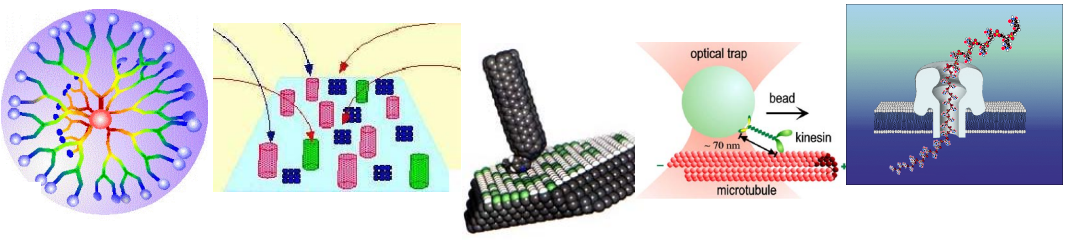
Nanoscience and Nanotechnology:

Shaping Biomedical Research

June 2000

Symposium Report



National Institutes of Health
Bioengineering Consortium

BECON

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Nanoscience and Nanotechnology: Shaping Biomedical Research

National Institutes of Health Bioengineering Consortium (BECON)
Natcher Conference Center
June 25-26, 2000

Foreword

Every once in a long while, a new field of science and technology emerges that enables the development of a new generation of scientific and technological approaches, and research and clinical tools and devices. Nanotechnology holds such promise. The essence of nanotechnology is the creation and utilization of materials and devices at the level of atoms, molecules, and supramolecular structures, and the exploitation of the unique properties and phenomena of matter at the nanoscale (1 – 100 nm). Two things must occur if the potential of nanotechnology for medical science is to be best realized. First, the biomedical community must be made aware of, and urged to participate in, this emerging field. Second, biomedical scientists and engineers must communicate effectively with each other and understand that biological systems provide excellent models for the development of these new technologies.

The symposium, *Nanoscience and Nanotechnology: Shaping Biomedical Research,* was structured to provide a forum where scientists, engineers, and clinicians from a variety of fields would be able to exchange their knowledge, further develop their vision for this new science and enabling technology, and identify collaborators for future research. The plenary talks, poster and exhibit presentations, and breakout sessions were also designed to assist the Institutes and Centers of the National Institutes of Health (NIH) in developing mechanisms and plans for incorporating nanoscience and nanotechnology into their research programs.

Members of the National Institutes of Health Bioengineering Consortium (BECON) organized the symposium. BECON was formed in 1997 as an NIH focal point for bioengineering research. In addition to representatives from each of the 25 Institutes and Centers that comprise the NIH, BECON includes participants from the National Science Foundation and the Department of Energy. BECON now operates from within the Office of Bioengineering, Bioimaging, and Biocomputing (OBBB), in the Office of the Director of NIH.

This was the third in a series of BECON symposia. The first symposium, *Bioengineering: Building the Future of Biology and Medicine*, was held in February 1998, and the second symposium, *Biomedical Imaging: Visualizing the Future of Biology and Medicine*, was held in June 1999. Each of these symposia has attracted substantial participation (in excess of 600) by scientists, clinicians, and engineers from diverse fields of study and from all areas of academia, government, and private industry. Reports from each of these symposia are available on the Internet at http://grants.nih.gov/grants/becon/becon_symposia.htm.

External Co-Chairs	*NIH Co-Chairs*
Lynn Jelinski, Ph. D. *Louisiana State University*	*Eleni Kousvelari, D. D. S., Ph. D.* *National Institute of Dental and Craniofacial Research*
Richard Zare, Ph. D. *Stanford University*	*Jeff Schloss, Ph. D.* *National Human Genome Research Institute*

Nanoscience and Nanotechnology: Shaping Biomedical Research

Contents

Executive Summary

The goals and objectives of the symposium, *Nanoscience and Nanotechnology: Shaping Biomedical Research,* convened by BECON/NIH were to:

- Develop a better understanding of nanotechnology as it pertains to biological and medical applications;
- Communicate recent developments and identify challenges and opportunities;
- Develop strategies for integrating nanoscience and nanotechnology with medical research and treatment;
- Discuss the vision and future of this interdisciplinary area;
- Ensure that NIH can facilitate nanoscience and nanotechnology research that will benefit biomedical science; and
- Make recommendations to NIH on areas of future investment.

These goals and objectives were primarily addressed and accomplished through breakout panel discussions, which were in turn stimulated by visionary and thought-provoking plenary presentations. These discussions addressed contemporary areas of nanoscience and nanotechnology pertinent to biomedicine:

- Synthesis and Use of Nanostructures;
- Applications of Nanotechnology to Therapy;
- Biomimetic Nanostructures;
- Biological Nanostructures;
- Electronic/Biology Interface;
- Devices for Early Detection of Disease;
- Tools for the Study of Single Molecules; and
- Nanotechnology and Tissue Engineering

The large number of attendees (650), representing academia, industry, and government laboratories, affirmed the vibrancy of the field and ensured that the nanoscience and nanotechnology communities were well represented. The mode of interaction provided by the panel discussions and the large number of poster presentations ensured that all attendees had substantial opportunity to provide input to the recommendations. These recommendations would address priorities among a wide range of biological, chemical and engineering sciences, and involve multidisciplinary teams of scientists working in public and private institutions.

Scientific Priorities

With the vision that nanoscience and nanotechnology can bring about fundamental changes when integrated seamlessly with biology and with devices at a continuum of length scales for the purpose of investigating fundamental life processes, combating disease, and improving the human condition, the conferees outlined a number of common scientific themes:

- **The development of new tools and new methods should be one of the most important scientific priorities.** The need exists to be able to selectively modify, isolate, manipulate, analyze and control nanosystems. Methods are needed for improved detection and analysis of single molecules, single cells, and single cellular compartments. Some important examples include laser tweezers for molecules and other nanostructures, and the development of single molecule NMR imaging technology.

- **Improvement of existing materials and development of new materials are required for continued progress in tissue engineering.** Materials capable of dynamic incremental control particularly as they involve organic/inorganic interface for hard tissue and for skeletal tissue repair and replacement, will benefit from nanoscience and nanotechnology.

- **A "marriage" of biological and inorganic systems is needed and can be achieved through research on surfaces, adhesion, and molecular recognition, leading to biotronics and the development of biocompatible nanofabrication processes.** A recent example is the coupling of inorganic nanoparticles to the moving components of a molecular motor.

- **Much can be learned from developing a further understanding of how nature designs, processes, assembles and disassembles molecular building blocks, and how transient structures of nanoscale assemblies relate to their functions.** A primary goal should be the synthesis of molecules that mimic biological systems but move beyond some of their restrictions. Systems from which much could be learned by understanding recognition, processing, self-assembly and templating include bone, muscle, brain, molecular motors, and receptors. The lessons learned can be applied for the design and fabrication of patterning, parallel production, and inexpensive assembly of robust nanostructures and nanodevices in two and three dimensions.

- **Nano systems for gene therapy and other methods of protein/drug delivery**, such as the production of non-immunogenic assemblies that approach and finally surpass viruses in efficiency for the delivery of genes, drugs, or nanoscale probes across membranes, and to specific sites, should be developed. Nanoparticles have already improved the availability and efficiency of delivery of some drugs.

Educational Priorities

Two common educational priorities emerged from the panel discussions.

- **Interdisciplinary teams of researchers will make the most rapid and significant progress in this field.** The people who will succeed will not necessarily know everything, but will know how to interact with researchers from other fields and how to build interdisciplinary teams.

- **A new generation of students should be trained, combining a rigorous disciplinary depth with the ability to reach out to other disciplines.** Novel education and training programs at the undergraduate, graduate, and postgraduate levels will be required to develop these "T-shaped individuals."

Challenges and Obstacles

While scientific and technological challenges can be overcome by innovative research, conferees participating in the discussion panels identified several general challenges and obstacles that currently impede progress. Solving these challenges and removing these obstacles are critical to fostering this immature discipline.

- Technology development and high-risk research need to be encouraged.

- The number of skilled personnel trained in both biological sciences and in the physical sciences and/or engineering must be increased.

- Novel mechanisms to foster and implement these emerging technologies and grow their new branches must be devised.

- Funding of new capital equipment and support for maintaining existing capital equipment must be increased.

Implementation Strategies

Several implementation strategies were recommended with consensus of the symposium participants. When existing mechanisms are inadequate, innovative implementation strategies should be devised.

- **Pursue the use of nanoscience and nanotechnology in biomedical research and clinical systems. The advances that will follow have the potential to revolutionize medical research and ultimately, the delivery of health care.**

- **Technology-specific peer-review panels, particularly for the unique aspects of nanotechnology, should be implemented.** Reexamine peer-review mechanisms broadly within NIH with the goal of encouraging support for technology-driven and high-risk nano-science and -technology exploratory research.

- **Particular attention needs to be paid to ways to enhance communication among all the disciplines, ranging from physical sciences to medicine.** Pursue bold, new, innovative education and training programs now, to promote this cross-talk among today's researchers and develop a new generation of researchers to whom this interaction is second nature.

- **Develop incentives that encourage team research as well as individual efforts.** Academic and industrial reward, promotion, and tenure systems should recognize individuals who make substantial contributions to research projects that cross traditional boundaries.

- Develop co-funding strategies involving different funding agencies with complementary missions.

- Encourage *genuine* collaboration among industry, academia, and government scientists.

Symposium Report

This report of the third NIH/BECON symposium, *Nanoscience and Nanotechnology: Shaping Biomedical Research*, held on June 25-26, 2000, summarizes the plenary lectures and overviews delivered by six distinguished speakers as well as the eight panel discussions led by sixty-three eminent scientists, engineers, and clinicians. Over 650 participants from universities and colleges, research foundations, national laboratories, government, and the private sector joined in the discussions and expressed their views. The insights and suggestions that are captured in these summaries comprise valuable input that the NIH will use as it formulates programs and procedures to facilitate creative and novel research into the fundamentals and applications of nanoscience and nanotechnology. This research will lead to a better understanding of the basis for disease and disability, and thus to better modes for their prevention, detection, diagnosis, and treatment. NIH welcomes additional suggestions for improving its mechanisms for funding and facilitating science and engineering research in general, and nanoscience and nanotechnology research in particular.

