths show little variation across the periodic table. Many of the heavier components of mixed or complex materials that are opaque to X-rays and that dominate X-ray scattering are then virtually transparent to neutrons. This property facilitates the lighter, hydrogenated components (polymers, peptides, proteins, lipids, nucleic acids, or solvents) of complex systems, composites, or phases to be highlighted

and analyzed in situ. At the atomic and molecular levels, neutron diffraction can pinpoint hydrogen atom positions in such materials. This feature can provide fundamental insight into catalytic processes in enzymes or of the proton shuttling/relay pathways involved in biological processes (Figure 4.6, left). In complex biopolymers such as cellulose, an important potential source of renewable ethanol, neutron diffraction experiments have mapped the detailed hydrogen bonding interactions that mediate its macroscopic material properties (Figure 4.6, right).

The enhanced visibility of hydrogen atoms in water, substrates, and proteins allows direct determination of protonation state and thus helps to provide a more complete picture of atomic and electronic structures in macromolecules. This is beneficial for determining enzyme mechanisms, for studies of ligand binding interactions and, since complete D_2O water molecules are prominent in neutron density maps, for detailed analysis of the structure and dynamics of water in hydration layers at the protein-solvent interface. Neutron diffraction can determine the pattern and extent of H/D isotope substitution in proteins, the solvent accessibility of individual amino acids, the mobility and flexibility of interesting domains, and the H/D exchange dynamics themselves.

Incoherent scattering from a sample containing H and D is strongly dominated by the motions of the H nuclei, which in neutron-scattering experiments mainly reflect the motions of the macromolecular side chains to which they are bound. Inelastic neutron-scattering experiments on dedicated time-of-flight and filter-

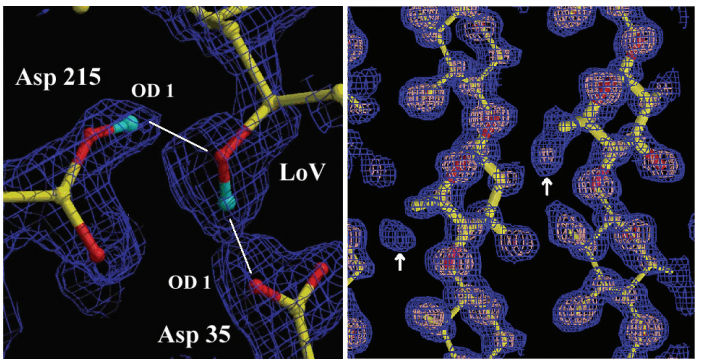

FIGURE 4.6 *Left:* Location of hydrogen or deuterium atom at the active site of endothiapepsin in a 2.1 Å resolution Laue diffractometer (LADI) structure. SOURCE: Dean Myles, Oak Ridge National Laboratory. *Right:* Hydrogen bonding interactions in cellulose from neutron fiber diffraction. SOURCE: Y. Nishiyama, P. Langan, and H. Chanzy, "Crystal structure and hydrogen-bonding system in cellulose I from synchrotron X-ray and neutron fiber diffraction," *Journal of the American Chemical Society* 124:9074-9082 (2002). Copyright 2002 American Chemical Society.

analyzer instruments provide high-quality data on the inelastic structure factor and vibrational densities of states in the energy domain from a few meV to a few hundred meV, which serve as constraints on dynamic models of atomic bonding and even their structures. The ability to systematically highlight, isolate, and probe the dynamics of specific H-labeled residues in situ within their natural environments is valuable for the study of biological and model biophysical/biotechnological applications.

At the mesoscale, neutron scattering is sensitive to the bulk hydrogen atom content and composition of materials and can be used to characterize and determine the structure of mixed and complex systems and phases. For example, the bulk neutron scattering characteristics of proteins, nucleic acids, lipids, and carbohydrates all differ significantly from one another (Figure 4.7). This natural difference in contrast can be exploited to locate individual components in functional biological assemblies such as histones or ribosomes.

Small-angle neutron scattering (SANS) experiments allow the influence of protein or chemical cofactors and ligands on both structure and dynamics to be monitored in solution and at near-physiological conditions. The packing interactions of natural or bioinspired materials with synthetic substrates are detectable at the interface with synthetic nanostructures and scaffolds. Applications at the intersection of biology and materials science include characterization of functionalized nanomaterials such as DNA-, protein-, or peptide-coated carbon nanotubes or polymeric assemblies. When such interactions occur at planar surfaces, interfaces, or layered phases, neutron reflectivity experiments can be exploited. These experiments can be extremely powerful in characterization and help to design new classes of biodevices that incorporate active biological or bioinspired agents in monitoring devices. The neutron contrast can allow marker, signaling, or receptor proteins, peptides, or nucleic acids to be discriminated from host substrates or supports that are composed of polymer or lipid matrices—information that can be difficult if not impossible to obtain from other techniques. In hybrid or functionalized materials and composites, neutron scattering allows the in situ structure and dynamics of D-labeled polymers and proteins incorporated into devices to be analyzed directly. For example, solar fuel-producing molecular photovoltaic structures are a promising future concept.

The availability of new neutron-scattering facilities will contribute greatly to these efforts. For example, the Spallation Neutron Source (SNS) at Oak Ridge National Laboratory will provide the world's most intense beam of cold neutrons for biomaterials research. The SNS will allow probing at length scales of nearly 10,000 Å and timescales of 400 ns. The order of magnitude gains in performance at such new facilities will make possible the analysis of whole new classes of macromolecular materials and processes and can be used to guide the design, synthesis, and assembly of natural and synthetic components into functional units.

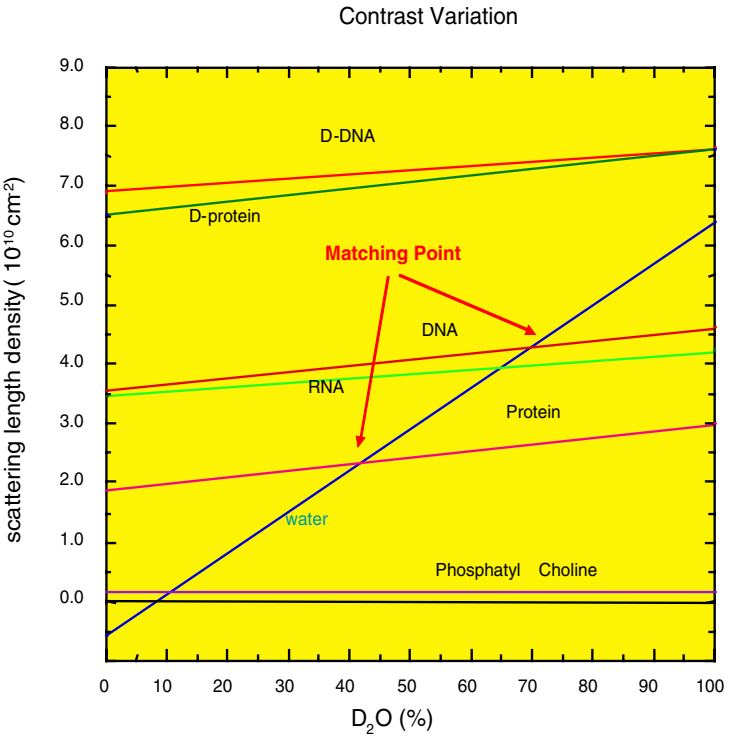

FIGURE 4.7 Contrast variation. Plot showing the neutron-scattering length density as a function of H_2O/D_2O solvent content for some common biomaterials. SOURCE: Dean Myles, Oak Ridge National Laboratory.

SINGLE-MOLECULE PROBES

A major recent advance for investigations of the macromolecules that make up biomolecular materials is the development of instrumentation and protocols to investigate physical behavior of individual molecules. So-called single-molecule biophysics reveals the mechanics, biochemistry, structural biology, and dynamics of biomolecular processes, complementing classical methods for understanding them. Some important aspects of biomolecular function are usually obscured in studies of ensembles of molecules due to the averaging of heterogeneities and dynamic variations among their individual functional units. This ambiguity is removed by detecting the reaction trajectory directly for each molecule under study. Signals from single molecules are noisy partly because the specimen is so tiny but also because the individual molecules exhibit large variations in their orientations and conformations due to thermal fluctuations and bombardment by solvent

molecules. This noisiness is a real expression of the random, stochastic nature of molecular interactions, so that the facile detection of such processes is fundamental to understanding biomolecular mechanisms, especially nanoscale phenomena.

Dramatic progress in understanding biological macromolecules, materials, and cellular function has derived from genetics, structural biology, and biochemical experiments on ensembles, solutions, and suspensions of proteins and nucleic acids and in live cells. High-resolution structures derived from X-ray crystallography and cryo-EM are revealing snapshots of particular states in the reaction pathways. These conformations must be fit into the context of the functional mechanism by measurements of the path, kinetics, and equilibrium of the enzymatic reaction sequence. Classical steady-state biochemistry, rapid reaction kinetics, and physiology provide much of this information, but important characteristics of the individual molecules that are crucial for their behavior are very difficult to detect in ensemble experiments. Individual chemical reactions, including the elementary steps of biological enzymatic pathways, are stochastic, which means that their stepwise progress is probabilistic rather than regular and clocklike. Thus virtually every dynamic characteristic fluctuates greatly. Researchers do not often detect the randomness of molecular events because observations usually average the behavior of billions of molecules. But all nanodevices, including natural biomolecules and human-made nanomachines, function in chaotic conditions due to these thermal and quantum fluctuations. Taking account of this aspect of physics at the small scale is a major challenge for designing biomolecular materials.

Some of the important characteristics of single biological macromolecules that are averaged out in ensemble observations are listed below. Each of them is directly accessed by single-molecule methods.

- *Conformational fluctuations.* The stochastic nature of individual reactions and randomness implies that each molecule follows a different temporal trajectory and possibly even a different reaction path. Surmounting an activation barrier for a reaction to proceed is a ubiquitous example. Some biomolecules harness thermal motions to gain brief access to the edges of their conformational distributions, thereby achieving a functional advantage.
- *Reversals.* Any isomerization or binding reaction that is not too far from its equilibrium undergoes many reverse reaction steps along with its forward progress (for example, most reactions in biological processes).
- *Pauses.* DNA-processing enzymes that expose and replicate the genome and the ribosome that translates the genetic code into amino acid sequences exhibit brief, sequence-dependent pauses with important regulatory consequences, such as termination or splicing.
- *Inhomogeneities.* Complex macromoleucles have individual chemical differences, such as (1) protonation, (2) redox state, and (3) post-translational chemical

modifications such as phosphorylation and methylation. Thus each molecule may display physiological variations from its nominally identical partners. These dynamic and structural variations are usually invisible in ensemble measurements.

In some specialized systems, single-molecule biophysics reveals special signals that are intrinsically difficult to obtain in bulk assays:

- *Rotational motions and wobble.* These are very common in enzyme mechanisms, but in a suspension of macromolecules the orientations of the individual molecules are random, and rapid tumbling tends to make the suspension isotropic.
- *Stepping distance.* The primary output of molecular motors and DNA-processing enzymes is progress along cytoskeletal or nucleic acid tracks. The individual motions are difficult to synchronize in an ensemble, so only the average progress is detected.
- *Mechanical forces and elasticity.* Molecular motors, DNA-processing enzymes, cytoskeletal filaments, and other subassemblies are the mechanical actuators and structural supports for the tissue. The mechanical characteristics of the cells and macroscopic tissue are a sophisticated composite of the individual molecular devices.
- *Conductivity.* Ion channels and transporters are ubiquitous membrane components that control and modulate the composition of cellular and tissue compartments. In addition, the electrical performance of these intrinsic membrane proteins produces sensory, neural, and cardiac signaling and many other receptor-effector responses. One of the earliest applications of single-molecule approaches used patch electrodes to measure the conductance and dynamics of single-ion channels. This development led to the 1991 Nobel prize in physiology or medicine.
- *Binding and folding energy landscape.* Spontaneous or assisted folding of proteins and RNA enzymes (ribozymes) are essential steps in their assembly into native, functioning macromolecules. Chemical interactions between most macromolecules and their smaller liganding partners produce the overall system response. The average behavior is the composite of the individual assembly and interaction events, which are seldom detectable except by studying one molecule at a time.

Single-Molecule Instrumentation

Some of the most prominent single-molecule techniques that have been successful for elucidating the dynamic and energetic aspects of biological macromolecules are the "optical trap," also called the "laser tweezers," single-molecule fluorescence microscopy, scanning probe microscopy, such as the atomic force microscope, and single-ion channel electrophysiological recording of membrane currents. The first two of the methods are described here as examples.

Optical Trap

Tightly focusing an optical beam by a high-numerical-aperture microscope objective forms an intense spot of light that attracts small objects having a higher refractive index than their surroundings. For instance, a 0.5- to 1-μm polystyrene bead with a refractive index *n* of 1.57 (water has an *n* of 1.33) acts like a tiny lens that deflects or scatters the rays of a tightly focused infrared laser beam. The transfer of momentum from the bead to the infrared photons when they are scattered implies a reaction force between the photons and the bead. This force causes the bead to move toward the position of highest intensity, the center of the focused spot of light. The force becomes zero when the particle is balanced in the center of the spot. Thus the tightly focused beam is termed an optical trap (or, alternatively, laser tweezers). When biological molecules are attached to the bead, the forces and stepping or folding motions that pull the bead away from the center of the trap can be measured at the functionally relevant scales of piconewtons and nanometers.

Optical traps have been used to study molecular motors, DNA-processing enzymes (Figure 4.8), the folding and unfolding of proteins, and ligand-receptor interactions. The forces and length steps of molecular motors and the enzymes that replicate and transcribe DNA, untwist it, and translate the DNA code are fundamental characteristics, necessary to understand the relationship among their enzyme activities, the energetics of their internal conformational changes, and their function in movement and progress along their biological tracks. Figure 4.8 shows the geometry and mechanical recordings from an experiment on the enzyme RNA polymerase (RNAP), the cellular macromolecular machine that transcribes DNA sequences into messenger RNA. The recordings (panels B and C) show the distance between two beads tethered together by the RNAP and a DNA molecule (panel A). This distance decreases as the enzyme translates along the DNA while synthesizing an mRNA molecule. The translocation sometimes pauses and backtracks, probably to correct errors (panel C). Detailed kinetics of these processes and the actual trajectory of the reaction waited until the technology progressed to resolve the individual events.

Total Internal Reflection Fluorescence Microscopy

Detecting the fluorescence from single organic dyes (for example, rhodamine), fluorescent peptides (for example, green fluorescent protein), or semiconductor nanocrystals (for example, quantum dots) has become a relatively routine laboratory procedure. Specific attachment of one of these probes to a biological molecule allows the tracking of its position and the measurement of its orientation and rotational motions, internal distances that change between conformations and interactions with neighboring molecules and binding partners. In order to

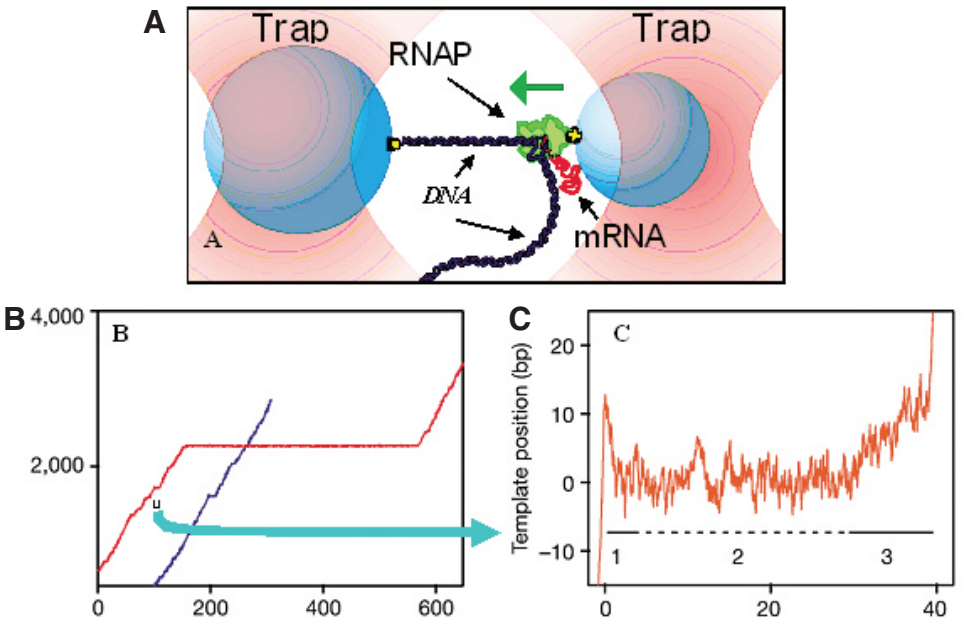

FIGURE 4.8 Single-molecule in vitro assay using dual optical traps to investigate RNA polymerase, the enzyme complex that transcribes DNA to synthesize messenger RNA. (A) Two tightly focused infrared optical beams are depicted as pink waists. The blue spheres represent small 0.5-μm and 0.7-μm polystyrene beads. During synthesis of the mRNA, RNAP (green) moves forward on the DNA (blue) as it elongates the nascent RNA (red). The smaller bead (right) is bound to a single molecule of RNAP by biotin-avidin (yellow cross), while the larger bead (left) is bound to the downstream end of the DNA by an antibody-antigen complex (yellow polygon). (B and C) Recordings of the distance between the two beads at a constant stretching force of 8 pN. At each position along the DNA template, RNAP may slide backward along the template (bracket in B), causing transcription to pause temporarily and recover. These events occur randomly or at particular DNA sequences. SOURCE: J.W. Shaevitz, E.A. Abbondanzieri, R. Landick, and S.M. Block, "Backtracking by single RNA polymerase molecules observed at near-base-pair resolution," *Nature* 426:684 (2003).

detect a single fluorescent molecule, it is electronically excited by a laser beam, and then the longer-wavelength photons that are subsequently emitted (fluorescence emission) are captured by a photon-counting detector or a sensitive video camera. The detector must be capable of registering 500-10,000 photons per second above spurious instrumental dark counts. This performance is achievable with commercial cameras, photomultipliers, and silicon avalanche photodiodes. The samples must be very clean to reduce background contamination down to the

level that fluorescence from contaminants is well below the emission of the target fluorophore.

