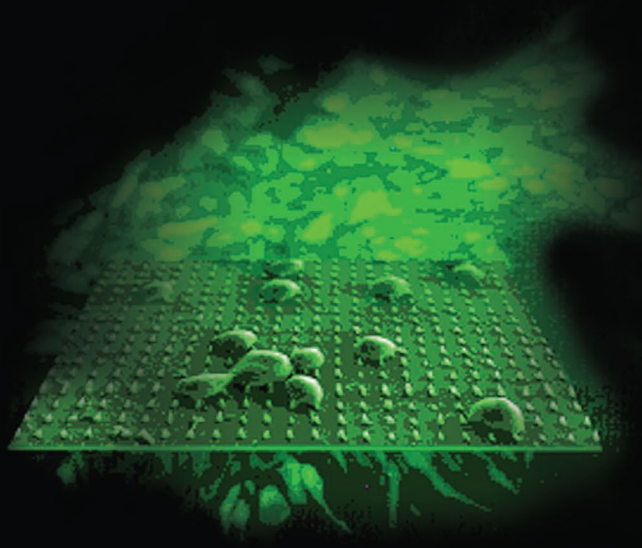
Nanobiotechnology

Report of the
National Nanotechnology Initiative Workshop
October 9–11, 2003



About the Nanoscale Science, Engineering, and Technology Subcommittee

The Nanoscale Science, Engineering, and Technology (NSET) Subcommittee is the interagency body responsible for coordinating, planning, implementing, and reviewing the National Nanotechnology Initiative. The NSET Subcommittee was established in 2000, replacing the Interagency Working Group on Nanoscience, Engineering, Technology (IWGN), which was formed in 1998. NSET is a subcommittee of the National Science and Technology Council (NSTC), which is one of the principal means by which the President coordinates science, space, and technology policies across the Federal Government. The National Nanotechnology Coordination Office (NNCO) provides technical and administrative support to the NSET Subcommittee and supports the subcommittee in the preparation of multi-agency planning, budget, and assessment documents, including this report.

For more information on NSET, see http://www.nano.gov/html/about/nsetmembers.html.

For more information on NSTC, see http://www.ostp.gov/NSTC/html/NSTC_Home.html.

For more information on the NNI, NSET, and NNCO, see http://www.nano.gov.

About this document

This document is the report of a workshop held under NSET auspices in October 2003 seeking input from the research community on the NNI research agenda at the intersection of nanoscale science and technology and biology, and particularly biomedical applications of nanotechnology. It was used as input for the NNI Strategic Plan released in December 2004. The meeting was jointly sponsored by the U.S. National Science Foundation, the National Institutes of Health, and, through the NNCO, the other member agencies of the NSET Subcommittee.

Cover and book design

Book design and layout by Roan Horning, Geoff Holdridge, and other NNCO staff members. Cover design by Kanako Yamamoto and Laura Garber of Affordable Creative Services, Inc.

Front cover: Central image (composite of two optical fluorescence microscopy images) shows functional integration of vertically aligned carbon nanofibers and Chinese hamster ovary cells. Green fluorescent (GFP) genes tethered to the nanofibers are expressed by the cells, indicating that the cells remain viable after insertion of the nanofiber. This demonstrates the controlled delivery of material to cells (courtesy of Michael L. Simpson, Oak Ridge National Laboratory).

Back cover: Two images depicting a conjugate of a "smart" photo- and temperature-responsive polymer and the enzyme endoglucanase 12A. The enzyme is in the "on" state at right, when the yellow substrate can enter while the attached polymer is in an extended state. A small change in temperature or light irradiation causes the collapse of the polymer to the state, on the left, where the enzyme is in an "off" state (courtesy of Patrick Stayton, University of Washington).

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Nanobiotechnology

Report of the National Nanotechnology Initiative Workshop
October 9–11, 2003, Arlington, VA

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ACKNOWLEDGMENTS

Thanks to the principal authors of this report listed on the title pages of each of the chapters. In addition, the sponsors wish to thank all the participants at the October 9–11, 2003, workshop held in Arlington, Virginia (see Appendix C), and particularly the workshop co-chairs, Viola Vogel of the University of Washington (now moved to ETH/Zurich) and Barbara Baird of Cornell University, as well as the other members of the workshop organizing committee, as follows:

Geoff Holdridge, National Nanotechnology Coordination Office
Eleni Kousvelari, National Institutes of Health
Soo-Siang Lim, National Science Foundation
Mike Roco, National Science Foundation
Jeff Schloss, National Institutes of Health

The presentations and discussions at that workshop provided the foundation for this report.

Credit is also due to other members of the NNCO staff who helped organize the workshop: Clayton Teague, Cate Alexander, Stephen Gould, and Sam Gill, and to Thomas Bartolucci and other staff members from NNCO and WTEC, Inc. who assisted in final production of the report. Special thanks to Anne Wenzel of Walrusworks for her editing work on the report.

Finally, thanks to all the members of the National Science and Technology Council's Subcommittee on Nanoscale Science, Engineering, and Technology, who co-sponsored the workshop with the National Institutes of Health and the National Science Foundation, and who reviewed the draft report before publication.

This document was sponsored by the National Science Foundation, the National Institutes of Health and, through the National Nanotechnology Coordination Office (NNCO), the other member agencies of the Nanoscale Science, Engineering, and Technology (NSET) Subcommittee of the National Science and Technology Council. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the United States Government or the authors' parent institutions.

PREFACE

This report on nanobiotechnology is one of a series of reports resulting from topical workshops convened during 2003 and 2004 by the Nanoscale Science, Engineering, and Technology (NSET) Subcommittee of the National Science and Technology Council's Committee on Technology through the National Nanotechnology Coordination Office (NNCO). The workshops were part of the NSET Subcommittee's long-range planning effort for the National Nanotechnology Initiative (NNI), the multiagency Federal nanotechnology program. The NNI is driven by long-term goals based on broad community input, in part received through these workshops. The NNI seeks to accelerate the research, development, and deployment of nanotechnology to address national needs, enhance our nation's economy, and improve the quality of life in the United States and around the world, through coordination of activities and programs across the Federal Government.

At each of the topical workshops, nanotechnology experts from industry, academia and government were asked to develop broad, long-term (ten years or longer), visionary goals and to identify scientific and technological barriers that once overcome will enable advances toward those goals. The reports resulting from this series of workshops inform the respective professional communities, as well as various organizations that have responsibilities for coordinating, implementing, and guiding the NNI. The reports also provide direction to researchers and program managers in specific areas of nanotechnology R&D regarding long-term goals and hard problems.

This workshop was convened to solicit input from the research community on the NNI research agenda at the intersection of nanoscale science and technology and biology, with a particular focus on research for biomedical applications of nanotechnology. The workshop was held in part in response to the 2002 National Research Council report on the NNI, *Small Wonders, Endless Frontiers*, which included a recommendation that the NNI increase its investments at the intersection of nanoscale technology and biology.[1] Research in this area is worthy of attention because there are many opportunities that have the potential to improve health care, longevity, and quality of life.

This report identifies four general areas of opportunity for research at the intersection of nanotechnology and biology, with particular emphasis on biomedical applications:

- Advanced imaging technologies
- *In vivo* analysis of cellular processes
- Understanding how cells work through bottom-up assembly of biological nanosystems *ex vivo*
- Nanotechnology and human health

The scope of this workshop did not fully encompass nonmedical biological applications of nanotechnology, or application of principles of biology to solving problems in other areas of nanotechnology research such as biomimetic approaches to self-assembly or nanomanufacturing.

The findings from this workshop were taken into consideration in preparation of the updated NNI Strategic Plan released in December 2004[2] and were input to the development of programs that make up portions of the Fiscal Year 2006 NNI budget requested for the National Institutes of

[1] http://www.nano.gov/html/res/smallwonder.html.

[2] http://www.nano.gov/NNI_Strategic_Plan_2004.pdf.

Health, the National Science Foundation, and other NNI participating agencies.[3] The NNI's investments at the intersection of nanotechnology and biology are growing, guided in part by the expert advice provided at this workshop.

On behalf of the NSET Subcommittee, we wish to thank Professors Viola Vogel and Barbara Baird for their creativity and hard work in conducting an outstanding workshop and in preparing this report. We also thank all the speakers, session chairs, and participants for their time and efforts to join the workshop, to make their individual contributions to the discussions at the workshop, and to draft this report. Their generous sharing of research results and insights ensures that this document will serve as a reference for the NNI.

Mihail C. Roco
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[3] See the NNI Supplement to the President's FY 2006 Budget: http://www.nano.gov/NNI_06Budget.pdf.

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EXECUTIVE SUMMARY

INTRODUCTION AND PURPOSE OF THE WORKSHOP

The National Nanotechnology Initiative Workshop on Nanobiotechnology was held on October 9–11, 2003, in Arlington, Virginia. Scientists, engineers, and physicians assembled to identify groundbreaking scientific opportunities for research at the intersection of nanotechnology and biology. The workshop was sponsored by the National Science Foundation, the National Institutes of Health, and (through the National Nanotechnology Coordination Office) the other member agencies of the Nanoscale Science, Engineering, and Technology (NSET) Subcommittee of the National Science and Technology Council's Committee on Technology. The findings and recommendations of the workshop included in this report also provided input to the December 2004 strategic plan (http://nano.gov/NNI_Strategic_Plan_2004.pdf) for the U.S. National Nanotechnology Initiative, which is coordinated by the NSET Subcommittee.

Substantial segments of the scientific community are confident that nanoscience and nanotechnology will revolutionize research on and applications in the areas of biology and medicine. Yet, much of the biomedical research community has at best a passing familiarity with the relevant discoveries that are emanating from the larger nanotechnology communities, ranging from physics and chemistry to engineering and the biosciences. Although many nanotechnologists develop new tools, they often have had a limited understanding of the needs of the biomedical communities or of the restrictions that biology (and medicine in particular) place on the proper design of nanotools or nanosystems. Nanotechnologists also require a much deeper understanding of biology to fulfill their goal of designing synthetic nanoengineered materials, devices, and integrated systems based on the design of biological systems. Conversely, biomedical communities often have had relatively little insight into what nanotechnology can potentially do and how they can take advantage of the ongoing technological advances. The U.S. National Nanotechnology Initiative Nanobiotechnology[4] Workshop convened thought leaders in biomedical and nanotechnology research to identify crosscutting scientific opportunities that can be realized only through effective collaboration among these communities.

Workshop participants were asked to identify fundamental changes that nanoscience and nanotechnology can bring to the study of life processes and that can lead to effective interventions for treating disease and promoting human health. To stimulate closer and lasting collaborations among biologists and nanotechnologists, this workshop was expected to identify key issues and far-reaching goals that cannot be achieved without effective teamwork. Finally, the participants were asked to recommend priority research areas for the National Science Foundation, the National Institutes of Health, and the other agencies participating in the National Nanotechnology Initiative.

MAJOR RECOMMENDATIONS

Nanotechnology—the ability to engineer systems with defined structure and function on the nanometer scale—is in the process of driving a new wave of medical innovation. The challenge for the future is to develop newer and more sophisticated nanotechnologies that can address problems associated with the diagnosis, treatment, and management of multigene diseases (e.g., cancer,

[4] See discussion on definition of the term "nanobiotechnology" in Chapter 1 of this report.

cardiovascular diseases, environmental diseases, Alzheimer's disease, etc.), organ dysfunction, and structural disorders (e.g., developmental diseases, degenerative diseases, tissue injury) that our aging population will experience over the next century. The report focuses on the following topics, in which major progress is expected from advanced nanotechnologies:

1. **New molecular imaging technologies** need to be developed to probe nanoscale physiological processes occurring in human organs, whether we try to diagnose cancer or any other disease in early stages, or whether the goal is to treat diseased tissues more effectively. This includes new molecular imaging probes and technologies that allow for a quantitative understanding of how biological systems work on the nanometer scale, and how these systems are integrated within cells, enabling recognition and processing of a myriad of spatiotemporal stimuli, which ultimately results in coordinated responses within and between cells. This also includes tools to observe molecular events and to track the distribution and kinetics of molecular probes, drugs, and environmental agents within the whole animal or conscious human under a variety of physiological conditions, as well as to observe where the relevant molecules are and how they interact in a dynamic way. Furthermore, tools are needed to image the fate of single molecules and how they interact with each other. This requires nanometer spatial resolution at rapid refresh rates, the development of advanced multifunctional nanoprobes, and the engineering of advanced genetically encodable markers (Chapter 2).
2. **Quantitative analytical tools** to learn how cell functions are regulated and how they can be manipulated in a predictable manner are urgently needed for the advancement of cell biology and medicine. This includes deriving the engineering principles that control and regulate cellular functions and the development of new technologies to derive a detailed understanding of how cells of all types sense external and internal signals within the context of their particular three-dimensional environments, and how these signals are processed to yield particular cellular responses, including stimulated secretion, growth, differentiation, motility, contractility, and apoptosis. Deriving such insights will also require the capability to engineer two- and three-dimensional spaces for cells on the nanoscale that allow systematic examination of specific cues between the cell and its local environment (Chapter 3).
3. Building on topics 1 and 2 above, the **quantitative integration of information** derived from the application of advanced nanoprobes combined with novel real-time imaging technologies is critical. For example, the integration of data over the nano, micro, and longer length scales within specific models of cell function by engineers and physical scientists, in close communication with life scientists, will rapidly advance our understanding of cellular systems by incorporating information from genomics, proteomics, and biochemistry to measure parameters and provide predictions that guide advanced experiments. Finally, all the experimental insights need to be integrated into predictive computational models. With increasingly realistic cellular models will come greater insight into spatial and mechanical control, multimodality, and hierarchical integration of processes within the living cell (Chapter 3).
4. Studies on a **physical model of the cell as a machine** are essential to understanding how the components of a cell work together to accomplish overarching functions. This line of study will give new insights into the physical relationships between cellular components and functional irregularities that trigger pathological abnormalities. Genomics and proteomics, combined with nanobiotechnology, will thus define defects associated with disease in a way not previously possible and will allow therapy to be targeted far more effectively. This includes the determination of the "assembly instructions" for a cell and then the implementation of these instructions to generate synthetic cellular components at the nanometer scale. This in turn will enable rebuilding of the functional modules *de novo* from the lower end of the nanoscale to

devices on the same size scale as organelles and cells. Such assembly processes will test hypotheses about the process of going from genes to live assemblies and will also produce useful materials and devices for the diagnosis and treatment of human diseases (Chapter 4).

5. Because a significant part of tomorrow's medicine will be based on early detection and prevention, **better *ex vivo* tests and improvements in current laboratory techniques** are required to allow measurement with greater sensitivity and specificities. This includes engineered nanosystems that can be integrated into biological systems, including sensors implanted within human tissues and cells that would provide real-time information on biological processes and functions, and that would potentially be suited for long-term *in situ* monitoring. Furthermore, microfabricated platforms with integrated nanoscale components are needed to build integrated, low-cost portable devices to analyze samples from body fluids and gases without major time delays. Finally, high-throughput systems need to be developed, if possible with single-molecule detection, for the analysis of biological materials (Chapter 5).
6. Advances are also needed in the field of **drug delivery and intelligent therapeutics**. More sophisticated "smart" systems for drug delivery have to be developed that sense and respond to specific chemical agents (e.g., releasing insulin in response to glucose), deliver drugs and genetic agents to specific sites, and are tailored to each patient on the basis of genotype. Multifunctional nanodevices need to be developed that simultaneously detect, diagnose, treat, and monitor response to the therapy (Chapter 5).
7. Longer-term goals have to be extended beyond our current definition of medical intervention, with advances leading to nanosystems that totally replace, repair, and regenerate diseased tissue. Ultimately, nanomedicine will go beyond restoring function to its normal healthy state and will provide a means to guide regeneration to tissues, organs, and organ systems with advanced capabilities for self-repair and disease prevention. The result will be a new era in health care (Chapter 5).

Additional Recommendations

- The issue of compatibility between biological and nonbiological nanotechnology materials warrants further exploration. A large gap still exists at the interface between biological and chemical/mechanical/solid nanomaterials. For example, the toxicity and compatibility of nanomaterials in biological systems (e.g., including humans and animals) requires careful scrutiny. Efficiently addressing this issue requires strong communication and collaboration among scientists in both biomedical and other nanomaterial sciences.
- Findings and raw data from research in nanobiotechnology should be shared rapidly through an information network, including inventory systems for cataloging the vast information obtained at different scales. Integration and organization of data from genomics, proteomics, biochemistry and biophysics within specific models of cell function by engineering, physics, and mathematical disciplines in close communication with biologists will rapidly advance understanding of cellular systems and will yield predictions to guide further experiments.

The research possibilities and medical outcomes envisioned at this workshop cannot be achieved without careful attention and research dedicated to the safety and possible toxicity of nanomaterials. At the same time, some of the capabilities developed through the research discussed at this meeting will be critical in addressing these safety and toxicity issues.

1. INTRODUCTION

Viola Vogel and Jeffery Schloss

SIGNIFICANCE

The science and engineering of nanobiosystems is one of the most challenging and fastest growing sectors of nanotechnology. Nanotechnology emerges from the physical, chemical, biological, and engineering sciences, where novel techniques are being developed to probe and manipulate single atoms and molecules—tools that already have enabled a myriad of discoveries of how the properties of matter relate to the atomic and molecular arrangements at nanometer dimensions. These discoveries already are revolutionizing manufacturing processes of many materials and devices that find broad applications in society, often by integrating sophisticated design functionalities. Controlling the architecture, and thus properties, of materials and devices at the nanoscale is made possible by exploiting self-assembly strategies that are frequently complemented by top-down engineering approaches. Substantial segments of the scientific community are now confident that nanoscience and nanotechnology also will revolutionize research in biology and medicine.

Meanwhile, in a parallel effort, the molecular biology community has sequenced the human genome and developed sophisticated biochemical tools to analyze molecular systems, greatly deepening our knowledge of biological phenomena and facilitating the development of many new diagnostic and therapeutic tools. As a consequence, biotechnology applications now have a vital role in several industrial sectors.

Despite these many advances, society will continue to be faced with daunting challenges in biomedicine and the delivery of health care unless major scientific and technological breakthroughs are fostered. These challenges, all of which will benefit from nanotechnology, include identifying the molecular origins of many diseases (including cancer), which is paramount for effective treatment; developing more selective therapies that minimize medical side effects (including the synthesis of new drugs and their targeted delivery); enabling health monitoring and early diagnostics of diseases (through novel diagnostic tool kits and advanced imaging technologies); and establishing the knowledge base needed for biocompatible prosthetics and regenerative medicine (from tissue to neuromorphic engineering). Many if not all of these biomedical challenges will depend on the development of a quantitative understanding of how biological nanosystems and, ultimately, all the subcellular constituents work in synchrony ("nanosystems biology"), and on the synthesis of new devices or systems that could, for example, serve for targeted drug delivery or recognize cellular responses or molecular processes associated with diseased states. As beneficial as this confluence of disciplines will be to biology and medicine, even broader benefits will be derived, as nanobiosystems are a source of inspiration for the engineer and provide insights into new design principles for man-made nanosystems.

How can rapid advances at the interface between nanotechnology and biomedicine be fostered?

CHALLENGE

The biomedical community has at best casual familiarity with recent advances in nanotechnology, whereas the nanotechnology community, envisioning potential applications for its newly developed tools, is largely unfamiliar with the major challenges in and the nuances of the biological sciences. Neither of these communities is sufficiently aware of the deeper questions that the other community is trying to answer, nor of the rich knowledge and insights that each can contribute to help answer those questions in an effective manner.

OBJECTIVE OF THE WORKSHOP

A major goal of this workshop was to bridge the gaps between these communities by inviting leaders in biomedical and nanoscale science and engineering research and asking them to identify cross-cutting scientific opportunities that can be realized only through effective collaboration among these communities. The agenda was designed to stimulate dialogue and create a more effective interface among outstanding investigators from the biological and clinical sciences and the physical sciences. By providing several concrete examples of opportunities and needs and by disseminating that information to a wider community through this report, it is hoped that the workshop will provide incentives for the formation of multidisciplinary teams in which participants will benefit from and contribute to each others' existing/ongoing investigations and ultimately open new lines of novel inquiry. Accordingly, workshop participants were asked to identify major challenges in medicine and health care, and to assess how nanoscience and nanotechnology can bring about major changes to the study of life processes as well as contribute novel technologies that lead to more effective interventions for treating disease and promoting human health. To stimulate closer and lasting collaborations among biologists and nanotechnologists, the final objective of this workshop was to identify key issues and far-reaching goals that simply cannot be achieved without effective cross-fertilization. The participants were thus asked to recommend priority research topics at the intersection of nanotechnology and biology for the National Science Foundation (NSF), the National Institutes of Health (NIH), and other agencies that participate in the U.S. Government's National Nanotechnology Initiative (NNI).

STRUCTURE OF THE REPORT

While planning for the workshop, the organizers requested input from the relevant communities to identify biomedical areas in which nanotechnology is poised to have considerable effect. This input was gathered during the workshop in five breakout sessions. Subsequently, the report authors have consolidated this input into four subtopics that emerged from these consultations, which comprise the following chapters of this report:

Chapter 2: Advanced imaging technologies: from nano to macro

Chapter 3: *In vivo* analysis of cellular processes at the nanoscale

Chapter 4: Understanding how cells work through bottom-up assembly of biological nanosystems *ex vivo*

Chapter 5: Nanotechnology and human health

Chapter 6 includes a summary of overarching themes from the workshop, including an overview of conclusions and recommendations from chapters 2-5. Appendices to the report include the written

abstracts provided by workshop participants in advance of the workshop, the agenda for the workshop, and a list of participants and their institutional affiliations.

Beyond outlining the opportunities at the interface between nanotechnology and biomedicine, and focusing on the topics outlined above, the format of each breakout session was organized around the following objectives:

1. *Identify major scientific challenges and technological limitations that the bioscience and medical communities are facing today. Identify key areas of impact.* Articulation of challenges and limitations would guide members of the nanotechnology community to put their expertise behind problems about which the biomedical community cares deeply. If technologies are developed to overcome the limitations listed here, they should have far-reaching effect on biomedical fields.
2. *Project how tools and technologies developed by the nanotechnology community can be used to bring new innovations to the biological sciences and medicine, and point to key areas of impact.* The major objectives are to help the biomedical communities to become better acquainted with the potential of the emerging technologies developed by the nanotechnology communities and to identify the key challenges the nanotechnology community has to overcome to realize some of the promising long-term applications.
3. *Identify and prioritize goals.* We defined as short-term goals (1–5 years) biomedical challenges that can be addressed effectively with *already existing* technologies in the nanotechnology community. Mid-term goals (5–10 years) are biomedical challenges that are likely to be addressed effectively with *emerging* technologies in the nanotechnology community, and long-term goals (10–15 years) are those that are likely to benefit us enormously if technologies can be developed to meet those challenges. These are problems that the nanotechnology community should strive to solve as the nanoscience knowledge base grows.

Various communities have held workshops in recent years to identify how nanotechnology can help to advance their missions. Our goal was to build on these reports rather than to duplicate recent activities. To gain a more comprehensive picture of the activities related to nanotechnology, the reader is referred to the reports from those workshops (see list on p. 30 of the NNI Strategic Plan, http://nano.gov/NNI_Strategic_Plan_2004.pdf). In addition, information on a recent series of workshops on nanotechnology research related to cancer detection and treatment is available at http://nano.cancer.gov/.

REGARDING A DEFINITION OF NANOBIOTECHNOLOGY

A topic that arose during the workshop and that, it was agreed, could derail more productive discussion and was therefore explicitly avoided, was to develop a definition of nanobiotechnology.

The NNI definition of nanotechnology serves as a useful starting point for integrating nanotechnology with biology and medicine:

> Nanotechnology is the understanding and control of matter at dimensions of roughly 1 to 100 nanometers, where properties of matter differ fundamentally from those of individual atoms or molecules or bulk materials. Encompassing nanoscale science, engineering, and technology, nanotechnology involves imaging, measuring, modeling, and manipulating

> matter at this length scale, to understand and create materials, devices and systems to exploit these phenomena for novel applications.[5]

While much of biology is grounded in nanoscale phenomena, NIH has not re-classified most of its basic research portfolio as nanotechnology. Studies are classified as nanotechnology projects if they (a) use nanotechnology tools and concepts to study biology or develop medical interventions, (b) propose to engineer biological molecules toward functions very different from those they have in nature, or (c) manipulate biological systems using nanotechnology tools rather than synthetic chemical or biochemical approaches that have been used for years in the biology research community. But for the purposes of this workshop, it was important to avoid defining precise boundaries. Instead, the desire was to stimulate the most productive research and development through communication among the various communities that have much to contribute to, and to gain from, collaboration across traditional disciplinary boundaries.

SAFETY ISSUES

The NNI and participating agencies are actively pursing the development of research to address the biocompatibility and safety of nanomaterials and the broader societal implications of the introduction of nanotechnologies. (Reports of some NNI meetings addressing these subjects are cited above.) These topics were, therefore, not explicitly addressed at this meeting. It was noted during our discussions that, on the one hand, the research possibilities and medical outcomes envisioned at this workshop cannot be achieved without careful attention and research dedicated to the safety and possible toxicity of nanomaterials. At the same time, some of the capabilities developed through the research discussed at this meeting will be critical in addressing these safety and toxicity issues.

[5] Based on *The National Nanotechnology Initiative: Strategic Plan*, p. ii (http://nano.gov/NNI_Strategic_Plan_2004.pdf).

2. ADVANCED IMAGING TECHNOLOGIES: FROM NANO TO MACRO

Principal Contributing Authors: Roger Tsien, Joanna Fowler, and Michael Phelps

MAJOR CHALLENGES IN BIOLOGY AND MEDICINE

New molecular imaging probes and technologies are needed to develop a quantitative understanding of how biological nanosystems work and how these systems are integrated within cells, enabling recognition and processing of a myriad of spatiotemporal stimuli, which ultimately results in coordinated responses within and between cells. From the biomedical perspective, new molecular imaging technologies are needed to probe nanoscale physiological processes occurring in human organs and their response to treatment, whether the intent is to diagnose cancer or any other disease in early stages, or whether the goal is to treat diseased tissues more effectively. New approaches to disease begin with a precise understanding of the changes in cellular circuits and pathways that are related to the pathology. From this knowledge, effective molecular therapeutic interventions can be developed that turn off the disrupted circuits or restore them to normal function. Imaging of disease processes in patients and experimental animal models is of even more direct clinical relevance.

Long-term success requires development of new techniques to probe molecules and molecular activities at the nanoscale with highest possible time, spatial, and chemical resolution and specificity; development of nanoscale sensors and environmentally sensitive molecular probes; and development of algorithms to interpret the measurements in three dimensions and in real time. Deriving a quantitative understanding will furthermore involve integrating information attainable from any one imaging mode with that from complementary imaging modalities and from genetics, biochemistry, physiology, anatomy, behavioral sciences, and clinical medicine. Sophisticated informatics and theory/modeling will be essential in such integration and for building a systems biology view of cell circuits and cell-based networks that establish the integrated functions of organ systems and the whole organism.

A huge number of problems in basic biology could be solved in a straightforward manner if only we could directly see where the relevant molecules are and how they interact in a dynamic way, rather than having to infer their behavior from indirect experiments. Similarly, optimization of clinical therapies would be advanced by improving our ability to predict how molecules behave in living systems—both those of the organism and those used as therapeutics. This understanding, in turn, would improve our ability to control the bioavailability of sensing agents and drugs and would thereby improve the diagnosis and therapy of individual patients. From the perspective of imaging, the ultimate goal would be to observe molecular events and to track the distribution and kinetics of molecular probes, drugs, and environmental agents within the whole animal or awake human under varying physiological conditions, using multiple modalities. Finally, a commitment to translate rapidly the new knowledge and methodologies coming from nanoscience investigations to materials and approaches that can be incorporated into the practice of health care will benefit enormously from application of advanced imaging technologies in the preclinical phase.

Figure 2.1 illustrates one such pathway, from *in vitro* optical imaging and nanosystems biology, to building knowledge and procedures that are then used for *in vivo* imaging in rodents with optical and microPET imaging, and subsequently applied to human patients via positron emission

tomography (PET) [1]. This example is one of many experimental pathways through which various molecular imaging technologies can drive science from the laboratory to patients.