Overview of Presentations

What is Nanotechnology?

Steven M. Block, Ph.D., Stanford University

An inevitable outgrowth of modern technology has been an increasing trend towards the miniaturization of components. In parallel with this, scientific developments have led to the increased study of material properties at the mesoscopic level and below. In areas as diverse as materials engineering, quantum physics, and molecular biology, new tools and approaches have been developed that enable both the fabrication and the study of molecular complexes, or even of single macromolecules. We therefore find ourselves at the dawn of an era where the forefronts of both engineering and scientific endeavor have reached the length scale of nanometers. This serves to define not only a new 'nanotechnology,' but equally a new 'nanoscience,' and the important distinction between these terms is worth noting. That said, nanotechnology advances are influencing many scientific areas in bioscience (Figure 1).

Against the backdrop of much exciting technical and scientific development, there are legitimate expectations for breakthroughs in areas such as computing/electronics, biotechnology, materials, and so on. In many cases, these expectations seem realistic and appropriate, and therefore merit the attention and support of government agencies and academia, as well as the private sector. In others cases, the expectations seem naïve and unrealistic: these are primarily being advanced by a vocal cult of futurists whose enthusiasm is beyond question, but whose agenda is not. Dr. Block argued that for the real science to proceed, nanotechnologists must distance themselves from the giggle factor and position themselves for the serious work of the 21st century. However, he pointed out (Figure 2) that several general challenges and obstacles needed to be removed in order to foster this immature discipline.

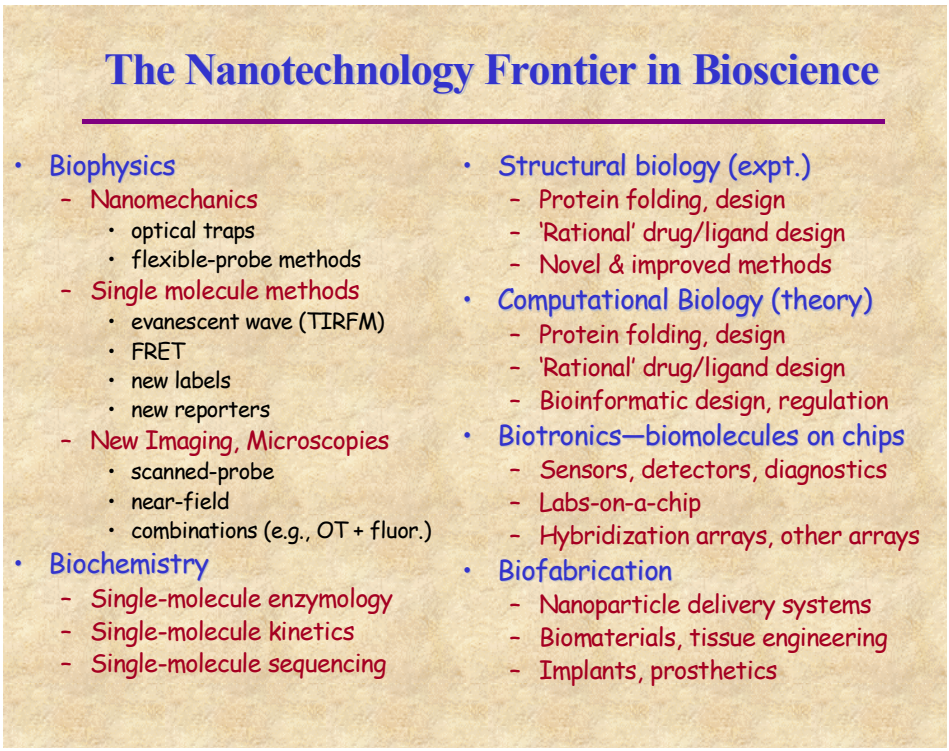


Figure 1. Nanotechnology frontiers in bioscience.

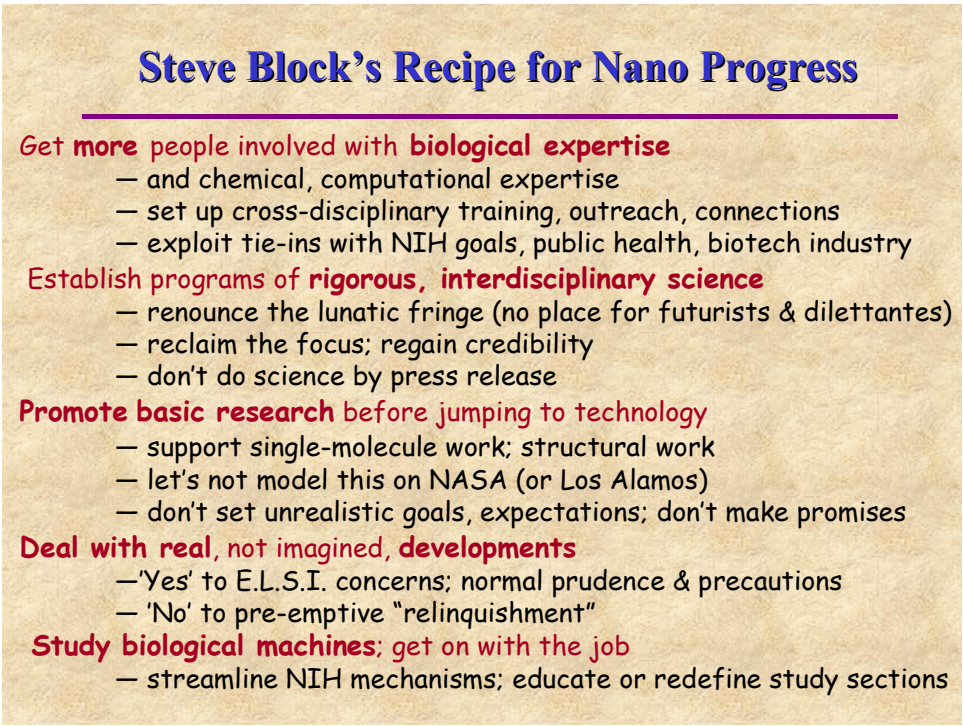


Figure 2. Five important goals for NIH progress in nanotechnology.

Challenges and Vision for Nanoscience and Nanotechnology in Medicine: Cancer as a Model

Richard D. Klausner, M. D., National Cancer Institute, NIH

The recognition of the molecular basis of cancers creates the opportunity for a future where cancers are detected, diagnosed, and treated based on the fundamental changes in the specific disease. Ongoing efforts target the definition of the genes and expressed gene products of the human genome, the discovery of sentinel biomarkers of the early presence of disease, and establishment of informative diagnostic classification systems based on the fundamental molecular changes. Closely linked are efforts to discover and exploit molecular targets for cancer prevention and treatment. New technologies are needed to speed the discovery process that are rapid, highly parallel, and cost effective. Reductions in the scale of analysis tools and automation are proving critical to enabling the discovery of the fundamental changes associated with the development of cancers, and insight in to the molecular processes of the cell.

The identification of molecular signatures of cancers will enhance our ability to identify and accurately diagnose disease. Our goal is to use this information to identify cancers or precancers at the earliest point in the disease process and intervene before symptomatic disease becomes apparent. Realization of this goal will only be possible if minimally invasive technologies exist that allow us to scan in the living body for the earliest signatures of emerging disease and support immediate, specific intervention. The ability to scan the body for molecular signatures will also allow us to monitor the progress of disease and effects of interventions.

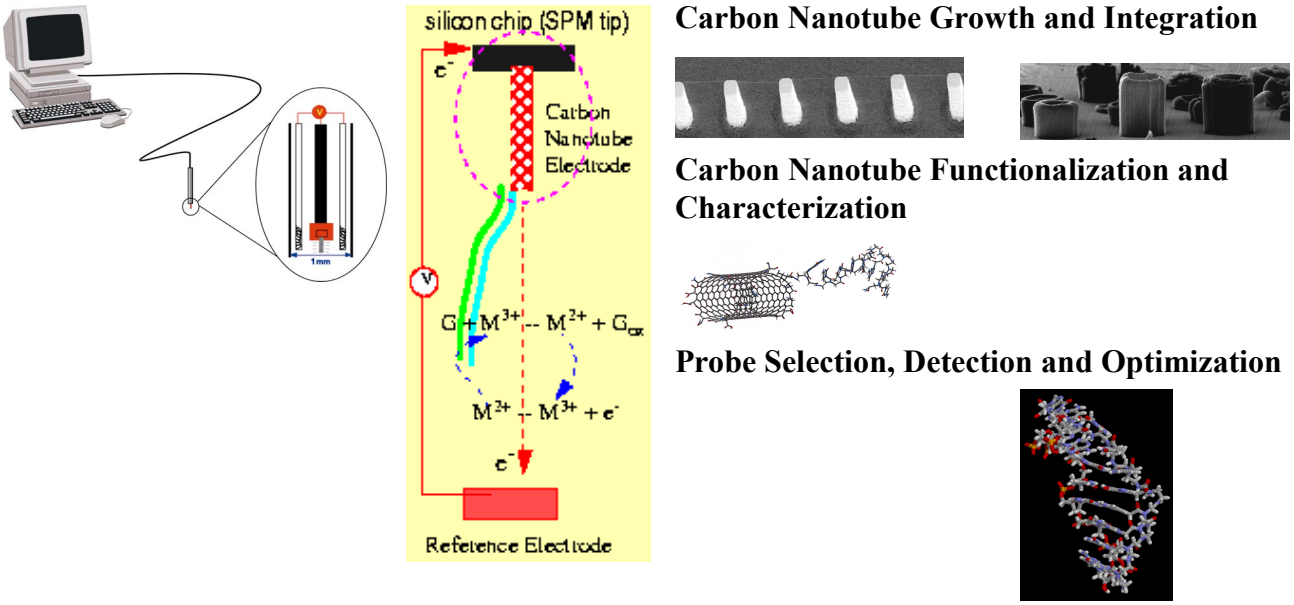


Figure 3. Dr. Meyya Meyyappan and collaborators at NASA Ames Research Center are developing a novel carbon nanotube-based biosensor technology for the Unconventional Innovations Program of the National Cancer Institute. The integrated platform will consist of molecular probes interfaced to microelectronic components through carbon nanotubes. Changes in electrical or electrochemical signal transduction upon recognition will be the basis of the recognition scheme.