Even with these characteristics, the sample volume that is interrogated by the instrument usually has to be limited to the region containing the fluorophores of interest to sufficiently lower fluorescence from contaminants. The excitation volume is often reduced by using the tightly focused exciting beam in a confocal microscope or the evanescent electromagnetic field that is present near a total internally reflective interface, giving total internal reflection. Figure 4.9a shows this geometry for a single-molecule total internal reflection fluorescence (TIRF) microscope. The evanescent wave extends only 100-200 nm into the aqueous sample compartment. Thus only fluorescent molecules on or very near the surface are excited for fluorescence.

The orientation of the fluorescent probe is determined by time-multiplexing the exciting input polarizations. Distances are measured by fluorescence resonance energy transfer (FRET) which is sensitive to the spacing between two fluorophores on labeled protein domains in the 30- to 70-nm range. The location of an individual molecule can obtained at 1- to 2-nm precision by fitting the distribution of emitted light to a Gaussian-shaped function, a method termed fluorescence imaging at one nanometer accuracy (FIONA). These signals have been used to determine the mechanism for stepping in several of the molecular motors, structural changes in RNA switches, and many other mechanistically important characteristics. In the example shown in Figure 4.9b, a molecular motor, myosin V, is walking along its cytoskeletal filament, actin. The alternating large and small translocation steps of a fluorophore located on one of the myosin "heads" observed in this kind of recording provide strong evidence for a hand-over-hand mechanism, as shown in the cartoon (Figure 4.9c).

THEORY AND COMPUTATION

Synergies between theory, computation, and experiment are well established in materials science and are beginning to emerge in the biological sciences. These past and recent successes, however, point to some pressing needs. Examples of challenges that need to be confronted and overcome in order for theory and computation to play an important role in the discovery and design process include the development and refinement of fundamental theoretical frameworks for describing properties far from equilibrium and in small (nanoscale) systems; the development of efficient, multiscale simulation methods that can describe the full complexity of biological and bioinspired structures and biomaterials and can be applied to the spatiotemporal evolution of many-component systems; ways to analyze information content in biomolecules; and providing the infrastructure (hardware, software, and communication) to support these activities.

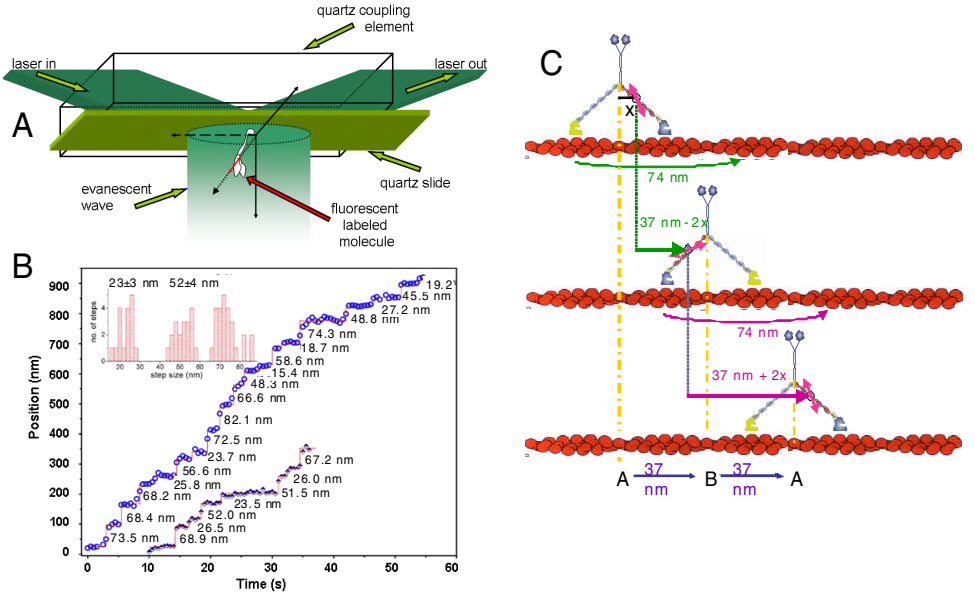

FIGURE 4.9 Single-molecule fluorescence imaging assay to monitor position at nanometer precision (FIONA). The experimental geometry is indicated schematically in (A). A laser beam is reflected from the coverslip-water interface, producing a nonpropagating, spatially decaying electromagnetic oscillation, termed an evanescent wave. This fluorescence excitation field extends into the sample compartment only 100-200 nm, thereby reducing background intensity relative to conventional epifluorescence or confocal excitation. (B) Position of a rhodamine fluorescent probe attached to a myosin V molecular motor while the motor translocates along a filament of the cytoskeletal protein, actin. The probe tilts and moves stepwise, either 74 nm at a time (blue circles) or with alternating 52-nm and 23-nm steps (dark, half-filled symbols). (C) Cartoon showing the origin of the alternating large and small steps when myosin V walks hand-over-hand along actin. The yellow and grey actin-binding motor domains swap places on each step along the filament. If the probe is located nearer the motor domain, it moves 74 nm every other step. SOURCES: Yale E. Goldman, University of Pennsylvania; and A. Yildiz, J.N. Forkey, S.A. McKinney, T. Ha, Y.E. Goldman, and P.R. Selvin, "Myosin V walks hand-over-hand: Single fluorophore imaging with 1.5-nm localization," *Science* 300:2061 (2003).

Advances in these areas and the unabated increase in processor speeds could make it possible for theory and computation to play a major role in the design of new superstrong materials with memory and recognition; cell-mimetic materials that exploit feedback and stochastic fluctuations for carrying out stimuli-responsive functions; sensors that detect hazardous molecules with unprecedented sensitivity and specificity; self-replicating materials; and systems yet to be imagined. Some specific topics worthy of consideration are outlined below.

Modeling and Computer Simulation

This section is focused primarily on classical computer simulations, but it is important to emphasize that results from such simulations usually need to be augmented by analytical approximations, quantum mechanical calculations, and phenomenological models in order to glean a proper understanding of mechanistic and dynamic principles.

Molecular Simulations

Molecules are the building blocks of all biomolecular processes and biomaterials, and so the simulation of molecular structure and molecule-specific properties is important. Typical molecular simulations are based on molecular dynamics, Monte Carlo methods, or Brownian dynamics methods. There are many examples of problems pertinent to biomolecular processes and biomaterials that could be addressed using these methods. Consider foldamers (Figure 4.10), polymers with monomer

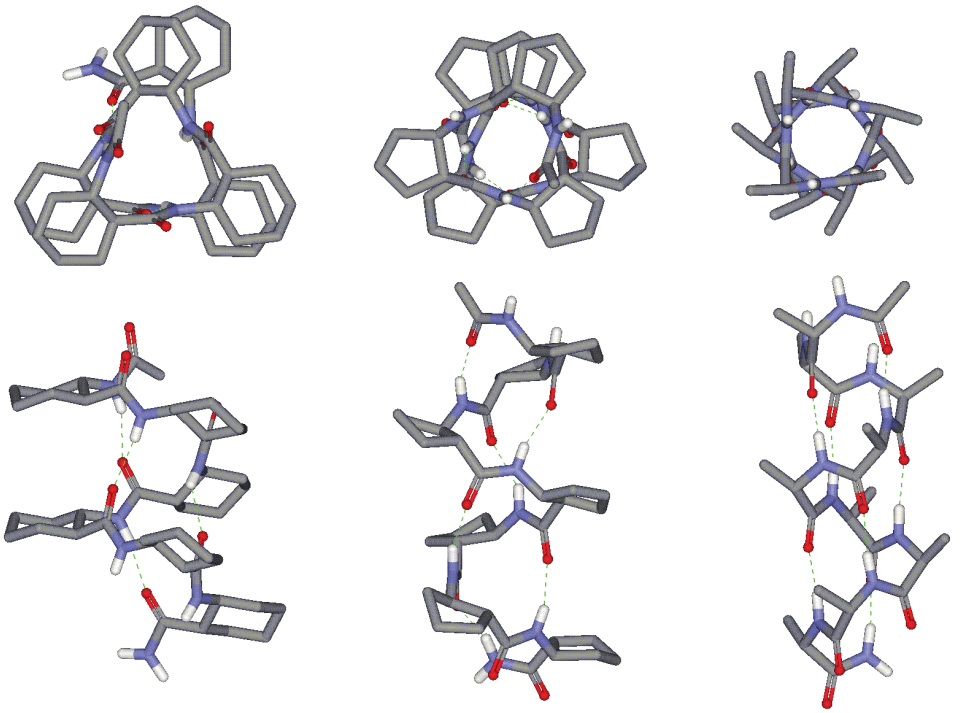

FIGURE 4.10 Examples of three beta-peptide foldamers, each of which forms stable helices in water. *Left:* 14-helix. *Middle:* 12-helix. *Right:* alpha-helix. SOURCE: Samuel H. Gellman, University of Wisconsin at Madison.

sequences that are designed so that they collapse into unique native conformations that can perform proteinlike functions such as catalysis, ion transport, and energy transduction. An outstanding question is how to determine the monomer sequence that will fold into a given target native structure. This is a computational challenge that is similar to the grand challenge of predicting protein structure from knowledge of the sequence of the peptide backbones.

There are several important bottlenecks for computations that could answer such questions. Many of the bottlenecks are the same as those identified for computational materials science, computational nanoscience, the chemical sciences, and energy research. For example, there is a need for more efficient methods for sampling large conformational spaces and for finding rare (but important) dynamical trajectories that traverse complex potential energy surfaces. More accurate atomistic force fields that include quantum effects and can treat heterogeneous materials, better models for water and polarizability, improvements in implicit representation of solvation, and faster algorithms for computing electronic transport using first-principles methods are also required.

The development of multiscale simulations that can accurately describe biomolecular assemblies beginning from their fundamental protein building blocks represents a new challenge in terms of both their underlying theoretical basis and their computational implementation. As one illustrative example, Figure 4.11 depicts the multiple scales that exist for the actin filament (the major component of the cellular cytoskeleton). These scales begin at the atomistic level with the basic actin monomer building block depicted at the right in Figure 4.11. As with most such biomolecular assemblies, the atomistic-scale is then coupled to one or more intermediate (meso-)scales, and this in turn is coupled to the near-continuum scale (in this case the cytoskeleton network). This multiscale coupling means that the

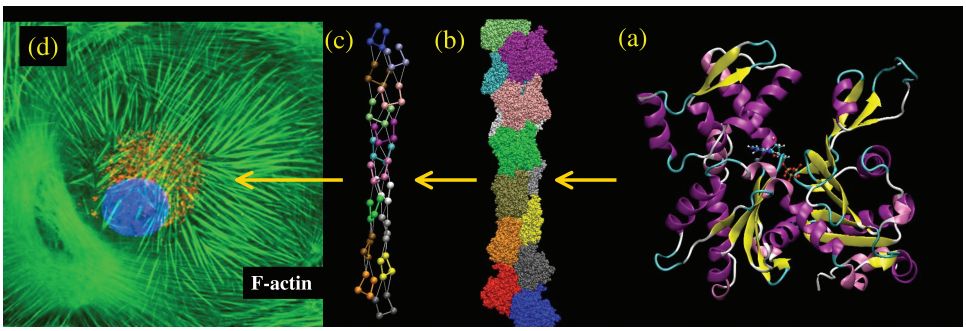

FIGURE 4.11 Example of a multiscale simulation approach to the study of an actin filament. The smallest scale is shown at right, and the model depicted becomes increasingly coarse-grained from right to left. SOURCE: Gregory A. Voth, University of Utah.

behavior at the level of the atomistic-scale (molecular) domains is ultimately crucial in determining the collective properties at larger scales. Such problems abound in biology and biomaterials. Some critical challenges in addressing such problems are developing better ways of constructing simplified mesoscale potentials starting from atomistic representations to make the computations tractable; improving multiscale methods for global optimization of structures; and developing scale-bridging algorithms incorporating Monte Carlo methods, molecular dynamics, or other simulation methods.

Modeling and Simulation of Cellular Processes

The molecular and mesoscopic models described above should interface with theoretical and computational studies focused on understanding how cells function and how these principles may be mimicked. In particular, information on biomolecular interactions emerging from such calculations and experiments are inputs to studies of dynamic phenomena in cells. Cellular functions, and that of future cell-mimetic materials, are the result of cooperative dynamic phenomena that involve a myriad of membrane-proximal and cytosolic components (see Figure 4.12).

Because experimental tools to interrogate such processes are becoming available, analogous computational studies are also emerging. Such calculations can be invaluable complements to the experiments in the quest for a mechanistic understanding of the underlying principles. Two classes of computational studies are being pursued. The most common is based on mean-field descriptions of the pertinent phenomena in terms of ordinary differential equations. Such an approach cannot account for the spatial organization of components or the effects of stochastic fluctuations, features that are often proving to be important in biological systems. It is expected that spatial organization of components and stochastic fluctuations will also play an important role in the design of the various biomimetic systems that were envisaged in preceding sections of this report. Currently, spatially resolved stochastic simulations of dynamical phenomena in cells are carried out using recently developed variants of the Gillespie algorithm (or continuous-time Monte Carlo methods). Some important advances were recently realized by bringing together such computational studies and experiments. However, these methods are extremely computation-intensive and difficult to apply as the complexity of the phenomena being studied increases. The understanding of biomolecular processes and concomitant design of biomolecular materials will be greatly aided by faster and more efficient algorithms for the simulation of spatially resolved, stochastic dynamic phenomena in cells. Another critical need is algorithms that can carry out parameter sensitivity and bifurcation analyses using such simulation tools (when the closed-form differential equations are not known). This is

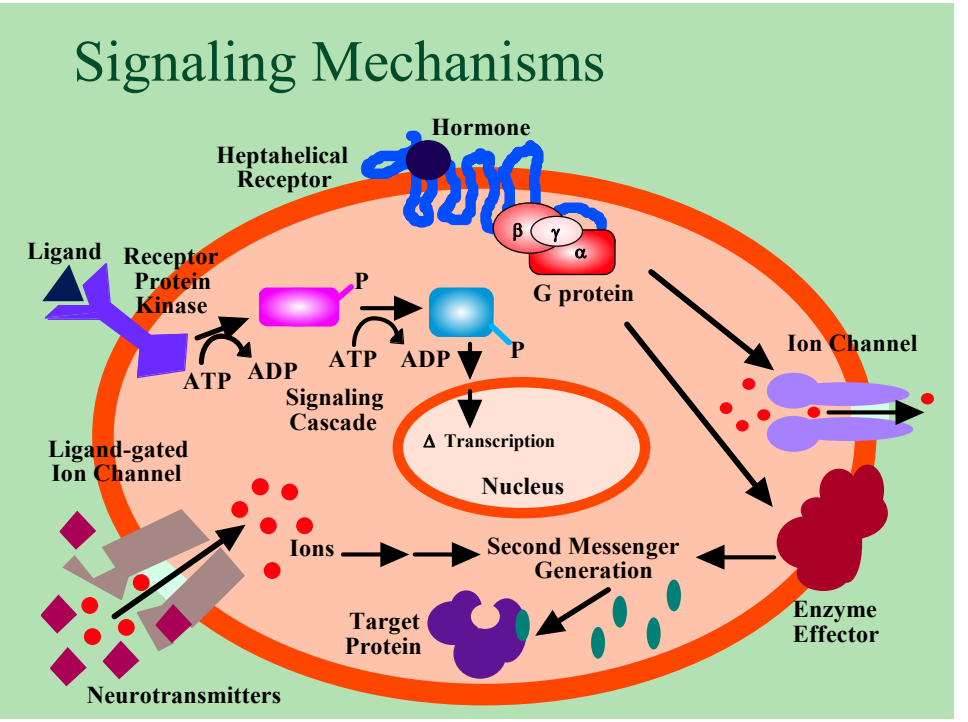

FIGURE 4.12 The complexity in a signaling system. SOURCE: Joyce B. Easter, Virginia Wesleyan College.

because in spite of advances in experimental technologies and molecular simulation methods, many molecular and mesoscopic parameters will be unknown, and it is imperative to establish the parameter range over which a mechanistic principle or design criterion is robust. It is possible that developing such methods will also impact computational investigation of phenomena that occur on larger scales (for example, collections of cells or macroscopic biomaterials with built-in nanoscale functionality).