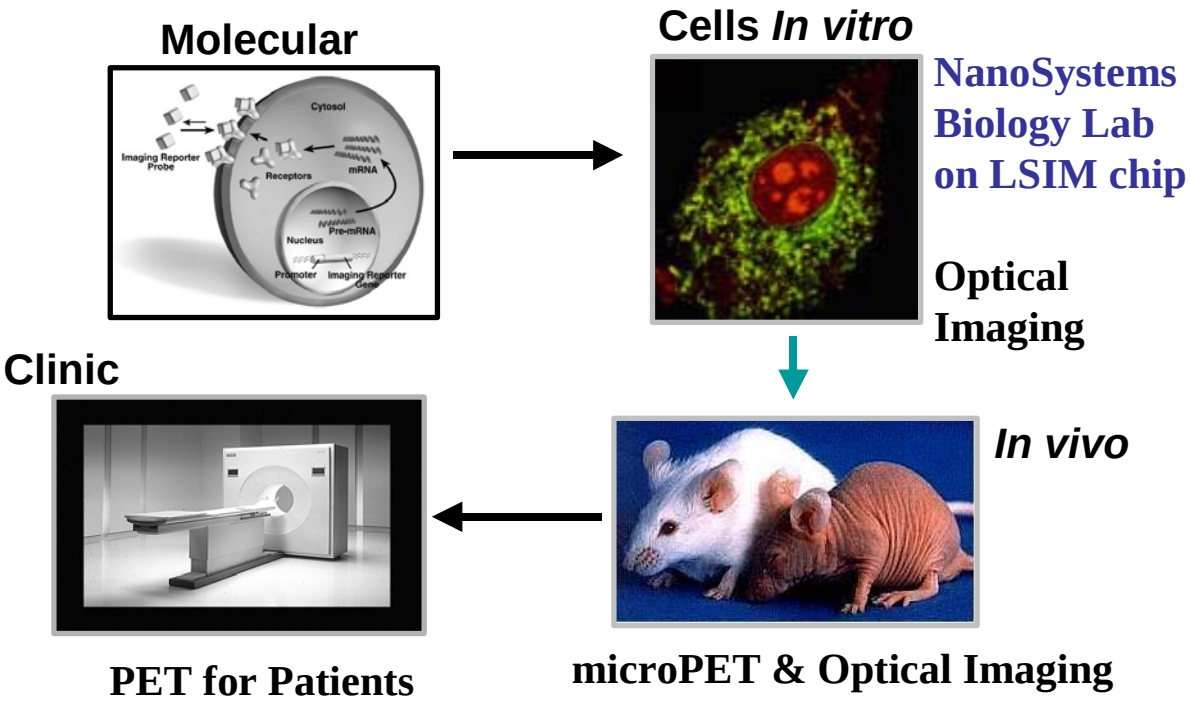

Figure 2.1. Example of a molecular imaging discovery pathway from *in vitro* to *in vivo*, including patients. The large-scale integrated microfluidics (LSIM) chip provides an environment for synthesizing, modifying, and screening diverse arrays of molecular imaging probes for various imaging technologies (optical and PET in example) within the context of the systems biology assay capability with nanotechnologies that also resides on the LSIM chip. These imaging probes are then used in optical imaging of *in vitro* cells and *in vivo* small animal models of disease, along with microPET. This research establishes, simplifies, and directs the molecular probe studies with PET in patients (courtesy of M. Phelps, UCLA; © 2003 Elsevier; reprinted by permission [1]).

NOVEL DEVELOPMENTS IN NANOTECHNOLOGY

Optical microscopy has been the major racehorse harnessed by biologists for imaging and probing dynamic events in living cells and transparent organisms. Imaging of organs and organ function *in vivo* has mainly been advanced by magnetic resonance imaging (MRI), PET, and ultrasound, although none of these technologies currently has nanometer spatial resolution. PET and MRI provide molecular selectivity. The greatest gap in imaging technologies is in the visualization of supramolecular biological assemblies that are too large or disordered for diffraction techniques or nuclear magnetic resonance (NMR), yet too small for optical microscopy. Examples include transcription factor complexes, splicing complexes, ribosomes, plasma membrane signaling domains, protein- and membrane-trafficking complexes including exo- and endocytic assemblies, and sequential multienzyme processes such as participate in the tricarboxylic acid cycle. PET and MRI can, however, isolate and measure the concentration and reaction rates of select components of these systems.

Probing physiological processes at the nanoscale requires novel imaging technologies that are emerging from the nanotechnology communities and are efficiently advancing resolution capabilities. Because many of these advances are highlighted in other NNI reports referred to in Chapter 1, this chapter will only briefly summarize some of the most promising developments of imaging techniques with their spatial resolution pushed toward the nanoscale. The reader also is referred to the special issue in *Nature Biotechnology* focused on optical imaging [2] and the special issue on Imaging in Cell Biology produced jointly by *Nature Cell Biology* [3] and *Nature Reviews in Molecular and Cell Biology* [4]. Imaging applications are further discussed in the subsequent chapters.

Techniques to Enable Optical Microscopy at the Nanoscale

The resolution of conventional optical microscopy is set by the diffraction limit of light (about 0.5 μm), restricting its capacity to probe the spatiotemporal organization of cells. Visualizing molecules and molecular assemblies involved in cellular activities demands new methods with nanometer resolution. Although electron microscopy provides such resolution, it is currently limited to using dried or frozen samples and so cannot monitor dynamic events in real time. Novel optical imaging schemes are being developed that circumvent the diffraction limit of light, thus resolving features of living cells at well below micrometer resolution. For example, near-field scanning optical microscopy (NSOM) is configured so that light is passed through an aperture whose diameter is much smaller than the wavelength of light. By scanning samples with the evanescent tail of light passing the aperture, a lateral resolution of 50 nm is currently possible [5]. Another promising new technique is stimulated emission depletion (STED) microscopy, which improves the axial resolution in confocal microscopy to better than 30 nm [6, 7]. Broad utilization of these new techniques by biomedical scientists will come after such instruments become more accessible to those who are addressing biological questions of interest.

Applicable to all optical microscopy techniques and currently used widely, fluorescence resonance energy transfer (FRET) can probe distances and distance changes between energy donors and acceptors in the 1–10 nm range, including those associated with conformational changes and molecular assemblies [8]. Fluorescence correlation spectroscopy (FCS), in conjunction with multiphoton excitation, can be used to monitor dynamic molecular associations that are spatially and temporally controlled by evaluating cross-correlations. Total internal reflection fluorescence microscopy (TIR-FM) can be used to observe fluorescence within 100 nm of a surface and, combined with dynamic measurements such as fluorescence recovery after photobleaching (FRAP) or anisotropy, can evaluate lateral and rotational mobility, respectively. Finally, optical tweezers can be used to grab and steer a nano- and microscale object by trapping it in a laser beam. In addition to moving the particle, which could be a biomolecule, an organelle, or a synthetic bead, optical tweezers also allow well-controlled mechanical forces to be applied to selected objects at the same time that their response is being recorded.

Atomic Force Microscopy

A major breakthrough in our capacity to probe structures at the nanoscale came with the invention of atomic force microscopy (AFM), in which the surface of interest is scanned with nanometer resolution by a sharp tip mounted to a cantilevered arm. AFM imaging provides a powerful advantage over electron microscopy because samples do not have to be dried, therefore enabling observation of both static and dynamic processes (limited by the scan rate). Furthermore, the mechanical properties of single molecules or molecular assemblies can be determined by studying their response to controlled perturbations. The tip of an AFM, although typically used to measure the surface topography of an object, also can be operated to apply a loading force on nano- and

microscale objects in a controlled manner and to record their responses. For example, AFM has been used to image proteins embedded in membranes, to probe the interactions among receptors and their ligands, and to probe the elasticity of biological samples or their surface chemistries. Thus, AFM provides unique capabilities to image and probe the physical properties and dynamics of biomolecules, as well as cellular and subcellular systems. Because the main contrast mechanism is surface topography or stiffness, means to resolve chemically distinctive markers need to be developed; for example, redox active proteins able to funnel electrons via tunneling to the tip. The capacity to genetically tag specific surface features with a distinctive contrast signal would greatly broaden the application of scanning microscopies.

CHALLENGES AND RECOMMENDATIONS

Complementing new imaging techniques is the requirement for smarter, more biocompatible, more precisely targetable, more flexibly manipulable probes to enhance the sophistication by which physiological processes can be examined, from biomolecular events to the coordinated functions of organs. Detailed challenges are discussed below.

Short-Term (1–5 years)

Multifunctional Imaging

No one imaging modality can cover all spatial, temporal, and molecular selectivity domains or all molecular events. Combinations of modalities will be necessary to image and understand most events and processes. To enable correlation of these different types of imaging, a primary approach is to construct molecular probes that can be visualized by multiple modalities at once. Examples include fusion proteins incorporating bioluminescence (BL), fluorescence (FL), and PET reporters (e.g., Fig. 2.2 below) [9]. Combining optical and electron microscopy is enabled by the tetracysteine-ReAsH system [10], in which a protein of interest is fused to a 6–12–amino acid, cysteine-rich sequence, which then binds the label. Because ReAsH is visible in a living or fixed cell, it can be used to correlate fluorescent video images with postfixation electron microscopic (EM) snapshots to provide higher resolution. More such combined labels should be engineered, with genetic encodability being a highly desirable feature.

The easiest generic approach to creating multifunctional probes is via polymers or nanoparticles that can be decorated with multiple types of labels as well as targeting/recognition motifs. Experimentally powerful particles currently include semiconductor “quantum dots” providing extraordinarily bright fluorescence, metal nanoparticles with ultrahigh extinction coefficients for labeling in colorimetric and surface plasmon resonance assays, and elongated “nanorods” for measuring anisotropy. To extend applications, new chemistries are being developed for particle modification and coating, improving biocompatibility and expanding targeting and other functional capabilities. Thus, a new generation of spectroscopic probes is steadily becoming available with considerable potential for biological researchers. Nanostructured biological probes, which can be a few nanometers in diameter (extended by any coating), can provide substantially enhanced signal and multiple functional groups and might eventually be less costly than common organic reagents. In addition, the specific photochemical reactions that cause organic probes to photobleach or crosslink nonspecifically with biological samples may be minimized for inorganic nanostructures. Research into tailoring the optical properties, surface chemistry, and biocompatibility of metallic and semiconductor nanoparticles, or creating polymeric (dendrimer) or silica particles with selected properties, all enable engineered nanostructures to be customizable substitutes for organic molecular probes for biological applications.

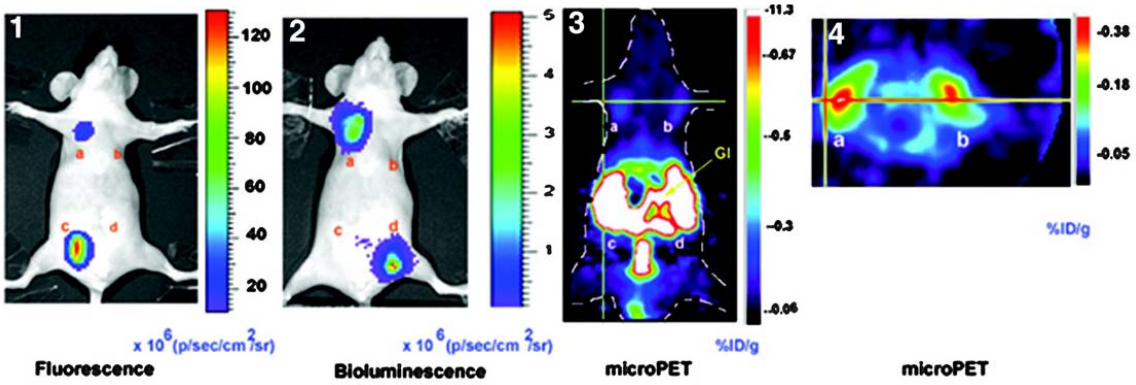

Figure 2.2. Results of imaging living mice. Fluorescence, bioluminescence, and micro–positron emission tomography (PET) imaging of hrl-mrfp-ttk expression in the same living nude mouse. Ten million 293T cells transiently expressing the CMV-hrl-mrfp-ttk, CMV-ttk, CMV-mrfp1, and CMV-hrl plasmids were implanted subcutaneously at four sites on the ventral side of a nude mouse and imaged the next day for fluorescence/bioluminescence and PET, using a cooled charge-coupled device camera and microPET, respectively (courtesy of R. Tsien, UCSD; reprinted by permission from [9]).

Even within one imaging modality, independent of the length scale, we often need to distinguish multiple structures or events occurring simultaneously. A familiar example is multicolor fluorescence to label different proteins. A limitation in multicolor imaging with standard fluorophores is that just a very few different molecules can be simultaneously tagged if their absorption and emission spectra overlap. An advantage of quantum dots is that their emission spectra are much sharper and their emission wavelength can be tuned by adjusting particle size, and all quantum dot sizes can be excited by irradiation with light in the near ultraviolet. Thus, investigators may be able to introduce a set of quantum dot probes including as many as 10 colors to visualize and distinguish among different classes of target molecules simultaneously.

Many other imaging modalities need to develop analogies to multiple colors. The conventional EM technique of immuno-gold labeling is capable of distinguishing two or more distinct molecular species simultaneously by using different sized gold particles, but the low percentage of antigens that get labeled makes detailed analysis of protein–protein associations difficult to measure with this approach.

Energy loss spectrometry offers a potential dimension along which different heavy element stains could be distinguished. Other imaging modes also have sophisticated signal dimensions to provide their analogies to multicolors, but the molecular probes and instrumentation needed to exploit these dimensions need much further development. For example, PET imaging [11], commonly used in both animals and in awake humans, can visualize and quantify many different molecular targets (enzyme activity, receptors, transporters, gene expression, synthesis, and metabolism) within the same individual in the same day because of the short half-life of the isotopes (20–110 min) [12]. In this way we can begin to construct a picture of various biochemical systems, how they interact, and how they are affected by drugs, environmental exposures, genetics, disease, and other factors. Drugs can also be labeled to image and measure their pharmacokinetics and pharmacodynamics throughout the body in animals and patients. This can dramatically change the drug discovery process because at present, pharmaceutical companies do not know whether a drug hits the intended target or whether it occupies the target to a sufficient degree to induce the desired pharmacologic effect in patients. These imaging methods are also amenable to assessing the effects of exposures to nanoparticles on specific biochemical pathways.

Multiple imaging instruments are also being combined into single instruments to provide simultaneous or near-simultaneous images of multiple properties. PET/computed tomography scanners provide molecular and structural images fused into one image. Optical/microPET (OPET) provides dynamic, spatial, and molecular images from optical and PET molecular probes simultaneously from mice. MRI can provide structural molecular images.

Bioconjugation and Targeting

Crucial to most probes or contrast agents is their mode of targeting to desired biological sites. In the extreme case of radioactive probes, molecular localization is their only mode of selectivity, because biology can never change the nuclear physics of decay. Achieving the desired bioavailability and selective delivery and localization on the whole organism level, even with small radioactive probes, is still a challenge that needs to be addressed systematically to facilitate radiotracer development for molecular imaging. More daunting yet is delivering molecular probes or contrast agents that are complex molecular entities to the correct location in the cytoplasm or nucleus, where the plasma membrane must be crossed, or in the central nervous system, where the blood–brain barrier must be traversed. Substrate analogs, drug analogs, and small molecules used in PET provide one means to circumvent this problem. The other alternative, which is the most generic and widely advocated solution, is the use of polycationic peptides such as those derived from HIV tat, or simpler multiarginine sequences. Despite much early promise, it remains unclear how these peptides work, why they sometimes fail, into what intracellular compartment they deliver their cargoes, and the final metabolic fate of the peptides and their cargoes. Basic mechanistic understanding of these peptides' mode of action on the whole-organism level will be crucial.

General approaches to target specific proteins or organelles currently are limited to genetically encoded probes or contrast agents, which exist only for a few modalities, most spectacularly fluorescence and bioluminescence. A striking example is green fluorescent protein (GFP). Molecular engineering of the GFP coding sequence has allowed optimized expression of GFP in different cell types, as well as the creation of GFP variants with varied spectral properties, including increased brightness, relative resistance to pH variation, and photostability [13]. More genetically encoded contrast agents, especially for nonoptical imaging, are sorely needed.

Even for probes and contrast agents targeted to extracellular non-CNS sites by means of antibodies or aptamers, significant problems remain of circulatory lifetime, steric accessibility of ligands within tortuous extracellular compartments, and immunogenicity. Some of these problems can be somewhat ameliorated by reduction of the antibody to single polypeptides containing the binding site (scFvs) and by converting to human-compatible sequences. However, these reengineering processes are both laborious and often proprietary, limiting their application to blockbuster therapeutic antibodies. Moreover, radioimmunotherapeutic agents will need to be developed with knowledge enabling prediction and control of their localization in various organs in the body to minimize toxicity to nontarget organs.

Faster means of synthesis and testing of probes and contrast agents would be highly desirable. One promising approach is integrate chemical synthesis and nanotechnology-based assays of biology onto integrated microfluidics chips to accelerate, diversify, simplify and lower the cost of producing and biologically screening molecular imaging probes and labeled drugs.

Engineered Fluorescent Proteins

GFP, horse radish peroxidase (HRP), and other genetically encodeable marker proteins have already revolutionized cellular imaging, but development of proteins with a wider range of properties (including greater environmental sensitivity, photostability, or smaller size, for example) should continue. An important new capability would be to generate singlet oxygen on illumination, because this highly reactive oxygen species allows for the controlled photoinactivation of proteins in the immediate vicinity of the singlet oxygen generator [14]. These species would also enable electron microscopic visualization via photooxidation of diaminobenzidine, as is currently done with HRP probes.

Quantum dots are enormously exciting new optical and electron microscopic contrast agents, but they still have significant problems with biocompatibility, toxicity, stability (including photostability), and complex photophysics such as blinking. Targeting to particular sites on or in living cells (other than the endocytic pathway) is an additional major challenge to overcome. Metal nanoparticles detected by scattering or reflectance are alternative contrast agents in which the stability and photophysics problems are alleviated, but biocompatibility and targeting remain key challenges.

An invaluable adjunct to imaging is the ability to suddenly perturb the biological system, preferably with the spatial and temporal precision of photochemistry. In some cases the biological metabolite is intrinsically photosensitive—e.g., DNA crosslinking, photooxidation of NAD(P)H—but in most cases photosensitive reagents—e.g., caged compounds—have to be designed and synthesized. Controlled, instantaneous perturbation is also a powerful mechanistic tool for interrogation in nuclear imaging of animals and humans in which a drug or other stimulus is given in a serial study to assess response.

Systems (Whole Animal and Patient) Imaging

On the whole-organism level, PET and SPECT (single photon emission computed tomography) radiotracers provide low spatial resolution (0.8–6 mm) but exquisite molecular resolution because of their high affinity and selectivity for specific molecular targets. For example, presynaptic elements (dopamine transporters, for example) and postsynaptic elements (e.g., dopamine receptors) can be distinctly visualized in the same individual even though they are only 10–50 nm apart because the radiotracers are selective for the transporter or receptor (see Fig. 2.3) [15].

One of the most promising means to extend high-resolution optical imaging to greater depths (> 1 mm) is via fiber-mounted endoscopic microscopes with confocal or multiphoton optical systems [16]. This is a ripe field for microelectromechanical systems (MEMS), especially at the tip of the fiber. Miniaturization, ruggedness, disposability, and sterilizability will be important criteria in addition to optical performance per se.

Even less invasive optical methods include fluorescence tomography, optical coherence tomography (OCT), and various photon-ballistic techniques. Although advances in all such methods would be valuable, one particular application of nanotechnology would be the development of genetically encoded or injectable nanoreflectors for OCT, as some organisms know how to biosynthesize efficient reflectors; for example, by assembling quarter-wave stacks of membranes [17, 18].

Promising new developments in MRI include labeling of cells such as macrophages or stem cells *ex vivo* with magnetic nanoparticles, then reintroducing such cells into the intact organism to

monitor their subsequent fate. High-field magnets are also making possible the imaging of physiologically important elements such as $^{39}K^{+}$, $^{23}Na^{+}$, and $^{35}Cl^{-}$, all of which have been relatively hard to observe until recently.

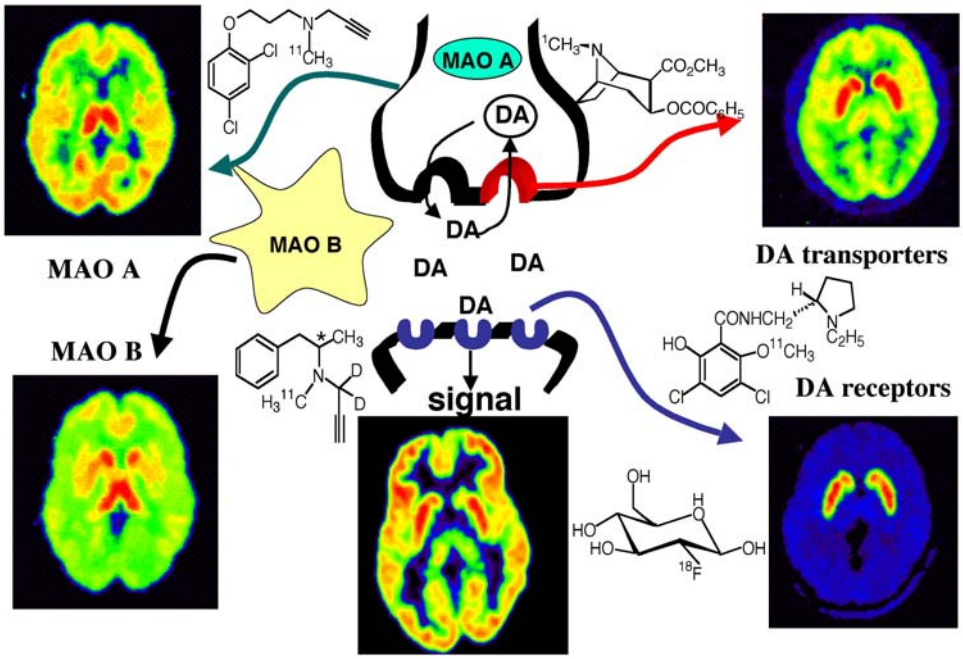

Figure 2.3. Diagram of the dopamine nerve terminal illustrating that the dopamine transporter (shown in red), the dopamine receptor (shown in dark blue), monoamine oxidase A (MAO A), and monoamine oxidase B (MAO B), as well as brain function can be imaged with a PET scanner, using radiotracers labeled with short-lived isotopes carbon 11 and fluorine 18. Images are of the human brain, using a rainbow color scale, where red indicates the highest levels and blue indicates the lowest levels. Note that dopamine transporters and dopamine receptors are only 10–50 nm apart (the approximate distance of the synaptic gap). They look similar in the regions that are intensely colored because they are in the same anatomical region of the brain. However, because they are on different cells and in different pharmacological concentrations, they do not interfere with each other's binding [15] (courtesy of J. Fowler, Brookhaven National Laboratory).

Major medical challenges for which nascent nanotechnology-based solutions may be beginning to appear include imaging of inflammation, metastasis, angiogenesis, atherosclerosis, amyloid fibril deposition, and cell degeneration (apoptosis and necrosis). For all these pathologies, the key is the invention of contrast agents that either home in on the disease-marking molecules or sense these molecules and become activated in their contrast properties (or both). The recurring challenge is to understand the variables that control the distribution and stability of contrast agents within the whole organism as a springboard for improving bioavailability and accomplishing specific targeting to selected sites.

At the other extreme from the endogenous or specifically engineered miracle machines of life, we also must be concerned with exogenous toxic nanoparticles, such as in air pollution and tobacco smoke (e.g., see Fig. 2.4), or airborne pathogenic organisms. Means for tracking their biochemical effects and their fate within cells, organs, animals, and humans, possibly requiring extrinsic labeling of the particles, will be of considerable practical importance.

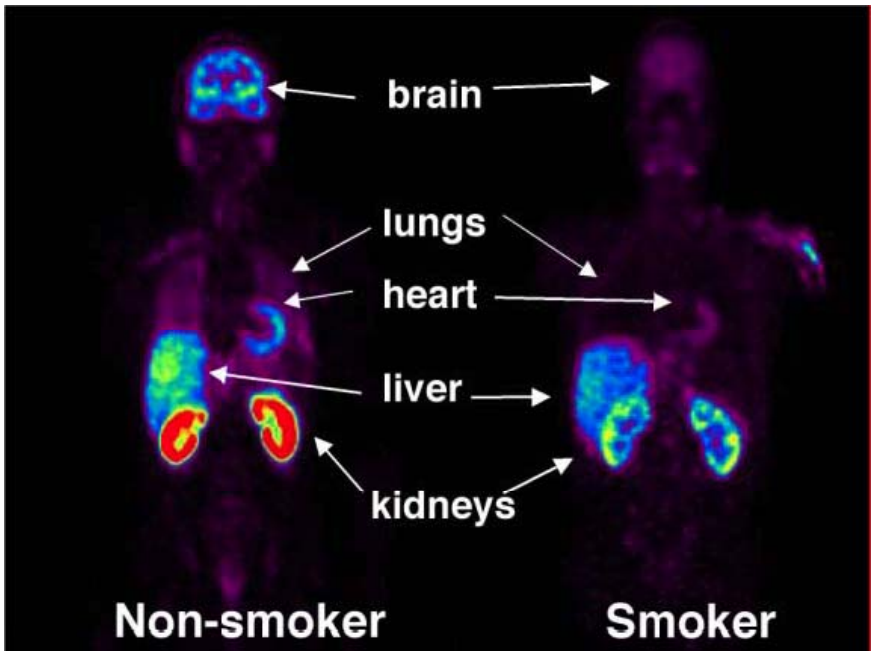

Figure 2.4. PET images of MAO B in the nonsmoker and the smoker, showing that MAO B is reduced in the brain, lungs, heart, and kidneys in the smoker. This illustrates how neuroimaging reveals the pharmacological effects of drug exposure on a particular molecular target as well as the fact that the pharmacological effects of tobacco smoke extend far beyond the lungs and that, in this case, the effects are not caused by nicotine (courtesy of J. Fowler, Brookhaven National Laboratory; © 2003 National Academy of Sciences; reprinted by permission [19]).

Mid- to Long-Term (5–15 years)

A long-term goal that can be reached with the contributions of nanobiotechnology is the development of a range of nanoparticles and other structures that can be targeted to specified locations in biological systems, probe the environment, serve as contrast markers, deliver chemicals, sense the response, and regenerate the surrounding tissue. Ultimately specifically designed nanostructures would be directed within a living organism (a human patient) and controlled by a remote operator, with communication occurring in real time. The enormous experimental possibilities offered by quantum dots are currently limited by biocompatibility and functionality. An audacious idea for harnessing quantum dots would be to evolve microorganisms or organelles able to synthesize quantum dots within themselves. Certain yeasts are known to be able to deposit intracellular cadmium sulfide particles of a controlled size of 1.8–2.0 nm [20]. If size and wavelength could be genetically controlled in a way transferable to other organisms, this would provide an ideal solution to the challenges of biocompatibility and targeting.

Other types of biocompatible structures with built-in functionalities or chemistries for modification may be drawn from nature. For example, a biomimetic challenge would be to harness the ability of organisms such as diatoms and corals to deposit intricate nanostructures of silica or calcium carbonate. One potential application would be to deposit refractive scattering objects for reflectance-based or ballistic imaging. An even more difficult goal would be to biologically generate *in situ* optical light guides to help channel light to and from deep sites of interest. This would be an important step if the current generation of miniaturized endoscopes, still wired to the outside, leads to highly sophisticated MEMS-based microscopes and cameras that are tetherless. These would need endogenous power sources, supremely miniaturized image sensors, and passive

or active means of homing to desired sites. The image data would have to be either recorded internally to be downloaded after recovery or transmitted continuously and wirelessly back to external recording devices.

For nonoptical imaging modalities, revolutionary new contrast agents may be possible. For example, the most important type of contrast for ultrasound imaging comes from gas bubbles. Although organisms cannot be expected to generate perfluorocarbons, one can easily make them nitrogen supersaturated simply by breathing air under pressure for a while and then bringing the ambient pressure back to normal. A targeted protein that catalyzed bubble formation would create local bubbles easily visible by ultrasound, which would last until the supersaturation dissipated (probably several hours, judging from decompression sickness, which is simply uncontrolled bubble formation). The medical utility of approaches like this would depend not only on the capacity to target the bubble-producing protein (possibly on a nanoparticle together with sensing and other functionalities) but also on controlling the outcome of the other part of the patient treatment—supersaturation with air.

For magnetic imaging modes, acceleration of current efforts to clone and characterize the proteins that control deposition of magnetite in magnetotactic bacteria [21] would be potentially very profitable. Such biocompatible single-domain nanoparticles would provide genetically encodable contrast agents for MRI, magnetoencephalography, and transcranial magnetic stimulation. Even if we are unable to isolate and control those genes, an alternative strategy would be to harness the intact bacteria. Already they naturally migrate to zones of low oxygen tension; perhaps they could be modified to allow introduction into animals or patients, where they would migrate to zones of hypoxia and increased endothelial permeability (i.e., metastatic tumors and infarcts). Of course, any possibility of pathogenicity and immunogenicity would have to be minimized.

New technologies are required to rapidly synthesize, modify, label, and biologically screen diverse arrays of molecular imaging probes. Initial work has begun on using LSIM chips made of various polymers. These chips allow large-scale (10^3–10^4) digital operations that are controllable and addressable from a personal computer (PC). These operations can yield a landscape on the chip composed of chemistry labs, molecular libraries for storing molecular probes and drugs, cell culture labs, and various nanotechnologies and conventional assays for measurements of transcription, translation, protein–protein or protein–probe interactions, and so on. All components within the chip are connected by fluid channels controlled by the PC [22]. These LSIM chips also collect systems biology information so that molecules can be evaluated within systems biology, and not just their interaction with a target. Research is needed to develop the nanotechnology assay technologies and produce new polymers for chip construction that are resistant to chemical solvents and reagents when compared to the water-based LSIM chips of today.

From the broadest perspective, the ultimate goal would be to observe molecular events and track the distribution and kinetics of molecular probes on the whole organism level under varying physiological conditions, using multiple modalities. Virtually all animal imaging is currently done under anesthesia. This has a profound influence on the behavior of chemical compounds in living systems, particularly in the brain. A challenge is to develop imaging technologies and animal preparations to probe these processes so that they can ethically be performed in awake animals without the confounding effects of anesthesia [23].