The NCI is currently seeking technologies that will support the earliest detection of the molecular signatures of cancer and serve as a platform for the seamless interface between detection, diagnosis and intervention. Platform technologies must integrate the ability to sense, signal, respond, and monitor. Nanotechnologies are emerging as enabling components to these goals. Ongoing efforts highlight that full systems will require the integration of new discoveries from a variety of fields including nanoscience, chemistry, photonics, computational sciences, and information science and technology. For example, Figure 3 shows a schematic representation of the contributions from different fields for the fabrication of nanotube-based biosensor technology.

Advances in nanotechnology have already been used for the development of smart nanodevice therapeutics based on dendrimers. An example of the work of Dr. James Baker and colleagues at the University of Michigan on the development of such devices is shown in Figure 4.

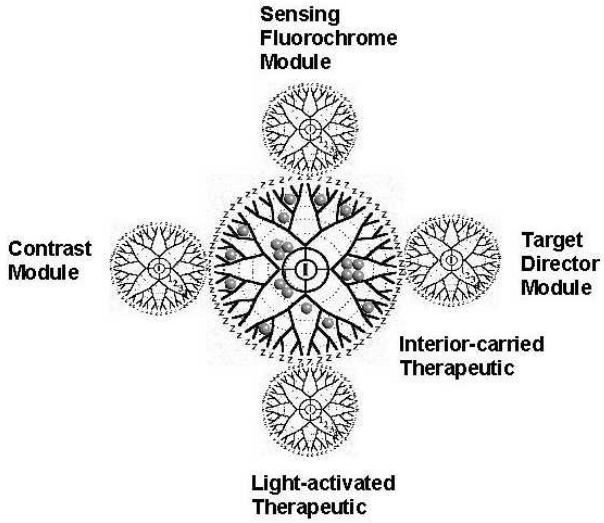


Figure 4. Dendrimer cluster agent (approximately 200 C in diameter) being developed as a smart nanodevice therapeutic. Each dendrimer module would serve a different function, such as molecular targeting, contrast enhancement, or therapeutic delivery to create a nanosystem that integrates capabilities for early detection, diagnosis, and treatment of cancer. Thus, such an agent could be used to target to specific tumor cells, image the tumor, sense tumor cells for pathophysiologic defects, select and deliver therapeutic agents based on tumor characteristics, non-invasively trigger release of therapeutics (with or without an external trigger), document response to therapeutics, and identify residual tumor cells.

Nanostructure Synthesis: Functional Polymers and Dendrimers

Jean M.J. Fréchet, Ph. D., University of California

Nanostructures based on functional polymers and dendrimers are already having a significant impact on nanotechnology research as they provide access to well controlled functional building blocks that may be used for a broad spectrum of applications from unimolecular devices or nanoreactors to sensing and targeted drug delivery. Aside from their near ideal size - ranging from 1 to10 nm - dendrimers benefit from their globular, sometimes near spherical shape, high

surface functionality, structural regularity and size monodispersity. The preparation of dendrimers is now well established with the original divergent procedure of Tomalia (1) as well as the convergent route of Hawker and Fréchet (2). The convergent approach is particularly well suited for the preparation of dendrimers to be used as building blocks for larger functional nanostructures as it provides access to dendritic "wedges" with differentiated reactivity at their focal point and chain ends.

Advances in nanotechnology have already been made using dendrimers and other polymeric building blocks with highly controlled structure and functionality. For example, using natural photosynthetic systems as a model, we have prepared nanometer size antennas each consisting of a single "engineered" dendrimer molecule that can harvest sunlight at multiple peripheral sites and transfer the harvested energy in near quantitative fashion to a single central location of the dendrimer where it can be "re-processed" into monochromatic light, or electrical or chemical energy. Figure 5a shows the schematic representation of an existing functional dendritic antenna based on a single molecule about 3 nm in size while Figure 5b shows a schematic photocatalytic system making use of the dendritic antenna to power a porphyrin-based catalytic site located at its core. Such light harvesting and light emitting antennas also show great promise as biological markers, while closely related dendritic nanostructures can be used as imaging agents.

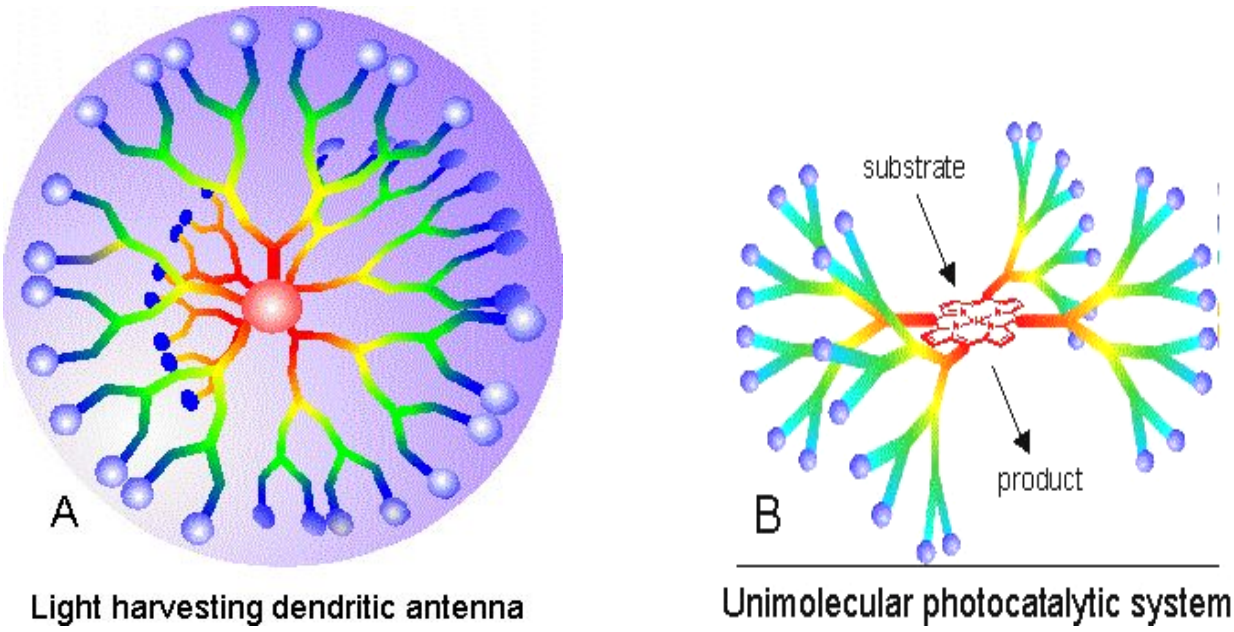
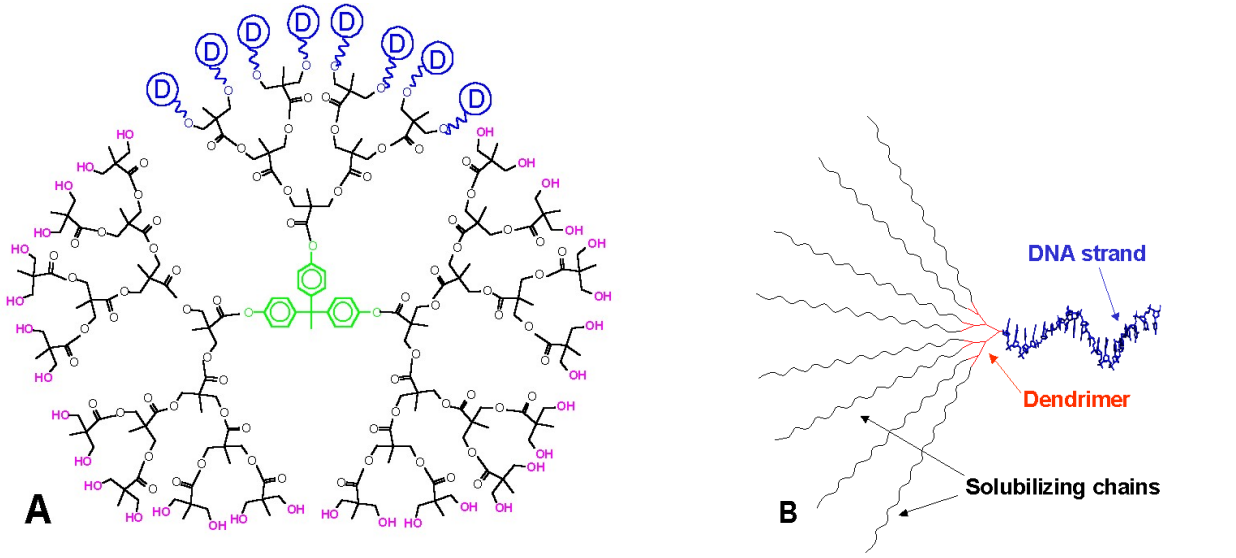


Figure 5. (A) Nanoscale light harvesting antenna based on a dendrimer. (B) Dendritic photocatalyst combining antenna and catalytic function.

Taking advantage of the spherical shape, and highly versatile functionality of dendrimers, highly reproducible and effective drug delivery systems can be designed (3). Preliminary work has already established that a high payload of anticancer drugs such as doxorubicin can be attached to a dendritic molecule that serves to solubilize the drug, while also assisting its penetration into cells and reducing drastically its toxicity until release is achieved in the targeted

area. Figure 6a shows a model carrier system based on the polyester dendrimers used in the early in vivo studies that demonstrated the very low intrinsic toxicity of the dendrimers and their ability to be excreted from the body. The design of this nanoscale dendritic assembly incorporates polyvalency as well as triggered release, for example through the enhanced permeation and retention effect.