Studies of biological systems, from gene regulatory systems to the response of cells to therapeutics, are benefiting from the computational approach of Bayesian networks—probabilistic graphical models that represent a set of variables and their causal influences. Combining this approach with models at molecular scales that provide input probabilities to the Bayesian network models is an important area for future growth.

Field-theoretic simulations have been developed to study complex pattern formation in soft matter such as polymers and other complex fluids. These simulations

typically start from a free-energy function derived from statistical mechanics that describes the thermodynamic properties of the material. These models are powerful because molecular details are included at a coarse-grained level for the prediction of structures on long length and time scales. Driven systems such as biological systems that are not described by a free-energy function pose challenges for this approach, and additional processes must be considered that include energy input and output. Such approaches sometimes map on reaction-diffusion models and are used to study the emergence of spatiotemporal patterns in complex chemical systems. Combining these two related simulation methods to describe the large-scale assembly of biological components is an important opportunity for future research. As described later, a proper theoretical understanding of nonequilibrium phenomena remains elusive.

Access to High-Performance Computing Environments

Supercomputer centers play an important role in computational research on bimolecular materials and processes. However, many computations for biomaterials design require faster swaps of information than can be provided by grid computing or require resources that are more dedicated than are available at current supercomputer centers. Thus there is often a need for large local computational resources. Various computing environments are being put to use to meet these requirements. Clusters of processors are now ubiquitous, and cost-effective ways of maintaining and operating them are emerging in various institutions (for example, centers for mid-range computing and co-location facilities). The field could be advanced if funding agencies and research institutions were to jointly develop mechanisms for providing and renewing local computational resources so that computational labs can remain at the forefront of the ever-evolving state of the art in computing environments. Another emerging and much publicized paradigm is distributed computing (for example, folding@home and SETI), which aims to harness computers all over the world to carry out specific computations. Its efficacy needs to be evaluated in the future as more data on performance become available.

The next several years will see the development of petascale computers able to perform computations at an unprecedented 10^{15} floating-point operations per second (petaflops). These fast machines will take advantage of increases in processor speed as well as sophisticated multichip architectures for the simulation of biological processes 1,000 times faster than is currently possible. This means that simulation will be able, in principle, to model molecular processes over a millisecond time scales with femtosecond resolution. However, to be able to use these ultrafast supercomputers will require the (unprecedented) development of software algorithms capable of efficiently utilizing these new architectures, and

the development of such software has yet to begin in a substantive way. Efforts in this direction must begin at once, so as to leverage the enormous investment now being made in petaflop computers.

Informatics and Data Mining

Storing, accessing, mining, and sharing large amounts (terabytes to petabytes) of information generated by simulations and experiments pose a continuing challenge for research in all fields of science and engineering. There are many examples of successful databanks in the biological sciences but far fewer in materials science, although many online resources do exist. Tools developed over the next decade, such as those based on Fedora, wikis, and the like, will revolutionize the sharing of data among researchers around the world as databanks increase in functionality and ease of access and use. The growing use of metadata tags in data files and all electronic resources should be encouraged, since metadata allow the creation of relationships among related data, which in turn allows for intelligent searching of related objects. Standards for interoperability, security, and data integrity will be required. The integration of federated databases with modeling and simulation codes will provide new opportunities for scientific discovery and prediction.

Public Domain Codes

While commercial software has an important place in scientific research, it is not always well suited to the needs of individual researchers and can limit the types of studies performed owing to its black box nature. At the same time, academic research codes developed by university research groups are not easily shared and are often used only within the group, leading to a duplication of effort. Thus the development and maintenance of public domain, open source codes are to be strongly encouraged. Interoperability and portability of codes within and across communities will necessitate the widespread adoption of standards and responsibilities.

The Need for Theoretical Advances

Infrastructure for computational research is essential because results emerging from such research complement experimental work. Computational studies allow elaborating the consequences of mechanistic hypotheses and the calculation of material properties for specific systems. While synergy between computation and experimentation can provide some insights that go beyond the specific system studied, the development of overarching principles that govern the behavior of classes of systems requires theoretical studies as well. An understanding of general

principles is crucial for enabling the a priori design of materials with desired properties. Therefore, theoretical studies should be strongly encouraged.

Theoretical studies can be divided into two broad classes. The first comprises research that employs known fundamental principles to develop a deep understanding of classes of phenomena. This type of research is often initiated by the experimental observation of a puzzling phenomenon but can turn into the formulation of new ideas and predictions that lead to further experiments. Research of several kinds will be invaluable. The understanding of collective dynamical phenomena in cells, especially how fluctuations are used and avoided, underpins many biological phenomena and should guide the design of biomimetic systems. Many interesting materials that are being studied and imagined today involve nanoscale systems. Understanding of the thermodynamics of such small systems is still not as mature as that of bulk materials. Fundamental studies in this regard will be a welcome addition to the knowledge base required for the design of biomolecular processes and materials that function on the nanoscale. Systematic and rigorous ways to bridge scales in computational studies will be important for the development of computational algorithms that could be used for design of biomaterials.

A second class of theoretical studies comprises efforts to establish fundamental new ideas that are currently not available. Such problems need not ever be driven by a specific class of phenomena but may impact understanding of a vast array of scientific and technological questions. As has been noted several times in this report, many functional materials work under conditions far from equilibrium. Developing an understanding of the theoretical principles that govern the behavior of systems at equilibrium or close to equilibrium constitutes one of the major theoretical scientific advances of the twentieth century. For example, the Renormalization Group Theory provides researchers with the concepts necessary to think about the behavior of systems near a critical point and classifies different systems into universal classes. Theoretical approaches to study the dynamics of systems close to equilibrium are also well established. Such general theoretical frameworks are not available today for systems far from equilibrium. This is largely true even for nonequilibrium steady states, although some advances in treating reaction-diffusion systems in this regard appear to be promising. Two challenges that hinder the development of such theoretical principles are the inability to a priori identify slow degrees of freedom and the lack of the equivalent of fluctuation-dissipation relations far from equilibrium. The latter difficulty implies that although noise correlations can be very important in determining properties, they can only be inferred phenomenologically or guessed. Fundamental theoretical advances that address these (and related) problems will be a very important component of the arsenal of tools for understanding biomolecular processes and for developing biomaterials with precise functional properties.

SYNTHESIS OF BIOMOLECULAR MATERIALS

Our knowledge of the reactions, transformations, and mechanisms that govern the chemical behavior and synthesis of small molecule systems is rich and diverse. Researchers have sufficient mastery over small molecule organic synthesis with stereo- and regiospecific control. Researchers have also gained the ability to fully characterize complex small molecular structures with a number of spectroscopic techniques. Chemists have successfully isolated, characterized, and fully duplicated the synthesis of many natural products that have played an extremely important role in ameliorating human health and quality of life. However, at the macromolecular, supramolecular, and nanoparticle scale a large gap exists; researchers have not gained nearly as exquisite a level of synthetic control. Translation of chemical concepts from the small molecule scale to the macromolecule scale is a difficult and often impossible task owing to the increased complexity of intricate material-based systems. Fully characterizing the sequence and conformation of such elegant and multifaceted architectures is also currently problematic, yet is of the utmost importance to accurately equating structure to function. At the most fundamental and essential level, materials researchers must acquire the ability to creatively synthesize, modify, and manipulate novel macromolecules and nanosystems with atomic-level control to broadly and accurately design and apply nanoscale materials to achieve revolutionary scientific advances.

Indeed, macromolecule synthesis and assembly have been mastered in nature. Biomolecular function arises from the sequence, structure, and conformation by both covalent bond formation and noncovalent interactions within and among macromolecules. Sequential atomic arrangement, hierarchical assembly (discussed in Chapter 2), three-dimensional conformation, and allosteric interactions play an incredible role in the performance and activity of biomolecules. For example, DNA and RNA are supramolecular polymers built from only four monomers. Yet, in addition to the monomer sequence, three-dimensional conformation is important for coding the synthesis and assembly of diverse functional proteins. Proteins, too, are created by the differential arrangement of only 22 amino acids linked together through identical amide bonds. Nature uses this simplistic monomer set to create an infinite and complex library of proteins with functions ranging from cellular signaling and transport to complex molecule synthesis, assembly, and degradation. These extraordinary features are completely dependent on molecular-level compositions and intramolecular and intermolecular interactions, which together in large numbers and over multiple dimensions determine the macromolecular and supramolecular conformation on the nanometer scale.

In contrast to the simplistic set of biological monomers that make up such complex biofunctional architectures, a large number and diversity of monomers are available to scientists to create synthetic systems. Yet, the materials, synthetic

techniques, and applications developed by scientists remain simplistic by nature's standards. Although significant progress has been made in assembling unique architectures of narrow dispersity, achieving nature's idealistic standard of synthetic control with an unlimited monomer set has remained elusive. Indeed, tremendous opportunities exist in marrying computational modeling and theory, new synthetic and biological engineering methods, and precise characterization techniques in this area. Coupling diverse yet complementary areas will certainly facilitate and allow the design of novel smart and dynamic materials with extraordinary function, far from equilibrium.

Synthetic Methods for Materials Synthesis

Extensive efforts have been devoted to modifying existing materials to perform various functions. Synthetically evolving materials for a desired application offers the most simplistic and rapid route for functional and novel material creation. This concept has been commonly applied in the field of sensors. Synthetic receptors are often formed by molecular imprinting of the most commonly utilized material, polyethylene. Ethylene and functionalized vinyl monomers are polymerized around a template and this analyte molecule can then be removed, which endows such systems with molecular recognition properties. Existing materials have also been commonly utilized and modified for drug and nucleic acid delivery. Polyethyleneimine (PEI) has been studied since the 1960s to aid biomolecule separation but is now widely studied for nucleic acid delivery. Many examples of modifying the PEI backbone exist, for example, via PEGylating (to reduce toxicity and promote serum stability) and conjugating carbohydrates for tissue targeting. Although readily available and scalable, off-the-shelf materials often suffer from polydispersity. Thus unlimited opportunities exist in developing novel synthetic methods and materials that encompass existing and newly synthesized monomers assembled by conventional and novel routes.

The advent of dendrimer synthesis has been a significant advance toward synthetic structural control. Macromolecules such as polyamidoamine (PAMAM) dendrimers can be synthesized via divergent or convergent routes (Figure 4.13), yielding completely monodisperse structures with well-defined architectural features that have been compared to artificial proteins. PAMAM is monodisperse, readily scalable, and commercially available. Thus much work has also focused on synthetically evolving this structure for a number of applications, ranging from drug delivery and synthetic vaccine development to disease diagnosis. Many elegant and diverse architectures are continuing to be designed and synthesized. For this reason, opportunities exist in this subfield to develop elegant structural architectures endowed with creative chemical functionality specifically and/or asymmetrically placed on monodisperse dendritic macromolecules.

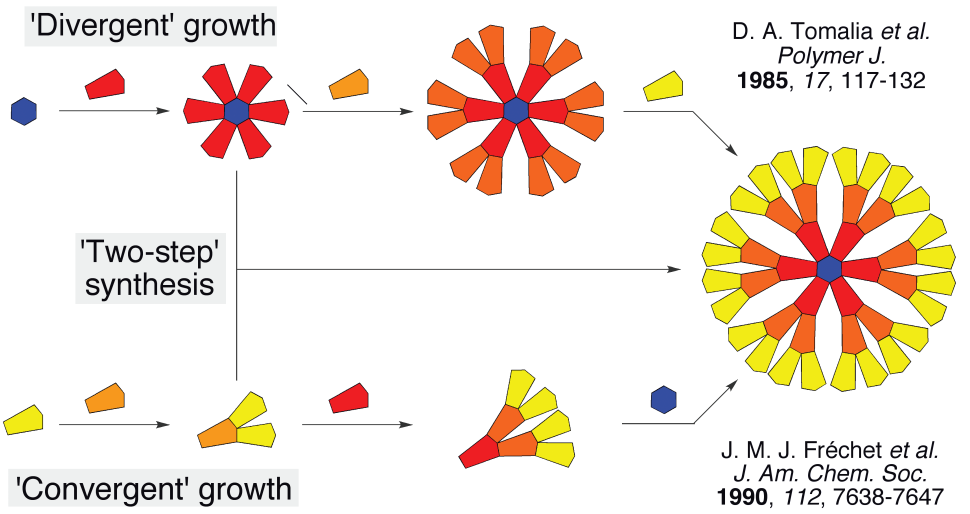

FIGURE 4.13 Methods of dendrimer synthesis via divergent (top) or convergent (bottom) means. SOURCE: Andrew Shipway. Available at http://www.ninger.com/dendrimer/.

Well-defined materials, control in dispersity and architecture, and understanding the role of sequence on macromolecular conformation and function are the paradigms for materials synthesis. A main emphasis is to develop catalysts that yield unique architectures with highly defined sequences and controlled molecular weight (low dispersity). To broadly apply these catalysts toward controlled materials synthesis, the systems must be compatible with a variety of functional groups, which can be a significant challenge. The discovery of Grubbs' catalyst and the subsequent generations of these unique ruthenium catalysts have facilitated the design and synthetic control over innumerable polymeric systems, ranging from adhesives to multivalent glycopolymers that are being used to understand cell surface receptor spacing and signaling interactions. In addition, the "click reaction," copper-catalyzed Huisgen azide-alkyne cycloaddition, is a high-yielding reaction that can be performed in aqueous conditions and has facilitated the synthesis of a rich library of functional macromolecules. Incredible opportunities exist in developing unique catalytic systems that promote coupling in a user-friendly and efficient manner and can be carried out in the presence of oxygen and water to create highly functional materials.

Materials Synthesis Using Natural Machinery

Although numerous metal-based catalysts offer some control over stereochemistry, regiochemistry, and dispersity, often the catalyst can contaminate the final material, which may cause unwanted toxicity or interfere with proper material function. In addition, these catalysts do not play a role in the structure, conformation, or supramolecular assembly of synthetic material architecture in solution, all of which have been shown to be essential for proper function and reproducibility. To this end, researchers have been inspired by the synthesis and assembly control allowed by the intrinsic biological machinery. Adapting and engineering cellular systems to manufacture, assemble, and scale up completely synthetic materials in a controlled and monodisperse fashion affords an exemplary model in synthetic materials chemistry. Researchers have shown that a number of enzymes, utilized by biological systems for degrading biomacromolecules, can catalyze polymerization reactions with nonnatural monomers. Functional materials can be formed easily and rapidly in a regio- and stereoselective manner with narrow polydispersity. Chemical control of this sort is very difficult or impossible on the macroscale using traditional chemical methods. For example, lipases, enzymes normally utilized to degrade ester bonds in lipids, have been shown to catalyze the formation of polyesters with low dispersity. Widespread opportunities exist in this field to understand and utilize existing enzymes and/or biological engineering of enzymes to facilitate specific coupling reactions and assembly of novel materials in aqueous and/or organic conditions. Evolving enzymes and biological assembly machinery such as chaperones to catalyze reactions and assemble materials in a reproducible manner would be powerful.

By hijacking and engineering the promiscuous cellular machinery, researchers have also shown that novel materials can be created by incorporating nonnatural amino acids into protein structures containing functional groups not normally found in biological systems. This allows the specific placement of reactive sites in a polypeptide such as alkynes and azides that can be utilized for specific and selective bioconjugation with, for example, the click reaction. The uses of cellular systems to synthesize these materials (as opposed to a protein synthesizer) also allows for the proper assembly and folding of polypeptides into precise protein conformations, which is essential for protein function. Extensive opportunities are available in engineering prokaryotic and eukaryotic cells not only to synthesize but also to assemble supramolecular structures. Such unique potential and promise represent the future of materials chemistry: control over synthetic sequence, dispersity, and conformation.

Materials Synthesis Using a Natural Toolbox

It has been shown that noncovalent interactions play as essential a role in function as do covalent bonds and are the ultimate model and inspiration for control and performance. By understanding the workings of the natural toolbox, researchers can model biological reactions, understand biological principles, and alter the chemistry to build unlimited functional materials. Utilizing amino acids, peptides, nucleosides, nucleotides, lipids, carbohydrates, and synthetically modified versions of these molecules, many bioinspired assemblies can be created by conventional and unique chemical routes. One approach is to utilize natural monomers because of their incredible biodiverse workings and mechanisms. Carbohydrates and polysaccharides alone store energy and promote and/or discourage cellular and biomolecule interactions, recognition, adhesion, and signaling. Simple and complex saccharide-based systems are therefore being exploited in the development of novel materials, ranging from sensors for shiga toxin to increasing specificity for targeting the delivery of various drugs. Moreover, their hydrophilic structures are highly biocompatible and can be consumed and degraded in biological systems. For this reason, carbohydrates are being utilized to build synthetic vehicles to increase intracellular delivery efficiency and lower toxicity of the drug and nucleic acid delivery process (Figure 4.14).