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3. *IN VIVO* ANALYSIS OF CELLULAR PROCESSES AT THE NANOSCALE

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MAJOR CHALLENGES IN CELL BIOLOGY

Deriving a quantitative understanding of how the genetic blueprints of cells ultimately regulate cell function and behavior remains an ultimate goal in cell biology [1]. Although the tools of biochemistry and molecular biology have provided impressive knowledge about many cellular components, the challenge of deciphering the hierarchical architecture of molecular networks and how they are regulated is daunting. Major challenges that need to be overcome include a detailed understanding of how cells of all types sense external and internal signals and how these signals are processed to yield particular responses, including stimulated secretion, growth, differentiation, motility, contractility, and apoptosis. Moreover, although all cells function as biochemical factories of a broadly similar type, the details of their molecular transactions are vastly different depending on the cell's identity and physical location (e.g., macrophage in the bloodstream, neuron within the brain, or hepatocyte within the liver). For substantial advances over the next decade, the community needs methods that enable a fundamental inventory of cell structures and functions at all levels of organization and that allow integration of information from all subcellular mechanisms simultaneously. This comprehensive, hierarchical knowledge base can provide biomedical/clinical researchers with the ability to diagnose and intervene at the minimal subcellular level when disease occurs. A grand challenge is to be able to identify cell pathosis, return the altered cell to normal operational parameters, or remove it before it causes the derangement of other cells, or to retrain deranged cells to return them to a developmental state for optimal repair or regeneration.

Cell growth, division, and differentiation involve the activity of numerous intracellular processes, such as DNA replication and transcription, protein synthesis and transport, regulated secretion, organelle and cytoskeletal assembly/disassembly, and apoptosis. These processes occur fundamentally on the nanoscale with interactions between DNA, RNA, proteins, carbohydrates, and lipids—the cell's basic molecular building blocks—that are integrated within a cell on the scale of tens of nanometers in molecular assemblies (e.g., chromosomes, ribosomes, receptor complexes, and proteosomes) and also on the micrometer scale in membrane compartments, cytoskeletal architecture, and other intracellular structures. Cell homeostasis, involving the dynamic interactions between these biochemical assemblies and compartments, is maintained by regulated gene expression and metabolism. Homeostasis is disrupted when the cell senses and responds to extracellular stimuli through cellular signaling pathways that are restricted in time and space. This can change the cell's own differentiation state as well as affect other cells in surrounding tissue and throughout the whole organism.

Investigations of the physical properties and functions of cells currently depend heavily on molecular imaging, which can reveal when and where genetically or biochemically defined molecules, signals, or processes are formed, transformed, and consumed in space and time (Fig. 3.1). GFP and other genetically encodeable probes have provided great versatility as reporters when attached to a protein of interest, while minimally perturbing the cell under investigation [2]. Although the tagged molecules must be checked for proper localization and activity, GFP and its variants have empowered real-time imaging and analysis of live cells. These probes have further

been used in advanced techniques, including FRAP, FRET, and FCS (see Chapter 1), which allow evaluation of protein diffusion, transport, turnover, and protein–protein interactions within living cells [3, 4].

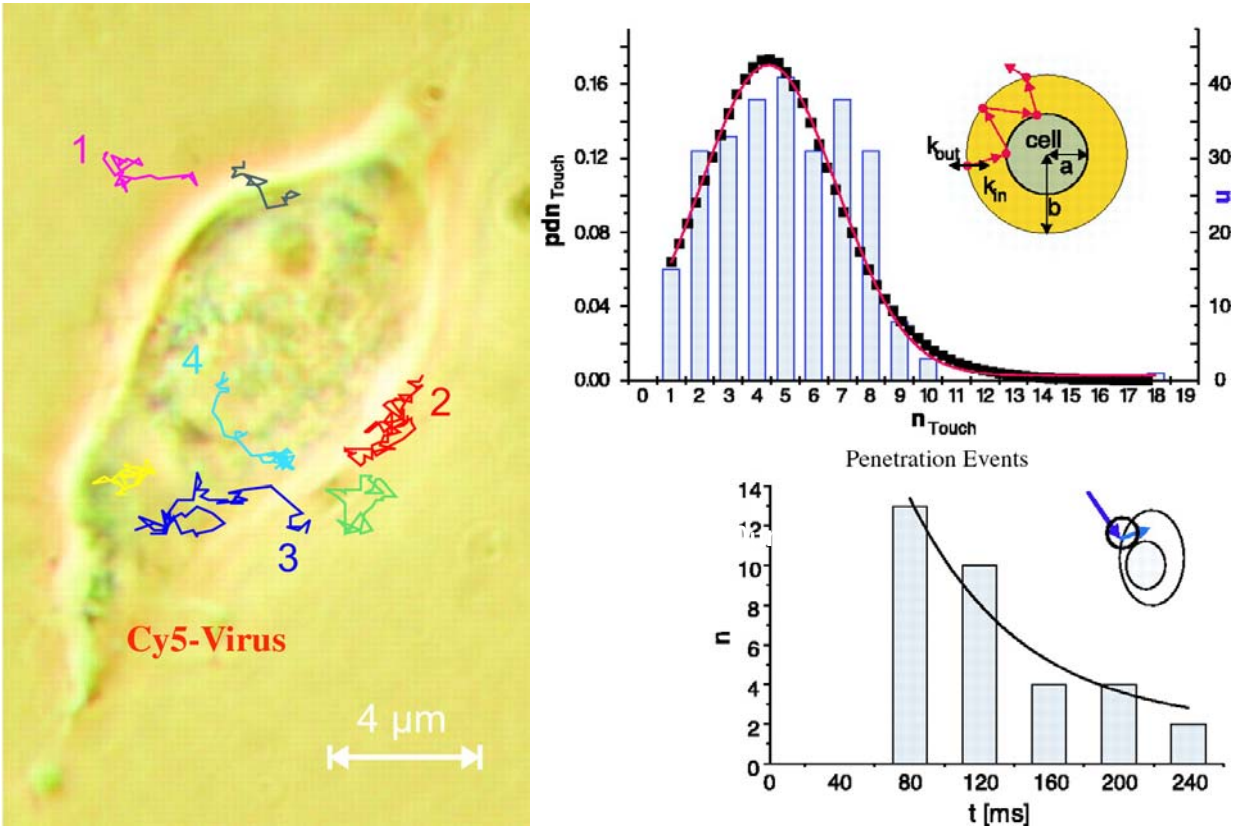

Figure 3.1. Tracing the trajectories of single adeno-associated viruses as they attack and infect a living HeLa cell (adapted from [5]; © 2001 AAAS; reprinted by permission).

The information obtained from these approaches is rapidly expanding our understanding of the dynamic spatial organization and function of molecules, molecular assemblies, and compartments within cells. However, substantial limitations remain both with the variety of probes currently available and with the spatial resolution provided by conventional optical microscopy (half the wavelength of the excitation light, typically > 200 nm). Electron microscopy can provide nanoscale spatial resolution [6] but is hampered by the need for fixatives or rapid freezing. NSOM and AFM are emerging as promising microscopies for nondestructive continuous imaging of living specimens with resolution below 100 nm (see Chapter 2). Development is lagging for independent and corroborative magnetic and other types of imaging techniques on reduced length scales. In addition to advanced imaging techniques, the field stands to benefit enormously from the development of multifunctional nanoparticles and other artificial probe molecules as well as supramolecular assemblies that effectively mimic critical biological functions and that can be reintroduced into living cells to manipulate and report about regulatory network structure and function.

Numerous micro- and nanofabricated tools are being used in studying the behavior of molecular components in the complex environment of the living cell. These include microinjection instruments that allow chemicals to be locally applied to cells, laser tweezers that allow cells to be physically manipulated, engineered transfection reagents, fabricated surfaces for cells to grow on, and devices for extracting cells for compositional analysis with mass spectrometry and other powerful techniques. As discussed below, new advances and applications of these nanotechnology-based tools offer tremendous promise for the future.

The field of cell biology also draws heavily from genomic and proteomic analysis, which can provide high dimensional data sets (e.g., from microarrays) but with limited informational context as to how these components work together in living systems. It is therefore necessary to integrate this growing database with the visualization of molecular distributions and dynamics at high spatial and temporal resolution in the physical context of living cells. To understand hierarchical mechanisms of cellular regulation over many length scales, the field must gain the capability to apply controlled chemistries and forces and sense the effect of these at the molecular scale while simultaneously reading out changes in molecular, supramolecular, and cellular function. Currently not available are (massively parallel) tools to simultaneously read out spatio-temporal dynamics and functional activities of multiple (thousands) of molecules in living cells, tissues, and organisms. Finally, the development of predictive computational methods to model complex cell behaviors requires detailed information on molecular and supramolecular interactions under dilute and crowded conditions. Advances in deciphering the complexity of the functional regulation of cells will be an iterative process integrating computational approaches with experimental work [7]. Computational models are needed to point to new experiments. They then need to be refined based on experimental data in an iterative process, and eventually be able to predict how cells function and misfunction.

Understanding how individual cells work is only part of the challenge. Another important question is how cells become organized within three-dimensional tissues with specialized form, function, and mechanics. Although most of our insights into cellular processes have been derived from the study of two-dimensional cell cultures, recent research points out that cells behave rather differently when cultured in two-dimensional environments than when cultured in three-dimensional environments [8, 9]. These observations demand reexamination of the significance of experimental data derived in two-dimensional cell culture and their significance in predicting the effect that certain drugs, for example, have on a targeted cell types in tissues and organs. Despite this recognition, investigations are limited by the lack of elaborate methods to control either two-dimensional or three-dimensional cell organization. This includes the lack of tools to define the mechanical and structural properties of tissues from the nano to the microscale. Other limitations are our limited capabilities to control extracellular matrix structure, function, and chemistry *in vivo* and to provide synthetic matrices that can serve as true three-dimensional biomimetic scaffolds for basic research and tissue engineering.

NOVEL DEVELOPMENTS IN NANOTECHNOLOGY

Many novel technologies are beginning to translate between the nanotechnology and cell biology communities that address one or another challenge discussed above. Emerging optical imaging technologies discussed in Chapter 1 are enabling visualization of the distribution and dynamics of proteins, with spatial three-dimensional imaging at the nanoscale and with short time resolution. New approaches are beginning to address increasingly difficult questions about how molecular composition changes in the physical context of living cells. The development of nanotechnology-

based tools for applying physiologically relevant stimuli to selected sub-micrometer cellular locations is beginning to allow the cell's responses to a variety of realistic physical perturbations to be measured and interpreted. These and other tools of great potential to the biomedical communities are being developed, based on advances in micro/nanofabrication, molecular assembly, synthetic nanosystems, and other approaches.

In Vivo Nanoprobes

Nanoprobes combined with optical detection schemes are emerging to evaluate the functional states of proteins and other biomolecules in living cells and to detect changes that occur as a result of defined stimulations. For example, single-GFP-based indicators have been engineered to respond to pH, halides, free Ca^{2+}, or redox potentials, and intramolecular FRET-based indicators have been engineered to monitor intracellular Ca^{2+}, cyclic GMP, GTPase, and kinase activities [10], as well as force-induced stretching of biomolecules [11]. Protein–protein interactions, cyclic AMP dynamics, and clustering in membrane domains are also being investigated with intermolecular FRET-based probes [12]. Alternate detection schemes allow for sensing of electrochemical processes, transmembrane voltages, ion fluxes, and many other cellular processes.

Determining a protein's kinetic properties can be accomplished with new techniques for highlighting specific populations of molecules. One recently developed technique is photoactivation of molecules to a fluorescent state with a brief pulse of high-intensity irradiation [13]. These fluorescent proteins can be followed as they reequilibrate in the cell (Fig. 3.2). The extent and rate at which this occurs can be quantified and used to describe the kinetic parameters of a protein, such as its diffusion rate, compartmental residency time, and speed of degradation. Photoactivatable proteins also can be used to study cell lineage and cell migration in developing or diseased organisms, and they can be used to highlight and later target selected cells within a heterogeneous cell population. An expanded set of photoactivatable proteins needs to be developed, including some with different spectra that allow multiple protein species to be highlighted and followed simultaneously within cells, or that can be used in FRET-based techniques to study protein–protein interactions in greater detail.

Tracing and Manipulating Single Molecules in Living Cells

Atomic force microscopy recently has been applied to studying the mechanical properties of selected molecules and molecular assemblies on the surfaces of living cells. This includes measurements of the forces, binding constants, and stoichiometry of individual molecules, and their interactions. Optical tweezers have been used to stretch biomolecules and to manipulate single organelles in living cells, potentially allowing for nanodissections of organelles for compositional analysis with mass spectrometry and other analytic methods. Magnetic methods also have been used for manipulating and probing molecules (with nanometer resolution) and for controlling cellular biochemistry/gene expression in living cells [14]. Another approach, chromophore-assisted light inactivation (CALI), uses photochemically generated, reactive oxygen species to inactivate proteins acutely [15]. Nanotechnology-based regulation of the photostability of spectroscopic probes combined with enhanced imaging capabilities, as discussed in Chapter 2, will further allow single molecules in living cells to be traced, potentially including their characterization and manipulation.

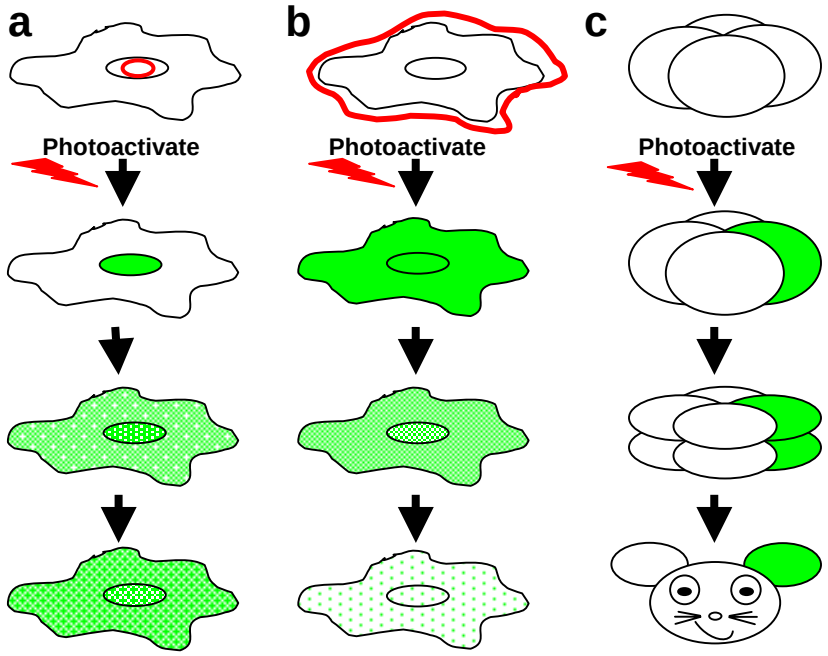

Figure 3.2. Photoactivation of fluorescent proteins. (a) Before photoactivation, cells expressing photoactivatable proteins display little fluorescence in the spectral region used for detecting enhanced fluorescence. After photoactivation of a selected region (indicated in red), an increase in fluorescence is observed. By directly highlighting specific populations of molecules, such as the nuclear pool of the fluorophore, the movement from this region throughout the cell can be monitored. (b) Alternatively, the entire cell can be photoactivated and the fate of the fluorescence followed over time. Because newly synthesized proteins are not detected or photoactivated during the imaging experiment, photoactivatable fluorescent proteins circumvent this possible artifact and might allow us to monitor the fate of fluorescently tagged proteins by optical pulse labeling. (c) Photoactivation of a single cell or population of cells can be used to monitor cell lineage within a developing organism (courtesy of J. Lippincott-Schwartz, NIH).

Microfluidic Devices with Integrated Nanofeatures (NEMS)

Novel schemes are being developed to integrate nanoscale pores or sensors into microfluidic devices to probe, measure, and manipulate the activity of single cells with high spatio-temporal resolution [16]. Although the effect of chemical stimuli on cells was typically studied by adding the stimulus to the cell medium and averaging over the response of a large cell population, more recent efforts interrogate single cells as they are responding to spatially controlled stimuli; for example, releasing a growth factor through a nanopore in close proximity to the cell surface. This also includes application of physiologically relevant stimuli (chemical, electrical, mechanical) to selected sub-micrometer locations both on and within cells. These devices must be able to operate on living cells while preserving their functionality. Integrating multiple nanoscale probes or sensors into microfluidic devices has the added advantage that various stimuli might be applied or that multiple readouts might be acquired in parallel from single cells. Integration of nanoelectromechanical systems (NEMS) devices with other analytical techniques provides many new opportunities. For example, analysis of the molecular content of single cells after cell lysis becomes possible in combination with two-dimensional electrophoresis (Fig. 3.3) or mass spectrometry. Detection of just a few copies of molecules that may serve important regulatory

functions can come from combining NEMS with single-molecule detection schemes. Advanced analytic methodologies that are being used in conjunction with nanotechnology are described further in Chapter 5.

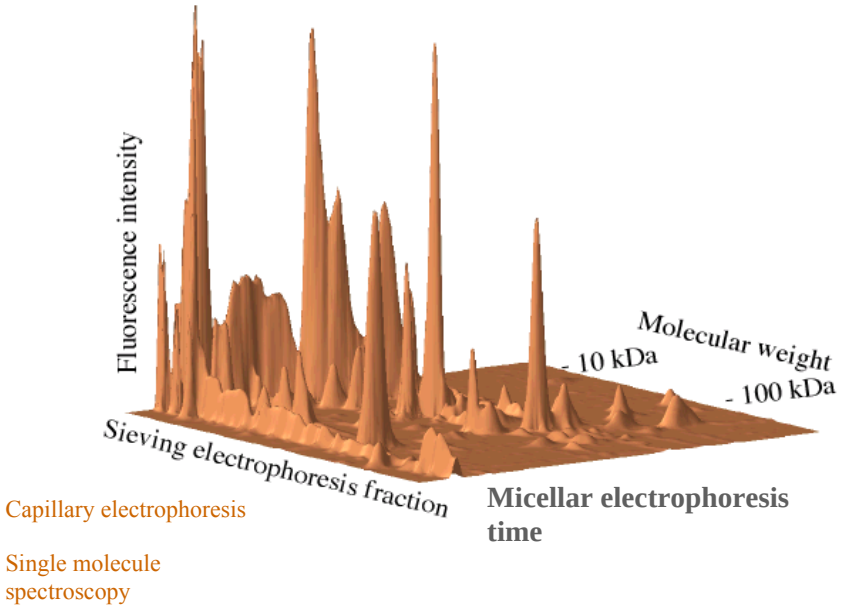

Figure 3.3. Two-dimensional capillary electrophoresis analysis of the protein content of a single osteoprogenitor cell. High-efficiency electrophoresis is coupled with high-sensitivity laser-induced fluorescence detection to monitor protein expression in a single mammalian cell. The cell contains roughly 2 fmol of protein, distributed among perhaps 10,000 different proteins. In the analysis, proteins are separated based on their molecular weight, using capillary sieving electrophoresis, and based on their hydrophobicity, using micellar electrokinetic capillary chromatography. Each protein generates a peak in the two-dimensional electropherogram, and changes in protein expression are revealed as changes in the electrophoresis profile (© 2004 American Chemical Society; reprinted by permission [17]).

CHALLENGES AND RECOMMENDATIONS

Life processes that occur intracellularly, intercellularly, and between cells and their fluidic and surface environments are complex. Quantitative analysis to learn how cell function is normally regulated and to determine how this can be manipulated will require many complementary and versatile technologies to be developed in parallel, As daunting as solving the complete puzzle appears, important advances in understanding critical pieces are being made as new technologies emerge.

Short- to Medium-Term (1–10 years)

Biomimetic Nanoprobes

Biomimetic nanoprobes are needed that accurately address the structures and functions of biomolecules, supramolecular assemblies, and organelles of living cells and that can provide read-outs on their activities. Broad use of advanced imaging techniques by cell biologists will depend on the ability to generate genetically encoded contrast agents or nanoparticles for visualizing organelles and other cellular structures. Better delivery systems for introducing these and other reagents into tissues and embryos can be achieved with improved transfection methods, including the use of nanobeads coated with antibodies or other molecules that are injected or taken up by

endocytosis into cells. Microfabricated cell culture systems that control delivery of stimulants, drugs, or other agents should allow detailed and systematic analysis of the specific cellular responses as detected with the targeted probes.

Engineering Cell-Surface Interactions

Nanotechnology offers many opportunities to control the interface of a cell with its environment (i.e., to expose cells to two-dimensional substrates and a three-dimensional environment with precise properties). For example, fabrication of structures with defined architecture or surface chemistry holds promise for learning how the spatial organization of matrix molecules and their derivatives regulates cell behavior and gene expression [18, 19]. Model substrates in general should be used to model the interface of a cell with neighboring cells or of cellular subsystems with other intracellular structures. Precisely defined, patterned stimuli can be used to test hypotheses about cell differentiation or other responses to extracellular signals, leading eventually to the capability to construct nanodevices that direct desired cellular responses. Early stages of success have been demonstrated for environmental control with fabricated molecules and surfaces, including use of molecular self-assembly on the nanoscale to fabricate cell substrates that control cell adhesion, shape, and functions [20]. Other examples include architecturally defined molecules used both for specific stimulation and inhibition of IgE receptor-mediated activation of mast cells, such as occurs in the allergic immune response [21]. Surfaces with micrometer-sized patterns of ligands specific for immunological receptors reveal spatial reorganization of cellular components that occurs during the activation process (Fig. 3.4).

Optical imaging of cells has been typically performed on cells that are cultured from tissue on plates. Although this has proved invaluable in determining the overall organization of cell machinery, most cells grow in a three-dimensional context within tissues or developing embryos, which affects their behavior and phenotype. To study the *in vivo* organization and function of cellular components in this context, new approaches are needed for introducing reporter proteins into tissues and embryos, for imaging deep into such specimens, for mimicking this environment using nanofabricated materials, and finally for improving the spatio-temporal resolution of imaging techniques.

Biomimetic probes in defined assemblies or environments are needed for the development of quantitative models of subcellular and cellular functioning, providing, for example, improved dynamic data on reaction rates in confined spaces or in close proximity to biomimetic organelles. Biomimetic chimeric proteins offer special opportunities to foster multiprotein assemblies and, potentially, to create novel cross-talk among pathways to alter cellular homeostasis and physiology. Nanoscale engineered probes used in defined combinations and conditions will furthermore allow analysis of complex relationships between different cellular components and compartments. It will be necessary to simultaneously model mechanical, structural, and signaling activities and their relationships to selected assemblies and then integrate these models to more fully understand cell function. Such engineered nanosystems are described further below.

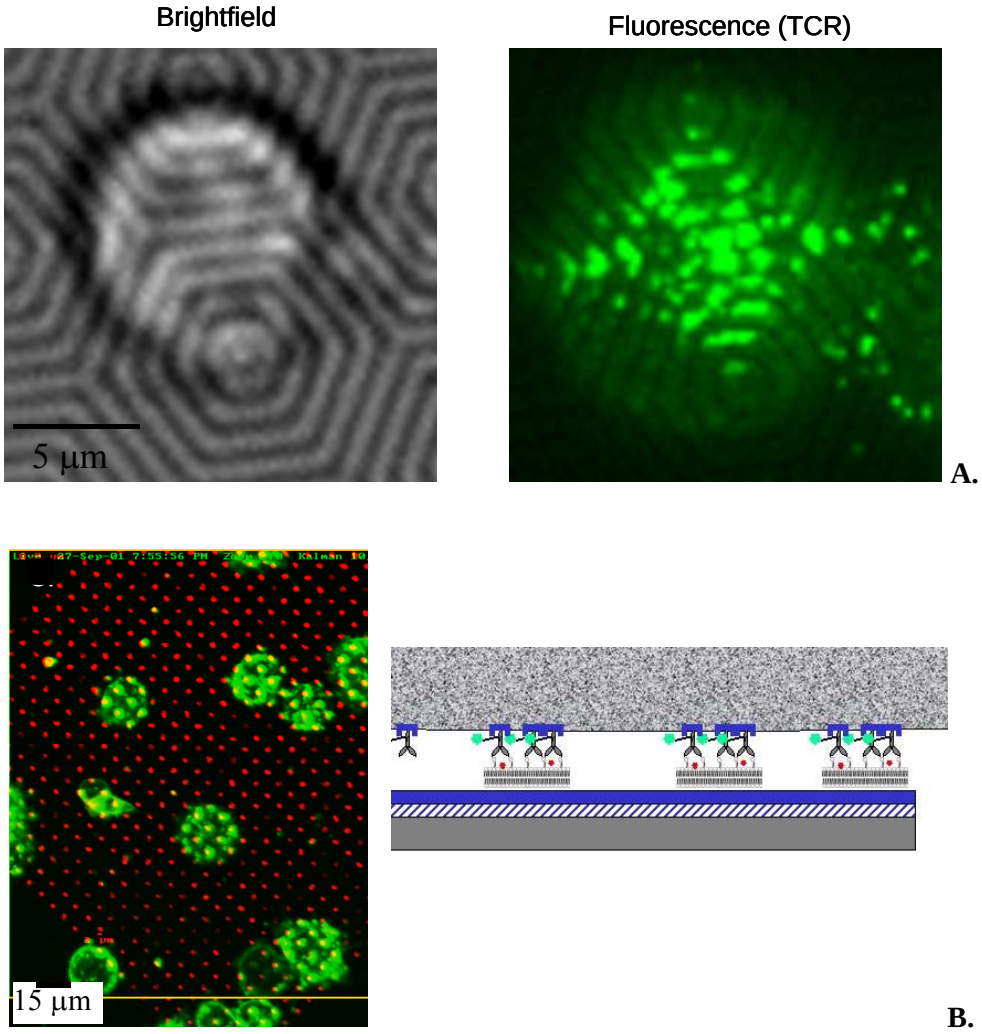

Figure 3.4. Shaping cellular responses with nanofabricated two-dimensional patterns. A. Antigen receptors (TCR) on T cells respond to a supported membrane containing peptide-MHC and ICAM. The TCR are specifically labeled with a fluorescent Fab fragment that is known not to interfere with TCR-pMHC binding and signaling. One-hundred-nanometer-wide chrome lines on the substrate impose a freedom-of-motion constraint [22, 23] (courtesy of K. Mossman and J. T. Groves, UC Berkeley, and M. L. Dustin, NYU). B. Mast cells respond to a patterned lipid bilayer. On the surface of the cells IgE-receptors labeled with fluorescein (green) cluster over micrometer-size lipid bilayers that contain DiIC16 (red) and DNP-cap-DPPE. The IgE is specific for DNP, and clustering of these receptors initiates transmembrane signaling and cell activation [24, 25] (courtesy B. Baird, Cornell Univ.; lower left figure © 2003 American Chemical Society; reprinted by permission [24]).

Engineered Extracellular Matrices

Extracellular matrices with defined nanostructure are needed to learn the basic biology of how cells interact with textured environments [26–28] and, beyond this, for a variety of applications ranging from tissue engineering, wound and organ repair, and eventually bionics. Further refinements of surfaces patterned with stimulating molecules will lead to an increasingly detailed examination of spatial organization that occurs with cellular signaling (e.g., cell adhesion complexes as well as immunological or neuronal synapses). Two- and three-dimensional surfaces that are structured with

micro- and nanoscale features that mimic natural environments with respect to structural, mechanical, and chemical properties could improve the compatibility, effectiveness, and functionality of interfaces between living cells and engineered materials. These new materials may further incorporate specific features that go beyond mimicking natural environments.

A central question concerns the organization and function of cells within three-dimensional tissues with specialized form, function, and mechanics. Two- and three-dimensional surfaces that are structured with micro- and nanoscaled features resembling some selected aspects of natural environments will be valuable to test models that involve specific cues between the cell and its local environment and, furthermore, to determine how environments between cells lead to tissue and organ development. Engineered adhesive substrates and surfaces patterned with various effectors also will benefit these purposes, and the design of particular chemicals that are incorporated to mimic specific ligands will be an important aspect. Although these engineered matrices mimic only a small set of cues presented to cells in their natural environment, the question arises to what extent the complexity of natural extracellular matrix can be reduced such that engineered synthetic matrices do indeed mimic their natural counterparts. Many past approaches to engineering synthetic matrices neglected the fact that cells have the ability to mechanically stretch extracellular matrix (ECM) proteins. Cells can thus self-regulate the chemical display of the recognition sites of ECM proteins [29] or dynamically reconfigure the spatial distribution of receptors [23]. Furthermore, cells actively organize and remodel the matrix through the tight regulation of ECM fibril assembly [30], which is likely to be impacted by a cell sitting either in a synthetic or a biological environment [31].