Yet another application of nanotechnology based on dendritic systems is directed towards genomics and gene therapy. While early work by Szoka *et al.* (4) and later, Baker *et al.* (5) has



Polyvalency and model biocompatible drug carrier

Nanoscale dendritic assembly for gene therapy

Figure 6. (A) Schematic representation of a unimolecular dendrimer based drug delivery "device". (B) Schematic representation of a dendritic assembly for antisense gene therapy.

demonstrated the raw potential of dendrimers for gene therapy, we have carried out preliminary experiments with hybrid structures consisting of dendrimers with a solubilizing corona for the transport of single strand DNA into cells. Figure 6b shows a schematic representation of this prototype nanodevice designed for its ability to penetrate and deliver DNA into cells.

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Analyzing Single Molecules, Vesicles, and Cells

Richard N. Zare, Ph. D., Stanford University

Advances in instrumentation have brought about the detection, characterization, and manipulation of single molecules, vesicles, and cells. For example, scanning probe techniques allow study of single molecules on surfaces, and laser fluorescence measurements allow their study in complex condensed environments. Single vesicles and single cells are readily trapped in solution by focused laser beams (laser tweezers). This new power opens many opportunities for addressing biomedical problems whose conventional study generally involves an average over a multitude of entities, an average that hides from view the record of individual events. With such techniques we are able to detect and observe inhomogenities in populations and relate these to the microenvironment that each member of the population experiences. Moreover, time-dependent phenomena can be studied without the need to synchronize the behavior of all members of a population. Examples abound for the power of these new methods, from the study of molecular motors, to the forces required to make or break protein-protein interactions (Figure 7), to the possibility of sequencing strands of DNA directly with molecular probes, to the manipulation of individual cells and vesicles with nanoengineered structures (Figure 8).

This overview is only able to touch on a few of the many highlights of recent work in this rapidly evolving research area. Nevertheless, it is hoped that these profound advances in analytical power can become an enabling method for solving many nanoscale problems encountered in understanding various aspects of life processes.

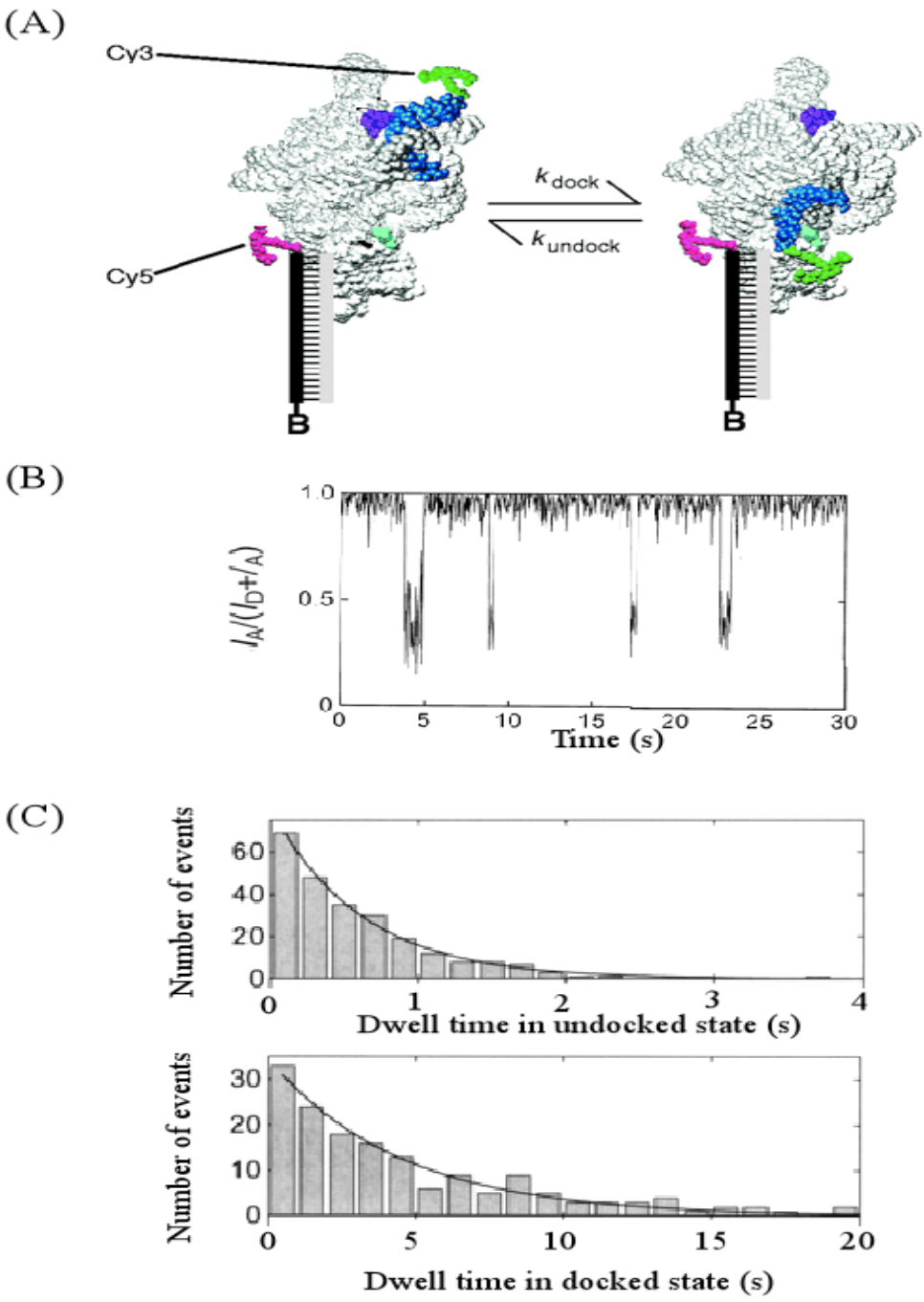

Figure 7. (A) Conformational change of RNA illustrating motion of the P1 duplex (shown in blue and grey) in *the Tetrahymena* Group I ribozyme. Cy3 dye attached onto P1 (green), and Cy5 (violet) attached on the other end of RNA, act as donor and acceptor, respectively. Fluorescent resonant energy transfer (FRET) between Cy3 and Cy5 signals conformational change occurring in the RNA molecule. (B) FRET time traces from single ribozyme molecules showing P1 docking and undocking. FRET is defined as $I_A/(I_D + I_A)$, where I_A and I_D are the fluorescence signals from acceptor and donor, respectively. (C) Histograms of the dwell times in the undocked (top) and docked states (bottom) obtained from the FRET time trajectories. The solid lines are single exponential fits of the data giving rate constants for docking (k_{dock} = 1.62 ± 0.08 s^{-1}) and undocking (k_{undock} = 0.224 ± 0.015 s^{-1}), respectively. Abstracted with permission from X. Zhuang, L.E. Bartley, H.P. Babcock, R. Russell, T. Ha, D. Herschlag, S. Chu, *Science* **288**, 2048-2052. Copyright 2000 American Association for the Advancement of Science.

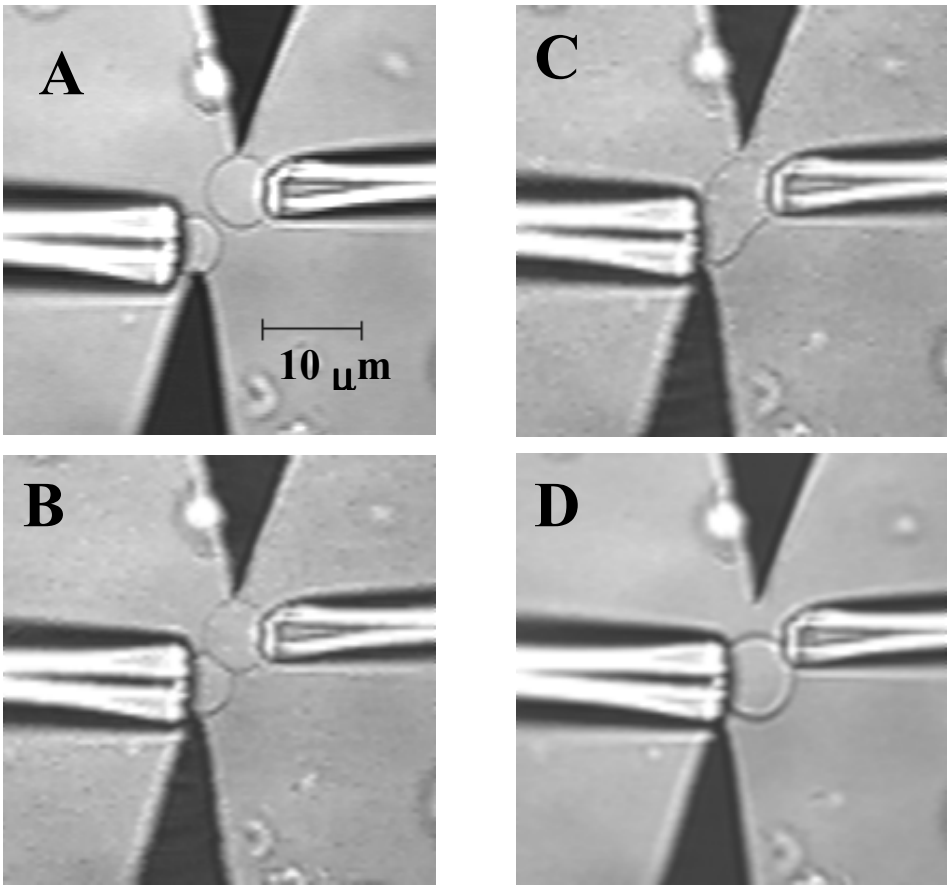

Figure 8. Electrofusion of liposome pairs using nanoengineered pipettes. The positioning of holding pipette-docked liposome pair and microelectrodes prior to electrofusion is shown in (A). Figures (B) and (C) are adjacent frames (separated by 0.03 s) acquired during electrofusion, demonstrating first the formation of a planar contact between liposomes (B) followed by fusion to a single aspherical liposome (C). The final fusion product is shown in (D). Taken from Clyde F. Wilson, Garth J. Simpson, Daniel T. Chiu, Anette Strömberg, Owe Orwar, Nestor Rodriguez, and Richard N. Zare (unpublished work).