Numerous opportunities are available to build biomimetic structures utilizing natural tools. For example, if synthetic viruses can be built to deliver nucleic acids into cells in a specific, efficient, and nontoxic manner, a paradigm shift from small molecule to macromolecular therapeutics could occur and revolutionize modern medicine. In addition, multivalent materials have been shown to serve as inhibitors and effectors for various biochemical pathways and have promise as novel drugs and research tools. Very subtle structural changes in such biomolecular systems are

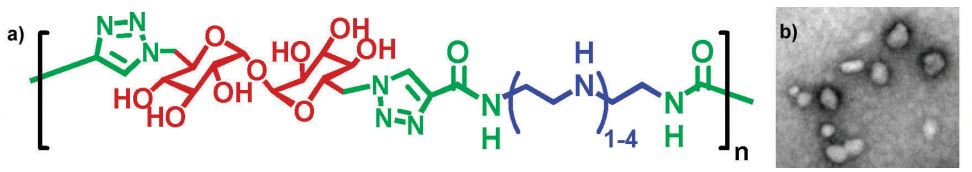

FIGURE 4.14 (a) The general structure of novel carbohydrate-based polymers (the length, *n*, can be varied between 56 and 100) that are efficient intracellular nucleic acid delivery vehicles. The synthetic structures, polymerized via click chemistry, contain a disaccharide for lowering toxicity and heterocycles, amides, and amines that facilitate self-assembly via electrostatic interactions with DNA into (b) viral mimetic nanoparticles. SOURCE: S. Srinivasachari, Y. Liu, G. Zhang, L. Prevette, and T.M. Reineke, "Trehalose click polymers inhibit nanoparticle aggregation and promote pDNA delivery in serum," *Journal of the American Chemical Society* 128:8176 (2006). Copyright 2006 American Chemical Society.

known to affect the performance and efficacy of biochemical pathways; a major challenge in this area is to elucidate the chemical structure-biological property relationships to develop advanced and bioresponsive systems. To this end, significant opportunities exist to understand advanced biomaterial structure-activity relationships (SAR) in a manner similar to traditional small-molecule medicinal chemistry to optimize biomaterial behavior.

Nucleosides and amino acids also provide researchers with a rich toolbox of monomers and macromers that can be linked by covalent or noncovalent means onto synthetic systems, providing advanced performance. Antibodies, essential for biological immunity, have been utilized extensively for sensor and bioassay development owing to their specific and selective recognition properties. Peptide sequences, such as the transactivator of transcription sequence derived from HIV, have endowed nanoparticles, drugs, and nucleic acid vectors with enhanced cellular penetration. Moreover, synthetic polymers formed with amino acid monomers (created either by conventional synthesis or by phage display) have been exploited to improve cellular uptake and delivery of drugs and promote cellular infiltration into biopolymer scaffolds for tissue engineering. Novel ligation chemistries with nucleosides and amino acids have also created completely new materials such as peptoids and peptide nucleic acids, which are not subject to enzymatic degradation in biological systems and may yield diverse applications from artificial transcription factors to antisense agents. The challenges with exploring and exploiting such materials lie in the fact that researchers are limited by the lack of biological knowledge and the unavailability of functional peptides and other biomolecules to enhance biomaterial performance. Thus many opportunities exist in understanding, creating, and modifying the tools and coupling chemistries to create unique protein and nucleic-acid-like materials and in exploration of these structures for sensor, therapeutic, and diagnostic development.

Macromolecular Assembly Routes

Intrinsically coupled with synthesis is the ability to assemble macromolecules into novel structures that control function. This ability to create accurate, specific, and reproducible three-dimensional structures is the holy grail of materials synthesis. To this end, understanding the routes and mechanisms of macromolecule assembly via noncovalent interactions is as important as developing new covalent coupling routes that link molecules together. Unique opportunities in materials synthesis are unfolding that exploit both covalent and noncovalent interactions such as hydrophobic, hydrophilic, and electrostatic interactions and metal coordination and H-bonding. For example, novel monomers can be polymerized via H-bonding and metal coordination (Figure 4.15a), and the backbone of a polymer can be functionalized via noncovalent interactions after polymerization (Figure 4.15b).

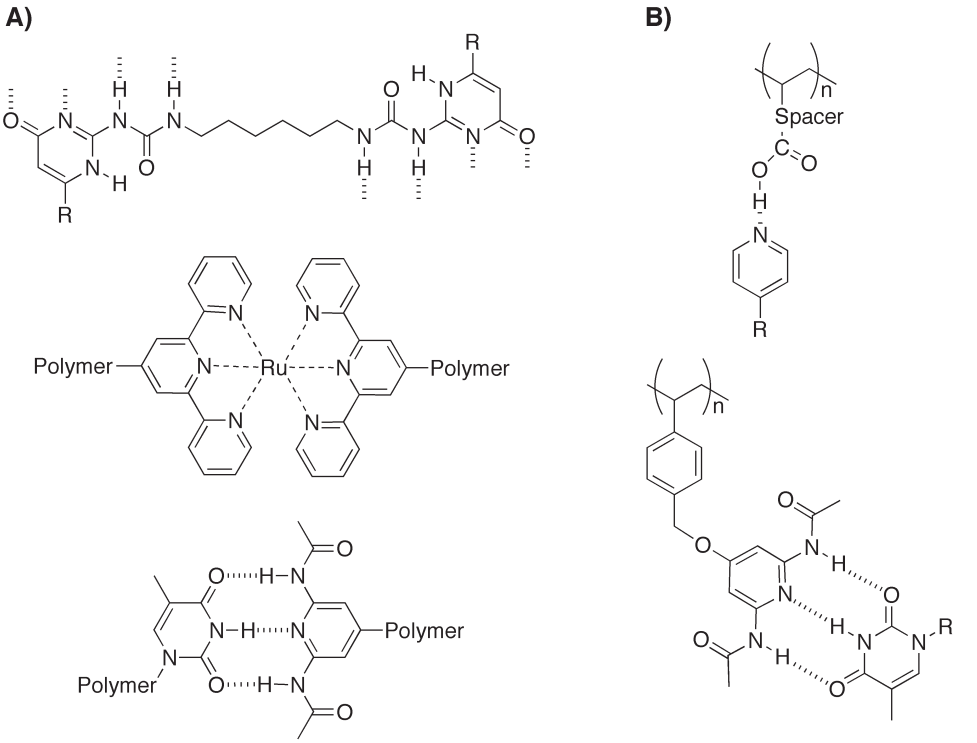

FIGURE 4.15 Routes to create supramolecular polymers via noncovalent interactions. (A) Main-chain supramolecular polymers and (B) side-chain supramolecular polymers. SOURCE: M. Weck, "Side-chain functionalized supramolecular polymers," *Polymer International* 56:453 (2007).

Many opportunities arise to form innovative materials by this plug-and-play design, whereby a library of structures can easily and rapidly be formed. Structures created via noncovalent synthetic routes also offer potential to create self-healing systems because the molecular structure can be self-corrected by atomic rearrangement due to the specificity and directionality of hydrogen or coordination bonds. Similarly, many possibilities also exist in template-directed polymerization strategies; for example, monomers could be assembled via H-bonding along a preexisting polymer backbone and then be polymerized. This strategy may allow the assembly of complex material structures in a manner similar to the transcription of DNA to RNA.

Other unique supramolecular materials can be formed by a combination of hydrophobic and hydrophilic interactions. Novel supramolecular polymers have been created by peptide amphiphiles (Figure 4.16). Such polymers are a product

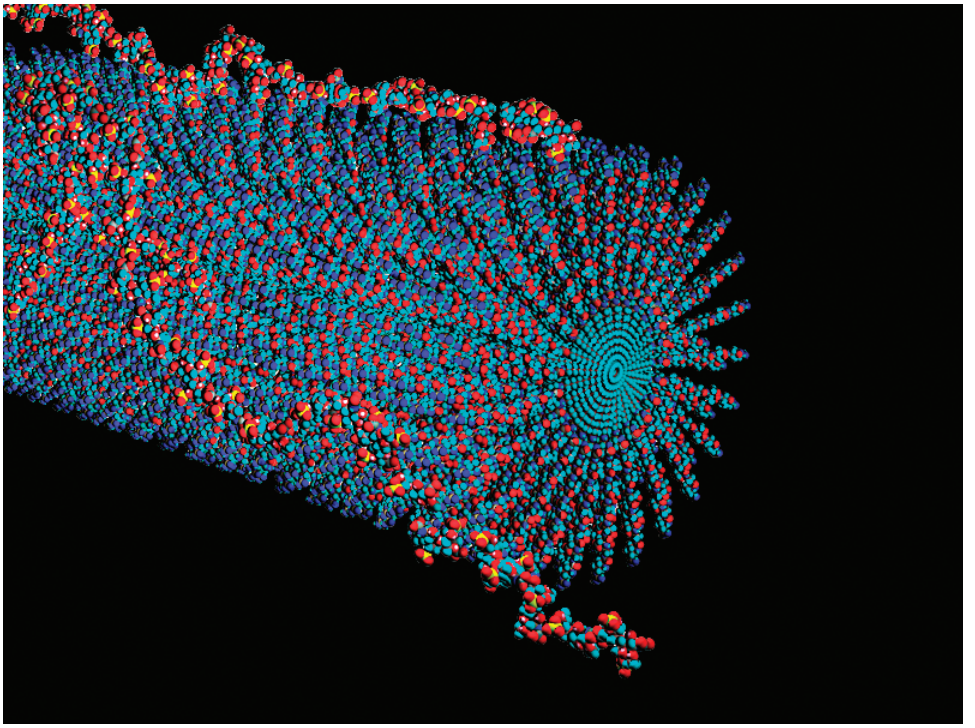

FIGURE 4.16 Peptide amphiphiles: The hydrophobic lipid tales are on the inside of the fibers and the peptides are displayed on the fiber surface. Each fiber is approximately 6 to 7.5 nm in diameter and 1 μm in length. Growth factors have been incorporated, which endows these fibrous structures with tissue regeneration properties. SOURCE: K. Rajangam, H.A. Behanna, M.J. Hui, X. Han, J.F. Hulvat, J.W. Lomasney, and S.I. Stupp, "Heparin binding nanostructures to promote growth of blood vessels," *Nano Letters* 6:2086-2090 (2006). Copyright 2006 American Chemical Society.

of the self-assembly of peptide-lipid hybrid molecules that have been shown to form micellelike fibers. Also, block copolymers have been shown to assemble into core-shell nanoparticles, polymer-based micelles with well-defined size and shape. As shown in Figure 4.17, chemical reactions can also be performed on the micelle shell (e.g., covalent crosslinking), creating a stable shell. The core may also be removed, yielding hollow nanocapsules that can house a variety of guest species such as drugs and diagnostic agents. These nanocapsules can also offer controlled release of these substances by incorporating degradable linkers in the capsule shell. Many other novel methods of monodiperse nanoparticle formation are beginning to unfold using novel molding and templating techniques. Tremendous opportunities exist in examining noncovalent polymerization and assembly methods

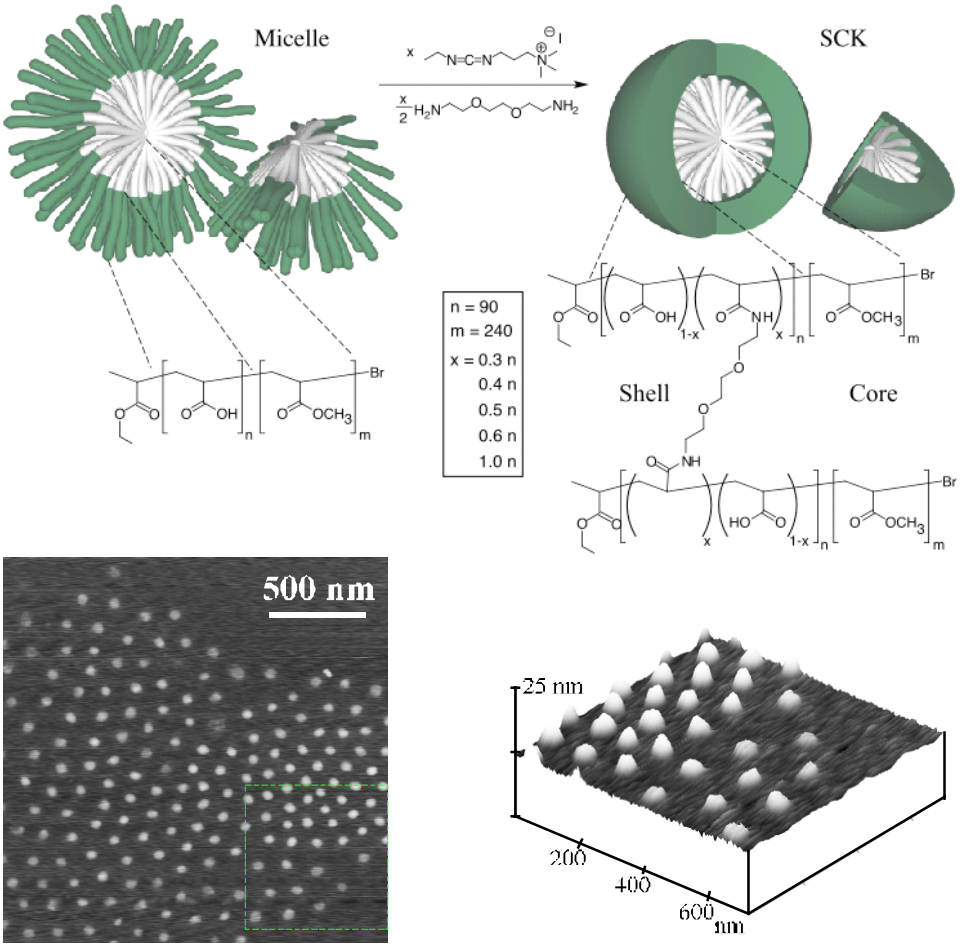

FIGURE 4.17 Polymer micelles can be formed by noncovalent interactions. *Top:* The shell can be chemically crosslinked to stabilize the nanoparticles. *Bottom:* Atomic force microscopy displays the nanoparticle shape and size. SOURCE: Karen L. Wooley, Washington University, St. Louis, Mo.

that yield biomolecular structures. Although challenges remain in understanding and controlling the creation, morphology, and reproducibility of such structures, exceptional properties will result once researchers understand and can manipulate these processes.

Lastly, the ability to pattern molecules on substrates offers another means to control the synthetic assembly of macromolecules in three-dimensional space.

Molecular patterning and lithography are a powerful means of assembling structures via both covalent and noncovalent methods on substrates. Lithography (dip pen, photo, electron beam, and so forth) has proven to be very effective at the nanoscale to design and develop a number of devices dependent on the control, placement, and conformation of macromolecules. For example, proper and accurate patterning, placement, and conformation of nucleic acids on substrates have offered powerful detection methods for applications ranging from gene sequencing to disease diagnosis. Opportunities in three-dimensional patterning of biomolecules such as growth factors in biocompatible gels and scaffolding will certainly play a role in creating novel tissue regeneration systems to promote cell signaling, angiogenesis, and tissue formation.

OPPORTUNITIES AND CHALLENGES

In this chapter, the committee discussed many emerging areas in the modeling and analysis of biomolecular materials and processes. New experimental tools are facilitating cutting-edge experiments that when closely coupled to theoretical and computational analysis, are providing new research opportunities in biomolecular materials and processes. Some of the challenges to further advancement are listed here along with some possible opportunities.