Molecular Mechanisms of Mechano-Transduction

The molecular mechanisms of how cells sense and respond to mechanical force are currently still poorly understood because of the complexity of the molecular players involved and the lack of appropriate tools to elucidate their roles [32, 33]. Novel technologies are need to apply mechanical forces locally to cells in a spatially and temporally well controlled manner, as well as to detect and quantify the effect of mechanical forces on the functional states of proteins that are part of the signaling complexes that link the extracellular matrix to the cytoskeleton. This involves the visualization of cell-mediated protein unfolding events in cell culture and the development of high-resolution structural models of how force-induced protein unfolding alters the display of its molecular recognition sites (Fig. 3.5). Additional tools also are needed to study the effect of mechanical forces on downstream cell signaling and, ultimately, gene expression. To probe the role mechanical forces play in cell differentiation and organization, it will be necessary to make measurements of the mechanical and structural properties of proteins and protein assemblies in living cells and in developing tissues with high spatial and temporal resolution. For example, nanoscale force sensors are needed that can be integrated into intracellular macromolecules or macromolecular complexes.

Engineered Nanosystems

Engineered nanosystems are needed to address a number of different challenges in cell biology. For example, genetically encoded nanoparticles can be envisioned to address specifically external or internal receptors (after internalization), and if they also contain fluorescence or a high electron density, these receptors can be detected at high spatial resolution by optical imaging or electron microscopy, respectively. Another type of nanoparticle, chemically synthesized multivalent ligands that are related to natural stimulants, have considerable potential in deciphering how molecular clustering regulates cell signaling or metabolic pathways. Also feasible are “tweezer” systems that can enter cells through endocytosis or other natural or artificial pathways and, either directly or

guided by externally applied fields, specifically manipulate intracellular contents. A likely development is nanotechnology-based knockout or knock-down approaches, as well as controlled and specific disruption of intracellular membrane trafficking.

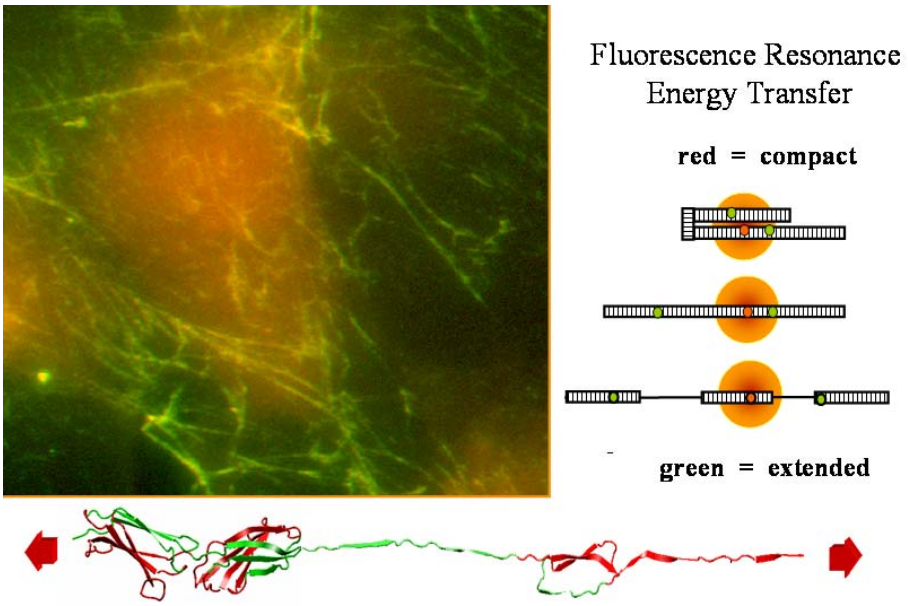

Figure 3.5. Deciphering the molecular basis of mechanotransduction. Intramolecular fluorescence resonance energy transfer [11] is used to visualize how fibroblasts stretch fibronectin into partially unfolded states (green), and steered molecular dynamics simulations are applied to develop high-resolution structural models of unfolding trajectories (© 2003 Annual Reviews; reprinted by permission [29]).

Further insights into the activity of supramolecular assemblies and organelles are essential and require the development of biomimetic devices that accurately represent these functional structures. Micellar nanocontainers that distribute to defined cytoplasmic organelles are one promising example [34]. To target and mimic other structures, such as focal adhesions, lysosomes, Golgi, neuronal and immunological synapses, mitotic spindle, centriole, lamellipodia, filopodia, or flagella, will require directed fabrication of two- and three-dimensional versions of these structures that provide readouts on their activities. These biomimetic devices will be useful in several ways. First, they can be used for developing testable models involving protein functions in close proximity to the biomimetic organelles. Second, they can be used to define the combinations and conditions for analyzing complex relationships between different cellular components and compartments. This information can then be used to make hypotheses and to test quantitative models of subcellular and cellular functioning. Finally, the biomimetic structures can be introduced into cells to perturb the operation of individual intracellular components. They could also be introduced into diseased or pathological cells to correct dysfunction or into normal cells to induce their differentiation or dedifferentiation (e.g., generate mature cell types from stem cells).

The *in vivo* analysis of cell function using these nanotechnology-based tools is likely to provide insights into cell-driven regulation of assembly (e.g., collagen assembly in corneas/tendons and organized smooth membrane assembly) that will be relevant for improving the effectiveness and economy of other nanofabrication processes. One example is the self-organization of endoplasmic reticulum membranes into crystalloid structures—an assembly process that requires only low-affinity interactions yet leads to compact and stable structures over time (Fig. 3.6). The new

discoveries will also likely be useful in improving the compatibility, effectiveness, and functionality of interfaces between living cells and engineered materials. Interfacing with dynamic cytoskeletal components may enable motile systems, leading to new approaches to drug targeting and delivery [35]. It is also likely that some of the fabricated materials used to analyze cell function will lead to more natural and long-lasting joint replacements, more natural limb and digit prostheses, or better functional repair or replacement for various organs. Bionics, or fabricated tissues that are better than what nature has provided, are also a possibility. In other applications, the fabricated materials might allow cells to interface with silicon or other inorganic materials for biocomputation and biosensing.

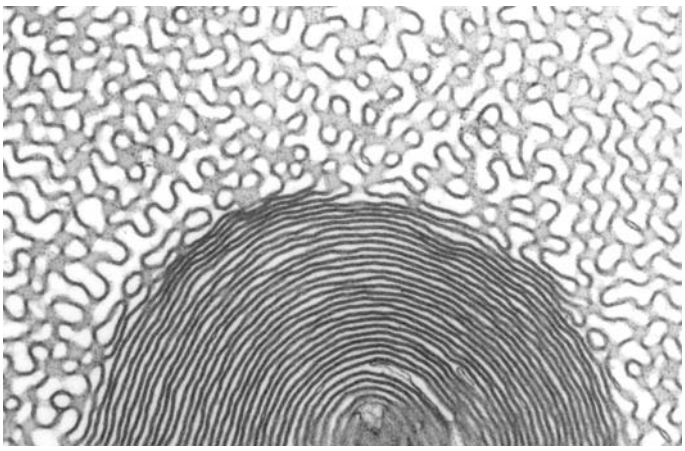

Figure 3.6. Example of organelle plasticity. In response to overexpression of proteins with GFP attached to their cytoplasmic tail, the ER remodels into whorls and crystalloid structures. This results from the low-affinity, antiparallel dimerization of GFP, which causes ER membranes to wrap up into tight parallel arrays. For details see [36] (courtesy of J. Lippincott-Schwartz, NIH).

Long-Term (10–15 years)

In the long term, novel real-time imaging technologies, including X-ray microscopy and high-resolution NMR imaging will be paramount to advancing cell biology. Furthermore, findings and raw data should be shared rapidly through an information network maintained by the nanobiotechnology community. Inventory systems for cataloging the vast information obtained at different scales will also be essential. Integration of these data within specific models of cell function by engineering, physics, and mathematical disciplines in close communication with biologists will rapidly advance the understanding of cellular systems by incorporating information from genomics, proteomics, biochemistry, and biophysics to organize parameters and provide predictions that guide experiments. Through experimental verification and correction, ever more elaborate and realistic cellular models will evolve, providing greater insights into spatial/mechanical control, multimodularity, and hierarchical integration of processes within the living cell.

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4. UNDERSTANDING HOW CELLS WORK THROUGH BOTTOM-UP ASSEMBLY OF BIOLOGICAL NANOSYSTEMS *EX VIVO*

Principal Contributing Authors: Michael Dustin, Gerald Seltzer, and Peixuan Guo

INTRODUCTION

Nanometer-scale spatial arrangement of molecular components within living cells gives rise to an enormous spectrum of emergent properties, including life itself. The observation, study, control, and assembly of spatially organized molecular systems is a critical aspect of nanotechnology and is at the same time crucial to learning how the large number of molecular activities are synchronized in living cells. Understanding how cells work through bottom-up assembly of biological nanosystems *ex vivo* will require an integrated effort from a variety of disciplines.

MAJOR CHALLENGES IN SYSTEMS BIOLOGY

To really understand how a mammalian cell works would be to understand life at a level at which the information is directly applicable to human health. This would be an accomplishment with the potential for translating the sequence of the human genome into a true working blueprint for the human machine. This is a great challenge because the field of biology lacks the tools to link the now-tangible information of the genome into the physical act of making a cell. However, the combination of biology and nanotechnology—a science of making things at the subcellular scale—has the synergistic potential of making a comprehensive understanding of a cell attainable; basically, to work out the previously unwritten rules that allow a genome to become a living organism. A physical model of the cell as a machine from the engineering perspective is needed to gain important insights into the critical design features beginning naturally at the nanometer scale [1]. This may start as a quantitative inventory of the components of cells. The concept is that form emerges at the micrometer level by integration of nanoscale modules, so this inventory will then be combined with an understanding of the dynamic spatial organization of the components [2]. The approach will be to use tools of engineering and nanotechnology to analyze functional assemblies in living cells [3]. Nanotechnology has provided and can continue to provide tools that bridge mechanical force and biological function [4].

The study of spatially organized biomolecular systems will draw together a number of conceptual areas. Mechanical and fluidic forces, diffusive and active transport, and self-assembly become intermingled with chemical processes at the nanometer length scale. The function of signaling structures, such as the immunological and neuronal synapses, are emergent behaviors that result from a superposition of all of these influences [5]. Developing an understanding of these processes at the level of complexity exhibited by cellular subsystems will require integration of physical ideas, quantitative experimentation, and theoretical modeling.

Through these efforts, a fundamental understanding of nanoscale modules of life will emerge (see Fig. 4.1). As this understanding begins to crystallize in many modules, the parallel process of rebuilding functional nanomodules from biological and inorganic materials can begin.

DE NOVO ASSEMBLY OF LIFELIKE NANOMODULES

The second part of the initiative will then be to rebuild the functional modules *de novo* from the lower end of the nanoscale to devices on the same size scale as organelles and cells.

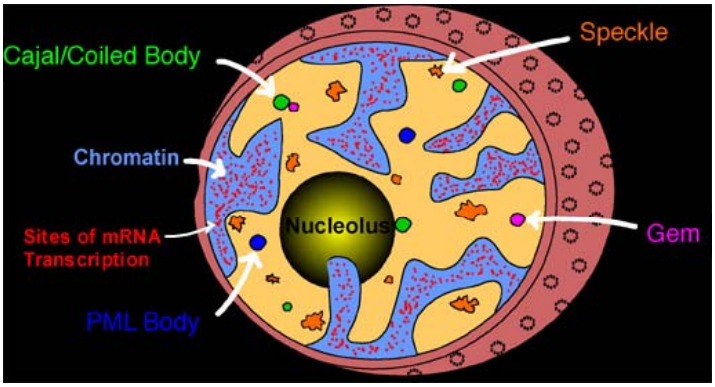

Figure 4.1. The cell nucleus contains a number of nanoscale structures that are encoded by the genome, but whose composition and assembly are not understood. An organized effort and new tools are needed to document, quantify, and determine the self-assembly principles of these nanoscale cellular structures [6] (courtesy of K. Borden, Mt. Sinai School of Med.).

The most basic element that we need to understand to be able to build *de novo* lifelike nanomachines is the dominant and versatile solvent of life: water. Although lacking complex emergent properties, water's ability to form hydrogen bond networks over the low end of the nanoscale and to drive self-assembly though the hydrophobic effect make water a basic facilitator of lifelike nanosystems. Attaining a detailed understanding of water at the nanoscale will require an investment. Fortunately, nanotechnology is well equipped to mount a systematic study of the nano- and mesoscopic properties of H_2O that are critical for nanobiotechnology, and of the role hydrogen bonding plays in regulating various functional aspects of biomolecules and their assemblies.

The nanoscale is the distinctive length scale at which structures cross over from the molecular realm to the realm of systems with emergent properties (Fig. 4.2). Protein and ribonucleic acid (RNA) molecules are paradigms of this distinctive character. They are molecules, but they also display the

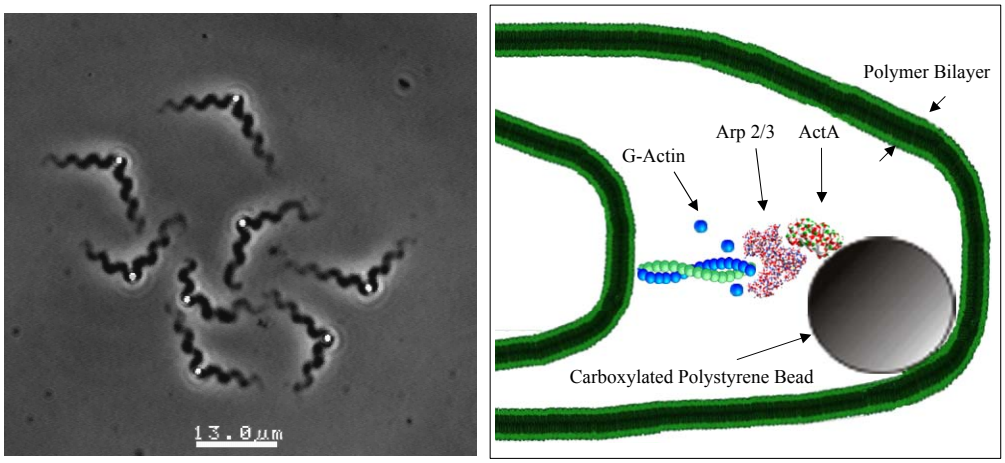

Figure 4.2. Actin self-assembly system as a propulsion mechanism for artificial cells [7]. The actin-based cytoskeleton of cells can be harnessed *in vitro* to propel beads and may be envisioned as moving artificial membrane–enveloped drug delivery systems. Rotary molecular motors also have been successfully linked to synthetic nanosystems to generate hybrid devices [8] (courtesy of M.-F. Carlier, CNRS, and C. Montemagno, UCLA).

character of functional systems when properly ordered or folded. The properties that emerge from proper nanoscale ordering in proteins and RNA include structural, mechanical, electron transport, catalytic, molecular recognition, light emission, and others. Self-assembly of simple protein structures can also enhance useful activities, as in the generation of novel antibiotics [9]. The "proper" ordering of protein and RNA for these functions is achieved by "bottom-up" self-assembly.

It is therefore logical, and quite likely very fruitful, for nanoscale engineering both to take inspiration from and to mimic via synthetic routes the most basic functional modules of biological nanosystems—catalytic proteins and RNAs. A very important challenge and a real barrier to the achievement of many of the goals of biologically inspired nanoscience and technology is the current inability to produce man-made objects with the catalytic function of enzymes. The general need for new catalysts, which is very important in its own right, is not the primary driver to meet this challenge. Catalytic activity is the *sine que non* of energy conversion in biology; in particular, it is the key to conversion of chemical energy into mechanical energy. Biological motors do not result only from clever mechanical structures produced by nature but also because of the efficient transformation of chemical into mechanical energy. Self-assembly of objects resembling proteins or catalytic RNAs in structure and function is a route to achieve this transformation, as is templating media to the contours of a desired substrate, though neither has yet proven capable to accomplish the goal of synthetic, enzyme-like catalysis. This will be an ambitious, but attainable, goal for the emerging nanobiotechnology field. RNA folding in a controlled way, or genetic engineering to give useful modules via biosynthesis, may be alternate routes.

Beyond the enormous challenge of artificial lifelike catalysts there lies the nanoscale challenge of larger-scale self-assembly. Metabolism, transcription, and other key life events require spatial and temporal order. The biological macromolecules that perform these functions are generally organized into functional assemblies that biologists refer to as organelles but that can also be seen as biological nanomachines or modules. At present, very simple systems can be reconstituted *ex vivo* from biological building blocks (Fig. 4.3). It will be important to define the critical features of modules at the nanometer level that enable efficient function and proper feedback control. Physical as well as chemical aspects of the functional modules need to be understood and regenerated. The modules assembled *in vitro* not only must represent a real structure of the living organism but must also possess the biochemical properties exhibited in the living system. Once modules interact with the other counterparts in the assembly pathway of the living system, they should be suitable to interact with other components of the pathway and to be converted into a more comprehensive functional assemblage identical to those in a living system. The pathway and potential regulation of artificial assemblies should incorporate some of these aspects of control and functional integration.

CHALLENGES AND RECOMMENDATIONS

The bottom-up assembly of nanodevices that recapitulate biological functions will serve two important purposes. First, our ability to create, using synthetic materials and purified biological components, functional artificial nanosystems that mimic cellular subsystems will test whether we truly understand those modules in the intact cell. Second, a number of useful devices will emerge from these efforts with important implications for human health such as the "animal on a chip" for drug testing (Fig. 4.4). A goal of nanotechnology is to control complex molecular processes, such as those mentioned above, and ultimately to be able to assemble them from scratch for our own purposes. This also will require interfacing biological components with solid-state structures, which can be addressed by a broad array of inorganic nanotechnologies. An example is provided by the hybrid live cell–supported membrane interfaces that are beginning to be used to understand mechanisms of signaling at intercellular synapses [10]. These structures allow solid-state nanolithography techniques, such as

electron beam lithography, to control the spatial organization and movement of soft biological components within living cells. Nano- and cellular-scale modules may be assembled into larger function systems that simulate the interactions of organ systems on a microscale [11]. This theme of engineering the living–nonliving interface is expected to be recurring.

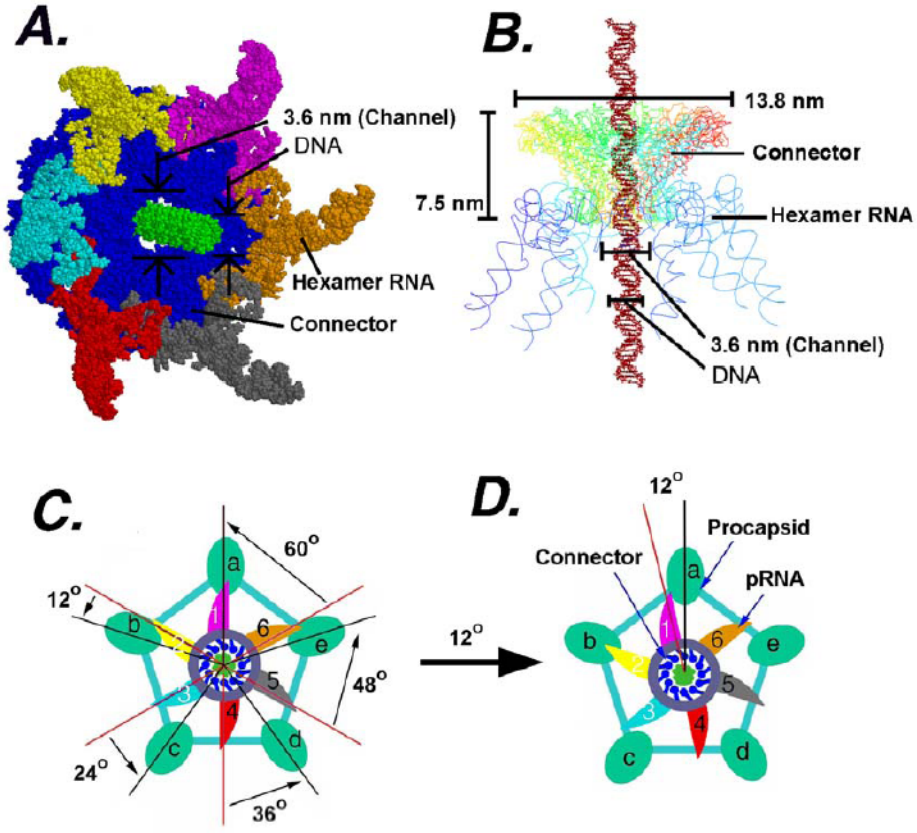

Figure 4.3. Construction of a controllable 30 nm DNA packaging motor of bacterial virus phi29. The motor is driven by an ATP-binding RNA (pRNA) hexamer, similar to the driving of a bolt with a hex nut. Conformation change and sequential action of the RNA with a fivefold (viral capsid)/sixfold (pRNA hexamer) mismatch could ensure continuous rotation of the motor with ATP as energy. The figure shows the tertiary bottom view (A) and side view (B) of the phi29 DNA packaging motor, which is embedded in a pentagonal capsid (the green pentagon in C–D) to enable the sequential action of pRNA in a manner similar to the sequential action of six cylinders of a car engine (courtesy of P. Guo, Purdue Univ., based on data derived from [12, 13]).

Short-Term (1–5 years)

The first level at which nanoscale molecular assemblies begin to show emergent properties of life is in the processes of energy conversion. Although these processes have been studied for many years in enzymology and structural biology, the understanding of biological machines based on data and modeling is reaching the level at which one could realistically think about trying to construct a molecular machine that uses biocompatible energy sources like ATP or electrochemical gradients. The efforts of nanobiotechnology at this level will require a detailed understanding of the nanoscale environment (water and surfaces at the nanoscale). The interface between materials and biological structures such as lipids, proteins, carbohydrates and nucleic acids will have to be refined to allow coassembly of these structures with covalent interactions.

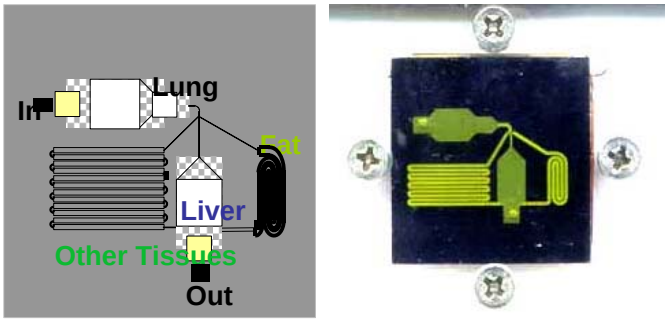

Figure 4.4. Animal on a chip. The need to use animal models for drug testing greatly increases the cost of drug development and raises ethical issues. Systems such as this one may allow complex interplay or organ systems to be evaluated on the micro scale to decrease the cost of new drug development (courtesy of M. Schuler, Cornell Univ.).

Another approach to energy conversion for nanoscale molecular assemblies is to borrow technology from biological systems. Virologists have been working on the bottom-up assembly for decades. The current achievement in virus assembly indicates that the bottom-up assembly to build a natural machine is feasible [14]. Research in this area will provide model systems for studies on motor function, energy conversion, component transport, mechanisms for the assembly of cellular machines, and the building of living modules at the nanoscale. For example, viruses make use of RNA in building nanomachines and gearing motors [15]. RNA will be an ideal medium for early generations of engineered enzymes, motors, and other machines.

In the first 5 years the effort to generate a physical model of the cell as a machine will be attainable. One goal could be a basic description of a normal cell at the level of an inventory of parts. In parallel, efforts to understand basic nanoscale building blocks will lead to a better understanding of materials and surface properties at the nanoscale. Engineered RNA nanomachines will be working on novel catalytic steps—a first step to *de novo* assembly of a fully synthetic nanomodule. The effort to generate artificial systems to communicate with live cells through molecular patterns will achieve sophisticated successes *in vitro*, perhaps turning off cancer cell growth or inactivating disease-inducing immune cells.

Medium-Term (5–10 years)

In 5–15 years, the physical model of the normal cell as a machine should be possible to the extent that the component parts and their physical relationships can be understood and extensions to diseased cells can be initiated. Genomics, proteomics, and this nanobiotechnology analysis will be able to define defects associated with disease in a way not previously possible without the physical understanding provided by nanobiotechnology. New insights from nanobiotechnology approach target therapy more effectively. The translation of genomic information into three-dimensional instructions for formation of cellular structures will be at an advanced stage. Fully synthetic lifelike catalysts and energy conversion systems are envisioned. Nanofabricated substrates may be created that control *in vitro* cell differentiation for use in human therapy. Biocompatible motile nanomachines may be generated on the basis of mimicking polymer-based motility systems by applying lessons learned from the study of biological models.

Long-Term (10–15 years)

In the long term, the nanobiotechnology cell model may raise our understanding to the level at which more intelligent treatment paradigms emerge that go beyond the drug paradigm. Examples of this may include using precise application of force to organize the molecular complexes that mediate cell adhesion and migration to promote wound healing and stimulate repair processes where no repair was previously possible. Progress in previously incurable diseases, such as severe spinal cord injury,

cardiovascular diseases, and osteoporosis, may be approached through a new understanding of the physical principles of cell function. Fully integrated energy conversion systems may be linked to other functional systems to create highly sophisticated nanomachines. These systems will provide the physical engines for many nanofabricated structures that can then operate in biological systems or other environments. Patterned artificial materials may be able to induce cell differentiation *in vivo*, thus correcting disease states with high specificity and minimal side effects.

CONCLUSION

Nanobiotechnology represents a coordinated effort to bring together the information of the genome, the intuition of biologists, and the know-how of nanotechnology and engineering to connect the abstract one-dimensional genome to the three-dimensional reality of the human body. An important focus of this activity will be on understanding the cell from engineering principles to determine how gene products guide assembly of functional nanoscale modules and to rebuild nanoscale modules *de novo* for useful applications. The initiative will bring investigators from diverse fields together and will coordinate activities to facilitate the essential synergy. Humanity will benefit through new treatment paradigms that will revolutionize medicine and preventive care.

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5. NANOTECHNOLOGY AND HUMAN HEALTH

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INTRODUCTION

Nanotechnology refers to science, engineering, and technology that involve the manipulation of atoms and molecules on the nanometer (one billionth of a meter) scale to engineer materials and devices that have novel properties because of their small size and uniform structure. Most past efforts in this area focused on the development of inorganic nanomaterials for nonmedical applications, such as the use of carbon nanotubes for microelectronics. Recently, however, there has been recognition of the potential impact on medicine of nanoscale materials and devices that can act on the same scale as molecules and cells. Parallel advances in molecular biology and genetic engineering have provided a separate and equally powerful means to engineer biological molecules with novel structures and functions on the nanometer scale. The convergence of these fields now offers exciting new possibilities for using nanostructures and intelligent nanoscale devices to improve human health, and hence to open a new era of "nanomedicine." In this chapter, we explore potential areas in which nanotechnology may have a significant effect on the future of medicine and discuss specific opportunities and challenges in this burgeoning field.

MAJOR CHALLENGES IN HEALTH CARE

Expenses for health care are increasing rapidly, threatening its availability to the average citizen. Today's medicine is based almost entirely on treatment; a significant part of tomorrow's medicine will be based on early detection and prevention. Genetic testing will identify a person's disease susceptibility at an early age, and ultrasensitive imaging modalities will be able to detect epigenetic disease alterations long before they are expressed clinically. In some cases, therapeutic vaccines will be administered to prevent the disease or pathology from occurring. Individuals will then be monitored for disease progression or pathogen exposure. Implanted sensors and noninvasive diagnostic tests are needed to look for physiological markers that define early-stage changes or progression to a disease state, and much of this monitoring eventually may be performed at home and recorded at a distant site by the attending physician. It is desirable that the technologies that sense these markers and lesions also will deliver prophylactic or therapeutic agents (either as closed-loop systems or as smart materials); in some cases, these smart devices will be embedded and responsive to the appearance of early-stage disease markers, so that problems are treated as they arise and long before symptoms appear.

The development of approaches to engineering systems with defined structure and function on the nanometer scale will enable this new wave of medical innovation. Importantly, this is not just a pipe dream. The Food and Drug Administration already has approved some nanotechnology medical products for use in humans. Examples include liposomes for drug delivery in cancer therapy, nanometer-sized magnetic particles for use as contrast agents for magnetic resonance imaging, and nanomaterials for use as bone void filler and for dental restoration. Future nanomedical technologies will be even more elaborate and might include, for example, "smart fishing nets" that assemble and disassemble to recognize, capture, and analyze disease markers from blood, saliva, or other body fluids, and intelligent delivery systems that will circulate through the body as innocuous materials until they are activated at injury or disease sites to deliver drugs

locally. In both cases, materials that can reversibly change their properties or catalyze ordered self-assembly reactions in response to distinct molecular signals will be used to provide these nanoscale systems with "intelligence."

Nanotechnology also can be used to design better multifunctional materials that are simultaneously diagnostics, therapeutics, and monitors of response to therapy. Nanostructured components also may be incorporated within micro- and even meso-scale systems that have additional functionalities, including components for power generation, movement, and self-assembly-based manufacturing [1]. This revolution in health care will be further accelerated by the use of nanoscale tools to greatly advance our understanding of the molecular and cellular origins of many diseases, which will open totally new avenues for medical diagnosis and therapy.