Microelectronic Array Devices for DNA Diagnostic, Pharmacogenomic, Combinatorial Selection, and Nanofabrication Applications

Michael J. Heller, Ph. D., Nanogen, San Diego, CA

A microelectronic array based system (Molecular Biology Workstation and NanoChip^{TM}) has been developed for single nucleotide polymorphism (SNP) analysis, DNA diagnostic, and pharmacogenomic research applications (see Figure 9). These active microelectronic devices combine the best attributes of both DNA array and "lab on a chip" technologies. These microarray devices are able to create re-configurable electric field transport geometries on the array surface, which allows charged reagent and analyte molecules (DNA, RNA, oligonucleotide probes, amplicons, antibodies, proteins, enzymes), nanostructures, cells, and even semiconductor structures to be moved to or from any of the microscopic test sites on the device surface. In the case of DNA hybridization analyses, these reactions can be carried out very rapidly and with high specificity in any hybridization format. A 100-test site active microelectronic chip and

cartridge device (NanoChip^{TM}) has been designed for the DNA hybridization-based applications. A programmable chip addressing component has been designed to provide the end-user with "make your own chip" capabilities. A fluorescent reader and controller component has been designed to rapidly and sensitively detect multiplex DNA hybridization for point mutation, SNP, STR, and gene expression applications.

A variety of other active microelectronic chips with 400, 1200 and 10,000 test sites have been fabricated for hybridization as well as other applications. The 10,000 test site active CMOS array device is being used to investigate a novel process for screening very large combinatorial peptide libraries. The peptides in these libraries are linked to a unique nucleic acid-like pairing molecule, which allows supramolecular structures and complexes to form. These combinatorial libraries and sub-libraries are composed of 10,000 different hexamer peptides, which have the potential to produce a trillion (10^{12}) different supramolecular binding structures. These peptide libraries are now being screened on the 10,000 site active CMOS array for unique three-dimensional ligand-binding complexes and for ultimate development of the array as a molecular descriptor device for drug discovery applications. In another area, prototype "samples-to-answer" systems are being designed which may ultimately have applications for point of care (POC), doctor's office, and field diagnostics.

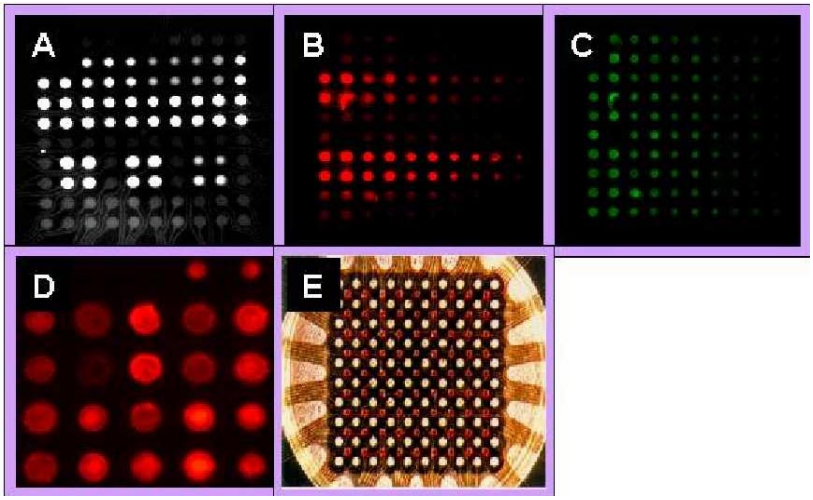

Figure 9. DNA diagnostic and pharmacogenomic applications (Nanogen's technology encompasses multiple formats and molecular biological techniques). (A) Short tandem repeats (STR – top) and single nucleotide polymorphism (SNP- bottom). For STRs, fast, multiplex analysis, in any hybridization format, high discrimination ratio's, high reliability for heterozygous calls. For SNPs, fast, multiple loci identification, forensics, human ID including microvariants (SNP's within repeat). (B & C) Gene expression. Two different probes labeled with red or green fluors for rapid expression analysis, with flexible probe and target formatting. (D) On-chip in-situ amplification. (E) Cell separation (research & POC systems).

For more long-term micro/nanofabrication and nanotechnology applications, we believe that microelectronic array devices can be used to carry out combinatorial selection processes. Such processes could be used for creating higher order mechanisms and for the directed self-assembly

of molecular (DNA based, etc.), nanoscale (carbon nanotubes, quantum dots, etc.) and microscale (lift-off) components into more complex structures. Electric field-assisted self-assembly using active microelectronic arrays is being investigated as a "Pick and Place Heterogeneous Integration" process for fabrication of two- and three-dimensional devices and structures within defined perimeters of larger silicon or semiconductor structures (see Figure 10). This technology has the inherent hierarchical logic of allowing one to control the organization, assembly, and communication of structures and components from the molecular scale, to the nanoscale, to microscale and macroscale systems.

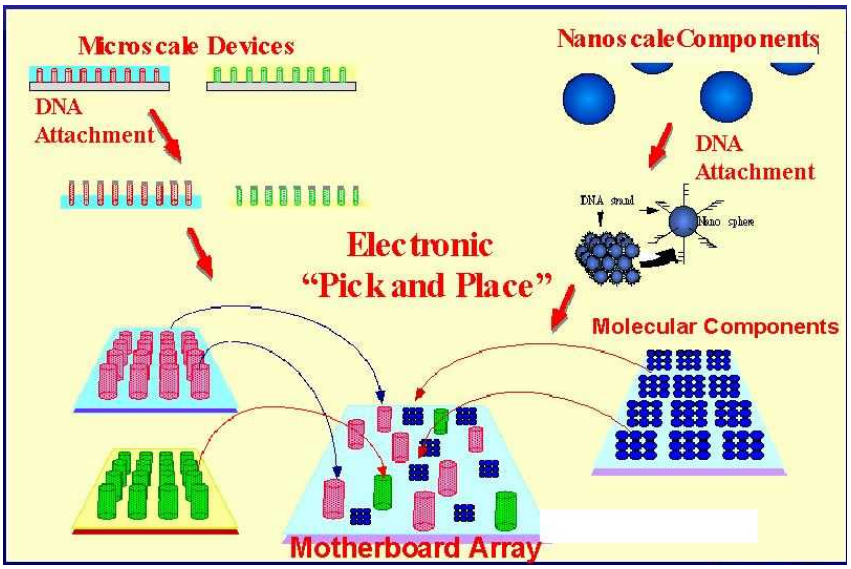

Figure 10. Heterogeneous integration process for micro/nanofabrication applications.

Reports of Panel Sessions

Synthesis and Use of Nanostructures

Moderators: Samuel I. Stupp, Ph. D., Northwestern University
George M. Whitesides, Ph. D., Harvard University

Panelists

David J. Beebe, Ph. D, University of Wisconsin
Charles M. Lieber, Ph. D., Harvard University
Joel F. Martin, Ph. D., Quantum Dot Corporation
John C. Miller, Ph. D., Oak Ridge National Laboratory
Milan Mrksich, Ph. D., University of Chicago
Erik Winfree, Ph. D., California Institute of Technology

The panel was charged with assessing current frontiers in the synthesis and fabrication of nanostructures, examining the requirements that the use of nanotechnology places on methods of synthesis and fabrication, and identifying where current methods do not meet future needs.

Broad Statement. Nanoscience holds great promise for biomedical applications in the future. The field of nanoscience, however, is still young and only beginning to develop the new tools that will allow rapid research progress and start to solve a number of biomedical puzzles. This emerging field requires a significant expansion of "discovery" research. Nanoscience encompasses structures with dimensions on the order of 1-100 nm. Above that range, the technology is properly microtechnology. This distinction, however, may not be particularly useful. The objectives of microtechnology may be defined better, but nanoscience is still in an exploratory stage and is only just beginning to understand and control its universe.

Vision

Nanoscience/nanotechnology will offer major contributions to biology and medicine in the 5- to 10-year future that will involve a broad range of techniques for synthesis, fabrication, and assembly of nanostructures into hierarchical structures:

- Proven techniques of chemical synthesis;
- New non-photolithographic procedures for structures with dimensions of 10 nm;
- Strategies to assembly nanostructure arrays; and
- Surface control of organic and inorganic structures

The wide range of phenomena that are scale-dependent – electron tunneling, quantum effects, near-field optical processes – and gaining an understanding of the astonishing examples of biological nanostructures (proteins, the ribosome, molecular motors) will help make these goals realistic.

Objectives

Scientific. New technical capabilities will form the basis of a new science of functional nano-objects that can be used in fundamental studies of biology and biochemistry, and in other areas from detection and treatment of disease to long-range opportunities such as neural/computer connections. The following are examples of scientific objectives and their possible outcomes:

- Analytical and diagnostic systems. The ability to harness nanostructures will lead to a range of new analytical and diagnostic systems for diseases as well as drug development. For analytical purposes in cell biology, nanostructures offer the means to explore biomolecules, organelles, cells, and tissues at the nanoscale. Scanning probe methods and quantum dot dyes are two such examples. Diagnostically, nanostructures could expand the variety of *in vivo* tools available for early detection of disease and dysfunction.

- Biomimetic strategies. There are a host of designs that, if understood, could provide clues for new types of non-biological structures. To date, the most successful example of "biomimetic thinking" is probably the derivation of artificial neural networks as an outgrowth of studying the cellular organization of the brain. Understanding nanoscale biological machines will help in the conception of new approaches to small synthetic machines. Such biomimetic systems could be useful as important probes in cell biology and as problem solvers in medicine. Examples include artificial cell ligands and receptors, or bone with nanoscale design.

- Methods for engineering interfaces. Controlling interfaces is a pervasive problem in nanoscience and nanotechnology. Solving this problem in the context of nanostructures will contribute strongly to solving it in a variety of other applications. An important example is the possibility of engineering interfaces between prosthetic and extracorporeal devices and tissues at the nanoscale.

- New materials for medicine. Control over nanostructure synthesis and assembly is also a potential strategy to design new materials. Medicine is in great need of new materials that are not only biocompatible but also have integrated functionalities to help repair or regenerate tissues.