- Challenge: Achieving angstrom resolution in electron and X-ray imaging
  —Opportunity: Unprecedented elucidation, at the molecular level, of the structural and operational principles of many important cellular processes
- Challenge: Studying the structure, dynamics, and kinetics of assemblies of biomolecular systems using X-ray scattering techniques
  —Opportunity: Elucidating the dynamical processes involved in RNA genomes packing into viral capsids and probing the collective behavior of motors moving on their natural tracks
- Challenge: Probing reactions using neutron scattering at timescales of microseconds or longer
  —Opportunity: The ability to probe dynamical behavior as well as structural correlations in biomolecular materials and processes
- Challenge: Developing techniques for seeing correlations in space and time (for example, neutron correlation spectroscopy and correlated neutron imaging) rather than in q (momentum) and frequency (energy) space, as is done in neutron inelastic scattering
  —Opportunity: Allow one to look at motions on a particular length scale, such as the size of some domain of a protein, and watch the time dependence of the motion in different environments

- Challenge: Improving the temporal and spatial resolution of single-molecule techniques and integrating them into studies of larger macromolecular complexes that approach the complexity of actual cellular machines
  —Opportunity: Designing artificial biomolecular machines from insights into folding and self-assembly of complex biomolecules
- Challenge: Predicting the native conformations of macromolecules and the mechanisms and rates of conformational transitions
  —Opportunity: Designing bioinspired macromolecules that can perform specific functions or serve as building blocks for functional supramolecular structures
- Challenge: Developing rigorous multiscale algorithms that bridge the molecular and mesoscopic scales
  —Opportunity: The ability to understand and predict how formation and function of supramolecular structures depend upon the molecular building blocks
- Challenge: Developing efficient, stochastic, spatially resolved simulation methods that can study dynamical phenomena characterized by cooperation and feedback
  —Opportunity: The development of stimuli-responsive materials, like cells, that can perform precise functions
- Challenge: Developing methods to study the thermodynamics of small systems and understanding how noise can be exploited or avoided in collective dynamical phenomena to effect a desired function
  —Opportunity: The design of nanoscale biomaterials through greater understanding of their thermodynamics and stochastic fluctuations
- Challenge: Developing a rigorous theoretical understanding of how to describe systems far from equilibrium
  —Opportunity: Greater understanding of all the topics outlined in this report since many biological systems and functional biomaterials operate far from equilibrium
- Challenge: Manipulating the organization of hierarchical assemblies (the secondary, tertiary, and quaternary structures) of biomolecular materials
  —Opportunity: The ability to create macromolecular, or even cellular, synthetic biomolecular materials from molecular building blocks
- Challenge: Having the ability to fuel directed assembly by novel reactions, templating agents, or hijacking the cellular machinery, such as chaperones (proteins that can assemble complex biomolecules into discrete structures)
  —Opportunity: Unprecedented control over the synthesis of new and complex biomolecular materials

SUGGESTED READING

Als-Nielsen, J., and D. McMorrow, *Elements of Modern X-Ray Physics*, West Sussex, England: John Wiley and Sons, 2001.

Bacon, G.E., *Neutron Diffraction*, Glasgow, New York: Oxford University Press, 1975.

Grunewald, K., O. Medalia, A. Gross, A.C. Steven, and W. Baumeister, "Prospects of electron cryotomography to visualize macromolecular complexes inside cellular compartments: Implications of crowding," *Biophysical Chemistry* 100(1-3):577-591 (2003).

Hawker, C.J., and K.L. Wooley, "The convergence of synthetic organic and polymer chemistries," *Science* 309:1200 (2005).

Langer, R., and D.A. Tirrell, "Designing materials for biology and medicine," *Nature* 428:487 (2004).

NRC (National Research Council), *Controlling the Quantum World: The Science of Atoms, Molecules, and Photons*, Washington, D.C.: The National Academies Press, 2007.

NRC, *The Role of Theory in Advancing 21st Century Biology: Catalyzing Transformative Research*, Washington, D.C.: The National Academies Press, 2008.

NSF, *Cyberinfrastructure Vision for 21st Century Discovery*, Arlington, Va.: NSF, 2007. Available online at http://www.nsf.gov/od/oci/CI_Vision_March07.pdf. Last accessed March 27, 2008.

NSF, *Simulation-Based Engineering Science: Revolutionizing Engineering Science through Simulation, Report of the National Science Foundation Blue Ribbon Panel on Simulation-Based Engineering Science*, Arlington, Va.: NSF, 2006. Available online at http://www.nsf.gov/pubs/reports/sbes_final_report.pdf. Last accessed March 27, 2008.

Neuman, K.C., and S.M. Block, "Optical trapping," *Review of Scientific Instruments* 75(9):2787-2809 (2004).

Ozin, G.A., and A.C. Arsenault, *Nanochemistry, A Chemical Approach to Nanomaterials*, Cambridge, England: Royal Society of Chemistry, 2005.

Tomalia, D.A., and J.M.J. Frechet, "Discovery of dendrimers and dendritic polymers: A brief historical perspective," *Journal of Polymer Science Part A: Polymer Chemistry* 40:2719 (2002).

Zlatanova. J., and K. van Holde, "Single-molecule biology: What is it and how does it work?" *Molecular Cell* 24(3):317-329 (2006).

5

Infrastructure and Resources

The effect of concept-driven revolution is to explain old things in new ways. The effect of tool-driven revolution is to discover new things that have to be explained.

—Freeman Dyson, *Imagined Worlds*, p. 50

There is a healthy tension between concept-driven and tool-driven learning. Biology relies on the available tools to develop new concepts; physics and chemistry bring concepts for rigorous thought and measurement. Advancement in biomolecular materials and processes will require both perspectives working together so that concepts and tools are integrated in common pursuits for understanding.

Work at the intersection of disciplines challenges traditional ways of conducting research and training researchers. Institutions will have to confront these challenges as they implement structures and mechanisms for supporting research that spans disciplinary and departmental boundaries, brings the academic and industrial sectors together with national laboratories, and spans both basic and applied questions. Some of these challenges include the following: Within academic institutions, how are collaborations with industrial partners managed? How are faculty given credit for their work, and how are their contributions considered within their discipline when it comes to tenure and promotion decisions? How are faculty positions allotted to different departments and who gets credit for teaching at the intersection of disciplines? The answers to these questions are important to advancing work in biomolecular materials and processes. But because they are not specific to this field, providing a thoughtful vetting of these issues is beyond the

scope of this committee. The Committee on Science, Engineering, and Public Policy (COSEPUP) of the National Academies recently addressed what is needed to facilitate interdisciplinary research in general[1] and other committees of the National Academies continue to conduct studies on research at particular intersections.[2]

There is also a tension in the education of students, which must be deep enough in traditional physics, biology, or chemistry to give identity and a strong foundation for later learning. However, that education must also be wide enough to allow an understanding of the questions and available techniques of other disciplines and to facilitate meaningful collaboration. The appropriate balance between breadth and depth is a challenge, because the time and attention given to one topic in a syllabus or curriculum necessarily restricts the time given to another topic.

Using new tools, scientists can teach themselves to think physically or chemically while learning from biological systems. Thus facilities that serve these different modes of thinking and learning are needed. In addition, industry, academia, and national laboratories bring unique strengths to the conduct and advancement of research. These strengths should be coordinated and exploited for the advancement of biomolecular materials research. All of these elements contribute to the progression from basic discovery to the development of a practical device.

EDUCATION AND TRAINING

To realize the opportunities in biomolecular materials research, the next generation of scientists and engineers should be taught to work at the intersection of disciplines and to build productive collaborations that span disciplinary boundaries. Institutions should take advantage of institutional strengths and needs in considering undergraduate and graduate curricula and should involve all relevant parties in these discussions. For example, departments of physics, chemistry, biology, engineering, and mathematics should work cooperatively to consider and reform their programs of study. One department should not reform its own curriculum without involving colleagues from other related departments or considering the increasing interdisciplinarity of science and the interests of students. Only by involving all of the players in curriculum (and course) development can a balance be achieved between focused study and general education in the relevant scientific disciplines. Education should be (1) deep enough in traditional physics or biology or chemistry to give identity and a strong foundation for later learning and

[1]COSEPUP, *Facilitating Interdisciplinary Research,* Washington, D.C.: The National Academies Press, 2004.

[2]For example, National Research Council (NRC), *Mathematics and 21st Century Biology*, Washington, D.C.: The National Academies Press, 2005; J.C. Wooley and H.S. Lin, eds., *Catalyzing Inquiry at the Interface of Computing and Biology*, Washington, D.C.: The National Academies Press, 2005.

(2) broad enough to allow an understanding of scientific questions and techniques and meaningful collaboration.

The correct approach is probably not to simply burden students with a large number of additional classes from existing offerings, tacking materials science courses onto a current biology major, for example. Rather, it will probably require retooling existing courses or creating new ones. For example, an institution might decide to provide a broad baseline for all students but offer in-depth preparation in one or more specific areas in which the institution has particular resources. The discussions of what to include and how to fit them into current undergraduate and graduate training will help institutions consider some of the fundamental questions at the intersection of these disciplines and what best builds on institutional strengths. How to achieve the appropriate breadth and depth will be different for each setting.

A variety of approaches are being explored around the country to achieve balance and cross-disciplinary perspectives in existing structures. In one model, examples from different disciplines are described in the context of traditional courses—for instance, using examples from the biological sciences in a traditional physics course. The NRC report *Bio2010* called for integrating introductory science courses by using examples from one discipline in another.[3] An extension of this is to offer a foundation experience that brings together students—and perspectives—from different disciplines explicitly, for example a capstone course of case studies that aims to reverse engineer biological systems using the principles of physics and chemistry. Another response has been the creation of entirely new disciplines, such as systems biology or biological engineering, that bring together aspects of physics, chemistry, and biology, with an emphasis on quantitation.

The essential interdisciplinary nature of the research demands careful consideration of the education of the next generation of scientists. Exactly how to accomplish such interdisciplinary education is best left to the universities. However, the need for interdisciplinary education must be emphasized, particularly at the graduate level, where the primary training of the next generation of scientists takes place. Some additional mechanisms can be used to encourage and enhance this, drawing on successful programs that have worked in the past. For example, block training grants for graduate students are a very effective means of encouraging the collaboration among students that is the hallmark of successful interdisciplinary research. For example, the National Institutes of Health (NIH) training grants for students and the National Science Foundation (NSF) Integrative Graduate Education and Research Traineeship (IGERT) program[4] are both successful methods for

[3]NRC, *Bio2010: Transforming Undergraduate Education for Future Research Biologists*, Washington, D.C.: The National Academies Press, 2003.

[4]Available online at http://www.nsf.gov/crssprgm/igert/intro.jsp. Last accessed March 27, 2008.

training students in interdisciplinary research. An evaluation of the IGERT program showed that these students were better prepared to work in multidisciplinary teams and communicate with people outside their own fields while maintaining the level of in-depth preparation in their chosen field.[5] One possible method of enhancing cross-agency interactions is to consider jointly funded training grants. These are mechanisms that are already in place at the different agencies, so the modifications to jointly fund them should be minimal.

In addition to integrating a variety of academic disciplines in education, institutions should strongly consider the need to incorporate the perspectives of different sectors. The field of biomolecular materials and processes has a particular need to integrate basic and applied approaches and the viewpoints from industry and national laboratories, as well as academic research. For example, it may be appropriate to include industrial advisors in discussions about the most appropriate training mechanisms and educational experiences.

Beyond education for the next generation of researchers, there is a need to provide opportunities for today's scientists to be able to work—and even simply talk—together. The different cultures and languages of the various disciplines can hamper collaboration. While some of these differences have deep historical roots, it would be relatively easy to provide additional opportunities for scientists from different disciplines to meet together for extended conversations and to learn to speak the same language. One promising idea is a sort of scientific "study abroad" to educate physical scientists and engineers in the tools and concepts of biology and biologists in the tools and concepts of the physical sciences. Following models such as intensive research courses and week-long conferences, the committee recommends the development of summer courses in which scientists can work across disciplines and learn ways of communicating across disciplinary boundaries.

Part of the challenge of different language is the degree of mathematical rigor. Biology has traditionally employed mathematical approaches different from many of those used in the physical sciences. It is likely that the appropriate level of mathematical sophistication necessary for work in biomolecular materials and processes is somewhere between that customarily used in biology and that used in the physical sciences. This need for a common mathematical perspective provides an opportunity for researchers and, especially, educators to develop courses and textbooks that can give biologists opportunities to learn the appropriate mathematical techniques and for physical scientists to appreciate the difficulties of modeling complex biological phenomena.

[5]National Science Foundation (NSF), *Evaluation of the Initial Impacts of the National Science Foundation's Integrative Graduate Education and Research Traineeship Program*, Arlington, Va.: NSF, 2006. Available online at http://www.nsf.gov/pubs/2006/nsf0617/nsf0617.pdf. Last accessed March 27, 2008.

To engage students in the culture of different disciplines, they should also be provided with a diverse collection of experiences, such as through graduate research rotations. Because much research in biomolecular materials and processes is applied in nature, it is especially important that both industrial and fundamental scientific perspectives are included, both in the classroom and in research settings. As this type of translational experience becomes more common in this field, it will be necessary to determine which approaches are most successful. Most interdisciplinary programs at various institutions are very new. It is difficult to evaluate successes or failures of these educational programs at the time of publication of this report. Federal and philanthropic grants for different kinds of interdisciplinary learning, and their evaluation, are crucial and are strongly encouraged. It will also be important to ensure that interdisciplinarity does not come at the expense of depth in specific skills and knowledge. Institutions could then use these evaluations to develop a successful approach to interdisciplinary education at the local level.

MECHANISMS FOR BRIDGING BIOLOGICAL AND MATERIALS SCIENCES

Research in the field of biomolecular materials and processes is inherently interdisciplinary. Funding for this type of research can often fall through the cracks of what is supported by different funding agencies. This is particularly true for many of the more speculative research areas identified in this report, which represent much higher risk but which also represent those areas likely to yield the most significant and far-ranging advances. Moreover, because the most important component is related directly to biotechnology and medical technology, research in this field transcends traditional materials research and strongly overlaps with the medical research field. As a result, there should in principle be funding available for this area from the traditional sources of support for the physical sciences, including the NSF, the Department of Energy (DOE), and the Department of Defense (DOD), as well as from the sources of support for the life sciences, primarily the NIH.

While there should be many sources of support for research in biomolecular materials, there is, in fact, an inherent problem in obtaining funding for such an interdisciplinary research field. In part, this is a result of the dichotomy in the underlying philosophies of the funding agencies that should fund this type of research. Work in the life sciences is typically supported by the NIH, where grantees are expected to propose relatively low-risk steps forward, with substantial proof of principle already obtained before a proposal is submitted. By contrast, work in the physical sciences is typically supported by NSF, DOE and, to some extent, DOD, where grantees are expected to take larger risks and to propose work with a much riskier vision. However, these agencies shy away from support of anything that may have direct medical applications, as this is viewed to be the realm of the NIH. Some of work identified in this report falls naturally into one or the other of these

basic funding models. However, there is also a great deal of crossover work, which transcends the individual agencies and the separate funding philosophies. Indeed, it is this work that might have the greatest potential for having a truly significant impact on both science and society.

This leads to significant opportunities being missed because the funding for them falls between the agencies. For example, there are many opportunities for research in materials and physical sciences to have significant impact on disease, biomedicine, or drug discovery, but these opportunities are not funded by NSF or DOE because of their overlap with the mission of the NIH. Similarly, some of the very physical and quantitative problems in biomolecular materials and processes, which intrinsically rely on the knowledge of the biological processes developed within the medical community, are not funded by NIH because the impact may not be sufficiently clearly related to the medical problems that are its purview.

There is clearly room for some funding of the research discussed here within the present boundaries of the funding agencies, and the committee strongly supports those funding mechanisms that currently exist. The committee also strongly supports any additional funding that is made available within the current funding constraints of the agencies; this is the surest way to seed new developments in the field. Although federal research support has been especially constrained in recent years, the time seems ripe for new investment. For example, the America Creating Opportunities to Meaningfully Promote Excellence in Technology, Education, and Science (COMPETES) Act (Public Law No. 110-69), passed in 2007, authorizes a doubling of the NSF budget over 7 years and a general desire to increase support for research agencies supporting the physical sciences.[6] If appropriations follow authorization, new resources might be made available for research in biomolecular materials and processes.

However, even with the current and potential sources of funding, the committee feels that the inherent interdisciplinary character of the work also requires a change in funding policies to cater to this class of research. For example, the composition of review panels and study sections may need to be altered to be sure that interdisciplinary proposals receive a fair review by true peers instead of a review structure tilted to a single discipline. The committee also feels that there should be a change in the attitudes of scientists who do get funded by any of these agencies to appreciate the nature of high-risk interdisciplinary research. Thus researchers in the physical sciences should recognize that materials research can play an incredibly important role in the life sciences even if it is not strictly funded by the NIH. Similarly, researchers typically supported by the NIH must recognize the impact that materials research can have on the medical field and must be more prepared

[6]A fact sheet on the America COMPETES Act is available at http://www.whitehouse.gov/news/releases/2007/08/20070809-6.html. Last accessed March 27, 2008.

to accommodate the more speculative nature of proposals in this area. The ongoing discussions at many agencies on how to foster additional investment in such risky research might be of help in this regard. NIH, for example, has established the NIH Director's Pioneer Award and the NIH Director's New Innovator Award to encourage creative, outside-the-box thinking and focuses on the promise of the individual rather than of a single detailed research proposal.[7] In addition, four of the NIH institutes have recently requested applications for Exceptional, Unconventional Research Enable Knowledge Acceleration (EUREKA),[8] a new program to foster exceptionally innovative research expected to have a high impact; programs such as these might help to foster not only transformative research within a discipline but also additional opportunities for groundbreaking work at the intersection of existing scientific disciplines.