Beyond addressing diseases that have been in the spotlight of the public for decades, including the major killers in the United States such as *cancer*, *cardiovascular diseases*, and *diabetes,* nanotechnology also will be critical to address other major health challenges. These include the following:

- *Environmental disorders*, for example, are an important cause of human disease and toxicity throughout the world [2]. Intervention in this group of disorders has the potential to provide a major global benefit from nanotechnology because environmental problems are of paramount importance in many less developed regions of the world [2, 3]. These disorders include a broad range of environmentally caused illnesses brought on by such diverse factors as infection (e.g., waterborne diseases), chemical toxicity, behavioral and health problems related to addiction or illicit substance use, and exposure to radiation through either natural or man-made sources. Although many of these disorders are better managed in the industrialized world, new types of infections, release of chemicals into the environment, and the development of new radiation sources may be unique problems for developed countries that also could be addressed by nanotechnology [4]. Issues related to global conflict and bioterrorism might also be addressed by nanotechnology [5].
- *Developmental diseases* such as congenital illnesses and developmental problems not related to genetics are a second area that may be ameliorated by nanotechnology. Interventions that improve these disorders offer the greatest benefit to the individual, because just one such intervention could prevent a lifetime of suffering for a person [6]. Although most of these disorders are a result of genetic causes, problems with nutrition and environmental deficiencies that cause altered development are also a major issue [7]. Approaches might include the use of nanomaterials to analyze and enhance the food supply and prevent the exposure of developing humans to environmental toxins. New nanotechnology approaches also potentially could guide tissue remodeling and regeneration through induction of hierarchical molecular self-assembly.
- Finally, *degenerative diseases* are an area in which nanotechnology may improve human health. Interventions in this area may achieve the most overall benefit in developed and industrial societies because of the tremendous financial implications of taking care of degenerative diseases in their aging populations [8]. Relevant problems include both normal and abnormal aging, trauma, and the results of chronic inflammation and autoimmune diseases. Although most current approaches to treating these diseases involve limiting their impact through attempts to suppress disease severity and replace function, something that would prevent or cure these diseases and result in complete restoration of function would be a remarkable accomplishment that would save society countless billions of dollars [9].

ENABLING NANOTECHNOLOGIES WITH APPLICATIONS TO HUMAN HEALTH

The interface of nanotechnology and biotechnology has produced some early successes. Examples include the use of nonbleaching fluorescent nanocrystals (e.g., quantum dots) in place of dyes, pulled glass capillaries with nanoscale tips implemented for cellular and subcellular electrophysiological monitoring for microinjection of membrane-impermeable molecules (e.g., proteins, DNA), and the use of oriented arrays of carbon nanotubes for the delivery of biomolecular components to cells [10–14] and "smart" biohybrid materials [15] that enhance bioanalytical and diagnostic technologies by providing new avenues for regulating the activity of protein and DNA components (e.g., Fig. 5.1).

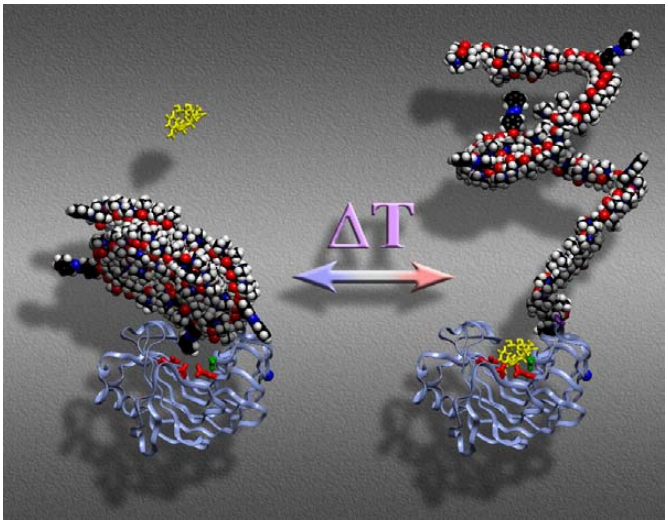

Figure 5.1. Molecular switches that turn enzymes on and off. The conjugate of a "smart" photo- and temperature-responsive polymer and the enzyme endoglucanase 12A is shown. The enzyme is in the "on" state at right, when the yellow substrate can enter while the attached polymer is in an extended state. A small change in temperature or light irradiation causes the collapse of the polymer to the state, on the left, where the enzyme is in an "off" state. The polymer thus serves as an antennae and an actuator to tune the enzyme activity reversibly (courtesy of P. Stayton, Univ. of Washington).

Nanofibers are promising tools for the controlled delivery of molecular materials (e.g., DNA) either in free form or tethered to nanofibers [10]. Figure 5.2 shows that nanofibers may be inserted into cells without affecting cell viability, as the cells continue to express a nanofiber-borne GFP gene long after insertion. Because this DNA is attached to the nanofiber, an intriguing possibility is that gene expression may be controlled by a nanofiber-mediated signal. It is envisioned that this approach could become a platform technology for systems biology, where nanofiber probes are used to decipher connectivity in gene pathways and networks, and eventually for therapeutics targeted to specific locations within these pathways and networks.

An example of a "smart" polymeric drug delivery system [16, 17] for protein and nucleic acid drugs and vaccines is shown in Figure 5.3. The polymer backbone combines pH responsiveness with membrane-destabilizing activity, so that it is activated only once the delivery system is inside the cell in the endosomal compartment. As the endosomal compartment pH drops, the backbone separates from the drug and destabilizes the membrane to enhance transport of the protein or nucleic acid to the cytoplasm. This synthetic system thus mimics biological drug delivery systems of viruses and pathogens that have evolved "smart" pH-responsive and membrane-destabilizing mechanisms. The new synthetic systems are designed to deliver protein therapeutics, antisense oligonucleotides, RNA interference drugs, and vaccines.

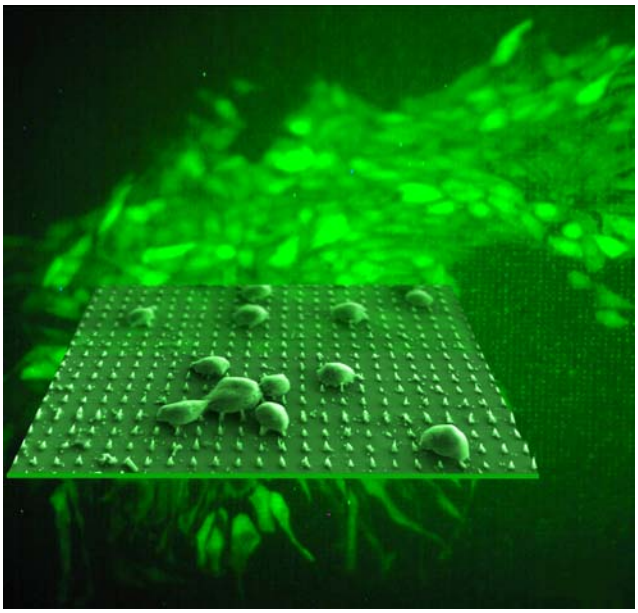

Figure 5.2. The functional integration of vertically aligned carbon nanofibers and Chinese hamster ovary cells is shown in a composite optical fluorescence microscopy image. GFP genes tethered to the nanofibers are expressed by the cells, indicating that the cells remain viable after insertion of the nanofiber. This demonstrates the controlled delivery of material (in this case DNA, but siRNA, enzymes, etc., are also possible) to cells (courtesy of M. L. Simpson, Oak Ridge National Laboratory).

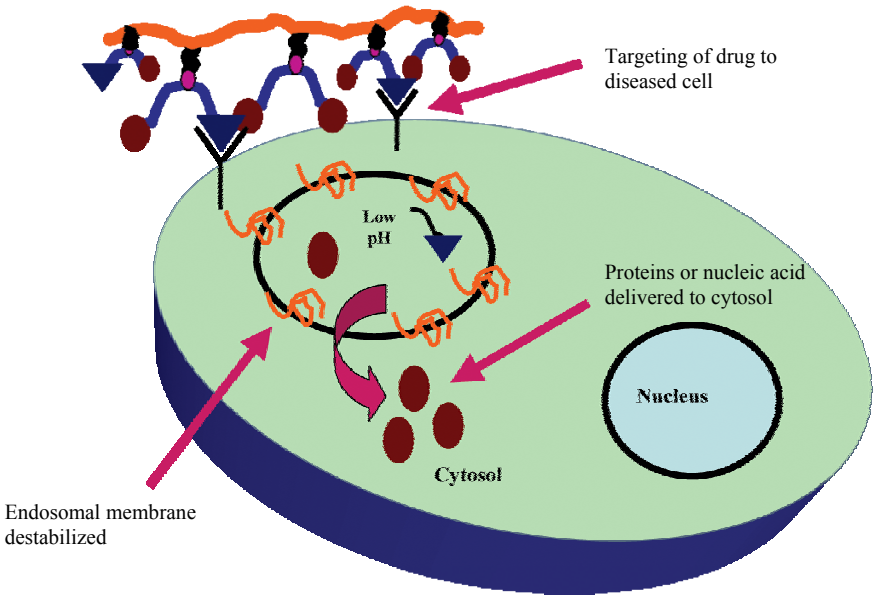

Figure 5.3. System activated at low pH to disassemble and enhance drug delivery (courtesy of P. Stayton, Univ. of Washington).

Selective photo-thermal tumor ablation has been demonstrated in mice by a simple, noninvasive procedure that uses gold-coated silica "nanoshells" that preferentially concentrate around leaky blood vessels characteristic of tumors and that heat up to destroy cancer cells when exposed to near-infrared radiation [18].

These early advances hold promise of new approaches for the diagnosis and prevention of disease such as cancer and for new targeted therapeutic strategies. However, it is far too early to speculate on how well any of these approaches will survive the scrutiny of extensive clinical trials that will be needed before they can be considered for use in regular medical practice. Nevertheless, the brochure on cancer nanotechnology recently released by the National Cancer Institute [19] reflects the optimism of many researchers in the field of nanotechnology.

GOALS AND RECOMMENDATIONS

Recent advances in materials science and molecular engineering are making the possibility of creating various types of nanoscale materials, structures, devices, and machines a reality. These nanostructured materials range from analytic tools to intelligent drug delivery/therapy programmable systems (Table 5.1). The following sections discuss potential areas in which these types of materials may have an effect on medicine over the next 5–15 years.

Table 5.1
Anticipated Advances from Nanotechnology

Nanostructured materials	Nanotools	Nanomanufacturing (that affects miniaturization)	Nanocomponents	Intelligent multifunctional nanodevices
Biomolecular self-assembly (e.g., liposomes)	Molecular detection and imaging of activities	Soft lithography used for MEMS and other analytic microsystems	Energy production	Nanomachines (integrated combinations of nanocomponents)
Biocompatible inorganic nanomaterials (e.g., magnetic nanoparticles)	Nanosurgery of molecular elements within cells	Nanopatterning-directed molecular assembly	Motors	Self-powered nanorobots with programmable functions and read-out capability
Membranes with nanopores		Molecular assembly–directed nanopatterning	Pumps	
Nanofilaments and networks			Synthetic factories	
Novel polymer structures Dendrimers Molecular Imprinting			Nanoassembly plants	
Self-assembling molecular mimetics and bionics (e.g., for tissue engineering)			Nanostructure-directed self-assembly	

Diagnostics

Approaches to health intervention begin with diagnostic tests that detect either the presence of the disorder or the susceptibility to disease. Major advances are likely to be seen in the near term because many of these diagnostic tests can be performed outside of the human body and therefore are not limited by biocompatibility issues, nor do they require full Food and Drug Administration

approval. This approach will allow early detection or even prevention of, for example, cancer and other diseases, which may be most effective in terms of limiting health care costs.

Short-Term (1-5 years)

Improvements in diagnostics will involve *ex vivo* tests and improvements in current laboratory techniques that would allow measurement with greater sensitivity and specificities [20]. Nanotechnology-based assays also may allow the identification of unique biological molecules, chemicals, and structures not addressable by current assays. To deliver on these promises, the following challenges need to be addressed.

Improvements of sensing and signal transduction modalities through engineered nanoparticles or nanosystems: Accomplishing this goal will require enhancing the selectivity of molecular recognition events and the development of new assembly strategies or nanostructures for recognizing and sorting biological molecules or nonbiological systems. Improving the sensitivity so that minute numbers of molecules can be detected will require nanoscale systems with new transduction properties. One of the most significant challenges associated with nanoscale approaches for diagnostics involves the ability to detect statistically significant levels of biomarkers in small sample volumes. Patient samples containing small numbers of cells or biomolecules may not be representative of a disease state, and therefore it is not sufficient to simply minimize the volume of material sampled and to use a smaller and more sensitive sampling device. It will be necessary to couple nanoscale sampling approaches with emerging techniques for concentrating analytes of interest. For example, signal enhancement might be accomplished through the synthesis of new components that assemble around target molecules to produce a strongly amplified signal in the presence of the target molecule. Such signals could be based on optical effects, including light harvesting and quantum dots, and on various energy transfer schemes. Furthermore, nanoscale systems might be designed that mimic enzymes but differ from natural enzymes as they generate easily sensed end-products. For this and other *in vivo* applications, the development of systems that disassemble, trigger their own degradation, or stimulate their own exocytosis once their task is completed is desirable [16, 17].

Finally, microfabricated platforms with integrated nanoscale components are needed to build integrated, low-cost portable devices to analyze samples from body fluids and gases without major time delays. For example, blood, saliva, and other physiological fluids need to be sampled and analyzed for their content with as little disruption of the patient as possible. Recent advances in the use of breath analysis to provide real-time diagnosis of patients in emergency rooms, mass casualty events, and other scenarios offer a totally noninvasive route for point-of-care diagnostics. These approaches may use spectrometric techniques capable of measuring part-per-trillion concentrations of analyte biomolecules indicative of host response to specific disease states. Nanoscale sample processing and measurement units will provide for enhanced sensitivity and specificity in the future. For example, microcantilevers and nanocantilevers have demonstrated highly sensitive detection of prostate cancer biomarkers through shifts in surface stress as a result of binding events.

Nanotechnology also might provide a dramatic decrease in the cost of genotyping and in the analysis of cells that are isolated from biopsies or surgical samples. This might provide a massively parallel method for obtaining high-dimensional information on the composition of complex cells and tissues and on how the composition changes in response to disease, infection, and stress. High-throughput systems, with single molecule detection if possible, for the analysis of content and its variations also need to be developed. This might include bioanalytical and diagnostic assays based on nanoparticles and nanobeads for analysis of the chemical composition of cells and organelles, as well as readout systems that are specific for these materials. It also may be useful to analyze the

formation of molecular binding events by measuring the ferromagnetic or electric behavior of assemblies of nanoscale magnetic particles, or the formation of catalytic assemblies that produce an easily detected molecular species. By being able to create uniform structures with defined shapes on the same size of individual molecules (with complementary shapes), nanotechnology offers the possibility to provide massively parallel analytic systems with enormous sensitivity. For example, all standard blood tests that require phlebotomy and tubes of blood today could be converted to "finger stick" tests that require only a small drop of blood in the future. If syringes can be made on the nanoscale, chemicals in blood or intercellular spaces may even be able to be sampled without compromising the skin.

Mid- to Long-Term (5-15 years)

Mid-term advances in diagnostics will involve diagnostics that are integrated into biological systems, and thus could be further miniaturized through use of nanoscale components. This would include concepts such as implanting sensors within human tissues and cells that would provide real-time information on biological processes and functions and that would potentially be suited for long-term *in situ* monitoring [21]. Systems developed for real-time monitoring of humans in any environment must have integrated devices with internal power sources that can communicate through wireless networks. This will allow the monitoring of human functions. The biological feedback signal could, for example, be used for controlled drug delivery on demand. Examples would be real-time control of insulin pumps in response to glucose monitoring, or warning alerts to reduce stress loads on joints to prevent orthopedic injuries. This also might allow the monitoring of brain function to help in managing problems like addiction or chronic pain.

Challenges to be addressed: Methods and pathways have to be developed to direct the nanosystems or nano-components to locate or self-assemble at a desired location within a cell, tissue, organ, or the cardiovascular system. Addressable systems need to be developed that can sense and communicate data to the outside. The surfaces of these nanoscale systems have to be engineered such that they can be specifically targeted to areas of interest without eliciting undesired biological responses such as inflammation or biofouling. Biomaterials can be nanofabricated or nanotextured to reduce biofouling by modifying the surfaces to alter surface free energy and inhibit the formation and adherence of proteins and biofilms. Predictive models have to be developed for physically distinguishing nanoscale parameters that can be used to bias their transport on user demand. These concepts in diagnostics would build on biologically integrated diagnostic approaches and would directly couple these sensor systems to treatment modalities [22]. One could furthermore envision a system that would monitor cellular functioning and that, if observed abnormalities occur, would release therapeutics that could resolve these abnormalities, as further discussed below [22]. Arrays of nanoscale sensors could be built for simultaneous analysis of many individual molecules.

In addition, cell sensors that monitor for genetic changes could observe the earliest events associated with cancer development and fix the genetic abnormalities before they reach the point at which they could cause tumors. These would be truly unique applications and, most importantly, could be seamlessly integrated into an individual once genetic disease susceptibility is identified. They would also lower the societal cost of illness because it would prevent illness and disability entirely. Finally, micro- or nanofabricated MEMS and microfluidic devices have to be developed that can be interfaced with biological nanosystems and cells. The ultimate goal is to detect appropriate parameters of human health and diseases in real time and *in vivo*.

Drug Delivery and Intelligent Therapeutics

The field of drug delivery already has provided the proof of principle for the value of nanotechnologies, with liposome-based formations of cancer therapeutics being one simple example. Ambitious projects that use nanotechnologies to couple drug delivery to recognition of undesirable biological compounds, such as glucose, triglycerides, cholesterol, or angiotensin, are underway. Molecular imprinting techniques also have been developed in which synthetic polymers are created that form complementary nanoscale binding pockets for small molecules (e.g., D-glucose) [23–26]. Development of new and creative approaches to identify nanoscale binding domains within molecules and to efficiently generate complementary ligands for selective recognition in aqueous environments will help to accelerate the development of intelligent biomolecule-modulated drug and protein delivery, as well as biomolecular recognition for micro–diagnostic devices, nanoscale manufacturing approaches, and modification of site- or ligand-specific interactions with cells and tissues.

New treatments for diseases are needed that have benefits far exceeding current therapies, or that build on newly derived information about how the nanoscale machinery of cells carries out its varied critical functions. This would include targeted medications that have greater benefits and fewer side effects, as well as individualized medications that would address patient-specific disorders; for example, those that are based on an individual's genetic susceptibility [27]. Membranous encapsulating materials for cells with nanoscale pores that permit cell engraftment and delivery of cell-derived products (e.g., insulin by beta cells) but that prevent immune rejection would be another example of how nanotechnology might affect the future of therapeutics.

The ultimate medical nanotechnologies, however, would be multifunctional nanodevices that simultaneously detect, diagnose, treat, and monitor response to the therapy. For example, injectable nanoparticles molecularly imprinted to recognize and bind to undesirable or toxic chemicals with high affinity may be used to clear these agents from the blood or to chemically deactivate these molecules. Information about changes in molecular concentration also may be transmitted to a "station" (e.g., a microchip or wristwatch) and from there be transmitted to the office of the attending physician. Recent developments in "smart delivery" or "intelligent therapeutics" show how far this field has come. The challenge for the future is to stimulate advances in nanotechnology to develop more elaborate devices with the ability to detect and locate pathogenic agents (e.g., toxic materials, microbes, and tumor cells), capture them, treat them locally (or eliminate them), monitor the response to therapy, and also provide distributed information flow (e.g., using radio frequency identification technology) to notify the physician located at a distance of the status of care in real time.

Short-Term (1-5 years)

New forms of drug delivery and therapeutics that need to be developed in the next decade will include smart therapeutics for the specific delivery of drug and genetic agents to specific sites. This would provide specificity in the actions of therapeutics that could thereby prevent systemic side-effects and allow much higher efficacy [28]. Examples include nanodevices that target vascular plaques and shrink them by removing accumulated lipids, new approaches to cancer therapy that can be given daily to control the growth of cancer without the nausea or hair loss, and individualized therapy for genetic disorders that targets the cell where the abnormal gene creates problems. This would improve current therapeutics and would be readily applicable to a broad range of disorders.

Challenges to be addressed: The delivery of drugs, genes and proteins is an area of tremendous interest. Nanotechnology can bring a revolution in the design of delivery systems that are small, smart, and potentially self-destructive by various means; for example, nanoscale assemblies (e.g., micelles, polymerosomes, liposomes, dendrimers, and others) with dimensions and chemical properties that will allow for delivery of drugs past biological barriers, such as cell membranes, nuclear membranes, the blood–brain barrier, the gastrointestinal tract, or skin. Each of these biological barriers will require unique schemes to allow drugs or drug carriers to pass nondestructively. However, the challenge is great, because particles will need to be less than approximately 8 nm in size to freely pass from the blood into the interstitial tissue space. Also, the biocompatibility of these materials and their ability to be cleared also must be considered. The first clinical targets of nanotherapies would therefore likely be sites that nanomaterials can access directly, such as the lining of the vascular system (when injected), nasal passages and lung (when inspired), and gastrointestinal tract (when ingested).

This effort will involve the development of new nanostructured "smart" delivery materials that change their properties in response to external or internal signals to deliver drugs, or of biohybrid materials that combine synthetic molecules and biomolecules to stabilize and deliver proteins and nucleic acids for therapy or vaccines. Finally, more sophisticated delivery systems engineered at the nanoscale are needed to deliver proteins and nucleic acids by oral and aerosol routes. However, the systems designed to deliver these molecules also should be stable in body fluids (blood, saliva) in addition to being able to cross biological membranes. They should be able to stabilize fragile drugs such as proteins and nucleic acids, deliver drugs selectively to target cells, and have inherent control over drug release at appropriate levels and time points. The materials must be highly biocompatible and either be cleared by standard physiological mechanisms (e.g., renal filtration) or be biodegradable.

Mid-Term (5-10 years)

Mid-range delivery agents and therapeutics will most likely involve combination therapeutics. One goal is to have controlled release systems that incorporate multiple drugs that may release at different levels and time points, or delivery systems for preventative or therapeutic vaccines that stimulate multifaceted immune responses. Another goal is to have drug delivery systems available that simultaneously include diagnostic imaging and interventional components. Microchips with nanoscale wells and nanoscale sensing elements will be capable of measuring the amount of drug dispensed from a delivery device, the concentration of the drug in the region of interest, and the resulting response to the therapy, all in a miniaturized and automated system. In another embodiment, one would be able to identify a predisposition to a disease, image whether or not the disease process has initiated, and then intervene with specific therapeutics based on the disease that was detected [29]. Examples of this could be the early detection and prevention of cancer, the identification and treatment of the earliest events of infectious disease, and the identification and prevention of genetic disorders. These approaches might also allow for the enhancement of current therapeutics by combining diagnostics and therapeutics in one agent or allowing other systems, such as implants, to function more efficiently.

Long-Term (10-15 years)

Longer-term goals would extend beyond our current definition of medical intervention, with advances leading to nanosystems that totally replace, repair, or regenerate diseased tissue [30]. This would begin with removal or modification of the disease process but would then follow with regeneration of normal tissue with a concomitant restoration of normal function. This could involve the self-assembly of molecular scaffolds for bone regeneration, wound closure, or correction of

developmental defects. Examples include rebuilding a heart that has developed abnormally because of viral infection or genetic disorder, and killing and removing a tumor with the subsequent replacement of the underlying tissue while simultaneously correcting the genetic defects that predispose that tissue to become cancerous. This type of therapeutic also could resolve the results of trauma such as healing a skull fracture and brain damage that occurs after a drunk driving accident, while correcting the underlying brain disorder that predisposes the individual to alcohol addiction and simultaneously monitoring this therapeutic response. Some would say that ultimately nanomedicine may go beyond regenerating function to its normal, healthy state, and lead to tissues, organs and organ systems with advanced (even bionic) capabilities for self-repair and disease prevention [31]. This might include such concepts as improved energy utilization by cells, better immunity to prevent infections, or modification of genetic abnormalities to prevent subsequent disease. Although these last concepts may seem to be far-reaching and distant, many of them could become reality within a 25-year time frame.

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6. OVERARCHING THEMES AND SUMMARY

The following sections summarize some of the overarching themes discussed at the workshop, and provide a summary of the conclusions and recommendations of chapters 2-5 of the report.

Major experimental and theoretical frameworks have to be established on which to base future technologies to address the goals outlined in this report. This includes the development of theoretical framework/models for sensing at the nanoscale, for transport processes in heterogeneous crowded environments, and for signal transmission and reception within nanometer-sized devices. Hardware tools that close the feedback loop between simulation and experiment also will be critical for success in this area. Such tools will provide a detailed understanding and characterization of the molecular-scale components and their interactions that together with higher-level network structures produce complex cell and tissue functionality. This knowledge and understanding will be used to engineer nanoscale devices that may provide the means to construct new tools for monitoring and manipulating cellular processes.

CHALLENGES TO IMPLEMENTATION

Effective Review of Nanomedicine Proposals

Many ground-breaking research programs in the nanotechnology area will come from groups and disciplines not represented or well understood by current biomedical study sections or review panels. One problem that arises is that review panels do not have the knowledge to discern whether the proposed research is truly new or technically sound. A related problem is that a purely nanotechnology-based review panel may not be able to effectively review proposals from the standpoint of the biomedical science or clinical perspective. The solution will require that the Federal agencies form review panels that represent areas such as materials science, engineering disciplines, and so on while also still incorporating biomedical reviewer expertise to ensure relevance and novelty. Furthermore, it is not sufficient to represent the various disciplines; investigators with experience working across disciplines need to be well represented and to recognize both the challenges and benefits of engaging in highly interdisciplinary research.

Need for Long-Term Investment

Much of nanotechnology development is at the embryonic level, and both long-term vision and sustained financial support (e.g., 10-year funding mechanisms) will be required to make these concepts a reality. One approach could be interdisciplinary centers founded on grand challenges to be solved by nanotechnology. However, the funding agencies need to provide financial support commensurate with the stated goals of this type of large-scale effort. Investments must be made that attract into medical research nanotechnologists who are currently developing tools for applications in defense, consumer electronics, telecommunications, energy, and other fields. These researchers will be critical members of teams when they join with clinical and biomedical researchers to bring about solutions to the nation's most challenging health care problems.

POLICY ISSUES

Although the concepts discussed in this report are truly far ranging in scope and timeline, they show the potential that nanomedicine has for revolutionizing health care in the future. However,

future technology will have to pass economic tests as well as efficacy tests if it is to be adopted. Therapeutics that increase the cost of health care may not be as widely adopted as we would like, no matter how attractive they may seem. Ethically, one of the most important considerations for the use of nanomaterials to affect human health is that future nanomaterials used in biomedical applications must be biologically compatible, as well as functional within biological fluids and tissues.

SUMMARY OF MAJOR CONCLUSIONS AND RECOMMENDATIONS BY CHAPTER

Advanced Imaging Technologies: From Nano to Macro (Chapter 2)

New molecular imaging probes and technologies need to be developed that allow for a quantitative understanding of how biological systems work on the nanometer scale and how these systems are integrated within cells, enabling recognition and processing of a myriad of spatiotemporal stimuli, which ultimately results in coordinated responses within and between cells. Continued research should lead to the development of new techniques to probe molecules and molecular activities at the nanoscale with highest possible time, spatial, and chemical resolution and specificity; the development of nanoscale sensors and environmentally sensitive molecular probes; and a quantitative understanding—through integration—of information from multiple complementary imaging modalities. Sophisticated informatics and theory/modeling will be essential in building a systems biology view of cell circuits and cell-based networks that establishes the integrated functions of organ systems and the whole organism.

A huge number of problems in basic biology could be solved in a straightforward manner if only we could directly see where the relevant molecules are and how they interact in a dynamic way, rather than having to infer their behavior from indirect experiments. Similarly, optimization of clinical therapies would be advanced by improving our ability to predict how molecules behave in living systems—both those of the organism and those used as therapeutics. This, in turn, would improve our ability to control the bioavailability of sensing agents and drugs and thereby improve the diagnosis and therapy of individual patients. From the perspective of imaging, the ultimate goal is to observe molecular events and to track the distribution and kinetics of molecular probes, drugs, and environmental agents within the whole animal or awake human under varying physiological conditions, using multiple modalities. Finally, a commitment to rapidly translate the new knowledge and methodologies coming from nanoscience investigations into materials and approaches that can be incorporated into the practice of health care will benefit enormously from applications of advanced imaging technologies in the preclinical phase.

Major medical challenges for which nascent nanotechnology-based solutions are beginning to appear include imaging of inflammation, metastasis, angiogenesis, atherosclerosis, amyloid fibril deposition, and cell degeneration (apoptosis and necrosis). For all these pathologies, the key is the invention of nanoprobes and contrast agents that either home in on the disease-marking molecules or sense these molecules and become activated in their contrast properties (or both). The recurring challenge is to understand the variables that control the specificity for selected sites, distribution, and stability of nanoprobes and contrast agents within individual cells to the whole organism. To image the fate of single molecules and how they interact with each other, advanced imaging techniques are needed that can image molecules and nanoprobes with nanometer resolution and on fast timescales. To reduce side effects of medication and to improve our ability in functional imaging, it is paramount that we learn how to precisely target probes, drugs, or contrast agents to desired biological sites. Simultaneous imaging of multiple functions from single cells to the whole patient will require the development of advanced multifunctional nanoprobes and the engineering

of advanced genetically encodable markers. Crucial for the early detection of disease, furthermore, is that high-resolution optical imaging techniques are extended to greater depths (> 1 mm), for example, via fiber-mounted endoscopic microscopes with confocal or multiphoton optical systems. It is desirable to direct designed nanostructures to specified locations in biological systems to probe the environment, serve as contrast markers, deliver chemicals, sense the response, and potentially even regenerate the surrounding tissue. Otherwise, engineered nanostructures would be directed within a living organism (e.g., a human patient) by a remote operator with communication occurring in real time.