Technological. The field needs flexible methods to construct a variety of nanostructures. Fabrication and synthesis currently underlie all the work in the field. Development is needed for the full range of possibilities – from molecular synthesis to biological synthesis, from x-ray photolithography to soft lithography, and from colloids to micelles. The biomedical community would benefit greatly by having numerous methods available for preparing nanostructures and for observing living processes. For example:

- Surface engineering for controlling the properties of nanostructures, in which the majority of atoms are at or near the object's surface;

- New types of analytical tools as well as improvement of existing tools, e.g., NMR, MRI, X-ray crystallography, AFM, STM, and capillary and gel electrophoresis;

- User friendly methods for the biomedical user;

- New probes and methods to examine the atomic-level composition and order of matter inside a non-crystalline solid;

- Methods of examining molecules *in vivo*;

- Methods for modifying and controlling living systems;

- Methods for observing subcellular structures and processes; and

- Methods for designing engineered biorecognition.

Obstacles and Challenges

- Insufficient mechanisms for grant application evaluation and funding. Exploratory/discovery research is not well supported by the NIH. The current peer review system does not handle applications in this area very effectively. NIH awards, to the extent that they fund this type of research, are often too small to support the scope and difficulty of the projects.

- Projects fall in between the missions of existing agency programs. In this new discipline, fundamental or device work is deemed outside the scope of NIH, but if it aims at a medical device or understanding disease, basic science agencies are less willing to fund.

- Weak interdisciplinary connections. Nanoscience will require strong connections between researchers in the physical and biological sciences if it is to develop in a way that provides maximum benefit to biomedicine. Not only are current connections weak, but also there may be too few investigators (especially world-class investigators) working in research that combines nanoscience and medicine for the community to be self-sustaining.

Recommendations

- Recognize that nanoscience requires exploratory and discovery research. A key aspect of "nano" is that it is an emerging science, not technology. Exploratory research is requisite to successful discovery of unanticipated phenomena. Because of its very nature, this type of research differs from that of molecular biology and molecular medicine where the background is more extensive.

- Develop mechanisms of support. The NIH must develop new mechanisms for supporting this research, i.e., new and continuing grant mechanisms and programs specifically targeted at supporting nanotechnology and the development of tools for nanoscience. Peer review panels need to be constituted specifically and populated to deal with these proposals; this science also needs an NIH administrative "home" that has both budgetary and funding authority.

- Develop mechanisms for funding medium-cost instruments for nanoscience/engineering. Nanoscience instrumentation is the foundation of advancement: SPR, STEM, IR, fluorescence, absorption and many others. The most important of these instruments, e.g., AFM instruments, microscopes, and lasers, range in cost from $100,000 to $500,000. The NIH should have mechanisms for considering and funding specific requests in this area.

- Co-invest with DoD and/or NSF. Both DoD and NSF have made substantial investments in nanoscience. The NIH could maximize its gains by co-funding with these agencies. Interagency coordinated efforts will also increase efficiency and will likely lead to collaborative work among scientists of disparate backgrounds and experience.

- Foster collaborations. Much of the exciting research in nanoscience requires collaborations between scientific communities that have not, historically, had much contact – the physical and engineering disciplines and the biomedical community. The NIH should encourage such collaborations.

- Strengthen educational programs. The strength of nanoscience ultimately depends on the skill and training of the people in the field. Education is an essential long-term investment.

- Build a self-sustaining community of researchers working in exploration, discovery, and tool development.

Applications of Nanotechnology to Medical Therapy

Moderators *Chad Mirkin, Ph. D., Northwestern University*
Nadrian Seeman, Ph. D., New York University

Panelists
Clifton Barry, Ph. D., National Institute of Allergy and Infectious Diseases/NIH
Eugene Cooper, Ph. D., Elan Pharmaceutical Technologies
Tejal Desai, Ph. D., University of Illinois at Chicago
Colin Gardner, Ph. D., Merck & Company, Inc.
Philip Leopold, Ph. D., Weill Medical College of Cornell University
Phillip Messersmith, Ph. D., Northwestern University

Broad Statement. In the last decade, nanotechnology has evolved in great part by exploiting a new set of synthetic and physical deposition tools and phenomena that exist on a size scale above the molecular scale, but below the microscale, in the nanometer range.

Vision

Nanotechnology has already had a positive impact in medical diagnostics in the areas such as sensors, selective filtration, and contrast imaging. With cellular- and molecular-level interactions occurring at the nanometer scale, nanoscience and nanotechnology can also be applied to medical therapy to provide substantial improvements over current treatment options.

Basic questions that need to be addressed include: what specific problems in medical therapy can be addressed using nanotechnology? What therapy problems can nanotechnology address that microtechnology cannot address? What devices and materials for therapeutic applications can be provided by nanotechnology?

Objectives

Specific areas where nanotechnology can provide near term benefits include drug discovery, production and delivery; molecular and cellular device applications (including gene therapy); and advanced and smart biomaterials.

Obstacles and Challenges

Specific research problems in this area include:

- Drug production, discovery and delivery via nanoparticle materials that offer significant improvements in bioavailability and efficiency through oral or injectable pathways. Related areas that need to be studied include bio-friendly fabrication methods at the sub-200 nm length scale, nanoparticle distributions (surface deposition vs. colloidal suspension), and efficacy of delivery by various pathways.

- Nanomaterial fabrication including synthesis or milling techniques, controlled and designed crystallization methods, large-scale methodologies suitable for manufacturing purposes, controlled particle aggregation, and nanoparticle coating techniques.

- The development of nanostructured biomaterials to replace hard and soft skeletal tissue, lipids for gene therapy vectors, and biocompatible materials for tissuegenesis. Related research areas include the development of biocompatible interfaces between organic and inorganic systems for tissue engineering, creation of nanoporous biocapsules for cellular therapy, and development of cell- and molecular-level biological recognition systems. In the biomaterials arena, the division between micro- and nano-scale applications is not always distinct, and micro/nano synergies must be used to their maximum advantage.

Recommendations

- Support research that involves interdisciplinary collaboration involving biomimetic materials using biologically-derived synthons (e.g., nucleic acids or peptides that are directed toward non-biological applications including nano-scale computation, DNA-based computing, and semiconductor discovery) to provide paradigms for biological systems and novel biomaterials. While the relationship of some of this research to biomedicine may not be readily apparent, consider the life saving role of the telephone in 20th century, or the computer in the 21st.

- Encourage research on the construction and production of artificial nanostructures that could be used within the cell as replacements for faulty naturally-occurring nanostructures or could serve other therapeutic purposes not found in normal cells.

- Establish trans-disciplinary training programs that encourage the individuals who develop the tools of nanotechnology and those who seek to use nanotechnology for therapeutic purposes to participate at all levels from undergraduate through professional career levels.

- Convene small meetings that focus on a particular nanotechnology tool (e.g., synthetic or physical deposition) and how best to apply it to solve therapeutic problems. These meetings should consist of representatives of both the technological and biomedical communities.

- Establish NIH study sections composed of experts in biomedicine and nanotechnology that emphasize the likely efficacy of proposed constructions and protocols rather than hypothesis testing.

- Encourage early, ongoing cooperation between academia, industry, and regulatory agencies, to smooth the path from nanotechnology research to biomedical application. Collaborations should develop a unifying perspective for employing nanotechnology tools in the context of macroscopic architectures. Interagency cooperation, particularly with regard to training programs, will facilitate these interactions.

Biomimetic Nanostructures

Moderators: David Tirrell Ph. D., California Institute of Technology
Viola Vogel, Ph. D., University of Washington

Panelists

Ilhan Aksay, Ph. D., Princeton University
Julio Fernandez, Ph. D., The Mayo Foundation
Linda Griffith, Ph. D., Massachusetts Institute of Technology
Daniel Hammer, Ph. D., University of Pennsylvania
Patrick Stayton, Ph. D., University of Washington
Dan Urry, Ph. D., University of Minnesota – Twin Cities

Broad Statement. Advances in the design and utilization of biomimetic nanostructures have been driven by two key objectives:

- Learning how nature designs, processes, and assembles/disassembles molecular building blocks, and how the structure of nanoscale assemblies relates to their function; and

- Utilizing design criteria and processing strategies obtained from biological systems for making new molecules, materials, sensors, and therapeutics.

Vision

These two objectives are intimately linked as the lessons learned from nature that will give crucial insights into nature's nanoscale materials and machines. The ultimate success in mimicking nature's approaches to systems engineering under *ex vivo* conditions will be the final proof of whether the underlying natural mechanisms are indeed understood.

The deliverables of an NIH investment into biomimetic nanostructures include the development of new molecules, materials, and technologies for diagnostics and therapy. Simultaneously, the study of biological nanoscale systems from an engineering perspective will provide fundamentally new insight into nature's approach to the engineering of complex interactive nanoscale systems that will lead to new discoveries in biosciences.

Objectives

Realization of this vision for nanoscale science and technology will require identification of both fundamental and practical objectives. A list of such objectives can be very long; the following presents just a few representative examples of a much larger set of challenging and important targets for research on biomimetic nanostructures of biomedical relevance.

Scientific. Research in biomimetic nanostructures must be guided by sound knowledge of the behavior of cellular and biomolecular systems. Such systems provide compelling examples of the power of molecular machines to accomplish efficient catalysis, exquisite molecular detection, selective molecular transport, and many other functions of obvious and direct medical relevance. Genomic information now allows increasingly rapid identification of such molecular machines, and successes in structural biology are providing detailed insights into the mechanisms by which ribosomes, transport systems, and molecular motors accomplish their tasks.

- Couple advances in structural biology with development-driven research in which targets for structural and mechanistic study are chosen at least in part on the basis of their relevance to the creation of novel biomimetic systems.

- Develop novel synthetic methods for generation of functional biomimetic nanostructures characterized by precisely defined architectures with architectural control of non-biological macromolecules in sequence, topology, and folding behavior.

- Develop control of molecular assembly in synthetic and biomimetic systems comparable to that effecting and modulating biological processes in fundamental studies of cellular behavior.

- Develop tools in nanotechnology to reveal how mechanical forces are utilized by nature not only to change conformational states, but also to regulate the functional state of biomolecules, cell signaling and transcription.

Technological. Whereas the scientific objectives of research on biomimetic nanostructures are relatively readily identified, the most important technological objectives are more necessarily speculative. A list of such objectives might include:

- Develop synthetic molecular systems that approach, and perhaps eventually surpass, viral vectors in their efficiency for intracellular delivery of genes, antisense oligomers, drugs or nanoscale probes that permit detailed study of intermacromolecular interactions, ligand-receptor binding, membrane transport and molecular trafficking.