To encourage this change in attitude, the committee recommends that workshops be held within both the life sciences and the physical sciences communities as well as jointly. The goal of these workshops should be to encourage the interdisciplinary research required for high-impact work and to educate the communities about both the opportunities and the research requirements. An important goal of these workshops would be to broaden the base of each class of research by exposing each community to the potential of interdisciplinary research. Thus, for example, in the physical sciences it is important to have a broader acceptance of the close interplay between the physical sciences and the medical sciences and to recognize the potential applications and outlet of the research in the biomedical fields. Similarly, in the life sciences, it is important to recognize the great potential that an understanding of biological processes can have on materials science and how exploiting this knowledge can also lead to great improvements in many areas of biotechnology. In addition, these workshops can help program managers gauge interest in the scientific community and help demonstrate the great potential of the field, especially as it impacts their own specific areas.

SHARED RESOURCES AND ESSENTIAL FACILITIES

Biomolecular materials and processes have been fortunate to be among the research areas included in a variety of interdisciplinary programs and centers. Perhaps most prominent are the NSF-funded Materials Research Science and Engineering Centers (MRSECs) that support interdisciplinary and multidisciplinary research and education addressing fundamental problems in science and engineering. Biomolecular and biomimetic materials are studied in several MRSECs.[9]

[7] http://nihroadmap.nih.gov/highrisk/.

[8] http://grants1.nih.gov/grants/guide/rfa-files/RFA-GM-08-002.html.

[9] See http://www.mrsec.org/research/biomolecular_biomimetic_materials/ for a list of research groups currently working in these areas.

MRSECs provide one model for structuring and supporting research in biomolecular materials. The National Academies' Board on Physics and Astronomy recently completed an assessment of the MRSEC program and recommended future directions and roles for the program.[10] It might prove valuable to carry out similar comprehensive reviews of other research centers conducting related research to learn which programs have been successful and why.

As described in Chapter 4, many of the advances in understanding the behavior and properties of biomaterials have come from the increasingly sophisticated experimental tools developed in recent years. Continued progress in solving the challenges of biomolecular materials research, highlighted in Chapters 2 and 3, will also depend on new tools and techniques. Indeed, state-of-the-art research will likely be restricted to those scientists who have ready access to sophisticated equipment.

Increasing sophistication inevitably increases the costs of acquisition and maintenance. Not all of these new developments require national-level shared facilities, such as a synchrotron or neutron source. Some of the most powerful new technologies (for example, a cryo-EM microscope and ancillary support) are feasible acquisitions for research universities or individual investigators (for example, single-molecule microscopic instrumentation). However, at each of these instrumentation scales, costs can rapidly become the main constraint on research.

As costs increase, federal funding agencies are less able to provide the bulk of the funding for purchase of experimental equipment. Moreover, funding agencies have rarely provided sufficient infrastructure support to operate and maintain equipment. This limited support holds particularly for highly skilled technical staff, a condition that is likely to impair ongoing use and development of the needed equipment.

The constraints on current federal funding might be loosened, at least in part, by new funding models. In particular, the wealthier American universities are beginning to put more of their own resources into shared on-campus research facilities. This is a funding model that is more common in some European countries, particularly Germany. For the United States, it is a new paradigm. While direct university support is of great value to the total national research establishment, relying on such local efforts might increase disparities in research quality between universities with larger endowments or clinical revenues and less well-off or state institutions.

The next level of single-university, shared facilities that impact biomolecular materials research are the cryo-electron microscopy, micro- and nanofabrication, and molecular expression and modification facilities. Core facilities for these ser-

[10]NRC, *The National Science Foundation's Materials Research Science and Engineering Center Program: Looking Back, Moving Forward*, Washington, D.C.: The National Academies Press, 2007.

vices exist in many locations, although modernization and enhancement with new tools is difficult. Both NSF and NIH have major shared-instrument funding programs for acquiring such cores, but demand for the funding of these is far outpacing the amount of money available, as more and more universities try to upgrade their research infrastructure with such equipment. Furthermore, maintenance of them as service operations after expiry of the seed grant is sometimes uncertain. Individual faculty start-up funds are often used to supplement such shared facilities, whereas a program for continued support of effective facilities would provide more continuity. Unfortunately, however, the pace of advance in instrumentation is such that equipment must be upgraded or replaced every 5 to 10 years.

Individual faculty commonly obtain high-end commercial equipment or build special-purpose instruments when they begin appointments or move between institutions. Securing funds for mid-level or senior investigators to obtain new instruments or enhance or upgrade machines is notoriously difficult. Funding overhead models also discourage applying for high proportions of equipment on renewal applications. Instrumentation funding for smaller purchases would allow productive investigators to leverage their investment in instrumentation and maintain state-of-the-art methodologies.

The cyclic nature of research funding availability forces agencies to adjust priorities to maintain both targeted programmatic and investigator-initiated research at appropriate levels. The committee encourages the funding agency representatives to realize the importance of methods and technology development for continued progress in advanced materials research and to gain the requisite biological and biophysical knowledge to utilize the striking features of biological systems for producing the new materials envisioned in this report.

Major infusions of funding have resulted in the commissioning of facilities such as the Spallation Neutron Source (SNS) at Oak Ridge National Laboratory and the BioCAT beam line at Argonne National Laboratory. Until the SNS comes on line, there is agreement that high-quality neutron radiation is sparse. For low-angle X-ray scattering, small-angle X-ray scattering, and imaging, experiments requiring the brightest and most collimated beams have few options. These facilities have an ongoing need to improve cameras, detectors, computational facilities, and maintenance by staff. Another very successful method of introducing students to the field is through intense summer workshops. Examples of these in other fields include the workshop on cell biology and physiology held each summer at Wood's Hole and the summer school in condensed matter physics held in Boulder. These programs are highly successful and the committee strongly recommends maintaining them.

PARTNERSHIP AMONG INDUSTRY, ACADEMIA, AND THE NATIONAL LABORATORIES

Translation of biomolecular material discoveries into useful applications has motivated an increasing number of industrial partnerships. While these partnerships are often nurtured by different sources (government, industry, academia), they all aim to improve the efficiency of knowledge transfer from discovery to development and to increase returns on investment.

Such returns can be defined in many ways, some more quantitative than others, but all entail increased involvement, communication, and contact between academic and industrial investigators and management. The expected products of these interactions include shared authorships on research papers, new intellectual property generation, as well as undergraduate and graduate training for new jobs in industry. While all of these outcomes are important, two—the generation of intellectual property and the translation of this property into licenses and product development within industry—are especially so.

A very productive partnership between national laboratories, academia, and industry has been provided by the sharing of large, capital-intensive resources that are housed and maintained at the national laboratories. Good examples of this are neutron beam lines, synchrotrons, and supercomputer centers. The continued development and sustenance of such facilities centered around large instruments will remain key to research and development in biomolecular materials and processes.

Another model for developing such partnerships that has recently been introduced is exemplified by the Molecular Foundry at Lawrence Berkeley National Laboratory (LBNL). Such shared user facilities are not built around one large instrument but rather include various experimental and computational platforms that are necessary to carry out research in a particular thematic area. For example, the synthesis, characterization, and computational user facilities required to create biomolecular nanostructures are grouped together. This model has not been in existence for a long enough time for the committee to have an informed opinion about its impact on the discovery process. Such an evaluation should be carried out in the near future.

How then to measure the return on investments for these partnerships? Certain measures, such as the number of peer-reviewed manuscripts or patents, are quantifiable. But these are not the only outcomes. It is difficult to measure the success of new research, of discoveries that have industrial value but that may not be realized for some time after the investment, or of the intellectual property and the commercial products that may derive from such long-term efforts. How does one measure the contribution of partnerships to the training of people who move between industry and academia? This is difficult to measure but is a very important

factor in the success of an industrial partnership. These partnerships continue to generate fundamental research across different sectors and remain a vital opportunity for biomolecular material research and development.

COMMERCIALIZATION OF BIOMOLECULAR MATERIALS

The application of useful biomolecular materials has long had a powerful impact on commercial product development. For example, in the food industry, bulking agents such as algae polymer (for example, carageenan) from marine sources are used in everyday foods such as ice cream. Bovine or porcine collagen is used as a structural material in medical devices. In these examples, the low cost of production compared to that of alternative, less functional synthetics, plus the abundance of the materials in nature, adds further to the advantages of biomolecular materials.

Biomolecular Properties, Processes, and Products

For the commercialization of biomolecular materials, it is useful to define biomolecular material properties used in the development of products and to relate the properties to particular industries (both current and future) that derive useful products from these characteristics (Table 5.1).

New tools have been developed around these applications to create useful products. They include the ability to process biomolecular materials such as through microfluid transport, assembly tools for two-dimensional and three-dimensional fabrication, and high-throughput mutagenesis or synthesis. Some of these tools have already been commercialized and play a major role in the translation of biomolecular material science and technology into product development. Examples are show in Table 5.2.

TABLE 5.1 Unique Properties of Biomolecular Materials Drive a Number of Important Applications and Products

Property	Product
Molecular recognition and binding	Sensors, medical diagnostics, drugs, and therapeutics
Mechanical and structural strength	Bulking agents in foods, medical devices, and biomaterials
High information content	Sensors, diagnostics, implants, storage devices
High energy content	Biobatteries, biofuels

TABLE 5.2 New Tools That Aid in the Development of New Products

Process	Tool
Transport of biomolecular materials	Microfluidics
Assembly of biomolecular materials	Lithography, stamping, polymerization, writing and capture tools
Biomaterial selection	Combinatorial methods, directed evolution

Manufacturability and Production

Some of the tools developed around the science and technology of biomolecular materials help to translate fundamental research into commercial development of the materials. One example is the ability to efficiently transport liquids (microliters to nanoliters) in high throughput for separating cells or biomolecular species. These devices permit the use of biomolecular species in sensor or diagnostic applications and are good examples of tools that have contributed significantly to commercialization efforts.

To make products based on biomolecular materials, large-scale manufacturing is required. In many cases—such as growing antibodies, producing recombinant proteins or other species, and bioprocesses for cell "expansion" (production in bulk)—large-scale production uses methods from biological sources while maintaining consistency of production during scale-up. For example, antibodies often change their activity based on production and process methods, large-scale protein production is hampered by inability to control protein folding and aggregation, and a bioorganic chemical industry is developing in order to make proteins, but cell phenotypes can be altered or lost during increased passage. An instance of the last mishap occurred during the effort to grow blood cells. While the biochemical triggers to derive useful blood cell lineages (red blood cells, platelets) have been identified, it is still necessary to engineer useful cell expansion methods that could efficiently yield a unit of therapeutic blood cells. The ability to grow a unit of blood efficiently and economically would qualitatively improve the practice of blood transfusion.

Specific Biomolecular Material Product Areas

Following on fundamental discoveries, biomolecular materials are candidates for new products such as sensors, diagnostics, prosthetics, fuels, and computers. In this section, current and future product areas are described that employ biomolecular materials.

Sensors and Diagnostics

The most mature application based on biomolecular materials is products that rely on the recognition and binding properties of biomolecular materials in sensor and diagnostic applications. These applications have created a large industry based on the practice of detection in the environment and in a clinical sample for predicting medical outcomes. Sensor and diagnostic products include devices that use antibodies, peptides, receptors and their antagonists, ribozymes, nucleic acids and biological cells to specifically and sensitively detect and report events as a result of molecular recognition and binding events. For well-established products that employ components such as antibodies, the development of useful diagnostics using biomolecular materials is a low-cost, high-volume technology that can be fabricated into easy to use kits, such as enzyme-linked immunosorbent assay (ELISA) for a number of diagnostic applications.

Medical diagnostics is a global industry practice with substantial contributions to human and animal health. This multibillion dollar global industry has moved into health screening, prognostics, and companion therapeutics, which consistently drive new products and revenues. These applications are an important example of the value of biomolecular science and technology and how it feeds commercialization efforts with significant societal impact.

The required biomolecular property specifications for sensor or diagnostic products will ultimately be set by market-required performance and regulatory and reimbursement practice. The current trend toward combining a diagnostic test with a therapeutic regimen to afford better individualized and more economically efficient medicine practice should facilitate the exploration of biomolecular material science and product development.

New sensor applications that employ biomolecular materials have also recently been motivated by the increased awareness and need in public health application driven by new or perceived threats in biodefense. In many cases, components or reagents (for example, antibodies) are being used in new biodefense applications. The performance specifications for biodefense can be more demanding, where the speed of response and its predictive value in the context of public health risk assessments are paramount. These assets also have implications for national security, public policy, and homeland defense.

Commercial practices such as DNA sequencing or genotyping are emerging rapidly. New service-based companies are expanding interest and growth in DNA microarray analysis. The tools to more accurately amplify and define nucleic acid sequence content of a sample have become much more robust, and there is substantial industrial activity in areas using PCR and genotyping. There have been substantial investments in high-throughput, cell-based diagnostics and in driving the information content from cells using microscopic and biochemical cell tests.

This has been motivated by the interest on the part of pharmaceutical companies in identifying targets and leads from combinatorial chemical libraries of potential drugs. High-throughput G-coupled protein cell assays to look for receptor binding are also common practice, based on the biomolecular assembled properties of the G protein system important in many cellular interactions. Other cell-based tests have utilized green fluorescent protein or luminescence to drive cell-specific information of interest. One test employs the use of B cells engineered with aquoerin (jellyfish protein) to create a commercial cell-based test for food contamination with *E. coli.*

Medical Devices

Biomolecular materials in the medical device industry have already achieved considerable penetration into the market. This includes the use of biomolecular polymers in a number of medical devices, including bandages, drug delivery vehicles, stents, orthopedics, and dentistry. These products capture structural properties of these materials (for example, collagen, ceramics, bioglass, dental polymers, and hydrogels), release properties (for example, hydrogels and coatings) and delivery of specific agents (for example, liposomes and imaging agents). They rely on careful identification of properties such as molecular interactions (collagen with growth factors or coagulation proteins), porosity and permeability, and surface modification for specific targeting of drugs and therapeutics.

Therapeutics

Biomolecules and their assemblies have also provided useful therapeutics for application in medicine. The use of assembled phospholipids in liposomes and other lipid emulsions as drug delivery vehicles has been widely practiced in the delivery of toxic agents (for example, antifungals) and antibiotics for extended release and targeting to specific sites in the body. Biomolecules as contrast imaging agents for PET or fMRI applications have also been very useful for in vivo medical diagnostics, and their use continues to grow rapidly. Other biomolecular properties exploited in useful therapeutic applications include adhesives such as thrombin (surgical glues) and absorbable biocompatible polymers (sutures made of polylactides and polyglycolides). These are mature examples of the utility of biomolecular material products and generate revenues of millions of dollars in commercial markets.

Challenges and Opportunities in Commercialization

Biomolecular science and technology enjoy wide application and generate correspondingly broad commercial interest. This interest is due to the unique

properties of the biomolecular materials described in this report, such as biomolecular recognition and communication, structural and dynamic strength, and high information and energy content. To maximize the commercial potential of these properties, the specific products that demonstrate these properties must be carefully assessed. Advancing a technology to product development often requires an understanding of the mechanisms of action and interactions in complex systems as well as the product's manufacturability. If they can meet a specific market demand, these systems will be commercialized.

Specific challenges for future commercialization efforts include translation to large-scale production and manufacturing (described earlier), increasing the long-term stability of products with biomolecular materials, and integrating the materials into devices and products (see Chapter 3). While long-term stability is not a problem for some materials, some biomolecules and their assemblies cannot be preserved easily or for long periods. The stability of other biomolecular materials (such as antibodies) can be lost through production methods. Further research and development in these three challenge areas are needed to fully realize the potential of biomolecular materials in commercial markets.

Public/private partnerships are one route to nurture commercialization efforts. The use of these enterprise zones to seed such efforts is warranted and should be encouraged. Careful attention to specific issues such as intellectual property and ways to increase its sharing and transfer should also be encouraged and used to evaluate the commercialization outcomes of these investments.

6

Conclusions and Recommendations

Over a decade ago, the National Research Council published an optimistic report assessing the field of biomolecular materials.[1] This committee's survey of biomolecular materials and processes has led to the conclusion that the situation today is qualitatively different from that which existed a decade or even 5 years ago. It is an especially exciting time at the intersection of the physical, materials, and biological sciences. New ways to measure, manipulate, and compute properties of biological systems are making it possible to learn the principles that govern their function. When coupled with the advances that occurred in hard and soft condensed matter research, this knowledge of principles offers the real possibility of creating materials that can perform diverse functions with biomimetic precision. These materials, in turn, will impact technologies that will further our nation's progress in areas such as energy independence, therapeutics and diagnostic tools, and devices for sensing biological and chemical threats. By pursuing the research outlined in this report, scientists are expected to gain a dramatically better understanding of basic principles underlying the complex emergent behavior of biological systems.

It will be difficult to realize this promise, however, if steps are not taken to evolve the infrastructure and organizations that support research and education in this field. It is the committee's view that the following issues demand immediate attention.

[1]NRC, *Biomolecular Self-Assembling Materials: Scientific and Technological Frontiers*, Washington, D.C.: National Academy Press, 1996.