In vivo Analysis of Cellular Processes at the Nanoscale (Chapter 3)

A unifying goal in cell biology is to derive a quantitative understanding of how the genetic blueprints of cells ultimately regulate cell function. Although tools of biochemistry and molecular biology have provided a large store of information about structures and activities of many cellular components, the challenge of deciphering the hierarchical architecture of molecular networks and how they are regulated is daunting. Whereas the field of cell biology draws heavily from genomic and proteomic analysis, current technologies of that community provide only limited information about how these components work together in living systems. New technologies thus need to be developed to derive a detailed understanding of how cells of all types sense external and internal signals within the context of their particular three-dimensional environments and how these signals are processed to yield particular responses, including stimulated secretion, growth, differentiation, motility, contractility, and apoptosis. Biomimetic nanoprobes are needed that accurately address the structures and functions of biomolecules, supramolecular assemblies, and organelles of living cells and that can provide read-outs on their activities. Nanotechnology-based tools need to be developed for applying physiologically relevant stimuli to selected sub-micrometer cellular locations. This will allow for measurement of the cell's responses to a variety of physical perturbations. Such a comprehensive, hierarchical knowledge base can provide biomedical researchers and clinicians with the ability to diagnose and intervene on the minimal subcellular level at the first indication of pathology. A grand challenge is to be able to identify cell pathosis and return an altered cell to normal operational parameters or remove it before it causes the derangement of other cells. Complementary technologies are beginning to translate between the nanotechnology and cell biology communities to address these challenges. Goals for the next ten years include the engineering of biocompatible nanoprobes and microfluidic devices, together with optical, scanning probe, or other detection schemes, to measure and manipulate selected biomolecular activities within individual living cells. Finally, further insights into the activity of biological nanosystems and organelles will be made possible with biomimetic devices that accurately represent these functional structures.

Understanding how individual cells work is only part of the challenge. Another important question is how cells become organized within three-dimensional tissues with specialized form, function, and mechanics. Although most of our insights into cellular processes have been derived from the study of two-dimensional cell cultures, recent research points out that cells behave rather differently when cultured in two-dimensional environments than in three-dimensional environments. Nanotechnology offers many opportunities to control the interface of a cell with its environment (i.e., to expose cells to two-dimensional substrates and three-dimensional environments with precise properties). Capabilities for engineering two- and three-dimensional spaces on the nanoscale with biomolecular interfaces are needed that allow systematic examination of specific cues between cells and their local environments. To address the importance of mechano-transduction, novel technologies will be needed for the application of mechanical forces locally to cells to quantify the effect of these forces on particular extracellular and intracellular proteins.

Extracellular matrices with defined nanostructure are needed not only to learn the basic biology of how cells interact with textured environments but, beyond this, for a variety of applications ranging from tissue engineering, to wound and organ repair, and eventually to bionics.

Advances in cell biology and medicine will thus depend on deriving the engineering principles that control and regulate cellular functions. Quantitative analysis to learn how cell functions are regulated and can be manipulated in a predictable manner will require many complementary and versatile technologies to be developed in parallel. This includes information derived from the application of advanced nanoprobes from cells interacting with materials and devices with nanoscale features and specificities, combined with novel real-time imaging technologies, including optical nanoscopy, X-ray microscopy, and high-resolution nuclear magnetic resonance spectrometry. Furthermore, the integration of data over nanometer, micrometer, and longer length scales within specific models of cell function by engineers and physical scientists, in close communication with life scientists, will rapidly advance the understanding of cellular systems by incorporating information from genomics, proteomics, and biochemistry to organize parameters and provide predictions that guide advancing experiments. Finally, all the experimental insights need to be integrated into predictive computational models. With increasingly realistic cellular models will come greater insight into spatial/mechanical control, multimodality, and hierarchical integration of processes within the living cell.

Understanding How Cells Work through Bottom-Up Assembly of Biological Nanosystems *Ex Vivo* (Chapter 4)

Nanometer-scale spatial arrangement of molecular components within living cells gives rise to an enormous spectrum of emergent properties, including life itself. Biotechnology has identified all the genes that are required to create a human being, but the manner in which these biological building blocks assemble and how this process can be constructively modified is still a mystery. This is a great challenge because the field of biology lacks the tools to link the now-tangible information of the genome with the physical act of making a cell. An important challenge for nanobiotechnology is determining the "assembly instructions" for a cell and then implementing these instructions to generate synthetic cellular components at the nanometer scale. The study of spatially organized biomolecular systems will draw together a number of conceptual areas. Mechanical and fluidic forces, diffusive and active transport, and self-assembly become intermingled with chemical processes at the nanometer length scale.

The next goal will be to rebuild the functional modules *de novo* from the lower end of the nanoscale to devices on the same size scale as organelles and cells. The bottom-up assembly of nanodevices that recapitulate biological functions will serve two important purposes. First, our ability to recreate functional nanosystems will test whether we truly understand the nanosystem modules in the intact cell well enough to recreate them from synthetic materials or purified biological components. This assembly process will both test hypotheses about the process of going from genes to live assemblies and also produce useful materials and devices for diagnosis and treatment of human diseases along the way.

Second, a number of useful devices will emerge from these efforts with important implications for human health. A goal of nanotechnology is to control complex molecular processes, such as those mentioned above, and to ultimately be able to assemble them from scratch for our own purposes. This also will require interfacing biological components with solid-state structures, which can be addressed by a broad array of inorganic nanotechnologies. A first level at which nanoscale molecular assemblies begin to show emergent properties of life is in the processes of energy conversion, where inspirations and technologies are borrowed from nature. Studies on a physical

model of the cell as a machine are essential to understanding how the components of a cell work together to accomplish overarching functions. This line of study will give completely new insights into the physical relationships between cellular components and functional irregularities that trigger pathological abnormalities. Genomics and proteomics combined with nanobiotechnology will thus define defects associated with disease in a way not previously possible and will allow us to target therapy far more effectively. Ultimately, we will understand the cell from the genetic, biochemical, and engineering perspectives and will be able to rebuild nanoscale modules *de novo* for useful applications. The biggest payoff, however, will be in going beyond the current drug paradigms for treatment of disease into new modalities of treatment that combine today's molecular agents with application of biomechanics at the nanoscale. Target areas are cancer therapy, tissue regeneration, and immune modulation—all areas that are essential to increased "healthspan"—the combined quality of life and life span.

Nanotechnology and Human Health (Chapter 5)

Expenses for health care are increasing astronomically, and soon we will reach the point at which health care will not be affordable for the average citizen. Today's medicine is based almost entirely on treatment; a significant part of tomorrow's medicine will be based on early detection and prevention. Genetic testing will identify a person's disease susceptibility at an early age, and ultrasensitive imaging modalities will be able to detect epigenetic disease alterations long before they are expressed clinically. Implanted sensors and noninvasive diagnostic tests will look for physiological markers that define early-stage changes or progression to a disease state, and much of this monitoring may eventually be performed at home and recorded at a distant site by the attending physician. The same technologies that sense these markers and lesions also will deliver prophylactic or therapeutic agents (either as closed-loop systems or as smart materials); in some cases, these smart devices will be embedded and responsive to the appearance of early-stage disease markers, so that problems are treated as they arise and long before symptoms appear. Nanotechnology also can be used to design better multifunctional materials that are simultaneously diagnostics, therapeutics, and monitors of response to therapy.

Improvements in diagnostics first of all will require better *ex vivo* tests and improvements in current laboratory techniques that would allow measurement with greater sensitivity and specificities. Nanotechnology-based assays also may allow the identification of unique biological molecules, chemicals, and structures not addressable by current assays. A longer-term goal is to engineer nanosystems that can be integrated into biological systems, including sensors implanted within human tissues and cells that would provide real-time information on biological processes and functions and would potentially be suited to long-term *in-situ* monitoring. One could furthermore envision a system that would monitor cellular functions and, if observed abnormalities occur, would release therapeutics that could resolve these abnormalities. To deliver on these promises, the following challenges need to be addressed. First, sensing and signal transduction modalities need to be improved through engineered nanoparticles or nanosystems. Second, nanoscale approaches for diagnostics also must be able to detect statistically significant levels of biomarkers in small sample volumes, including the detection of single molecules. Third, nanoscale systems have to be designed that mimic enzymes but differ from natural enzymes, as they generate easily sensed end-products. Fourth, microfabricated platforms with integrated nanoscale components are needed to build integrated, low-cost portable devices to analyze samples from body fluids and gases without major time delays. Finally, high-throughput systems need to be developed, if possible with single-molecule detection, for the analysis of content and its variations. With the development of these integrated technologies, nanotechnology also will enable a dramatic

decrease in the cost of genotyping and in the analysis of cells that are isolated from biopsies or surgical samples.

Advances also are needed in the field of drug delivery and intelligent therapeutics. More sophisticated "smart" systems for drug delivery have to be developed that sense and respond to specific chemical agents (e.g., that release insulin in response to glucose), deliver drugs and genetic agents to specific sites, and are tailored to each patient on the basis of their genotype. Over time, these new diagnostic nanotechnologies need to be leveraged to create implantable sensors with integrated devices containing internal power sources that can communicate through wireless networks to provide real-time monitoring of biological processes and functions within human cells and tissues *in situ*. Multifunctional nanodevices need to be developed that simultaneously detect, diagnose, treat, and monitor response to therapy. Through these efforts, someday it will be possible to detect and locate pathogenic agents (e.g., toxic materials, microbes, tumor cells), capture them, treat them locally, monitor the response to therapy, and also transmit information (e.g., using radio frequency identification technology) to notify the physician at a distance. Use of the imaging capabilities of these nanodevices to provide a real-time readout of the response to the therapy provided by the same device also will shorten the time required for human clinical trials and regulatory approval.

Longer-term goals extend beyond our current definition of medical intervention, with advances leading to nanosystems that totally replace, repair, and regenerate diseased tissue. Ultimately, nanomedicine will go beyond restoring function to its normal healthy state and will provide a means of guiding the regeneration to tissues, organs, and organ systems with advanced capabilities for self-repair and disease prevention. The result will be a new era in health care.

CONCLUSION

The introduction of biocompatible materials and devices that are engineered on the nanometer scale that interact with biological molecules and cells and provide specified diagnostic, therapeutic, and imaging functions will utterly change the way in which health care is provided in the future. Through ultraminiaturization, a single nanodevice may provide more than one function and potentially even act as a self-powered unit with real-time sensors, transmitters, and responders. Because of their small size and precise control of the packaging, such devices may be introduced with minimal disruption of living tissue. However, there are many challenges to be met before nanobiotechnology can blossom. Most of the greatest advances in nanotechnology have occurred in the area of inorganic materials science—carbon nanotubes and quantum dots are two well-known examples. However, because of biocompatibility issues, they are not likely to be useful for *in vivo* applications. Thus, nanotechnology researchers must be educated about the specific needs involved with the placement of these materials in the human body. At the same time, although bioscientists have made enormous progress in terms of being able to engineer individual molecules, they do not think like engineers or nanotechnologists, who focus on issues such as materials properties and systems integration. Thus, for nanobiotechnology to prosper, there needs to be a true unification of the sciences, which will require a multidisciplinary approach on the part of funding agencies relating to both the structure and review of relevant grant proposals. Finally, because of the broad and varied potential impact of these developments, this entire effort needs to involve the nonscientific and political communities from the beginning and to be executed with the highest level of openness. However, the potential opportunities are well worth the efforts required to overcome these challenges.

APPENDIX A. ABSTRACTS SUBMITTED IN ADVANCE BY WORKSHOP PARTICIPANTS

BARBARA BAIRD, CORNELL UNIVERSITY

Abstract

My research investigates transmembrane signaling mediated by cell surface receptors in immune responses. The signal is initiated by receptor engagement of specific molecules, and is rapidly translated to rearrangement of the membrane and regulated assembly of signaling complexes and pathways leading to a whole cell response. Understanding how this complex system operates fundamentally requires synthesizing information coming from a broad range of biological and physical approaches that collectively address multiple molecular interactions. Nanotechnology offers exciting opportunities to probe this and other biological systems on the molecular and longer length scales at which they operate. During the next ten years, effective collaboration among life scientists, physical scientists and nanotechnologists will yield biocompatible devices that control the interactions of molecules and cells such that the naturally occurring structures and mechanisms can be scrutinized in great detail. Understanding derived from these rigorous analyses will lead to exquisite diagnostics and intervention. By this means we shall be able to sensitively detect pathogens in complex milieu and we shall be able to deliver payloaded particles to targeted sites within tissues and cells.

JAMES R. BAKER, JR., UNIVERSITY OF MICHIGAN

Abstract

The application of nanotechnology to the prevention and treatment of human diseases holds great promise, but has great hurdles. Nanomaterials must be biocompatible, non-toxic, functional in biologic (wet) conditions and well enough defined to pass the scrutiny of regulatory agencies. Early applications of nanomaterials will likely involve the development of medications that take advantage of unique aspects of nanostructures to achieve or enhance therapeutic activity. Examples will be provided for the design, synthesis and analysis of therapeutic nanomaterials where distinct kinds of attached molecules allow for unique therapeutic functions. These applications include antimicrobial compounds, drug and gene delivery, and functional imaging. Concepts of future nanotechnology applications such as cellular engineering, human performance augmentation, and genetic manipulation for the treatment of human disease will be addressed.

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SANGEETA N. BHATIA, UNIVERSITY OF CALIFORNIA, SAN DIEGO

Biography

Sangeeta N. Bhatia, M.D., Ph.D., is an associate professor of bioengineering and medicine at the University of California at San Diego. Her research uses microtechnology and nanotechnology tools to interface live cells with inorganic platforms for applications in cell-based sensing, stem cell biology, and tissue engineering. She is the author of *Microfabrication in Tissue Engineering* and coauthor of *Tissue Engineering*, the first undergraduate textbook in the field. In recognition of her work, Dr. Bhatia was awarded the YC Fung Young Investigator Award, NSF Career Award, David and Lucile Packard Fellowship, and TR100's Young Innovator Award. She received her Sc.B. in biomedical engineering from Brown University, her M.S. in mechanical engineering from MIT, her Ph.D. in medical engineering and medical physics from Harvard-MIT Health Sciences and Technology Program, and her M.D. from Harvard Medical School.

Abstract

To date, the majority of biosensor technologies utilize biomolecules such as antibodies or nucleic acids as recognition elements; however, live cells and tissues offer unique advantages over inanimate sensors. In particular, cell-based sensors offer the potential to serve as integrated sensors that report on disease risk, status, and progression. My laboratory has adapted tools from the semiconductor manufacturing industry to interface live mammalian cells with inorganic platforms, manipulate their function, and perform high-throughput assays. Nanotechnology offers the potential capability to interface with cells over biologically relevant length scales in order to build sensors that can: control cell fate *ex vivo* (proliferation, apoptosis, differentiation), interrogate cells, detect cellular events, and extract data that relates back to human health.

KWABENA BOAHEN, UNIVERSITY OF PENNSYLVANIA

Biography

Kwabena Boahen is an associate professor in the bioengineering department at the University of Pennsylvania, Philadelphia. Boahen received a Ph.D. in computation and neural systems from the California Institute of Technology (Pasadena) in 1997 and both a B.S. and a M.S. degree in electrical and computer engineering from Johns Hopkins University (Baltimore, MD) in 1989.

Abstract

With this multidisciplinary training, I am studying and exploiting functional, structural, *and* developmental principles of nervous system design by *morphing* biophysical neuronal specializations and identified neural microcircuits into mixed analog–digital microelectronic systems. Microelectronic technology enables micrometer-sized transistors to function as excitatory or inhibitory synapses or as gap junctions, thereby recreating biological circuits at a similar physical scale. The time scale and energy dissipation can be matched as well by operating these transistors in the subthreshold region, where they conduct nanoamperes or even picoamperes, just like small populations of ion channels do. By recreating neural circuits and computations at an energy efficiency and physical scale comparable to biology, these *neuromorphic electronic systems* offer a fully implantable solution for neural prostheses.

Nanoelectronic technology promises to cram a trillion devices onto a 1 cm^2 chip. How do we harness all these devices? Abstraction, which has been used until now, is becoming increasingly inadequate as microelectronic chips approach a billion transistors. We can learn from biology, which handles complexity through developmental processes that elaborate a relatively simple starting recipe into a complex mature structure. By borrowing from nature, we have developed two self-configuring neuromorphic microelectronic chips. The first utilizes a model of activity-dependent axonal remodeling to automatically wire a topographic mapping based solely on input correlations. Its silicon growth cones migrate up neurotropin gradients, represented as charge diffusing in transistor channels. The second utilizes transistor heterogeneity—introduced by the fabrication process—to generate feature maps similar to those imaged *in vivo*. Its recurrently connected excitatory and inhibitory silicon neurons give rise to hot spots of activity that, when perturbed, cause distinct groups of cells to respond to stimuli of different orientations. Capturing the ability of epigenetic development to generate feature maps and autoroute connections between them provides a powerful alternative to handling complexity in nanoelectronic systems.

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KATHERINE BORDEN, NEW YORK UNIVERSITY

Abstract

My laboratory's interest lies in understanding the structure and biochemical functions of a subset of organelles found in mammalian cells referred to as PML (promyelocytic leukemia protein) nuclear bodies. Biomedically, the structural integrity of these 0.1–1 μm size bodies are of interest because their disruption is associated with leukemias and neurodegenerative conditions. We found that the ~60 residue zinc-binding RING domain of PML was required for its formation into nuclear bodies *in vivo*. Using standard biophysical strategies *in vitro*, we observed that the RING domain alone could form structures of similar size and morphology to those observed *in vivo*. Biochemically we observe that these RING bodies scaffold other proteins on their surface and that the associated biochemistries of these RINGs become more efficient than when in a monomeric form. Thus it appears that the surfaces of these RING bodies are catalytically active. We find that other RING

containing proteins, including the breast cancer associated protein BRCA1, function similarly to PML. BRCA1 also forms nuclear organelles in response to stress, and at least one of the familial mutations in breast cancer results in a loss in the ability to form these bodies *in vitro*. These studies address a long-standing question in cell biology, whether the dot like structures found in the nucleus are active organelles or merely storage sites for unused components.

Future in Nanobiotechnology

We are concerned with how supermolecular assemblies form and function within the cell. The biomedical relevance behind this is clear but in many ways the standard biochemical, biophysical and structural methodologies become limiting for studies within an intact cell. For instance, we can easily manipulate an *in vitro* system in terms of components to monitor how this changes the relevant biochemistries. However the tools to test this *in vivo*, so that the cellular context and complexity are maintained, are absent using traditional methods. Nanotechnology may allow the development of tools to study these structures *in situ*. Further, many proteins have self-assembly properties such as RINGs or amyloid proteins. The exploitation of naturally occurring proteins/protein domains as molecular building blocks for the design of particular biochemistries should be of interest to nanobiotechnologists, structural biologists, biophysicists and biochemists.

DANIEL BRANTON, HARVARD UNIVERSITY

Biography

Daniel Branton is a cell biologist whose education included undergraduate degrees in math and physics and graduate degrees in pomology and plant physiology. A tortuous educational path, perhaps, but one that served him well for 40 years of investigating the structure and function of molecules and molecule complexes that make up biological cell membranes and their underlying self-assembling dynamic cytoskeletons. During the last 4 years, Branton's laboratory has been collaborating with physics professor Jene Golovchenko to develop membranes with a nanopore that is used to probe, characterize, and eventually sequence biopolymers such as DNA and proteins at very high speeds.

Abstract

There are many ways in which nanotechnology can contribute to human health, but as a cell biologist/biophysicist who has only recently come to appreciate the potential contributions that inorganic nanoscale materials can make in biology, I will focus on two topics I suspect may not be addressed by others at this conference.

1. *Molecular behavior in confined spaces***.** Much of what we understand of human health and cell function comes from investigating molecules *in vitro*. But just as mechanical forces play a critical role in regulating cell behavior, so too do the confined spaces within a cell play a critical role in defining molecular behavior and interactions. We need new imaging tools that can visualize aspects of this behavior within a cell and improved nanofabrication methods, materials, and nanoscale applicable probing methods to develop models of such confined spaces outside the cell where molecular behavior can better be explored than has, for example, been done in a gel.
2. *Overcoming the limitations of today's technology*. The prototype nanopore we used for initial experiments was a self-assembled protein channel in a lipid membrane. Overcoming the limitations of this labile biological model, we learned how to fabricate robust, solid state

nanopores. To do this, we used the ancient phenomenon of feed-back control typical of biological homeostasis. And doing so revealed a completely unanticipated material science phenomenon that has since made it possible for us, and others, to fabricate a variety of structures at the otherwise hard-to-achieve single nanometer scale. This wonderful example of the interplay between biology and nanoscience also served to make it clear to us that a major dilemma facing nanoscale science is that it remains for the most part far from a "technology." Many anticipated nanoscale phenomena remain to be explored and many *unanticipated* nanoscale phenomena are still to be discovered. Consequently, I see a major challenge is convincing NIH that curiosity-driven research in the physics, material science, and optical aspects of science (nanoscience in particular) will eventually yield as high payoff in advancing medicine as has supporting hypothesis-driven research in molecular biology, cell biology, and chemistry/biochemistry.

THOMAS F. BUDINGER, UNIVERSITY OF CALIFORNIA, BERKELEY

Biography

Thomas F. Budinger, M.D., Ph.D. has training in chemistry, oceanography, physics, and medicine and is currently a professor at the University of California, Berkeley, and the medical center at San Francisco, with research centered on imaging technologies at the Center for Nuclear Medicine and Functional Imaging at the Lawrence Berkeley National Laboratory. Previous work relevant to this workshop includes research on imaging methods and on the safety of applying technologies to human research so as not to hinder progress.

Abstract

Two contributions for discussion at this workshop are:

1. The potentials for non-invasively imaging the trafficking of progenitor cells and cells associated with immune system response and inflammation using radioisotopes or magnetic nanoparticles;
2. The applications and potentials of magnetic resonance at fields of 12 Tesla for human studies.

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MARIE-FRANCE CARLIER, CNRS (FRANCE)

Abstract: Biomimetics of Actin-Based Motility

Living cells move and change shape, in response to signals from the outside world, by making protrusions. These elementary motile processes are driven by site-directed polarized assembly of actin filaments, which pushes the plasma membrane forward at rates of 1–10 μm/min. They play

crucial roles in essential physiological events like organism development and morphogenesis, cell locomotion and metastasis, wound repair, synaptic plasticity and immune response. An actively extending cellular protrusion is an example of a modular, self-organized system.

The self-organized actin-based machinery that operates at the front of the protrusion is reconstituted *in vitro* from a minimum number of components. Sustained actin-based propulsion of a functionalized particle (e.g. a polystyrene micro-sphere) is monitored in a synthetic motility medium that contains actin and 4 pure regulatory proteins that are required and sufficient for movement. This biomimetic motility assay provides insight into the molecular mechanism responsible for the generation of force and movement in cells by local polymer assembly against the plasma membrane. Movement results from site-directed catalytic branching of filaments in a dendritic array whose polarized transient growth is arrested by capping proteins. Polarized filament growth is fed by the regulated, dissipative (ATP-consuming) treadmilling of actin filaments. Force development and velocity are linked both to the growth of actin filaments and to the cycle of attachment-detachment of filaments coupled to autocatalytic branching. The rate of filament detachment from the immobilized branching enzyme controls force and velocity.

Reconstitution of the protrusion of a functionalized liposome is the next step in our biomimetic approach of motility. Several technical challenges are involved. Liposomes must be functionalized in a spatially controlled fashion and filled with the reconstituted motility medium. Such objects are potentially amenable to fusion with fractionated cell membranes carrying lipid rafts equipped with defined signaling pathways, thus generating mini cell-mimicking and cell-targeting machineries with curative properties (e.g., in wound healing).

In an ultimate goal, liposomes should be engineered to adhere to a matrix in a controlled fashion so as to reconstitute actual autonomous migration. This endeavor first requires the biochemical knowledge of the dynamics of focal adhesion assembly and of the mechanism ensuring the concerted turnover of actin filaments in protrusive and adhesive structures.

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CHRISTOPHER S. CHEN, JOHNS HOPKINS UNIVERSITY

Biography

Christopher S. Chen, M.D., Ph.D., is an assistant professor in the departments of biomedical engineering and oncology at Johns Hopkins University. As director of the Tissue Microfabrication Laboratory, Chen has published substantially on using semiconductor manufacturing tools to control interactions between cells and their microenvironment.

The goal of Chen's research is to identify the underlying mechanisms by which cells coordinate with each other to build the blood vessels that oxygenate tissues and to apply this knowledge in the development of tissue-engineered implants as well as the treatment of cancer. In recognition of his work, Chen has been awarded the Presidential Early Career Award for Scientists and Engineers and the Office of Naval Research Young Investigator Award. He serves on the board of trustees for the Society for BioMEMS and Biomedical Nanotechnology. He received his A.B. in biochemistry from Harvard, his M.S. in mechanical engineering from MIT, and his Ph.D. in medical engineering and medical physics from the Harvard-MIT Health Sciences and Technology Program. Chen earned his M.D. from Harvard Medical School.

Abstract

Cells respond to many signals from their local environment, as a result of contact with solid-state surfaces, soluble agents, neighboring cells, and mechanical forces. My laboratory has been studying how the spatial organization of these signals and of the cells themselves defines how cells respond to these signals. We have used numerous tools adapted from the semiconductor manufacturing industry in order to manipulate and investigate this physical relationship between the architecture of the environment, cellular organization, and cell function. Nanotechnologies promise to provide numerous new synthetic, manipulation, and analytical techniques to investigate these structure-function relationships. We believe that the interface between nanoscience and cell biology may provide the first hints as to how biological systems operate across so many length scales to provide a control system that links their structural, mechanical, and biochemical functions.

VICKI COLVIN, RICE UNIVERSITY

Abstract: The Health Effects of Engineered Nanomaterials

As society uses nanomaterials in greater quantities, and in consumer products, a public debate on the relative merits of nanotechnology has developed. The central question is whether the unknown risks of engineered nanoparticles, in particular their environmental impact, outweigh their established benefits for society. It is a challenge to approach this problem technically at this time, as there is little literature directly relevant for engineered nanostructures. However, given the unique chemical and physical properties of nanoparticles it is reasonable to expect that their biological effects may be quite distinct from either molecular species or larger particulate matter. Our own data on the environmental chemistry and toxicology of carbon nanostructures supports this viewpoint, though it is not possible to generalize these results to all nanostructure classes. Clearly, the technical community needs to address this problem directly so that there is better information for policymakers to use when handling the complex issues associated with regulation and public perception. How this debate evolves may be among the most important factors in defining the trajectory of nanotechnology commercialization.

MICHAEL DUSTIN, NEW YORK UNIVERSITY

Abstract

We have been focusing on the problems of cell adhesion and intercellular communication within the immune system. The immune system provides an outstanding model for cell-cell communication because the cells are readily isolated and many molecular pathways that control immune cell activation are well understood at the genetic and biochemical level. We have been interested in how molecular structures like the T cell antigen receptor and adhesion molecules work together during T cell activation. We discovered that a modification of the glass supported planar phospholipid bilayer (planar bilayer) system of McConnell allowed us to visualize and quantify molecular interactions. We fluorescently tagged purified, glycolipid anchored ligands for T cell adhesion molecules and inserted them into liposomes that were used to form planar bilayers. T cells adhered specifically to the bilayers containing the adhesion ligands and we could visualize and quantify accumulation of ligands in the interface. We found that we could measure 2D affinity of molecules' receptor-ligand interactions and study lateral segregation of two different types of adhesion molecules that differ in size. Insertion of major histocompatibility complex (MHC) proteins with agonist peptides allowed stimulation of T cells with complementary T cell receptors. Visualizing this process led to the discovery of dynamics of immunological synapse formation: T cell receptors interact with MHC in the periphery of the contact and then translocate to the center, while the adhesion ring starts as a central disc perforated by T cell receptor clusters and then inverts into a ring over several minutes. Replacement of the antigen-presenting cell with an artificial construct creates an opening to manipulate the system using tools of nanotechnology, which offers biologists a way to dissect the process of signaling in structures like the immunological synapse. The immunological synapse system offers the nanotechnologist an accessible biological-synthetic material interface with established function.

JOANNA S. FOWLER, BROOKHAVEN NATIONAL LABORATORY

Abstract

My research is on the design, development and application of radiotracers labeled with short lived positron emitting isotopes for positron emission tomography (PET) imaging in living humans and animals. The radiotracers are typically small organic compounds labeled with carbon-11 ($t_{1/2}$: 20.4 min) or fluorine-18 ($t_{1/2}$: 110 min) which are designed to track biochemical transformations or drug pharmacokinetics in living systems.

The coupling of PET to nanoscience is important both in developing new radiotracer materials as well as the application of PET to problems such as the following:

- Assessing the effects of nanomaterials on living systems (e.g. toxicity)
- Tracking the distribution and kinetics of nanomaterials which may be developed as drug or contrast vehicles in living systems (providing that these nanomaterials could be labeled with a PET isotope)

In addition the imaging field is a prime example of multi-disciplinary research where scientists (chemists, physicists, biologists, physicians, mathematicians, engineers and others) integrate their efforts on specific scientific/medical problems. In this sense it could provide a model for multi-disciplinary research and cross-disciplinary training of students, which will probably be needed to fully exploit nanoscience.