- Increase understanding of intermacromolecular interactions, ligand-receptor binding, membrane transport, and cellular trafficking to make such systems plausible targets for nanostructure research.

- Develop nanoscale antenna systems for signal amplification from single molecules that extend advances in the control of molecular topography and functionality and the study of molecular photophysics.

- Develop nanopatterned substrates on programmable surfaces for the capture, maintenance, and expansion of therapeutically-useful cells and to improve understanding of the role of mechanical forces in cell signaling processes.

- Engineer templates for the restoration and repair of hard and soft tissues and fabricate nanoporous capsules for implantable medical devices for difficult medical and surgical problems.

Obstacles and Challenges

- Exposure to and education in the biosciences by physicists, chemists, and engineers who develop analytic tools and microprobes of single molecule detection and analysis for *ex vivo* and *in vivo* studies needs to increase.

- Integration of experimental and computational approaches to the understanding of the structure, function, and driving forces that lead to the assembly of biological and synthetic molecules in water needs to be encouraged and fostered.

- Understanding of how nature utilizes non-equilibrium conditions for assembly, regulation, and signaling of biological structures needs to be enhanced.

- Availability of biomimetic systems using nanoscale materials and devices that display controllable time dependent behavior and display predicable aging behavior or degrade with predictable and controlled kinetics needs to increase.

- Nanoscale materials and devices need to be fabricated and integrated into addressable devices to understand how synthetic materials in contact with tissue are remodeled and/or degraded over time by cells.

Recommendations

- Foster the integration of biologists into the interdisciplinary community that crosses disciplines from physics to medicine through workshops and educational programs at all levels from faculty to students, so that biologists can adopt newly developed technological advances in nanoscience without hesitation.

- Create a new study section in "bioengineering and nanotechnology" that includes participation from multiple disciplines in the evaluation of interdisciplinary proposals in nanoscale science and technology.

- Encourage and develop co-funding strategies among different funding agencies with complementary missions, particularly for support of exploratory, high-risk research.

Biological Nanostructures

Moderators: Carlo Montemagno, Ph. D., Cornell University
Bernard Yurke, Ph. D., Lucent Technologies

Panelists
Susan Gilbert, Ph. D., University of Pittsburgh
Shuming Nie, Ph. D., Indiana University
Michael Roukes, Ph. D., California Institute of Technology
Joseph Sanger, Ph. D., University of Pennsylvania
Jacek Tuszynski, Ph. D., University of Alberta

Broad Statement. The molecular machinery of even the simplest bacterium is amazingly complex and functions essentially as nanomachines. These molecular machines take the form of motors, pumps, and valves that provide structural support, catalyze chemical reactions, store information, and transport material within, into, and out of the cell. These molecular machines have been removed, produced, and modified outside the cell to successfully study their properties. Recently, the first steps have been taken to applying these marvelous machines to engineered devices. As a result, it is evident that there is great potential in using systems constructed with these machines to improve significantly our understanding of basic biology and physiology. More importantly, this emerging technology offers the promise of molecular scale therapies and diagnostics. The challenge is how best to facilitate the realization of that potential.

Vision

Develop engineered systems that integrate seamlessly with life processes for the purpose of investigating fundamental life processes, combating disease, and improving the human condition.

Objectives

- Devise a simple rule-based technology that facilitates the self-assembly of complex hybrid living-non-living systems. Such a technology may permit the construction of smart drug delivery systems, vectors for gene therapy, and cellular level diagnostic tools.

- Integrate active biological molecules such as molecular motors and membrane pumps with engineered systems to create "living" machines for therapeutic treatment.

- Develop traceable standards for nanoscale force and distance measurements.

- Create a new class of non-interactive single molecule reporters for the study of basic biological and physiological processes.

- Develop a single molecule NMR imaging system using MEMS/NEMS technology.

Scientific Priorities

- Develop integrated biomolecular powered engineered devices.

- Create engineered structural molecular recognition technology.

- Refine the microscale-nanoscale communication and control interface.

- Improve *in vivo*, single molecule detection methodologies.

Obstacles and Challenges

- Funding for capital equipment and support for existing capital equipment need to increase substantially.

- Support for technology development and high-risk research is needed.

- Skilled personnel in both the biological and physical sciences/engineering must be trained.

- Single molecule detection/measurement methodologies need to be improved and developed.

- A controllable method for single molecule/nanostructure manipulation needs to be developed.

- Biocompatible nanofabrication processes need to be developed.

Recommendations

- Institute abbreviated selection mechanisms for funding of high-risk research at NIH.

- Support technically-driven proposals to develop critically needed measurement tools necessary for the successful application of nanoscale science to medicine

- Continue to fund microscale technology that is instrumental in the development of the interface between nanoscale devices and the macro world.

Electronic/Biology Interface

Moderators: Angela Belcher, Ph. D., University of Texas - Austin
Jeff M. Byers, Ph. D., Naval Research Laboratory

Panelists

Adam Arkin, Ph. D., University of California - Berkeley
Elias Greenbaum, Ph. D., Oak Ridge National Laboratory
Ron Miles, Ph. D., State University of New York-Binghamton University
William Shain, Ph. D., Wadsworth Center
Jeff Stuart, Ph. D., Syracuse University
Bruce C. Wheeler, Ph. D., University of Illinois

Broad statement. The fundamental biomolecular processes of life create, manipulate and propagate information with high density and fidelity. The large-scale genomic analyses begun in the 1990's and the emerging field of bioinformatics provide evidence that the future of biology is a quest to understand the processing of information encoded in molecules by cells. Silicon-based electronic information processing is partially responsible for enabling this revolution in molecular biology. The convergence of man-made and natural information processing systems will drive efforts to understand how to more closely couple electronics to cellular and biomolecular functionality.

Vision

The sequencing of genomes produces a parts list. This is an essential, but early step toward a comprehensive analysis of the detailed spatio-temporal interactions of proteins in the control networks that govern key cellular functions. To enable medicine to move beyond statistically based clinical trials requires a means of detailed monitoring and manipulation of regulatory and signaling networks at both the molecular (< one micrometer) and cellular levels (> ten micrometers). But the tools needed to measure molecular interactions on a large scale, interpret that information, and then intervene in the networks do not exist. The development of *in vitro* research tools and then *in vivo* medical devices capable of functional imaging of and interfacing to the information processing networks of life constitutes the central goal of work directed towards medical applications at the electronic/biology interface.

Objectives

A key scientific advance offered by the development of an electronic/biology interface would be the means to understand and intervene in the spatio-temporal choreography of biomolecular

interactions that constitute information processing in molecular biology. Biological organisms have developed micro- and nano-systems to solve many problems. The machinery of life can provide a blueprint for the development of nanomachines and synthetic molecular processors that carry out complex functions. To achieve this, scientific investigation of biological processes should move away from ensemble measurements of properties towards nanometer scale measurements on microsecond time scales. This information can be utilized to build nano- and micro- structures and systems to integrate with, and provide information input/output connections to biological systems. Examples of objectives include:

- using paradigms from nature – e.g., photovoltaic proteins in plants that extract electronic energy from light energy, or insect hearing organs 1 mm apart that have highly directional sound source localization sensitivity – as models for, or components of nanosystems that accomplish other functions;

- using biological machines to assemble micromachines;

- integrating biological machines with micromachines;

- using proteins to construct nanomaterials with useful properties;

- modify the cell-electronic interface so that the cell recognizes the electronics as self and the electronics recognize signals from the cell; and

- understanding how information gets into and out of a neural system and how the neurons interact with each other.

Obstacles and Challenges

Many obstacles exist to interfacing electronic technology, as it currently exists, with biology. As an illustration of the difficulties consider the mainstay of electronics, the field-effect transistor (FET). The FET is essentially a two-dimensional structure composed of layers defined by lithography. The resulting integrated circuit forms a fixed two-dimensional grid of devices (approx. ten FETs/micron^2). Addressing of the individual devices is achieved by the use of electric fields and hard-wired interconnections. In contrast, biology performs its information processing in an aqueous three-dimensional medium with diffusion of messenger molecules playing the role of 'wires.' The fixed-space addressing scheme of electronics is replaced by a more subtle shape recognition scheme embodied in receptor-ligand interactions. This biological addressing technique is far slower than that of electronics but exceeds its man-made counterpart in the sheer density of functionality. Engineering a robust and effective interface between such different technologies as biology and electronics will require a significant effort using tools from a myriad of disciplines. Some near-term paths may include the incorporation of biological molecules into otherwise electronic devices, mimicking biological structures in fabricated devices, and the incorporation of lessons learned from biological signal processing into the logic of electronic systems.

Beyond the challenging technical demands lies the obstacle of breaking down the conventional means of carving the world into academic departments and rigid review panels. These structures tend to be conservative and impede developments that do not clearly lie within established disciplines. Universities, in general, do not produce the kind of researchers needed to accomplish engineering work requiring fluency in physics, chemistry and biology. The trend toward establishing multi-disciplinary institutions within universities may correct these problems in time.

Recommendations

- Fund the development of device fabrication tools specifically designed for application to biological system interfacing rather than simply adapting devices the electronics industry has developed for semiconductor processing.

- Support efforts that broaden education in biology and medicine so that future students will understand the possibilities that modern materials processing and electronics provide.

- Involve a larger portion of the biological research community in micron-scale fabrication technology. For example, support the development of cheap, easy-to-use, wet micro-printing techniques for widespread use in molecular biology laboratories.

- Do not skip the development of micro-scale technologies in the enthusiasm to explore and exploit the nanoscale.

- Take more risks in the types of research funded and diversity the groups of people who review the proposals.

Devices for the Early Detection of Disease

Moderators: Harold Craighead, Ph. D., Cornell University
David Rakestraw, Ph. D., Eksigent Technologies

Panelists

Jeffrey A. Kant, M. D., Ph. D., University of Pittsburgh Medical Center
Martin A. Philbert, Ph. D., University of Michigan
John Sninsky, Ph. D., Roche Molecular Systems
Basil Swanson, Ph. D., Los Alamos National Laboratory
Tuan Vo-Dinh, Ph. D., Oak Ridge National Laboratory
David Walt, Ph. D., Tufts University

Broad Statement. Nano/micro technology will 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.