SUPPORTING INTERDISCIPLINARY RESEARCH

Fundamental new insights into how biological systems function and how bioinspired materials and processes can be created require contributions from different disciplines. Such interdisciplinary research efforts are growing organically in the scientific community at a fast pace and will undoubtedly lead to important advances. Several Nobel prizes (many to European scientists) have been awarded for work at the crossroads of these disciplines. However, the U.S. research community has not yet developed a culture that adequately supports interdisciplinary science.

Recommendation 1: The Department of Energy (DOE), the National Institutes of Health (NIH), the National Science Foundation (NSF), and other relevant departments and agencies should jointly sponsor programs of innovative research at the intersection of different disciplines. Initiatives of this type will provide incentives for universities to work across traditional departmental boundaries. The Office of Science and Technology Policy (OSTP) should take the lead in coordinating such programs.

Currently, no federal agency has ownership of research at the intersection of disciplines. For example, NSF and DOE do not support research that impacts mitigation of disease, which is viewed as the purview of the NIH. At the same time, the NIH often looks somewhat warily at research that includes a strong component rooted in the physical sciences. This situation makes it difficult to advance some of the most promising research efforts at the crossroads of disciplines. Some important efforts have been made by individual agencies (for example, the NIH Roadmap Initiative[2]), but these efforts are necessarily small because resources for the fields traditionally supported by a particular agency are shrinking. A comprehensive plan that involves the main federal agencies and avoids budgetary duplication is required. The committee recommends that the Office of Science and Technology Policy (OSTP) take the lead here, because it can work with the Office of Management and Budget (OMB) and the federal funding agencies to maximize taxpayer investment in research at the crossroads of disciplines—a type of research that the committee believes is critically important.

[2]More information on the NIH Roadmap Initiative is available at http://nihroadmap.nih.gov/. Last accessed March 27, 2008.

DEVELOPING AND EVALUATING PROGRAMS FOR INTERDISCIPLINARY EDUCATION

The U.S. higher education system has been dominant in the world for over eight decades. An important reason is that education and research are inextricably intertwined at U.S. universities. Interdisciplinary research should be accompanied now by the development of educational programs that train engineers and scientists who are easily able to cross disciplinary boundaries. Such programs are important because success in fundamental interdisciplinary research and its translation into commercial products will not be possible without such a pool of scientists and engineers. A knowledge-based economy will be important for the future in the United States, and interdisciplinary education will be one of the pillars supporting this enterprise.

Recommendation 2: University physics, chemistry, biology, materials science, mathematics, and engineering departments and medical schools should jointly examine their curricula, identifying ways to prepare scientists and engineers for research at the intersection of the physical sciences, engineering, and the life sciences. The educational programs being created should be evaluated from a wide range of viewpoints, including input from leaders in industry and at the national laboratories.

Efforts to educate students on topics in multiple disciplines are currently under way, and they are based on disparate philosophies. One extreme is to have a discipline-free education that exposes a student to a wide variety of subjects that are of current societal relevance and of interest to that student. This approach could produce graduates who have no in-depth knowledge of any particular area of science or engineering. Such a deficiency could be problematic since knowing how to learn a topic in detail will allow us to one day learn another topic in depth. At the other extreme is an in-depth education in a traditional discipline, but with an emphasis on exposure to other fields of inquiry. For example, some universities are experimenting with requiring a secondary major. This necessarily means fewer courses in the primary major, which impedes the design of a curriculum that provides both depth in one field and adequate exposure in others. Another recent development is the emergence of educational units (for example, biological engineering departments) that aim to bring together parts of other disciplines. Yet other programs are developing courses based on case studies. Issues related to the balance between breadth and depth of knowledge acquired by students are pertinent for these models as well.

It is too early to assess the strengths and weaknesses of these different models, but planning for such assessments should be initiated soon. An important quality

of the evaluation process is that it should be continual, and an important component would be direct input from outside the universities. Industry input is crucial because that sector plays such an integral role in this field, and the need to prepare graduates who can step into industrial jobs is so critical. Developing and evaluating interdisciplinary education models is essential for achieving the leadership goals of the America Creating Opportunities to Meaningfully Promote Excellence in Technology, Education, and Science (COMPETES) Act, Public Law No. 110-69.[3]

It will also be important to continue to develop short, but intense, courses to train physical scientists in the methods and principles of the biological sciences and to train biologists in the tools and approaches of the physical and engineering sciences. A few successful programs of this type are available (at for example, Woods Hole, the California Institute of Technology, and Cold Spring Harbor), but as interdisciplinary research flourishes, more such programs will be required. There is a pressing need for courses that communicate fundamental physicochemical concepts to biologists using the mathematical knowledge common to that community. Such courses would facilitate meaningful dialogue between biologists and physical scientists and engineers.

Recommendation 3: DOE, NIH, NSF, and other relevant departments and agencies should support the development of 1- or 2-week summer courses to train physical scientists and engineers in the tools and concepts of biology and medicine and, conversely, biologists in the tools and concepts of the physical sciences. Special attention should be given to finding ways of communicating fundamental physicochemical concepts to biologists using the mathematical knowledge common to the biology community. Such summer courses would help bridge the physical and life sciences communities, allowing them to exploit research opportunities at the intersection of the fields.

Federal funding agencies should make available resources to support and encourage the universities and individuals who wish to develop such courses. Similarly, real incentives need to be provided for the writing of textbooks for such courses. A particularly attractive model would be a book co-written by individuals who were trained in disparate fields but are working in the same interdisciplinary research field. The current academic system does not provide enough reward for writing such a badly needed book.

[3]A fact sheet on the America COMPETES Act is available at http://www.whitehouse.gov/news/releases/2007/08/20070809-6.html. Last accessed March 27, 2008.

EMPHASIZING BOTH FUNDAMENTAL AND APPLIED SCIENCES

Research in biomolecular materials and processes will impact society and technology in the ways described in Chapters 2 through 5 of this report. As such, both fundamental and applied research should be emphasized.

Recommendation 4: DOE, NIH, NSF, and other relevant departments and agencies should collaborate to link fundamental research with commercial applications. While it is imperative to recognize and exploit the connections between fundamental advances and opportunities to transition them into practice, curiosity-driven fundamental research on outstanding unsolved questions should be encouraged, because it could lead to unforeseen technological advances.

The committee especially emphasizes the importance of fundamental research. In recent years, the connections between fundamental and applied research have been encouraged, and this trend should continue. But fundamental research in the physical sciences has not been supported adequately. Yet, as described in Chapters 2 through 5 of this report, some fundamentally new advances are required (for example, understanding materials far from equilibrium) which are expected to elucidate important basic questions pertinent to biological function and bioinspired materials. This knowledge could provide the United States with the capability of developing revolutionary new technologies. It is important to emphasize that the recommendation for increased support of the basic sciences does not imply there should be a lesser emphasis on applications—basic and applied research are two sides of the same coin. The United States cannot afford to lag behind countries in Europe and Asia in applied research, and it can aim to continue to be the singular leader in paradigm-changing fundamental research. Other nations are increasing investments in both these categories.

DEVELOPING AND EVALUATING NATIONAL FACILITIES BASED ON MIDRANGE INSTRUMENTS

National instrumentation facilities have greatly aided the scientific enterprise in the United States. In the past, most such facilities were built around a single, large centralized resource (for example, a synchrotron light source or a nuclear reactor that produces neutrons). Interdisciplinary research in biomolecular materials and processes calls for diverse instrumentation not usually available in a single laboratory. Interdisciplinary collaboration between researchers with complementary expertise is one solution to this problem. Some universities and research centers are building private facilities that house instrumentation shared by the local com-

munity of researchers. But many researchers have not had access to such facilities. Now, however, national facilities that house clusters of small to midrange instrumentation and associated human expertise are beginning to provide this access.

Recommendation 5: DOE should continue to evaluate the effectiveness of recently created facilities to provide access to midrange instrumentation and computational facilities for the advancement of interdisciplinary research in nanoscience and technology. Based on what is learned from this evaluation, analogous, but distinct, centers could be created to facilitate research in biomolecular materials and processes.

Careful evaluation of the successes and failures of recently created facilities at DOE laboratories (for example, the Molecular Foundry at Lawrence Berkeley National Laboratory) will be important for gauging the effectiveness of this model. This evaluation should address questions that include whether the facility is effective in aiding research across the country (rather than just the local area), and whether universities that otherwise lack access to such facilities are benefiting from them.

Appendixes

A

Statement of Task

The goal of this study is to assess current work and future promise at the interfaces between biology and materials physics, and to recommend actions to realize the identified opportunities.

The committee is charged with the following tasks:

1. Identify the most compelling questions and the emerging scientific opportunities at the interface between biology and condensed matter and materials research—the biomolecular domain.
2. Suggest strategies to best meet the identified opportunities.
3. Consider connections to national priorities including health care, security, workforce, economic, and other societal needs.

B

Biographies of Committee Members

Arup K. Chakraborty (*Chair*) is the Robert T. Haslam Professor of Chemical Engineering, Chemistry, and Biological Engineering at the Massachusetts Institute of Technology (MIT). He obtained his Ph.D. in chemical engineering at the University of Delaware. After postdoctoral studies at the University of Minnesota, he joined the faculty at the University of California at Berkeley in December 1988. He rose through the ranks and ultimately served as the Warren and Katherine Schlinger Distinguished Professor and Chair of Chemical Engineering, professor of chemistry, and professor of biophysics at Berkeley. He was also head of theoretical and computational biology at Lawrence Berkeley National Laboratory. In September of 2005, Dr. Chakraborty moved to MIT. The central theme of his research today is the development and application of statistical mechanical approaches to study how T lymphocytes, orchestrators of the adaptive immune response, function. A characteristic of his work is that it directly impacts experimental immunology, and he collaborates extensively with immunologists. Dr. Chakraborty's work has been recognized by many honors that include the Allan P. Colburn and Professional Progress awards of the American Institute of Chemical Engineers, a Camille Dreyfus Teacher-Scholar award, a Miller Research Professorship, a National Young Investigator award, an NIH Director's Pioneer Award, and the E.O. Lawrence Memorial Award for Life Sciences. Dr. Chakraborty is a member of the National Academy of Engineering and a fellow of the American Academy of Arts and Sciences.

Joanna Aizenberg is the Gordon McKay Professor of Materials Science in the School of Engineering and Applied Sciences and the Susan S. and Kenneth L.

Wallach Professor at the Radcliffe Institute for Advanced Study, both at Harvard University. She was previously a member of technical staff at Bell Laboratories/Lucent Technologies. She received her B.S. and M.S. in chemistry from Moscow State University and a Ph.D. in structural biology from the Weizmann Institute of Science. Her research interests are in biomaterials and biomimetics. Dr. Aizenberg's select honors include the Ronald Breslow Award for Achievement in Biomimetic Chemistry from the American Chemical Society (ACS); Industrial Innovation Award, ACS; New Investigator Award in Chemistry and Biology of Mineralized Tissues; Arthur K. Doolittle Award of the ACS; Award of the Max-Planck Society in the field of Biology and Materials Science. Dr. Aizenberg is a member of the Board of Directors of the Materials Research Society. She is a fellow of the American Association for the Advancement of Science.

Annelise E. Barron is associate professor of bioengineering at Stanford University. She received her B.S. from the University of Washington, Seattle, and her Ph.D. from the University of California, Berkeley, both in chemical engineering. She was an NIH-NRSA postdoctoral research fellow at the University of California, San Francisco. Her research program involves the development of protein and peptide mimics based on biostable foldamers as well as novel materials for genetic analysis on microfluidic devices. She is a member of the advisory committee to the director of the NIH and serves on the NIH Synthetic and Biological Chemistry B study section. Dr. Barron's honors include the DuPont Young Professor Award, the Camille Dreyfus Teacher-Scholar Award, the Presidential Early Career Award for Scientists and Engineers, and the Beckman Young Investigator Award.

Ken A. Dill is professor of pharmaceutical chemistry and biophysics and associate dean of research for the School of Pharmacy at the University of California, San Francisco. Dr. Dill earned S.B. and S.M. degrees in mechanical engineering from MIT, a Ph.D. in biology from the University of California, San Diego, and was a postdoctoral fellow at Stanford University. His research group uses computational biology and statistical mechanical modeling to explore proteins, their physical properties, and their folding processes, in addition to the structure and properties of water. Dr. Dill has served as president and councilor of the Biophysical Society, councilor of the Protein Society, and as a member of the Executive Committee of the American Physical Society (APS). He currently chairs the Public Affairs Committee of the Biophysical Society. He is a fellow of the American Association for the Advancement of Science, the APS, the Institute of Physics, and the Biophysical Society, and serves on the editorial and advisory boards for Physical Biology, Protein Science, Protein Engineering, Biopolymers, Multiscale Modeling and Simulation, and Structure. Dr. Dill is the founder and codirector of the Bridging the Sciences Coalition, which brings together 16 basic research societies representing

280,000 scientists to support innovation at the interface between the life sciences and the physical sciences.

Sharon C. Glotzer is professor of chemical engineering, materials science and engineering, and physics at the University of Michigan. She also holds the titles Professor of Applied Physics and Professor of Macromolecular Science and Engineering and is a faculty affiliate in the University of Michigan's Center for Theoretical Physics, Center for the Study of Complex Systems, Center for Computational Medicine and Biology, and the Michigan Nanotechnology Institute for Medicine and Biological Sciences, where she serves on the executive board. She received her B.S. in physics from UCLA and her Ph.D. in physics from Boston University. Dr. Glotzer is a computational scientist specializing in soft matter and nanomaterials theory, with a special focus on self-assemby and phase transformations in liquids, glasses and jammed materials, nanoparticles and colloids, liquid crystals, polymers and other complex fluids. Dr. Glotzer received the APS's Maria Goeppert-Mayer Award, the Presidential Early Career Award for Scientists and Engineers (PECASE), and the Department of Commerce Bronze Medal and was a Sigma Xi lecturer. Dr. Glotzer served as chair of the APS Forum on Industrial and Applied Physics and chair of the American Institute of Chemical Engineering's Nanoscale Science and Engineering Forum. She is a fellow of the APS.

Yale E. Goldman is professor of physiology and director of the Pennsylvania Muscle Institute at the University of Pennsylvania. He received his B.S. from Northwestern University (1969) and M.D. and Ph.D. degrees from the University of Pennsylvania (1975). Dr. Goldman's research interests are understanding the mechanism of molecular motors and protein synthesis by the ribosome; mechanochemistry; and structural dynamics. His research group uses novel biophysical techniques, including laser photolysis of caged molecules, bifunctional fluorescent probes, single-molecule fluorescence polarization, and optical traps to map the real-time domain motions of proteins. Dr. Goldman is former president of the Biophysical Society. His honors include the Upjohn Achievement Award from the University of Pennsylvania; a research fellowship from the Muscular Dystrophy Association; the National Research Service Award the Research Career Development Award, and the MERIT Award (all three from NIH); the Bowditch Lecturer of the American Physiological Society; the Lindback Foundation Award for Distinguished Teaching; and the Lamport Lecturer of the University of Washington School of Medicine.

Elias Greenbaum is a corporate fellow and group leader at Oak Ridge National Laboratory and an adjunct professor in the University of Tennessee's Genome Science and Technology program. He received his B.S. degree in physics from Brooklyn College and Ph.D. in experimental nuclear physics from Columbia Uni-

versity. Dr. Greenbaum's main area of research is in the fields of photosynthesis and materials science and their applications to artificial sight, nanoscale science and technology, biosensor development, and renewable hydrogen production. Oak Ridge National Laboratory named him Scientist of the Year in 2000. He received the Department of Energy's Biological and Chemical Technologies Research Program Award in 1995 and several UT-Battelle, LLC, and Lockheed Martin Energy Research Corporation awards. He co-founded DOE's artificial sight program. Dr. Greenbaum led the team whose AquaSentinel Real-Time Water Supply Protection Monitoring Biosensor System won the Federal Laboratory Consortium Award for Excellence in Technology Transfer (2005). He is a fellow of the American Physical Society and the American Association for the Advancement of Science. He served as a member of the publications committees of the Biophysical Society and the American Institute of Physics and is currently editor in chief of the book series *Biological and Medical Physics/Biomedical Engineering*, published by Springer. Dr. Greenbaum was a Watkins Visiting Professor at Wichita State University, where he presented a series of lectures on photosynthesis, biotechnology, and renewable energy production.

W. John Kao is professor of biomedical engineering and pharmaceutical sciences at the University of Wisconsin at Madison. He received a B.S.E. in biomedical engineering from Johns Hopkins University and an M.S.E. in biomedical engineering and a Ph.D. in macromolecular science from Case Western Reserve University. He was a postdoctoral research associate in the Institute of Biomedical Engineering at the Swiss Federal Institute of Technology in Zurich, Switzerland, and in the Division of Chemistry and Chemical Engineering at the California Institute of Technology. Dr. Kao's research focuses on the role of biomaterials in the management of various pathological conditions. Specifically, he is elucidating the mechanisms of cell adhesion and activation on biomaterials, delineating the critical factors in material biocompatibility and biodegradation, and developing enabling technology for the synthesis of novel materials for tissue engineering. He is chair of the Society for Biomaterials' Education and Professional Development Student Affairs Task Force.