ROLF GRUETTER, UNIVERSITY OF MINNESOTA

Abstract

Our research has focused in the brain on attempting to elucidate the ability to characterize the metabolism of neuronal and glial cells. These cell types are (based on literature) distinctive in their rate of metabolism, but the location of certain enzymes also is reported to be asymmetrically distributed. For example, glutamine synthetase and pyruvate carboxylase are in the glial compartment. Evidence suggests that glutamine is mostly in glial cells and glutamate mostly in neuronal cells. In addition, brain glycogen, a store of fuel whose importance has been underestimated, also is localized to the glial cell. Furthermore, the lactate shuttle hypothesis supposes that glucose in activation is metabolized in the glia, then much of it is exported to the neuron in the form of lactate for oxidative generation of energy.

Because of the differential distribution of these enzymes and metabolites, *in vivo* NMR spectroscopy now offers an unprecedented opportunity to study their metabolism separately and *in vivo* and to quantify neurotransmission and glutamate excitotoxity, all of which have been implicated in the pathogenesis of key neurodegenerative diseases. With the advent of realistic therapies for many of these diseases, early detection of cellular dysfunction is critical not only in diagnosis, but also in the guidance of available treatment regimens.

While measurements such as the aforementioned do not allow the study of individual cells, they provide a tissue average characterization of these different cell types. Moreover, compartmentation of most of these metabolites has been based on *in vitro* work of, e.g., cell cultures or brain slices, which may or may not reflect the *in vivo* condition.

Nanotechnology approaches can help in furthering the imaging modalities in several ways. First, by aiding the cellular (or even subcellular) localization of metabolites and enzymes with the introduction of smart reporter molecules (molecular imaging) that selectively affect, e.g., the NMR signal of these metabolites without perturbing normal cell function. Alternatively, it would be exciting to have compounds (radioactively labeled) that would exhibit a high selectivity to specific cell types, enzymes or metabolites, that could be imaged non-invasively, but a post-mortem characterization of their localization would also be helpful.

Second, an exciting area of research that enhances sensitivity is the use of hyperpolarized compounds. However, in order for these enhancements to benefit cellular NMR measurements *in vivo*, the hyperpolarized compound has to be administered non-invasively and it has to be transported via the blood and blood-brain barrier to the brain cells. When dissolved in solution, most hyperpolarized compounds rapidly lose the hyperpolarization, which limits their usefulness at this point. A vehicle for transporting the compound to the brain cell, while preserving its hyperpolarization, is very much desired.

PEIXUAN GUO, PURDUE UNIVERSITY

Abstract: Bottom-up Assembly of Biological Materials and Incorporation into Nanodevices

Living systems contain a wide variety of nanomachines and ordered structures, including motors, arrays, pumps, membrane pores, and valves. The novelty and ingenious design of such machines have helped inspire the development of biomimetics. Research in bottom-up assembly is being devoted to make these machines function outside their native environment. Further research in the near future will focus on incorporating these imitating nanomachines into nanodevices for drug or

gene delivery, gearing of nano-equipment, driving of molecular sorters, building of intricate arrays and chips for diagnostics, molecular sensors, and actuators in electronic or optical devices.

Dr. Peixuan Guo, professor of molecular virology at Purdue University, is interested in viral structure and assembly. His lab has assembled infectious and replicating virions of bacterial virus phi29 through the exclusive use of synthetic RNA, DNA, and purified recombinant proteins. He discovered an ATP-binding RNA molecule (pRNA) that forms a hexameric ring to drive the DNA-packaging motor. The imitating 30 nm motor constructed in his lab has been tested and found to be the strongest biomotor studied to date. The formation of ordered structural arrays of the motor and its components, the retention of motor function after pRNA modification and extension, and the ease in manipulating the shape and size of pRNA dimers, trimers, and hexamers make this motor and its components promising building blocks for nanodevices.

1. DNA and proteins have been investigated extensively to further elucidate the mechanism underlying their bottom-up assembly and their possible applications in nanotechnology. RNA has largely been ignored as a potential building block in nanotechnology. In fact, RNA can be manipulated to form a variety of flexible shapes and can form dimeric, trimeric, and hexameric arrays. Stable RNA complexes or arrays can be made through the use of RNase-resistant nucleotide derivatives. Thus, RNA is another class of important materials in bottom-up assembly.
2. Virologists have been studying bottom-up viral assembly for decades. Recent achievements in this field indicate that the assembly of nanomachines from the bottom-up is feasible.
3. In order to incorporate natural nanomachines or biological materials into nanodevices, it is necessary to connect these biological materials with other nonbiological nanomaterials such as nanotubes and nanogold particles. One immediate task is to search for methods to link biological with nonbiological nanomaterials. Collaboration and interaction between biologists and material scientists are essential to such endeavors.

MATTHEW A. HOWARD III, UNIVERSITY OF IOWA

Biography

Matthew Howard is a neurosurgeon and medical device inventor. His undergraduate degree was in physics, and this background in a fundamental science has strongly influenced his work as a device inventor. In collaboration with other neurosurgeons and physicists, Howard developed the Magnetic Surgery System (MSS). This system was developed to address a fundamental problem with medical device interventions—difficulty in controlling the tip of flexible implants. This problem was addressed by using a magnetic field to guide and control magnetically tipped implants. By forming effective multidisciplinary working groups of physicians, physicists, and engineers, a wide range of technical hurdles was overcome and a practical medical device evolved.

Abstract

In the last year there has been explosive growth in the clinical utilization of MSS technology. The system makes interventional procedures more efficient, removes health care workers from X-ray fields, and enables physicians to carry out interventional procedures that cannot be performed using manual techniques.

It has been my experience that successful medical device inventions require that a medical problem be clearly defined and that creative minds from multiple disciplines engage in effective

collaborative work. The more that innovative clinicians and scientists know about each others' fields, the greater the probability of success. My knowledge of nanotechnology is very limited, but my intuition tells me there could be useful device applications in neurology and neurosurgery. As a conference participant I hope to learn more about this technology, and provide information on the medical challenges we face.

DON INGBER, HARVARD MEDICAL SCHOOL/BOSTON CHILDREN'S HOSPITAL

Abstract

My laboratory is interested in the process by which cells decide between fates, such as growth, differentiation, motility, contractility and apoptosis, during morphogenesis. We have used nanotechnology-based self-assembly techniques to create defined culture environments to study how extracellular matrix and cell shape regulate cell fate switching. We also have developed micro- and nano-magnetic methods to probe the molecular and biophysical basis of cellular mechanotransduction, and to quantitate the viscoelastic properties of macromolecular adhesion complexes in living cells. In parallel studies, we have used fluorescent molecular read-outs to define the way in which cells control their shape and mechanics through natural self-assembly of cytoskeletal filaments on the nanometer scale. A nanosurgical method utilizing femtosecond lasers also has been developed to selectively vaporize nanodomains within living cells, without compromising surrounding structures or cell viability. Ongoing studies focus on: development of nanomagnetic approaches to non-invasively control signal transduction and gene expression in living cells, engineering of novel FRET-based nanomolecular fluorescent readouts of stimulus-response coupling, mechanical analysis of nanotechnology-based cytoskeletal mimics, and computational modeling of cell fate switching.

Molecular cell biologists are interested in understanding the structure and function of molecules on the nanometer scale. However, the reality is that most of the critical functions of living cells emerge within multimolecular complexes that appear on the micrometer scale. The classic biological approach that involves dissection of these structures into their component parts loses aspects of these supramolecular structures that are critical for their function (e.g., architecture, mechanics, prestress, position). However, when analysis is carried out in whole cells, the complexity of these systems complicates interpretation of the results. New advances in nanotechnology from the physical and engineering sciences provide the ability to assemble defined bio-inspired systems that mimic complex properties of cells or subcellular components. Nanotechnology thus offers an exciting new "bottom-up" approach to attack these types of problems. Nanoscale tools based on optical and magnetic approaches also provide the ability to physically probe individual molecules within the structural context in which they normally function, while simultaneously reading out changes in systems-wide activities. Combination of these approaches with computational modeling may therefore provide new insight into the greatest challenge we face today: explaining how complex behaviors at the micrometer scale emerge from collective interactions among thousands of interacting nanoscale components.

REBECCA RICHARDS-KORTUM, UNIVERSITY OF TEXAS AT AUSTIN

Biography

Rebecca Richards-Kortum holds the Cockrell Family Chair in Engineering and is a professor of biomedical engineering at the University of Texas at Austin. The goal of her research group is to develop optical tools for the early detection of cancer and its precursors. She is developing tools to

image the biochemical and morphologic features of cancer *in vivo* and nanoparticle-based contrast agents to extend the range of molecular changes of cancer that can be imaged *in vivo*. In collaboration with Dr. Michele Follen at the University of Texas M.D. Anderson Cancer Center, Richards-Kortum has carried out clinical trials of optical spectroscopy and imaging to detect cervical precancer in more than 1,500 women.

Abstract

Results show that optical technologies can image precancer with dramatically improved specificity, and have the potential to significantly reduce the costs associated with cervical cancer screening. In collaboration with Dr. Michael Descour at the University of Arizona, Prof. Richards-Kortum has developed fiber-optic-based confocal microscopes to image subcellular morphology *in vivo* in real time. Images obtained *in vivo* show a detailed view of the nuclear structure and the changes that are characteristic of cancer and its precursors. Clinical trials are underway to test the ability of this technique to detect precancers, and to image tumor margins *in vivo*. Their groups are working to develop MEMS-based pen-sized confocal microscopes for wide-scale *in vivo* use. Most recently, she has worked with Dr. Kostia Sokolov at the UT MD Anderson Cancer Center to develop a new class of optically-active contrast agents to enable *in vivo* molecular imaging of cancer-related biomarkers. These contrast agents consist of optically active nanoparticles coupled to a probe molecule that provides molecular specificity. Images of cells, tissue cultures and organ cultures indicate that these agents can be imaged in real time with confocal microscopy, yielding dramatic images of the expression patterns of cancer-related biomarkers. Recent animal trials show that these agents can be delivered topically to the tissue at risk to image biomarker distribution with subcellular resolution *in vivo*.

Cancer is a major public health problem. Currently, the clinical classification of cancer and its precursors is based on phenotypic markers, such as nuclear to cytoplasmic ratio and extent of invasion, which are then used to select therapy. In the last decade, enormous progress has been made to understand the molecular events that accompany carcinogenesis. The identification of unique molecular markers of cancer and the associated processes they modulate has led to the development of new molecular cancer therapies that affect these processes, such as chemoprevention, chemo-radiation, gene therapy, and immunotherapeutics. There is an important need to image the molecular features of cancer *in vivo*. Progress toward a molecular characterization of cancer would have important clinical benefits, including (1) detecting cancer earlier based on molecular characterization, (2) predicting the risk of precancerous lesion progression, (3) detecting margins in the operating room in real time, (4) selecting molecular therapy rationally, and (5) monitoring response to therapy in real time at a molecular level. *Coupled advances in nanobiotechnology, MEMS and imaging science are required to achieve these important goals.*

Imaging the molecular features of cancer requires molecular-specific contrast agents that can safely be used *in vivo* as well as cost-effective imaging systems to rapidly and non-invasively image the uptake, distribution and binding of these agents *in vivo*. Radiographic imaging modalities such as CT scan and MRI, although useful for delineating the deep extent of advanced carcinomas, are not sufficiently sensitive to detect small, intraepithelial lesions. Optical imaging is a relatively new modality that enables real time, high resolution imaging of epithelial tissue. Optical imaging of tissue can be carried out noninvasively in real time, yielding high spatial resolution (less than 1 micrometer lateral resolution). Optical imaging systems are inexpensive, robust and portable because of advances in computing, fiber optics and semiconductor technology. Confocal microendoscopes that image near-infrared radiation reflected light have been used to image sub-cellular features in epithelial tissue at video rate to depths exceeding 400 micrometers. Optical

imaging systems are ideally suited for early detection of intraepithelial disease and for assessing tumor margins and response to therapy.

A comprehensive strategy is needed to develop inexpensive, rugged and portable optical imaging systems for molecular imaging of cancer, which couples the development of *nanoparticle-based,* optically active contrast agents with advances in functional genomics of cancer. Our group is working to develop optically active contrast agents that can be applied topically to areas of tissue at risk to monitor the three-dimensional profile of the targeted biomarkers as well as morphologic and architectural biomarkers such as nuclear to cytoplasmic ratio. We believe these contrast agents and imaging systems will have broad applicability to detect and monitor many types of cancer. At the same time, we are developing *MEMS-based* inexpensive, portable optical systems to image the morphologic and molecular signatures of neoplasia noninvasively in real time. These real-time, portable, inexpensive systems can provide tools to characterize the molecular features of cancer *in vivo*.

PAUL C. LETOURNEAU, UNIVERSITY OF MINNESOTA

Abstract

I study the motility of axonal growth cones and how growth cone movements are regulated by extrinsic molecules that guide developing axons to their synaptic targets. My research involves approaches to clarify how guidance molecules act in growth cones to regulate the functions of actin filaments and microtubules, the dynamic cytoskeletal elements that determine growth cone behavior and axonal elongation. A key feature of guiding axonal elongation, which is general to cellular movements *in vivo*, is to establish directed cell movement; when and where to start, what direction to move, when and where to stop. Investigation of these issues is where I see my uses of nanotechnology.

In my view this research will be advanced by nanotechnology methods for producing gradients or other precise patterns of extrinsic molecules. This could be at a cellular and multicellular scale, such as for patterning neurons or cells to study cell populations or for potential clinical applications, and at the subcellular scale to locally stimulate and analyze cellular physiology in limited regions of a cell. Other advanced nanotechnologies will allow localized live cell manipulations, sensing and visualization of intracellular physiology to probe the mechanisms by which cell movements are directed. These methods might allow high resolution tracking of motile proteins and other components within growth cones, detection of local fluxes of ions, cyclic nucleotides, protein phosphorylation, protein interactions, enzyme activities, or production of mechanical forces. A key aim of these studies is to clarify how cell movement is locally activated and regulated.

FRANCES LIGLER, NAVAL RESEARCH LABORATORY

Biography

Frances Ligler is the U.S. Navy's Senior Scientist for Biosensors and Biomaterials. Building on a background in biochemistry and immunology, she has spent the last 18 years developing portable biosensors for on-site identification of biological and chemical hazards in a wide variety of sample matrices. In addition to biosensors, she also leads research projects in microfluidics and proteomics.

Abstract

Bioanalytical capability is the primary lure for developing wet nanotechnologies. Capabilities such as analyzing the behavior of living cells, measuring changes in the molecular composition of cells in response to environmental forces or disease processes, or sequencing individual molecules are already envisioned. Within the next decade, these capabilities will become viable methods and configured in nanosystems for parallel processing. Additionally, new biological and biomimetic materials will be fabricated with complex structures highly organized at the molecular level for sensing and reaction to chemical or physical stimuli.

TERRY A. MICHALSKE, SANDIA NATIONAL LABORATORIES

Abstract

The National Nanotechnology Initiative (NNI) has grown into a major U.S. investment involving over twenty Federal agencies. As a lead Federal agency, the Department of Energy (DOE) is developing a network of Nanoscale Science and Research Centers (NSRC). NSRCs will be highly collaborative national user facilities associated with DOE National Laboratories where university, laboratory, and industrial researchers can work together to advance nanoscience and technology. The Center for Integrated Nanotechnologies (CINT), which is operated jointly by Sandia National Laboratories and Los Alamos National Laboratory, has a unique technical vision focused on integrating scientific disciplines and expertise across multiple length scales going all the way from the nano world to the world around us. It is often said that nanotechnology has the potential to change almost everything we do. However, this prophecy will only come to pass when we learn to couple nanoscale functions into the macroscale world. Obviously coupling the nano- and micro-length scales is an important piece of this challenge and one can cite many examples where the performance of existing microdevices has been improved by adding nanotechnology. Examples include low friction coatings for MEMS and compact light sources for μChemLab spectrometers. While this approach has produced significant benefit, we believe that the true potential will be realized only when device architectures are designed "from the nanoscale up," allowing nanoscale function to drive microscale performance.

JOHN A. PARRISH, HARVARD UNIVERSITY

Biography

John A. Parrish, M.D., is the chairman of the department of dermatology at Harvard Medical School (HMS), chief of the Department of Dermatology Service at Massachusetts General Hospital (MGH), professor of dermatology at HMS, and professor of health science and technology at MIT. Although his original training was in internal medicine, dermatology, and clinical research, Parrish has spent the last 25 years conducting and directing basic research in photobiology, biological effects of lasers, and cutaneous biology. Dr. Parrish developed a novel treatment (oral psoralen photochemotherapy, or PUVA), which is now used worldwide to treat a variety of skin diseases including psoriasis. His research group at MGH introduced laser lithotripsy of kidney stones, selective laser therapy of vascular birthmarks, and other novel techniques for laser-based diagnosis and treatment.

Dr. Parrish organized and directs the first, and now the world's largest, multidisciplinary research group to systematically study the basic nature of laser effects on tissue, the MGH Wellman Laboratories of Photomedicine. Dr. Parrish is also director of the MGH-Harvard Cutaneous

Biology Research Center (CBRC), a research center committed to fundamental research in cutaneous biology as broadly defined. Dr. Parrish directs the Center for Integration of Medicine and Innovative Technology (CIMIT), a multidisciplinary and multiinstitutional research effort to improve patient care by bringing together scientists, engineers and clinicians to catalyze implementation of innovative technology, emphasizing minimally invasive diagnosis and therapy. Dr. Parrish is a member of the Institute of Medicine, National Academy of Sciences.

Dr. Parrish has over 300 publications, many of which describe new treatments and diagnostics. He has written eight books, most of which are textbooks or scientific monographs but also include a book on baseball, a book on the Vietnam War, and a book on skin for the layman.

Abstract

Nanotechnology will have a major impact on biosensors, medical imaging and diagnostics. Synthesis of new biomolecules, coached self-assembly, and further miniaturization of microsystems will merge with the insights and technology used in modern molecular biology. The lag time for impact of this technology in medicine could be very long and the path could be somewhat haphazard. We will present one model of structured interactions designed to facilitate, accelerate and focus the capture of technology to solve clinical problems.

Meetings such as this workshop will develop national strategies to provide the collaborations needed from technologists, biologists and health care professionals. We will describe a local Boston-based collaboration designed to be a crucible in which teams of clinicians, scientists and engineers identify difficult problems, generate new ideas and develop innovative technological solutions. Nanotechnology provides our latest and greatest challenge and opportunity.

CIMIT (Center for Integration of Medicine and Innovative Technology) is a non-profit consortium of academic institutions founded by Partners HealthCare System, Massachusetts General Hospital, Brigham and Women's Hospital, Massachusetts Institute of Technology, and Draper Laboratory. CIMIT's mission is to improve patient care by bringing together scientists, engineers, and clinicians to catalyze development of innovative technology, emphasizing minimally invasive diagnosis and therapy. CIMIT is designed to form the academic base for interactions with government and industry

The complex barriers to rapid successful capture of nanotechnology by medicine are enormous, exceeded only by magnitude of the potential.

NICHOLAS PEPPAS, UNIVERSITY OF TEXAS AT AUSTIN

Biography

Nicholas A. Peppas is the Fletcher S. Pratt Chair of Chemical Engineering, Biomedical Engineering, and Pharmaceutics at the University of Texas at Austin. He is a world leader in the fields of controlled drug delivery, biomedical engineering, biomaterials, molecular modeling of protein structures in contact with biomaterials and tissues, modeling of biomedical devices, bionanotechnology, and molecular recognition processes. Among other medical devices, he has developed, patented, or commercialized delivery systems for oral administration of insulin to type I diabetic patients, systems for oral delivery of calcitonin for treatment of postmenopausal women suffering from osteoporosis, new contact lenses that do not need to be replaced but once a week, intraocular lenses for cataract patients, improved materials for cartilage replacement, biogels for

epidermal release of growth factors to improve wound healing, new materials for artificial heart linings, and materials for vocal cord replacement or reconstruction.

Peppas was educated in chemical engineering at the National Technical University of Athens, Greece (Dipl.Eng., 1971) and at the Massachusetts Institute of Technology (Sc.D., 1973). He has served as a visiting professor at the Universities of Geneva, Paris, Parma, Hoshi University, Hebrew, Naples, Berlin, Santiago de Compostela, and Complutense of Madrid, Spain. He has received honorary doctorates from the University of Ghent, Belgium; the University of Parma, Italy; and the University of Athens, Greece.

Peppas is the author of 850 publications, 27 books and volumes, 220 abstracts, and 19 U.S. and international patents. He is president of the Society for Biomaterials, past-president of the Controlled Release Society, and past-director of AIChE. Peppas has been elected a founding fellow of AIMBE, American Physical Society, AIChE, SFB, AAPS, and the American Association for the Advancement of Science.

He has been recognized by more than 60 national and international awards including the 2002 Dale E. Wurster Award in Pharmaceutics of the American Association of Pharmaceutical Scientists, the 2002 Newsmaker of the Year of the American Chemical Society, and the 2002 recognition as a Pioneer in Biomedical Engineering from the IEEE Engineering in Medicine and Biology Society.

Abstract

My research contributions have been in several areas of bionanotechnology, drug delivery, biomaterials, biomolecular engineering, and polymers. The multidisciplinary approach of my research in biomolecular engineering blends modern molecular and cellular biology with engineering to generate next-generation systems and devices, including bioMEMS with enhanced applicability, reliability, functionality, and longevity. The fundamental studies of my group have provided valuable results on biomaterials design and development. Physiologically-controlled and disease-responsive feedback control-based devices require the operation/function of electrical and mechanical parts as a result of on-line measurement of physiological variables of the body, blood, or other biological fluids. We have utilized the basics of biomedical transport phenomena, control theory, and kinetic behavior to design novel devices and to optimize their behavior in the body or in contact with the body. Adjustment of appropriate components of these devices has been based on simple or sophisticated control or other physiological based models.

My research has led to the development of a number of biomedical polymers and devices including hydrogels in drug delivery applications, the first pH-sensitive and temperature-sensitive systems for modulated release of streptokinase and other fibrinolytic enzymes, and novel buccal and vaginal controlled release devices. More recently, our group has announced new nanoparticulate systems for oral protein delivery systems and new biomaterials.

ROBERT V. SHANNON, HOUSE EAR INSTITUTE

Biography

Robert Shannon received his B.A. in mathematics and psychology from the University of Iowa in 1971 and a Ph.D. in psychology from the University of California, San Diego, in 1975. Since 1978 he has worked to develop cochlear implants and auditory brainstem implants, which restore some degree of auditory function via electrical stimulation of the auditory nerve or cochlear nucleus in deaf people.

Abstract

Prosthetic electrical stimulation of the human nervous system can be divided into three classes: global, coarse, and fine, referring to the level of stimulation specificity required for the task. Electrical stimulation for pain control or for relief of Parkinson's symptoms targets a global region, and neural activation is not very spatially specific (global). Electrical stimulation of the cochlea has been highly successful in restoring speech understanding to deaf people, so that most deaf people with cochlear implants can converse freely on the telephone. This type of stimulation requires only coarse spatial specificity. However, complex auditory perception and visual perception require a much higher degree of stimulation specificity that cannot be achieved presently with existing prosthetic devices and approaches. New electrode designs incorporating nanotechnology may improve the stimulation specificity by spatially controlling the current flow, or by actively promoting the growth of the remaining neurons to establish intimate contact with the electrical substrate.

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MICHAEL L. SHULER, CORNELL UNIVERSITY

Biography

Michael Shuler received his undergraduate degree in chemical engineering from the University of Notre Dame in 1969 and a Ph.D. in chemical engineering in 1973 from the University of Minnesota. He has taught at Cornell since 1974. He is currently the Samuel B. Eckert Professor of Chemical and Biomolecular Engineering and the director of the Biomedical Engineering Program. His research has covered a broad spectrum of topics in bioengineering including better processes for biomanufacture of proteins from recombinant DNA technology, production of the anticancer agent, Taxol, from plant cell culture, detailed computer models of living cells, aspects of drug delivery, bioenvironmental engineering, and surrogate animal systems for toxicity testing using microfabricated devices. Many concepts from Shuler's research have been commercialized. He has served on numerous advisory boards for the government, publications, national professional organizations, other universities, and corporations. He has received numerous professional honors for both teaching and research. He has been elected to membership in the National Academy of

Engineering and the American Academy of Arts and Science. He was named a NYSTAR Distinguished Professor in 2001 by New York State.

Abstract

Nanobiotechnology holds promise in two interrelated areas: (1) as a source of novel devices for medical intervention, homeland security, food safety, and a host of practical applications, and (2) as a platform technology to probe how biological systems function at the molecular and cellular level. In terms of practical devices, one can easily foresee biosensors, high throughput devices for genomic/proteomic and related data acquisition, high throughput surrogate *in vitro* systems for drug discovery and evaluation (to reduce and complement animal studies), neural probes (and possibly prosthesis, but maybe in more than 10 years), improved drug delivery systems, other medical probes, microsurgical tools, possibly (not sure, about 10 years) devices to couple metabolic reactions to provide energy for implantable mechanical devices, etc. In terms of biological function, nano- or micro-devices will provide tools to assess effects of extracellular signals (both mechanical and chemical) in intra- and inter-cellular signal transduction and on understanding cell-to-cell and cell-to-matrix interactions in constructing artificial tissues. Small-scale, cell-level analytical measurements can be combined with appropriate computer models to provide a firmer basis for systems biology and realization of functional genomics.

Success in making effective devices and in extracting insight into biological systems depends on development of appropriate physio-chemical tools. Understanding surface chemistry effects to develop bioactive materials with sub-micrometer structure, macromolecular/colloidal interactions, chemical-to-mechanical energy transduction, and microfluidics all present challenges to accomplishing goals in device development and to effective experimental design for probing cellular and subcellular biology. Further experimental devices to probe cellular behaviors should be coupled with theoretical/computational structures to obtain maximum benefit from such experiments.

MICHAEL L. SIMPSON, OAK RIDGE NATIONAL LABORATORY

Biography

Dr. Simpson is Thrust Leader for Nanofabrication Research Laboratory, Center for Nanophase Material Science at the Department of Energy Nanoscience Research Center at The Oak Ridge National Laboratory (http://www.cnms.ornl.gov/). He is also a participating faculty member, Center for Environmental Biotechnology at the University of Tennessee (http://www.ceb.utk.edu/), as well as a participating faculty member, Tennessee Advanced Materials Laboratory (TAML) at the University (http://www.phys.utk.edu/taml/).

Abstract

My group focuses on measuring, analyzing, modeling, simulating, mimicking and interfacing to information processing pathways in cells. Of particular significance for this workshop is our development of functional viable cell/synthetic nanostructure hybrids. We have demonstrated the controlled synthesis and directed assembly of deterministic arrays of vertically aligned carbon nanofibers (VACNFs) grown on a wide variety of substrates (including quartz and glass slides) with wide bases that provide mechanical strength while still generating a small diameter tip appropriate for insertion directly into cells. The viability of the cells after VACNF insertion has been demonstrated by the long-term expression of a constitutively-expressed GFP gene carried on nanofiber-borne plasmid molecules. This hybrid combination of cell and nanostructure has

accomplished gene expression from plasmid DNA tethered to the VACNFs. In the near term we will see these synthetic nanoscale tools interface directly with a wide variety of intracellular biomolecular processes; allow the introduction of controlled stimuli directly into pathways, circuits and networks; and provide transduction of responses with both spatial and temporal resolution. Over the next several years, we envision such structures becoming the hardware tools that close the feedback loop between simulation and experiment in systems and synthetic biology, providing a deeper understanding of gene circuit and network structure and function. The emerging understanding of gene circuit/network architecture and function highlights the information processing density in the complex systems from which cellular (and ultimately tissue, organ, and organism) function emerges. Synthetic systems that operate on comparable size and complexity scales may be our best hope of unraveling the operation of these systems and perhaps intervening in processes gone awry (e.g., disease) at the molecular level.

MALCOLM L. SNEAD, UNIVERSITY OF SOUTHERN CALIFORNIA

Biography

Malcolm Snead received his D.D.S. degree from Loyola University and then studied pathology, earning a doctoral degree from the University of Chicago in 1981. As a graduate student, he fell in love with the extracellular matrix and regulated gene expression as they relate to biomineralizing systems such as bones and teeth, as well as in developmental biology, with the problem of pattern formation and cell identity. His interests now focus on protein self-assembly in the extracellular space and the biofabrication of a matrix that directs its own replacement by a calcium phosphate mineral and the differentiation of specialized epithelial cells that direct mineral formation.

Abstract

We are pursuing the role of epithelial-derived proteins to undergo self-assembly into nanospheres that then guide the crystallite habit of the mineral phase. We have explored the ability of selected domains from the dominant proteins involved in this process to alter the biomineralization process. We have engineered these proteins with the goal of providing gain of function and loss of function to their physiologic role, testing the engineered proteins in whole animals using genetic manipulation of the mouse genome. We have also undertaken a discovery program seeking to better understand the patterning of the extracellular matrix and its inherent information content.