Vision

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 will revolutionize disease research and diagnosis.

Objectives

- Develop new research tools to help identify the markers of disease using micro- and nanotechnology approaches.

- Develop devices to detect identified disease markers for use in the clinic. Attributes of such devices include the following:
  - Continuous non-invasive real-time monitoring of disease processes in patients;
  - Analytical tools to assess biological responses and trends in healthy versus diseased patients;
  - At-home sensors to monitor characteristics of wellness;
  - Real-time sensing of cell function during surgery.

- Develop single cell, analytical tools for basic research of cellular processes and functions, keeping in mind statistical limits for accurate measurements.

- Develop new methods for high-throughput cell sorting using nanotechnology-based particles or tools.

- Use nanotechnology to create more cost-effective methods for early diagnosis.

Obstacles and Challenges

- Fundamental knowledge about molecular signatures of disease and surrogate markers to identify these diseases must be increased.

- Standard markers and analytical methods for new diagnostics using nanoscale technology need to be developed.

Recommendations

- Create a panel of pre-qualified clinical samples to aid the development of new diagnostic approaches in conjunction with long-term clinical (and animal model studies, where necessary) to provide validation for newly identified disease markers.

- Develop databases of known markers for diseases and expand with information about new markers as they emerge.

- Where known, identify diseases with characterized markers for near-term diagnostic device development.

- Support long-term research in new tools and analytical approaches for both biomedical research and diagnosis, keeping in mind that concomitant development of diagnostic/ analytical tools and biological studies must be done.

Tools for the Study of Single Molecules

Moderators: Paul Alivisatos, Ph. D., University of California - Berkeley
Jay Trautman, Ph. D., Praelux

Panelists

Dan Branton, Ph. D., Harvard University
Louis Brus, Ph. D., Columbia University
Stephen Empedocles, Ph. D., Quantum Dot Corporation
Jay Groves, Ph. D., Lawrence Berkeley National Laboratory
Lydia Sohn, Ph. D., Princeton University
Sunney Xie, Ph. D., Harvard University

Broad Statement. Single molecule and related high-sensitivity measurements will be critical to fundamental and practical studies in biology and medicine. The development of single molecule methods is a central theme in nanotechnology research and will be used to facilitate progress in all sub-areas of nanotechnology. Applications of single molecule methods extend over a wide variety of systems and include, for example, the study of molecular motors, enzyme catalysis, protein and RNA folding, complex protein-protein interactions, local structural changes in single ion channels, and signal transduction.

Vision

Current methods in single molecule detection depend primarily on fluorescent, mechanical, or scanning probe techniques, with less emphasis on techniques using magnetic, vibrational, or electrical properties of molecules. Single molecule measurements should enable one to examine individual members of a population and to identify, sort, and quantitatively compare subpopulations and substructures within a cell without averaging across the ensemble. Ultimately, any cellular process, such as exocytosis, channel flux or trafficking, could be observed in real time, *in vivo*, without perturbing the cell by using single molecule methods.

Objectives

- Integrate and develop technology to enable *in vivo* studies of cellular processes in real time.

- Develop new tools in the nanometer range for the study of single molecules to provide new ways to observe biological events individually and simultaneously.

- Develop new tools that will allow imaging of cellular processes such as signal transduction, protein-protein interactions, and gene expression as they occur *in situ*.

- Develop modeling, simulation, and statistical theory to describe single molecule behavior to parallel empirical observation.

- Develop better probes and instruments for non-invasive subcellular and single molecule imaging.

- Improve communication about the tools and methodology of single molecule techniques so that more researchers will have access to them.

- Adapt existing technologies developed through other venues (e.g., physics, materials science) to biological problems. This may require parallel adaptation of new materials to achieve biological compatibility.

Obstacles and Challenges

- Better probes need to be developed for single molecule detection with improved characteristics such as increased spectral sensitivity, decreased blinking, compatibility with physiological conditions inside the cell, and decreased quenching. Clonable tags for *in vivo* imaging experiments, such as GFP, are highly desirable, but rare.

- "Off the shelf" instruments for single molecule studies need to be developed. Current instruments must be custom designed and tailored individually by highly skilled investigators for specific experiments, making access impossible for a wide spectrum of users.

- Collaboration between experts in single molecule biophysics, biology, chemistry, and clinical research needs to be fostered and encouraged.

Recommendations

- Support cutting-edge single molecule research to enable technologies for use in fundamental biology, with the ultimate goal of understanding disease and developing better diagnostics and therapeutics.

- Extend the use of single molecule technology to analytics and the early detection of disease.

- Train and involve biologists in nanotechnology development and applications, which is currently the realm of physicists.

- Support collaborations that foster distribution of cutting-edge technologies to the wider community of biomedical researchers.

- Share the funding responsibilities of single molecule research with other federal agencies, not only to distribute the costs, but also to take advantage of a wider range of scientific expertise. This may require the creation of new paradigms for collaboration and cost sharing.

- Create/adapt study sections with appropriate compositions to integrate nanotechnology with biomedical research.

Nanotechnology and Tissue Engineering

Moderators: Ann M. Mayes, Ph. D., Massachusetts Institute of Technology
Robert M. Nerem, Ph. D., Georgia Institute of Technology

Panelists

Jeffrey Bonadio, M. D., Selective Genetics, Inc.
Kathleen Chesmel, Ph. D., Therics, Inc.
Kevin Healy, Ph. D., University of California - Berkeley
Buddy Ratner, Ph. D., University of Washington
Mehmet Toner, M. D., Massachusetts General Hospital
Richard Vaia, Ph. D., Air Force Research Laboratory

Broad Statement. Researchers involved in tissue engineering strive to repair lost tissue or organ function through the transplantation of living cells, cells delivered in many cases on a bioresorbable scaffold. Exciting advances have been made in the regeneration of cartilage, skin, bone and blood vessels, yet significant challenges remain in the regeneration of complex organs that exhibit full metabolic function. Pioneers in this area now recognize that many of the hurdles could potentially be overcome through more deliberate scaffold design, using materials that can better address structural requirements and guide the activity of seeded cells. In light of the rapid developments in synthesis and processing of tailored organic and hybrid nanostructured materials, the goal of this panel was to identify how such nanoengineered materials might play a role in advancing tissue engineering. Further, the panel considered how the NIH might foster interdisciplinary ties between scientists in the forefront of nanotechnology and the biologists, clinicians, and bioengineers actively developing new therapies.

Vision

For the immediate future (five to ten years), tissue engineering stands to benefit most from our growing ability to fabricate complex nanostructured materials.

Objectives

Cell responses *in vivo* are guided by chemical "cues" of nanometer dimensions. Synthetic tissue engineering scaffolds designed to elicit specific cellular responses (and eliminate non-specific responses) through the incorporation of signaling ligands (e.g., growth factors, adhesion peptides) or DNA fragments are viewed as particularly promising near-term strategies. Examples include materials with spatially-clustered ligands that can induce receptor clustering and consequent intracellular signaling events. Beyond the goal of producing scaffolds that "talk"

to cells lies the possibility to design scaffolds that "listen" to the *in vivo* environment through integrated molecular sensors, and structures that exploit the dynamic nature of cellular processes.

Hybrid organic/inorganic nanocomposites are emerging as a class of materials that could potentially yield bone scaffolds with vastly improved mechanical performance, and enhanced bioresorption/remodeling rate. Such approaches exploit the unique mechanical and transport properties afforded by inherently high amounts of interface per unit volume. Particular promise is seen for fabrication methods that yield hybrid nanostructures akin to approaches that are commercially and clinically relevant.

Cells and tissues are inherently hierarchical in nature, with structure-function relationships generated over multiple length scales. Long-term strategies for designing scaffolds from nanostructured materials must be compatible with three-dimensional device fabrication methods that allow for simultaneous structural control at the micron scale, to facilitate cell ingrowth (e.g., controlled porosity) and direct the organized growth of multiple cell types. Strategies of practical worth must employ materials and methods likely to encounter minimal regulatory barriers, and have scale-up manufacturing capacity. Nanotechnologists are increasingly able to achieve high spatial control of molecules, but only for relatively SMALL numbers. To move to microscopic device production will likely require novel supramolecular assembly or self-organizational strategies. Fabrication and surface modification approaches for tissue engineering scaffolds with controlled structure, that were discussed by the panel and appear promising in this regard, include: amphiphilic (ex. block) copolymers, electrostatic (layer-by-layer) assembly, contact printing, polymer-intercalated oxides, surface segregation, 3D printing, electrospun fibers, molecular templating, and imprint lithography.

Obstacles and Challenges

Investigators need to develop an understanding of the relevant molecular/cell biology to exploit the growing expertise in nanostructured materials fabrication. For example, a better understanding of the relationship between nanoscale topography and cell function is needed. Such knowledge will become available as tools that are now under development evolve to be an integral part of the discipline. Such resources include:

- Biological assays of the "nanomachinery" of cells;
- Advanced characterization methods – particularly those relevant to water-based environments and single molecules (e.g., NSOM, force spectroscopy, etc.);
- Computational tools for structure analysis.

Recommendations

- Reduce inherent "language" barriers and distinct cultures of these two groups of researchers that impede the integration of nanotechnology and biomedicine. Identify means by which the interdisciplinary ties between physicists, chemists, and materials scientists at the forefront of

nanotechnology and bioengineers, biologists, and clinicians actively developing new therapies (e.g., tissue engineering) can be fostered. Some such means might include:

- Development of an accessible databank and chat room;
- Small, interdisciplinary conferences;
- Mini-sabbatical support – particularly for young investigators; and
- Interdisciplinary training grants.

- Create within NIH, an administrative structure to facilitate the integration of nanotechnology and bioengineering. Such administrative integration would likely improve peer review mechanisms and facilitate the development of new programs to foster bioengineering partnerships.

- Develop a program for small technology development grants ($50K in direct costs) that can be reviewed rapidly.

Participants

Symposium Co-Chairs

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