Nanobiotechnology Center
Forging the Genesis of New Insights, Devices, and Systems through Research in Nanotechnology and Biological Systems

In the past few years, nanobiotechnology has emerged as an exciting scientific and technological opportunity to meld nano-microfabrication and biosystems to the benefit of both. Nanobiotechnology is the genesis of substantial new insights into the structure and operation of biological systems, which leads to the design of new classes of micro- and nanofabricated devices and systems.

Featuring close collaboration among life scientists, physical scientists, and engineers, the Nanobiotechnology Center (NBTC) enters its third successful year as a National Science Foundation-supported Science and Technology Center. With Cornell as the lead institution, the center draws on the talents of biological researchers at the Wadsworth Center of the New York State Department of Health and academic scientists from Clark Atlanta, Howard, Princeton, and Oregon Health and Sciences Universities. It also involves the active collaboration of K-12 educators at the Sciencenter in Ithaca, NY, as well as interactions with industry and government laboratories.

The center has made strong progress in the development of a fully integrated research and education effort with active participation of NBTC faculty, postdoctoral associates, and students. Many interdisciplinary research projects funded by the center are yielding exciting results as can be highlighted with some ongoing projects.

**Protein Folding**

To examine how proteins achieve their exquisite three-dimensional structures, Lois Pollack, Applied and Engineering Physics, leads a project using small angle x-ray scattering (SAXS) with time resolution of hundreds of microseconds. Within living organisms, proteins are synthesized by the addition of amino acids, one at a time, onto a growing chain. This chain rapidly “folds” into the specific shape that allows the protein to carry out its biological function. Even the smallest proteins are composed of thousands of atoms, yet some of them find their native structures on time scales as short as milliseconds, literally in the blink of an eye. This project seeks to uncover the physical interactions that drive protein folding and probes the connection between the protein’s one-dimensional amino acid sequence and its three-dimensional, biologically active structure. In the absence of cellular machinery, protein folding can be initiated by varying an external parameter such as solution pH or the concentration of a chemical denaturant.

In this research, the folding of β-lactoglobulin protein has been triggered by employing microfabricated fluid mixers to produce a rapid change in the chemical denaturant concentration. Using a microfabricated flow cell in conjunction with synchrotron x-rays, global structural changes in protein conformation on time scales less than 1 millisecond after the initiation of folding have been observed. These studies provide valuable and previously unobtainable insight into the role of compaction in protein folding. The unfolded protein is an expanded chain; a folded protein is a compact object. A major step in the folding process must therefore involve significant compaction, or collapse. Small angle x-ray scattering allows time-dependent compaction of different classes of proteins to be monitored on time scales as short as hundreds of microseconds. These measurements...
make it possible to determine when, during the folding process, these significant structural changes occur. They also allow experimental verification of previous models of protein folding, which were not testable.

Other groups that have contributed to this project include Carl A. Batt, Food Science; Sol M. Gruner, Physics; and Robert H. Austin, Physics, Princeton.

**Nanofluidic Structures**

Other NBTC projects advance the fabrication of nanofluidic structures, which have been used successfully for sorting DNA molecules and may be used to synthesize and process microscopic quantities of chemicals in “lab-on-a-chip” and other nanoscale devices. A project involving the research groups of Geoffrey W. Coates, Chemistry and Chemical Biology, and Harold G. Craighead, Applied and Engineering Physics, has produced nanometer-scale fluid channels with a new technique, using heat-depolymerizable polycarbonate (HDP) in a sacrificial layer configuration. Although the standard sacrificial layer technique gives excellent channel height uniformity, earlier work with polysilicon suffered from the disadvantage of requiring a lengthy chemical immersion to remove the sacrificial layer. In contrast, HDP films dissociate into a nontoxic vapor when heated above 300º C.

To form these nanofluidic channels, HDP is spun onto the front surface of a silicon wafer substrate and patterned directly with e-beam lithography. The polymer is removed from the exposed regions by a short immersion in isopropanol, and the patterned surface is then covered with a thermally stable oxide capping layer. The sample is then inverted and thin silicon nitride membranes on the back surface are etched away to provide venting ports for the HDP. These ports can also serve as fluid inlet and outlets in final nanofluidic devices. Heating to remove the polymer material leaves tubes that are typically 140 nm high, 1 micron wide, and 1 mm long.

This new technique will be developed for fabrication of more complex nanofluidic circuits and two-dimensional obstacle arrays with a wide range of applications including separation and analysis of DNA, proteins, and other biomolecules as well as chemical synthesis and processing.

**Researching Cellular Responses**

A recently initiated project by the research groups of Barbara A. Baird, Chemistry and Chemical Biology, and Harold G. Craighead, Applied and Engineering Physics, examines a central problem in immune cell signaling—spatial control of signaling events that are stimulated by receptor-ligand interactions at the cell-cell interface. The interaction between mast cells expressing antibody (IgE) receptors and their ligands, which are patterned on a surface, will allow the assembly of cellular signaling complexes on the membrane and within the cell to be examined. For example, the effect of ligand density on cellular response, the binding kinetics, and the redistribution of signaling components after the initial binding events can be elucidated. These will lead to a better understanding of signaling mechanisms as they occur in immune responses and thereby possible means of intervention with the design and development of new drugs.

A new parylene lift-off method will be employed to create ligands in patterns of self assembled monolayers or lipid bilayers on a silicon substrate. This parylene lift-off technique is a rapid, precise, and economical method to create micro- and nanometer-scale patterns on substrates. In addition, parylene lift-off patterning is biocompatible because the addition of bio-material is an independent step after fabrication of the pattern, when aqueous solutions may be used. Biomaterials are not denatured by this process, and thus retain both their structure and function. This provides a highly isolated pattern of ligands, offering a means for spatial control of binding across a cell surface. Other scientific advances provide the means of detecting the signaling events: both exogenous and endogenous cellular components can be labeled with fluorescent tags that can be followed with quantitative optical microscopy. The mast cells being studied serve as a model system for other immunological and physiological cells and responses. For example, spatially controlled aggregation of receptors at an interface occurs during cell-cell recognition at the initiation of an immune response. One outcome of these encounters is secretion of chemical mediators such as histamine from mast cells in the allergic response. Another NBTC project, headed by Manfred Lindau, Applied and Engineering Physics, has been set up to address this aspect both for immune cells and for nerve cells. Electrode arrays are being fabricated to localize and quantify the secreted solutions.

NBTC continues to expand and enhance interdisciplinary research. Faculty who recently joined the center include Ulrich B. Wiesner, Materials Science and Engineering; Kelvin H. Lee, Chemical and Biomolecular Engineering; and Larry P. Walker, Biological and Environmental Engineering, at Cornell, and Michael Koonce, Structural and Cell Biology, at the Wadsworth Center in Albany. This brings the total faculty membership to 32. NBTC currently supports 30 graduate students and 14 postdoctoral associates.

Barbara A. Baird
Director of the Nanobiotechnology Center and Professor of Chemistry and Chemical Biology

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For more information:
Nanobiotechnology Center
101 Biotechnology Building
Cornell University
Ithaca, NY 14853
(607) 254-5393, Fax: (607) 254-5375
http://www.nbtc.cornell.edu