Uncertainties and opportunity areas for nanotechnology and biomedicine under investigation include the following:

- *Bone bioceramic*. Biology issue: identity of the short-range interactions among proteins required for bone matrix assembly; tendon-bone interface. Nanotechnology: termination of crystallites; modifier of bone bioceramic behavior and crystallite behavior in materials science application; lamellar organization of bone with intrinsic positional information appropriate to comply with functional load (Wolfe's law); optimized interfacial design.
- *Cell polarity*. Biology issue: incomplete understanding of extracellular matrix clues for activation and maintenance of secretory face; manipulation of secretory pathways. Nanotechnology: optimal matrix texture to induce polarity; intracellular tools to modify secretory activity and vectorial transport.
- *Cell identify and positional information*. Biology issue: how do cells come to know where they are and interpret clues and signals for their identity and positional information? Nanotechnology: mimicking such positional information likely critical to all biomedical

applications; tissue and organ specificity for unique matrix constituents. Is there a "universal" matrix?

- *Biomineralization (enamel and dentin)*. Biology issue: interfacial biology remains poorly understood; how are dissimilar tissues integrated for optimal materials properties? Nanotechnology: organize the interface for optimal material properties by biomimetic design?

PATRICK STAYTON, UNIVERSITY OF WASHINGTON

Biography

Stayton serves as professor in the Department of Bioengineering at the University of Washington, where he directs the Molecular Materials Group at the UW Engineered Biomaterials (UWEB) Center.

Abstract

Our research group is interested in elucidating the fundamental mechanisms of biomolecular recognition and applying the unique capabilities of biological molecules to biotechnologies. In the nanotechnology area, we are developing "smart" biomolecular materials that merge the molecular recognition capabilities of biomolecules with the responsiveness and chemical versatility of functional polymers. These new biohybrid materials are designed for drug delivery and bioanalytical applications.

Challenges and opportunities for nanobiotechnology:

1. *Drug delivery systems for biomolecular drugs*. While there have been striking advances in the identification of interesting drugs based on DNA, RNA, and proteins, the ability to effectively deliver them has proven to be a major challenge. Nanoengineered delivery systems in nature can achieve delivery of such molecules quite effectively, and there is thus an opportunity for technology development where new synthetic materials engineered on the nanoscale might begin to challenge the biological systems without their problems.
2. *Bioanalytical technologies for drug discovery*. There is a strong need for new technologies that allow high throughput drug screening in biological systems that more accurately reflect real pharmaceutical activity. The opportunity is to develop new molecular toolsets where nanoengineered "spies," "reporters," and the like can be applied to report on relevant cell processes that are altered by potential drug candidates, as well as device formats that allow high-throughput screening.
3. *Bioanalytical technologies for medical diagnostics*. There are strong needs for simple-to-operate diagnostic devices for point-of-care usage in the home, in the field, or in resource-poor settings. While they need to be simple and inexpensive, the underlying device and componentry will require sophisticated engineering at the nanoscale. Current challenges lie in upstream processing of complex fluids for the purification and concentration of targets, signal amplification to enhance specificity, and multiplexing within simple diagnostic formats. Corresponding opportunities are for the development of new molecular componentry engineered for the specific device formats, i.e., modified proteins and DNA designed for better manipulation in microfluidic devices.

JONATHAN V. SWEEDLER, UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

Biography

Jonathan V. Sweedler is the Lycan Professor of Chemistry and holds appointments in the Neuroscience Program and in the departments of Integrative Physiology and Bioengineering at the University of Illinois at Urbana-Champaign. The Sweedler group's major research involves analytical neurochemistry and bridges the fields of neuroscience and analytical method development. Major efforts involve developing methods to probe nanoenvironments in and around neurons in well-defined neuronal networks. Techniques under development include methods to increase the mass sensitivity and reduce the required volumes of analytical methods, including capillary separations, laser-based detection methods, MALDI sampling techniques, and nanoliter volume NMR, and to use these approaches to study the distribution and the dynamic release of neurotransmitters and elucidate new neurochemistry including novel neuropeptides and classical neurotransmitters.

Abstract

The University of Illinois has embraced the concept of integrating nanotechnology and biology with the creation of two new UIUC cross campus initiatives—*the Cell as a Micromachine* (http://www2.uiuc.edu/initiatives/micromachine.html) and *PharmaEngineering* (http://www2.uiuc.edu/initiatives/pharmaengineering.html). Both initiatives are designed to use advances in nanoengineering and biotechnology to study the basic processes occurring in the cell. These are campus-level cross-cutting interdisciplinary efforts that employ nanotechnology development, imaging technologies combined with cellular biology and physiology, and aim to offer novel insights and knowledge about cellular function and fate, and in the case of the PharmaEngineering initiative, a multi-level model, from molecule to tissue, with a novel drug-discovery base.

MATTHEW TIRRELL, UNIVERSITY OF CALIFORNIA, SANTA BARBARA

Abtract: Protein Analogous Micelles

Short, bioactive peptides are being used increasingly in applications from therapeutic, to biomaterials, to new materials synthesis. Even when these peptides are derived from sequences in proteins with ordered secondary structures, the peptides alone usually are disordered molecules in solution. With the idea that this disorder may diminish the bioactivity of peptides, we began a study of peptide-lipid conjugates, in which a peptide amphiphile is produced by linking a hydrophobic tail to a hydrophilic peptide. We have found that these molecules can be made to have very low critical micelle concentrations and, most interestingly, in the micellar state the peptide headgroups are often in highly ordered secondary structures. It is as if the micellization creates an artificial tertiary structure for the short peptides, constraining their translational entropy as in the full protein. This has further led to the idea of using these micelles as artificial proteins. We are exploring these synthetic objects for several kinds of protein activities, including DNA-binding, as might be useful in artificial transcription factors, and anti-microbial activity.

ROGER TSIEN, UNIVERSITY OF CALIFORNIA, SAN DIEGO

Abstract

My background comes from 31 years of attempts to build small and large molecules to probe signal transduction, mostly in live mammalian cells. Our recent emphasis has been on genetically encoded indicators based on fluorescent proteins and on hybrids where synthetic organic molecules are targeted to small genetically encoded motifs. Some remaining challenges I see for the field include: (1) better integration of imaging modalities from nanometers to meters and from milliseconds to days; (2) better readouts and perturbants of neuronal signaling; (3) more genetically encoded contrast agents for imaging modes other than fluorescence; (4) smarter delivery systems to concentrate contrast agents and therapeutic drugs in or on cells displaying fundamental molecular signatures of pathology.

LINDA TURNER, HARVARD UNIVERSITY

Abstract

My expertise is bacterial motility and behavior. I work with cells 1 μm in diameter powered by rotary motors only 50 nm in diameter. In addition to trying to understand how these cells process sensory inputs and move in a purposeful manner, my colleagues and I are trying to interface them with microfluidic systems. In collaboration with Kenny Breuer and Tom Powers, hydrodynamicists at Brown University, and Greg Huber, a theoretical physicist and modeler at U. Mass Boston, we have attached bacteria to solid surfaces, forming "bacterial carpets." These carpets pump fluids and propel small objects. In collaboration with Willow DiLuzio, a graduate student in George Whiteside's lab, we study the motion of bacteria confined to microchannels. Willow has developed specific geometries that guide and control bacterial motion. So we envisage an era in which microscopic organisms can direct or be directed by small mechanical devices. For example:

1. *Microorganisms and components*. Evolution has solved many of nanotechnology's problems. Nano-bio technologists could exploit both the organism and the diversity of structures and functions found in the microorganism. Why build complex sensory devices when bacteria have exquisitely sensitive receptors coupled with detectable behaviors? Why not harvest the microorganism's physical components: rotary motors, bushings, and universal joints?
2. *Microorganism-powered chips*. Microorganisms are the native inhabitants of the low Reynold's number hydrodynamic environment, and are the natural beasts-of-burden for microfluidic systems. They come with a myriad of functions and sensitivities. Biological diversity could be exploited to develop robust, self-contained, inexpensive, microfluidic chips whose function is carried out by the microorganisms.
3. *Microchanneled fabrics*. Most microchannels appear as shallow geometric channels confined to a 2-D surface in a thin film. By designing channels optimized for a specific function, then stacking the films, purposeful fabrics could be made. In the future, I imagine fabrics used for implants or bandages that absorb and sequester bacteria from their environment.

VIOLA VOGEL, UNIVERSITY OF WASHINGTON (now at the Swiss Federal Institute of Technology, ETH Zurich)

Abstract

We study the engineering/construction principles of biological nanosystems aiming at technological applications of biological concepts. Central questions include how cells sense mechanical forces and transform them into biochemical signals (mechanotransduction), how mechanical forces alter the molecular recognitions sites of proteins, and how motor proteins could be applied to transport cargo in an active way. This research in bionanotechnology asks for transdisciplinary techniques such as molecular self-assembly, molecular spectroscopy of single molecules, atomic force microscopy computer simulations (steered molecular dynamics), high-resolution optical microscopy as well as cellular and microbiology. The insights of this research can be used for tissue engineering, for developing new methods preventing bacterial infections, and for the development of new materials and pharmaceutical products.

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DAVID R. WALT, TUFTS UNIVERSITY

Abstract

We have been working in the field of optical fiber sensors for 20 years, with applications in the fields of medicine, the environment, oceanography, and public safety. Our present work is in the areas of high-density nanoarrays, medical diagnostics, cell-based biosensing artificial olfaction, and optical tweezers.

Nanobiotechnology offers tremendous opportunities for analysis in general and biosensors in particular. Nanoscale sensors will enable the development of extremely high-density sensor arrays with the ability to collect enormous amounts of data. Nanotechnology offers the potential for realizing genuine universal sensing capabilities. In addition, and perhaps counterintuitive, nanoscale sensors provide enhanced sensitivity and promise to facilitate single molecule detection. Problems with nanoanalytics and nanobiosensors include data management and the generic problem of connecting nanoscale systems to the outside world.

APPENDIX B. WORKSHOP AGENDA

Location: National Rural Electric Cooperative Association (NRECA)
4301 Wilson Blvd., Arlington, VA
Oct. 9–10, 2003
and
National Science Foundation
4201 Wilson Blvd., Arlington, VA
Oct. 11, 2003

THURSDAY OCTOBER 9, 2003

Morning Plenary Session, NRECA Rm. CC1

7:30 Registration; coffee and pastries available
8:00 Welcome, Purpose of the Workshop, NSET and NSF Perspective (Mike Roco, Chair, NSET; NSF/Directorate for Engineering)
8:05 NSET and NIH Perspective (Jeff Schloss, NIH/National Human Genome Research Institute)
8:10 PCAST Perspective (M. Kathleen Behrens, President's Council of Advisors on Science and Technology)
8:20 Introduction; workshop outline and approach (Viola Vogel, University of Washington, and Barbara Baird, Cornell University, Workshop Co-Chairs)

Session 1: Understanding how cells work through bottom-up assembly of biological nanosystems *ex vivo*

8:30 Plenary speaker, nanotechnology perspective: Michael Shuler, Cornell University
9:00 Plenary speaker, biomedical perspective: Michael Dustin, New York University

Session 2: Nanotechnology to enhance human health

9:30 Plenary speaker, biomedical perspective: James Baker, University of Michigan
10:00 Plenary speaker, nanotechnology perspective: Nicolas Peppas, University of Texas at Austin
10:30 Coffee Break

Session 3: *In vivo* analysis of cellular processes at the nanoscale

11:00 Plenary speaker, nanotechnology perspective: Viola Vogel, University of Washington
11:30 Plenary speaker, biomedical perspective: Jennifer Lippincott-Schwartz, NIH/National Institute of Child Health and Human Development
12:00 Lunch (provided in room)

Breakouts for Sessions 1–3

Session 1 Breakout (Understanding how cells work through bottom-up assembly of biological nanosystems *ex vivo*): NRECA Room CC1

1:15 Introductions, objectives: Gerald Selzer, NSF/Directorate for Biological Sciences and Michael Dustin, Session Coordinators
1:20 Marie-France Carlier, CNRS
1:25 Katherine Borden, NYU
1:30 Carlo Montemagno, University California at Los Angeles
1:35 Peixuan Guo, Purdue University
1:40 Michael Sheetz, Columbia University
1:45 Jay Groves, UC Berkeley
1:50 Klaus Schulten, University of Illinois
1:55 Matt Tirrell, UC Santa Barbara
(Angela Belcher, MIT, will participate on Oct. 10 only)
2:00 Open discussion, impromptu presentations by other participants invited
4:40 Wrapup, summary of outcomes: Gerald Selzer and Michael Dustin

Session 2 Breakout (Nanotechnology to enhance human health): NRECA Room CC2

1:15 Introductions, objectives: Christine Kelley (NIH/National Institute of Biomedical Imaging and Bioengineering) and James Baker, Session Coordinators
1:20 Thomas Meade, Northwestern University
1:25 Vicki Colvin, Rice University
1:30 Dan Branton, Harvard University
1:35 Samuel Stupp, Northwestern University
1:40 Matt Howard, University of Iowa
1:45 Linda Turner, Harvard University
1:50 Michael Simpson, Oak Ridge NL
1:55 Robert Shannon, House Ear Institute
2:00 Kwabena Boahen, University of Pennsylvania
(Larry McIntire, Georgia Tech., also will present if available)
2:05 Open discussion, impromptu presentations by other participants invited
4:40 Wrapup, summary of outcomes: Christine Kelley and James Baker

Session 3 Breakout (*In vivo* analysis of cellular processes at the nanoscale): NRECA Room CC3

1:15 Introductions, objectives: Jennifer Lippincott-Schwartz and Catherine Lewis (NIH/National Institute of General Medical Sciences), Session Coordinators
1:20 Barbara Baird, Cornell University
1:25 John Sweedler, University of Illinois
1:30 Donald Ingber, Harvard University
1:35 Malcolm Snead, University of Southern California
1:40 Paul Letourneau, University of Minnesota
1:45 Christopher Chen, Johns Hopkins University
1:50 Open discussion, impromptu presentations by other participants invited
4:40 Wrapup, summary of outcomes: Jennifer Lippincott-Schwartz and Catherine Lewis

Plenary Session: Reporting back from breakouts (Rm. CC1)

5:00 Session 1: Michael Dustin
5:20 Session 2: James Baker
5:40 Session 3: Jennifer Lippincott-Schwartz
6:00 Reception
7:00 Dinner (Arlington Hilton Hotel banquet facility)

FRIDAY OCTOBER 10, 2003

Morning Plenary Session, NRECA Rm. CC2

7:30 Coffee and pastries available
8:00 Synopsis of Day 1, Plan for Day 2 (Barbara Baird, Workshop Co-Chair)
8:10 General plenary speaker, biomedical perspective: Kenneth Yamada, NIH/National Institute of Dental and Craniofacial Research
8:40 General plenary speaker, nanotechnology perspective: Rodolfo Llinas, New York University

Session 4: Nanoanalytics and biosensors

9:10 Plenary speaker, nanotechnology perspective: Norm Dovichi, University of Washington
9:40 Plenary speaker, biomedical perspective: John Parrish, Harvard University
10:10 Coffee Break

Session 5: Advances in imaging technology

10:30 Plenary speaker, biomedical perspective: Roderic Pettigrew, NIH/National Institute of Biomedical Imaging and Bioengineering
11:00 Plenary speaker, nanotechnology perspective: Roger Tsien, University California at San Diego

Breakouts for Sessions 4-5

Session 4 Breakout (Nanoanalytics and biosensors): NRECA Room CC2

11:30 Introductions, objectives: Eleni Kousvelari (NIH/National Institute of Dental and Craniofacial Research) and John Parrish, Session Coordinators
11:35 David Walt, Tufts University
11:40 Patrick Stayton, University of Washington
11:45 Fran Ligler, Naval Research Laboratory
11:50 Sangeeta Bhatia, University California at San Diego
11:55 Terry Michalske, Sandia National Laboratory
12:00 Edward Cox, Princeton University
12:05 Michael Heller, University of California at San Diego
12:10 Lunch (on your own, list of local restaurants provided; breakout groups encouraged to continue discussion over lunch)
1:30 Open discussion, impromptu presentations by other participants invited
3:50 Wrapup, summary of outcomes: Eleni Kousvelari and John Parrish

Session 5 Breakout (Advances in imaging technology): NRECA Room CC3

11:30 Introductions, objectives: Roger Tsien and Soo-Siang Lim (NSF/Directorate for Biological Sciences), Session Coordinators
11:35 Rebecca Richards Kortum, UT Austin
11:40 Paul Alivisatos, UC Berkeley and Lawrence Berkeley National Laboratory
11:45 Tom Budinger, UC Berkeley and Lawrence Berkeley National Laboratory
11:50 Michael Phelps, University of California at Los Angeles
11:55 Joanna Fowler, Brookhaven National Laboratory
12:00 Robert Balaban, NIH/National Heart, Lung, and Blood Institute
12:05 Rolf Gruetter, University of Minnesota
12:10 Lunch (on your own, list of local restaurants provided; breakout groups encouraged to continue discussion over lunch)
1:30 Open discussion, impromptu presentations by other participants invited
3:50 Wrapup, summary of outcomes: Roger Tsien and Soo-Siang Lim

Plenary Session: Reporting back from breakouts (Rm. CC2)

4:10 Session 4: John Parrish
4:30 Session 5: Roger Tsien
4:50 Open Plenary Discussion: Highlights of the workshop and what should be in the report
5:30 Adjourn for the day
7:00 Dinner for report authors (i.e., participants in report drafting session following day) (Dan and Brad's Restaurant, near NSF)

SATURDAY OCTOBER 11, 2003 -- REPORT DRAFTING SESSION

Location: National Science Foundation, 4201 Wilson Blvd., Arlington, VA, Rm. 375
8:00 Coffee and pastries available
8:30 Discussion on organization and content of the workshop report (led by Viola Vogel and Barbara Baird)

"Highlights" presentations by breakout session leaders; proposed report content for each session chapter

9:00 Session 1: Michael Dustin
9:10 Session 2: James Baker
9:20 Session 3: Jennifer Lippincott-Schwartz
9:30 Session 4: John Parrish
9:40 Session 5: Roger Tsien
9:50 Open session for drafting of summaries of entire workshop and of session chapters (computers, staff assistance available)
11:50 Concluding remarks; plans for follow-through, writing assignments
12:00 Adjourn

SESSION OUTLINE FOR THE JOINT NSF/NIH/NNI WORKSHOP ON NANOBIOTECHNOLOGY

Deliverables

Substantial segments of the scientific community are confident that nanoscience and nanotechnology will revolutionize research on biology and medicine. Yet, much of the biomedical research community has at best passing familiarity with the novel relevant discoveries that are

emanating from the larger nanotechnology communities, ranging from physics, chemistry, to engineering and biosciences. The purpose of this workshop is to convene thought leaders in biomedical and nanotechnology research, and to identify crosscutting scientific opportunities that can be realized only through effective collaboration among these communities. While many nanotechnologists develop new tools, they often have a limited understanding of the needs of the biomedical communities, or of the restrictions that biology (and medicine in particular) place upon the proper design of nanotools or nanosystems. Nanotechnologists also require a much deeper understanding of biology to fulfill their goal of designing synthetic nanoengineered materials, devices, and integrated systems based upon the design of biological systems. Conversely, the biomedical communities often have little insights what nanotechnology can potentially do and how they can take advantage of the ongoing technological advances. Workshop participants will enumerate fundamental changes that nanoscience and nanotechnology can bring to the study of life processes, as well as leading to effective interventions for treating disease and promoting human health. To stimulate closer and lasting collaborations among biologists and nanotechnologists, this workshop will identify key issues and far-reaching goals that simply cannot be achieved without effective teamwork. Finally, the participants will be asked to prioritize recommendations for the National Science Foundation (NSF), the National Institutes of Health (NIH), and the other agencies participating in the National Nanotechnology Initiative (NNI).

Sessions

Understanding How Cells Work Through Bottom-Up Assembly of Biological Nanosystems Ex Vivo

Reconstitution *in vitro* of biochemical pathways and of motility and transport systems has long been a fruitful approach to understanding how cells carry out complex functions. Nanotechnology offers powerful new analytical and conceptual tools to developing knowledge of how the biomolecules that perform functions such as mechanical force generation, energy production and conversion, chemical catalysis, etc., self-assemble to create multimolecular and hierarchical structures that are responsible for the complex behaviors (e.g., signal transduction, movement, growth, transport, metabolism, etc.) that are exhibited by living cells. A fundamental goal of nanotechnology is to understand, visualize, probe and manipulate the assembly of atoms and molecules to reconstitute and create new functions at the molecular and supramolecular level. By reconstituting function through the bottom-up assembly of biomolecules, based on understanding of biological system design, nanotechnology offers completely new insights into systems biology at the subcellular level. These same capabilities will allow the construction of engineered systems, using a mixture of biological and non-biological materials, for a variety of uses, some of which have utility in medical diagnosis and treatment. This session will also include discussion of emerging technologies, including the development and application of computational tools and experimental approaches to study the dynamics of large biological complexes, of reverse engineering concepts, how to build architectures over multiple length and time scales, and how to integrate biological and synthetic hybrid systems.

Nanotechnology to Enhance Human Health

The nanotechnologist wants to learn from biology how to create more useful nanoscale materials and integrated devices with improved functions. Biological molecules offer biochemical processing and sensing capabilities exceeding those of most inorganic materials. They also are amenable to molecular selection and evolution strategies that can quickly create materials with properties that are specifically tailored for an application. Nanotechnology provides new avenues to engineer materials and devices in support of or to enhance organ functions, to aid in the regeneration of injured tissue, and to create engineered tissue scaffolds, synthetic or cell-based, that might

ultimately replace at least in part functions of complex organs. Nanotechnology also develops new methods of drug delivery to better target selected tissues and cells, and to improve on the efficiency of drug activity in the cytoplasm or nucleus. Furthermore, many different avenues are being explored to convert signals at the nanoscale from one energy form into another using inorganic and organic nanoscale systems and to integrate these sensors into microscale devices that could potentially be inserted into patients (e.g., as microsensors) for the very early detection of disease or to monitor treatment efficacy. Yet, very little work has been done on how these inorganic-based transducers can interface with living systems and continue to operate for extended time periods. This session will first assess early successes in translating nanotechnology from the bench to the bedside, such as the use of nanoparticles as drug delivery systems and MRI contrast agents in patients. Furthermore, new opportunities and challenges for using nanotechnology to fundamentally alter medical diagnosis and treatment will be explored.

In Vivo *Analysis of Cellular Processes at the Nanoscale*

Recently, important advances in light and electron microscopy, new technologies in molecular biology, and the availability of new optical and (bio)chemical probes addressed specifically to molecules inside the cell provided essential tools for biologists to obtain the current level of understanding of the dynamics of biological systems at the cellular and subcellular level. Today, emerging nanotechnologies (e.g., molecular self-assembly, photolithography, optical tweezers and scissors, force spectroscopy, and many others) are providing novel ways to probe, manipulate and image molecular structure and function within living cells. Nanotechnology enables novel measurements on single molecules and molecular assemblies, and will change, potentially even revolutionize our views on how subcellular systems are designed and how they function *in vivo*. For example, single molecule imaging is at the verge of being able to track single molecules in cells and tissues, thereby giving detailed insights into the eventful life of a biomolecule, from its synthesis to degradation. This includes processes of molecular transport in cells, the lifetime of molecules, pathways of posttranscriptional modifications, and molecular degradation. This session will highlight several nanotechnologies for the study of biomolecular function and interaction, and novel discoveries that have been made with their use. It will also identify important questions to which biologists seek answers, and for which today's tools are inadequate, but that nanotechnologies have the potential to solve. Nanotechnology furthermore gives much new precision in the spatial and temporal control by which a biological system can be perturbed, as well as to probe the resulting responses. This includes chemical and mechanical perturbations, and multipoint measurements in space and time. Integrating these tools and concepts with the large datasets emerging from comprehensive (i.e., genomic) studies of biological systems, and powerful computational methods, will pave the way for understanding normal cellular processes and determining how to intervene in abnormal or diseased states.

Nanoanalytics and Biosensors

Nano/micro technology can have a tremendous impact in *ex vivo* sensing and medical diagnostics. They can provide the ability to measure a wide range of analytes efficiently, reliably, and quantitatively. Such new methods of diagnosis are amenable to small samples, are less invasive, and will be faster and more sensitive than current technologies. Advanced detection nano-based technologies will replace time-intensive laboratory methods and permit point of care diagnosis. Based on the tremendous impact that DNA microarrays have already had on basic and clinical research, it is clear that expansion of micro- and nanotechnology for measuring a broader range of biomolecules in complex media will revolutionize disease research and diagnosis. The session will address the challenges to develop new research tools that help to identify the markers of disease using nanoscale technologies. Topics should also include single molecule analysis, multivariant

array analysis of low affinity interactions, evolutionary strategies to enhance the performance of nanosystems, integration of nanosystems into MEMS devices, interfacing cells with the synthetic world, including neural systems, biosensors made from subcellular components or cellular networks, and the management of biosensor networks.

Advances in Imaging Technology

The challenge for biomedical imaging is the development of new imaging tools and procedures for early detection of disease (e.g., cancer) and evaluation of therapeutic efficiency, capturing and merging anatomic and molecular information. Imaging needs to be performed in real time without the need to remove and process a specimen. Phenotypic characteristics such as proliferation kinetics, virulence, angiogenesis, and heterogeneity of progression are important parameters that molecular imaging could provide noninvasively. Nanoscience and nanotechnology can provide necessary "smart" contrast agents and tools for real-time imaging of a single cell, biomolecules, organelles, and tissues at the nanoscale as well as real-time imaging of cell function during surgery. Advances in the imaging sciences could change the face of medicine, making it possible to non-invasively detect, diagnose, and guide therapy for a large variety of diseases. The goal of this session is to provide an update on imaging technologies today and expand on future developments using nanoscience and nanotechnology principles to image and visualize coherent and three-dimensional physiological processes, and how functional entities perform their work in a synergistic manner.

APPENDIX C. LIST OF WORKSHOP PARTICIPANTS AND REPORT CONTRIBUTORS*

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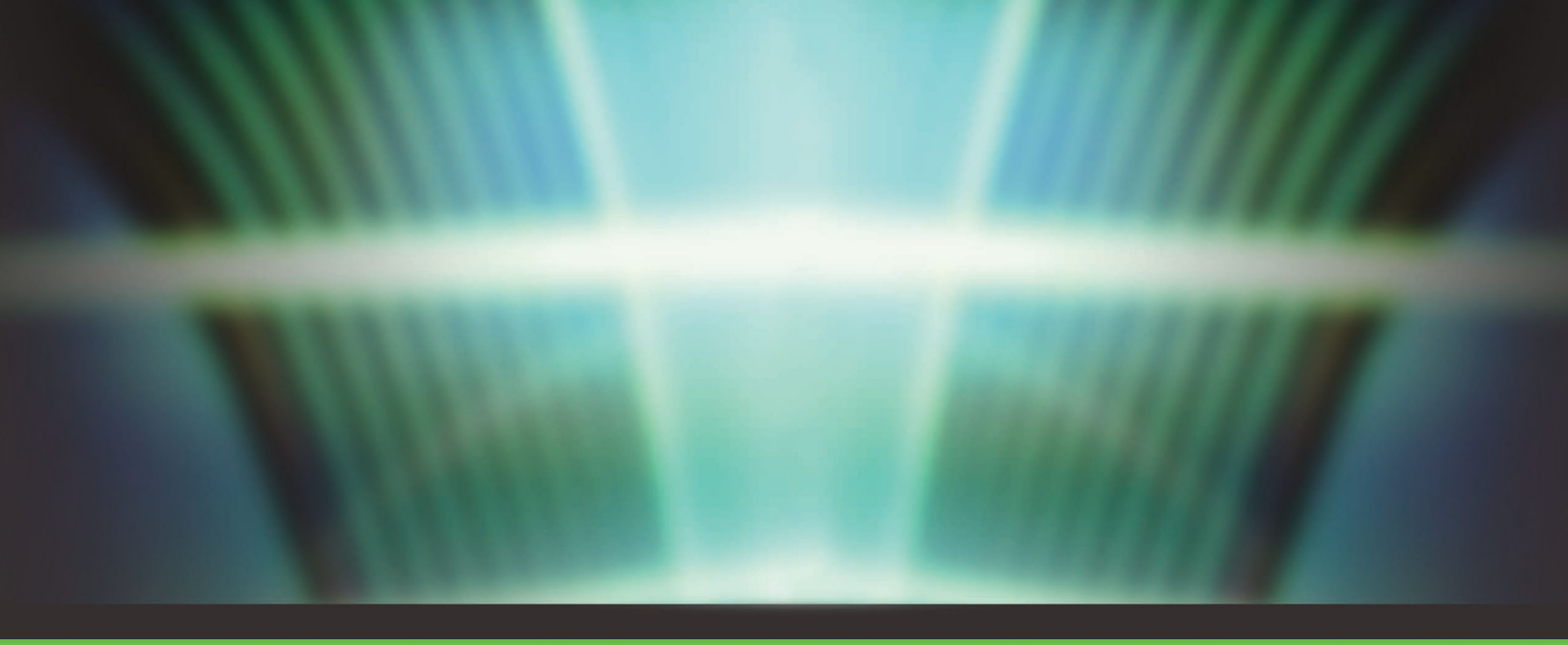

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