David Needham is professor of mechanical engineering and materials science at Duke University. He also holds appointments as associate professor of biomedical engineering; associate professor, Center for Cellular and Biosurface Engineering; and associate professor, Duke Comprehensive Cancer Center. He received a B.S. in applied chemistry from Trent Polytechnic and a Ph.D. in physical chemistry from the University of Nottingham. Dr. Needham's research interests are the material properties of lipid monolayers, bilayer membranes, hydrogels, wax particles, emulsions, gas bubbles, and cells; and adhesion and repulsion involving molecular

structures at interfaces including water-soluble polymers and receptor-mediated cell adhesion. His honors include the F. I. R. S. T. Award from the National Institutes of Health, the Alfred M. Hunt Faculty Scholarship, the NATO/SERC (England) Fellowship, and the Oppenheimer Research Fellowship.

V. Adrian Parsegian is chief of the Laboratory of Physical and Structural Biology at the National Institute of Child Health and Human Development. He received an A.B. in physics from Dartmouth College and a Ph.D. in biophysics from Harvard University. His research has provided fundamental contributions to the study of intermolecular forces in biological systems through measuring, formulating, computing, and gauging the consequences of forces that organize biomolecules. His work has included the theory and measurement of intermolecular forces, ion transport across cell membranes, protein conformation, colloids, aqueous interfaces, liquid crystals of lipids, protein, and nucleic acids. He is a fellow of the Biophysical Society and its former president, and he received the Society's Distinguished Service Award in 1995. He is also the recipient of the NIH Director's Award, which is NIH's highest award. He has just published a book, *Van der Waals Forces: A Handbook for Biologists, Chemists, Engineers, and Physicists*, the first of an intended series of texts to make the physics of intermolecular forces accessible to those without a background in advanced physics.

Alan Rudolph is president and CEO of Adlyfe, Inc. Dr. Rudolph has led R&D programs in biological self-assembly for over 20 years. He is currently spearheading an effort to develop novel technologies in the private sector as an active CEO of a small biotechnology start-up, and nurture emerging technologies through his consulting practice and board positions on foundations. Prior to this assignment, Dr. Rudolph was chief of biological sciences for the Defense Sciences Office at DARPA, where he managed a $40 million life sciences portfolio in academia and industry, focusing on early risk reduction in the context of prototype development and technology transfer. His position at DARPA was to invest and manage high-risk, high-payoff multidisciplinary R&D projects in biotechnology. These programs included the design and fabrication of useful interfaces for biological molecules, cells, and tissues for working devices (e.g., diagnostics, sensors, prosthetics). He has also explored the development of wireless devices with biological systems in order to better understand and develop emerging technologies. Dr. Rudolph is the author of 100 technical publications, including seminal work in *Nature* and at the National Academy of Sciences and holds 15 patents, of which 2 are licensed for commercial development (drug delivery and medical imaging). He received his B.S. with highest honors in biology from the University of Michigan, a Ph.D. in cell biology from the University of California, and an M.B.A from the George Washington University.

Cyrus R. Safinya is a professor in the Department of Physics and the Department of Molecular, Cellular, and Developmental Biology at the University of California at Santa Barbara and in the Department of Materials in the University's School of Engineering. He received a B.S. in physics and mathematics from Bates College and a Ph.D. in physics from the Massachusetts Institute of Technology. Before joining the faculty of the University of California at Santa Barbara, he was a member of the technical staff at Exxon Research and Engineering Company in New Jersey and conducted research on the structure of complex fluids and biological membranes. Currently, his group's research is focused on elucidating structures and interactions of supramolecular assemblies of biological molecules. This includes the elucidation of key parameters that control the interactions between proteins derived from the eukaryotic nerve cell cytoskeleton and lead to hierarchical structures, with the ultimate goal of relating structure to function; understanding DNA interactions with oppositely charged biomolecules in the context of DNA packing; and developing synthetic carriers of genes and short-interfering RNA for gene delivery and silencing applications. He was a Henri De Rothschild Foundation Fellow, awarded by the Curie Institute, in 1994. He is a fellow of the APS and the American Association for the Advancement of Science.

Charles F. Stevens is a Howard Hughes Medical Institute Investigator and the Vincent J. Coates Professor of Molecular Neurobiology at the Salk Institute for Biological Studies in LaJolla, California. Previously, he was professor and chair of the Molecular Neurobiology Section at Yale University School of Medicine. Dr. Stevens's research centers on mechanisms responsible for synaptic transmission in the central nervous system, using a combination of molecular biological, electrophysiological, anatomical, and theoretical methods. He is a member of the National Academy of Sciences and the American Academy of Arts and Sciences. Dr. Stevens has chaired and served on a number of NRC committees. In addition to his publications in the field of neurobiology, he authored the book *The Six Core Theories of Modern Physics*, which summarizes the basic theoretical structures of classical mechanics, electricity and magnetism, quantum mechanics, statistical physics, special relativity, and quantum field theory. Dr. Stevens serves as an advisor to a telecommunications firm on the possible health effects of cell phone use. He received his M.D. from Yale University School of Medicine and his Ph.D. from Rockefeller University.

David A. Weitz is the Mallinckrodt Professor of Physics and of Applied Physics at Harvard University. He received a B.S. in physics from the University of Waterloo and an A.M. and a Ph.D. in physics from Harvard University. Dr. Weitz's research group studies the physics of soft condensed matter. Before coming to Harvard, Dr. Weitz was a professor of physics at the University of Pennsylvania and a physicist with Exxon Research and Engineering Co.

C

Committee Meeting Agendas

FIRST MEETING
WASHINGTON, DC
MARCH 16-17, 2006

Thursday, March 16, 2006

CLOSED SESSION

8:30 a.m.	Opening Remarks —Arup Chakraborty
8:45	Discussion of Committee Balance and Composition —Donald Shapero
9:30	Introduction to the NRC and Overview of Study Process —Natalia Melcer
9:45	NRC study "Forefronts of Science at the Interface of the Physical and Life Sciences" —Timothy Meyer

OPEN SESSION

10:15	Perspective from 1996 NRC Study: "Biomolecular Self-assembling Materials" —Philip A. Pincus

11:15	Perspective from 2004 NSF Workshop: "The Role of Theory in Biological Physics and Materials"
	—Michael Thorpe
12:15 p.m.	Lunch
1:15	Perspective on Biomolecular Materials and Charge from the SSSC
	—Sol Gruner

CLOSED SESSION

2:15	Committee Discussion
5:00	Adjourn for the Day

Friday, March 17, 2006

OPEN SESSION

8:30 a.m.	Charge from DOE/BES
	—Harriet Kung
9:15	Charge from NSF/DMR
	—Lance Haworth

CLOSED SESSION

10:15	Committee Discussion
12:00 noon	Lunch and Adjourn

SECOND MEETING
BERKELEY, CALIFORNIA
JUNE 18-19, 2006

Sunday, June 18, 2006

OPEN SESSION

8:00 a.m.	Opening Remarks
	—Arup Chakraborty
8:30	Designing Materials for Biology and Medicine
	— Robert S. Langer (by teleconference)
9:00	Probing Biomolecular Materials with Neutron Scattering
	—Roger Pynn
10:00	Methodologies for Reverse Engineering
	—David Needham

CLOSED SESSION
10:45 Committee Discussion
12:00 noon Working Lunch in Breakout Groups

OPEN SESSION
1:30 Remarks from Robert Full
—Robert J. Full

CLOSED SESSION
2:30 Committee Discussion
5:00 Adjourn for the Day

Monday, June 19, 2006

CLOSED SESSION
8:30 a.m. Committee Discussion
12:00 noon Lunch and Adjourn

THIRD MEETING
WASHINGTON, DC
NOVEMBER 29-30, 2006

Wednesday, November 29, 2006

CLOSED SESSION
1:30 p.m. Opening Remarks
—Arup Chakraborty
1:45 Committee Discussion
5:30 Adjourn for the Day

Thursday, November 30, 2006

CLOSED SESSION
8:00 a.m. Review of First Day
—Arup Chakraborty
8:15 Committee Discussion
12:00 noon Lunch
1:00 Committee Discussion
5:00 Adjourn

D

Glossary

actin: Globular structural protein that serves as the monomeric subunit of microfilaments, one of the three major components of the cytoskeleton, and of thin filaments that are part of the contractile apparatus in muscle cells. Actin participates in many important cellular functions, including muscle contraction, cell motility, cell division and cytokinesis, vesicle and organelle movement, cell signaling, and the establishment and maintenance of cell junctions and cell shape.

actuator: Device that reads a signal and converts it to a physical action.

adenosine triphosphate (ATP): Multifunctional nucleotide that is the main energy source for the majority of cellular functions. ATP transports chemical energy within cells for metabolism. It is produced as an energy source during the processes of photosynthesis and cellular respiration and consumed by many enzymes and a multitude of cellular processes, including biosynthetic reactions, motility, and cell division.

adenosine triphosphatase (ATPase): Class of enzymes that catalyze the decomposition of adenosine triphosphate (ATP) into adenosine diphosphate (ADP) and a free phosphate ion. This reaction releases energy, which the enzyme (in most cases) harnesses to drive other chemical reactions that would not otherwise occur. This process is widely used in all known forms of life.

allosteric interaction: Interaction involving an enzyme that has two binding sites:

the active site and a site into which another molecule fits. The binding of a molecule changes the shape of the enzyme and alters its activity.

amphiphile: Molecule possessing both hydrophilic (water soluble) and hydrophobic (water repellent) regions.

amyloid: Insoluble fibrous protein aggregations sharing specific structural traits.

angiogenesis: Physiological process involving the growth of new blood vessels from preexisting vessels.

antigen: Molecule that stimulates an immune response.

bioderivation: Using a biomaterial with desired properties to create a hybrid material, such as incorporation of biologically derived proteins for targeted drug delivery.

bioinspiration: Observing a particular function performed with precision by a biological system, and then attempting to create a synthetic system that performs the same function for technological applications. The strategy devised to achieve this goal can be quite different from that employed by the biological system.

biomimicry: Learning the mechanistic principle used by living systems to achieve a particular function and then attempting to copy the same strategy to achieve biomimetic function.

biomineralization: Process by which living organisms produce minerals, often to harden or stiffen existing tissues. Examples include calcium carbonates in seashells and bone in mammals and birds.

Boolean logic: Algebraic system of logic developed by George Boole in the mid-19th century. It is the algebra of two values—usually 0 and 1—and three operations—AND, OR, and NOT.

Brownian motion: Random movement of particles suspended in a fluid or the mathematical model used to describe such random movements.

cell signaling: Part of a complex system of communication that governs basic cellular activities and coordinates cell actions.

chirality: Phenomenon in which an object or molecule cannot be superimposed on its mirror image.

cytoskeleton: Cellular "scaffolding" contained within the cytoplasm that maintains cell shape, enables cellular motion, and plays important roles in both intracellular transport and cellular division.

dendrimer: Repeatedly branched molecules that are characterized by their high symmetry and narrow distribution of molecular mass (low polydispersity).

directed assembly: Application of external fields, such as electric, magnetic, or shear, to align assembling particles into a larger structure.

fluorophore: Functional group in a molecule which will absorb energy of a specific wavelength and re-emit energy at a different (but equally specific) wavelength. The amount and wavelength of the emitted energy depend on both the fluorophore and the chemical environment of the fluorophore.

foldamer: Discrete chain molecule or oligomer that adopts a secondary structure stabilized by noncovalent interactions. It is an artificial molecule that mimics the ability of proteins, nucleic acids, and polysaccharides to fold into well-defined conformations, such as helices and β-sheets.

gene regulation: Cellular control of the amount and timing of changes to the appearance of the functional product of a gene.

histone: Chief protein components of chromatin. It acts as spool around which DNA winds, and it plays a role in gene regulation.

hydrogel: Network of polymer chains that are water-insoluble, sometimes found as a colloidal gel in which water is the dispersion medium. Hydrogels are super-absorbent (they can contain over 99 percent water) natural or synthetic polymers. Hydrogels possess also a degree of flexibility very similar to natural tissue, due to their significant water content.

lithography: Technique used to pattern or construct features on a surface.

macromolecule: Molecule of high relative molecular mass the structure of which usually consists of multiply repeated units that are derived—actually or conceptually—from molecules of low relative molecular mass; particularly a molecule of this kind that is of biological origin.

macrophage: Cell within most tissue that originates from specific white blood cells. Its role is to engulf and then digest cellular debris and pathogens either as stationary or mobile cells and to stimulate lymphocytes and other immune cells to respond to the pathogen.

magnetic resonance imaging (MRI): Noninvasive technique based on nuclear magnetic resonance (NMR) for imaging the interior of objects, often used in medicine.

microtubule: Long hollow cylindrical structure composed of the protein tubulin. It is one of the components of the cytoskeleton, serves as a structural component within cells, and is involved in many cellular processes, including mitosis, cytokinesis, and vesicular transport.

molecular motor: Biological molecular machine that consumes energy in one form and converts it into motion or mechanical work; for example, many protein-based molecular motors harness the chemical free energy released by the hydrolysis of ATP in order to perform mechanical work. In terms of energetic efficiency, these types of motors can be superior to currently available man-made motors.

natural killer (NK) cell: Type of cytotoxic lymphocyte which is a major component of the innate immune system. NK cells play a major role in the rejection of tumors and cells infected by viruses. NK cells kill by releasing small cytoplasmic granules of proteins that cause the target cell to die by apoptosis.

nuclear magnetic resonance (NMR): When an atomic nucleus in a magnetic field is exposed to photons that have an energy corresponding to the difference in energy between two possible orientations of its magnetic moment, it will resonate—that is, its magnetic moment will rapidly change orientation, in the process first absorbing energy and then radiating it. The frequencies at which resonances are seen in some specified magnetic field not only identify the kinds of atom responsible for them but can also provide valuable information about the molecular environment in which the atoms are found.

nucleotide: Molecule that consists of three portions: a heterocyclic base, a sugar, and one or more phosphate groups. Nucleotides are the monomers of nucleic acids, with three or more bonding together in order to form a nucleic acid. Nucleotides are the structural units of RNA, DNA, and several cofactors.

oligonucleotide: Short sequences of nucleotides, typically with twenty or fewer bases.

organelle: Specialized subunit within a cell that has a specific function and is separately enclosed within its own lipid membrane.

peptoid: Small proteinlike chain designed to mimic a peptide. Peptoids are closely related to their natural peptide counterparts but differ chemically in that their side chains are appended to nitrogen atoms along the molecule's backbone, rather than to the α-carbons (as they are in amino acids).

polymerase chain reaction: Technique used to amplify the number of copies of a specific region of DNA by the use of sequence-specific primers and multiple cycles of DNA synthesis, each cycle being followed by a brief heat treatment to separate complementary strands.

polymersome: Bilayered membranes of amphiphilic synthetic polymers. Polymersomes exhibit increased stability and reduced permeability compared to natural liposomes.

quantum dot: Semiconductor nanostructure that confines the motion of conduction band electrons, valence band holes, or excitons (bound pairs of conduction band electrons and valence band holes) in all three spatial directions. The confinement can be due to electrostatic potentials (generated by external electrodes, doping, strain, impurities), the presence of an interface between different semiconductor materials, the presence of the semiconductor surface, or a combination of these.

ribosome: Complexes of RNA and protein found in all cells that mediate the translation of messenger RNA molecules into polypeptide chains or amino acids.

self-assembly: Spontaneous organization of preexisting components by the forces acting among the components. Self-assembly is generally considered a reversible process, tunable by varying a thermodynamic parameter such as temperature or density, and one that can be controlled through judicious design of the components. Typically, self-assembled structures form based on thermodynamic principles in which free energy is minimized.

spectroscopy: (Usually) experimental study of the energy level of materials. More generally, a spectrum is a display of the dependence of some property of a sample as a function of some other parameter—for example, energy absorption versus energy or abundance versus molecular mass. Any experimental activity that generates such plots can be described as spectroscopy.

stochastic process: Opposite of a deterministic process in probability theory. Instead of dealing only with one possible "reality" of how the process might evolve under time (as is the case, for example, for solutions of an ordinary differential equation), in a stochastic or random process there is some indeterminacy in its future evolution described by probability distributions. This means that even if the initial condition (or starting point) is known, there are many possibilities the process might go to, but some paths are more probable and others less.

T cell: Member of a group of white blood cells known as lymphocytes which plays a central role in cell-mediated immunity. T cells can be distinguished from other lymphocyte types, such as B cells and NK cells, by the presence of a special receptor on their cell surface that is called the T cell receptor (TCR). The abbreviation "T", in T cell, stands for thymus since it is the principal organ for their development.

transcription factor: Protein that binds to specific parts of DNA using DNA binding domains and is part of the system that controls the transfer (or transcription) of genetic information from DNA to RNA.