

BIOTECHNOLOGY CAPABILITIES

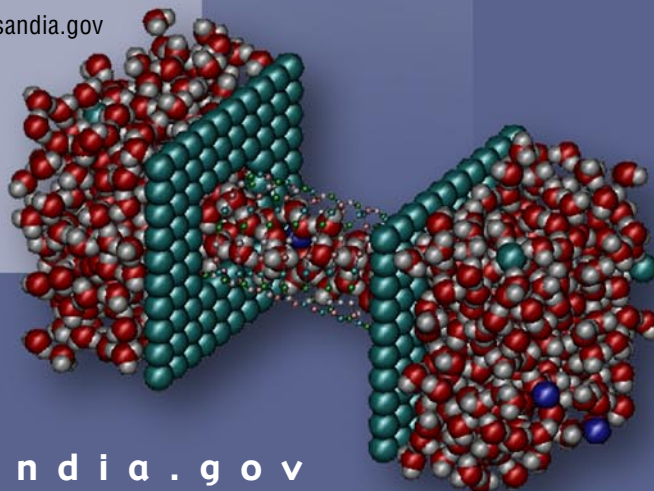
Sandia National Laboratories is a multiprogram engineering and science laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the U.S. Department of Energy's National Nuclear Security Administration. We design all non-nuclear components for the nation's nuclear weapons, perform a wide variety of energy research and development projects, and work on assignments that respond to national security threats—both military and economic.

In the future, Sandia's national security missions will increasingly depend upon state-of-the-art capabilities in biotechnology. Our biological and chemical detection and security programs extend the nation's capabilities via the integration of the traditional engineering, information, and physical sciences with molecular and structural biology, biochemistry, and microbiology. We encourage and seek partnerships with appropriate U.S. industry and government groups to collaborate on emerging technologies that support our mission (<http://www.sandia.gov/partnerships>). We seek to partner with universities, government agencies, and industry to further our biotechnology research for national security needs, and to license our discoveries to industry for commercialization.

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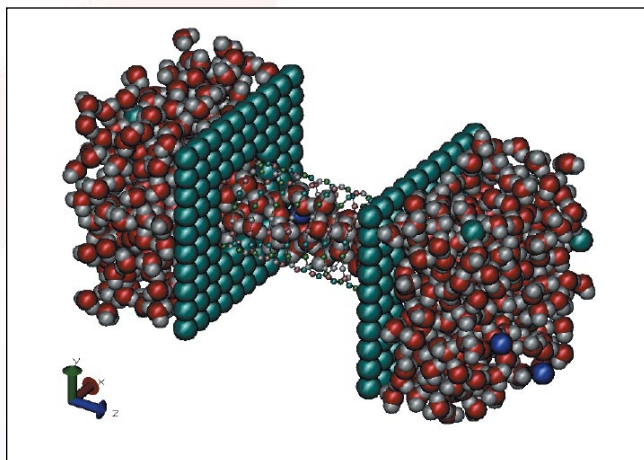
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BIOINFORMATICS & COMPUTING

Computational Science

Sandia is employing its world-class computing and computational science capabilities on computational biology problems. Such applications include computational tools for analyzing and understanding data from high-throughput experimental methods (bioinformatics), relating protein molecular structure to function (molecular biophysics and chemistry), and modeling cellular behavior



Snapshot of a molecular dynamics model where the selectivity of different ions in an ion channel was studied.

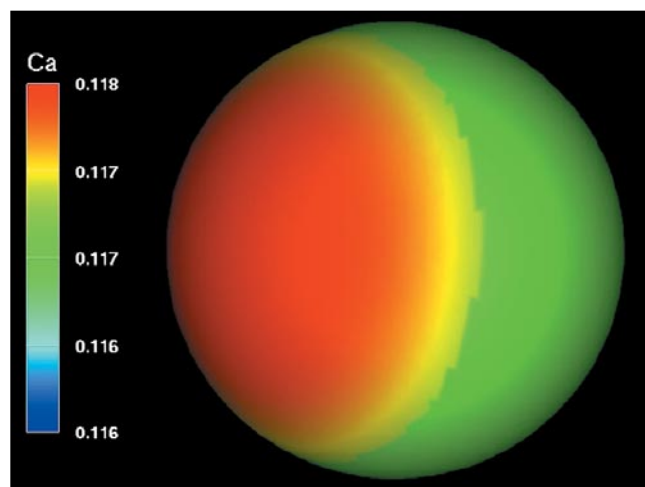
as a whole (complex system modeling). Many of the computational capabilities developed for Sandia's historical missions (e.g., parallel I/O, massively parallel computing architectures, algorithms and enabling technologies, molecular physics and chemistry, and complex system modeling) have found immediate applicability to life science and computing for life science problems. Sandia works with a wide variety of universities and nonprofit institutions and are funded primarily through DOE and other federal agencies.

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Modeling and Simulation

Modeling and simulation will have a significant impact on advances in biotechnology and medicine. It can extend from instrument and device design to modeling of complex biological systems such as entire cells to gain understanding of biomolecular processes to predict likely behaviors of organisms under varying conditions.

Sandia offers a broad range of expertise and a variety of modeling and simulation tools that can analyze complex biological systems. For example, Sandia's GOMA code, with its capability to treat moving boundaries, capillary and electrostatic forces, can simulate multi-species transport within the human body. The MPSalsa code has been used for micro-chem-lab design, analyte gaseous transport, and other biomedical areas of interest.



*Density plot of calcium ions in a *Xenopus Laevis* frog egg immediately after fertilization.*

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Scientific Data Discovery

The pattern recognition technique of "ensemble classifiers" is generally recognized as the best means of applying parallel processing to mining scientific data. Sandia is a leader in applying this technique to scientific data, which generally presents a unique combination of challenges. The data we handle is huge, noisy, and the regions of interest in the data are comparatively tiny; the problem is much like looking for a needle in a wind-swept hayfield.

As a result, the practical and analytical tools provided by ensemble classification are essential. In our computational modeling of molecular recognition, for instance, ensemble classifiers improved our overall sensitivity and specificity, allowed us to select the sensitivity specificity trade-offs we wanted, and provided confidence intervals.

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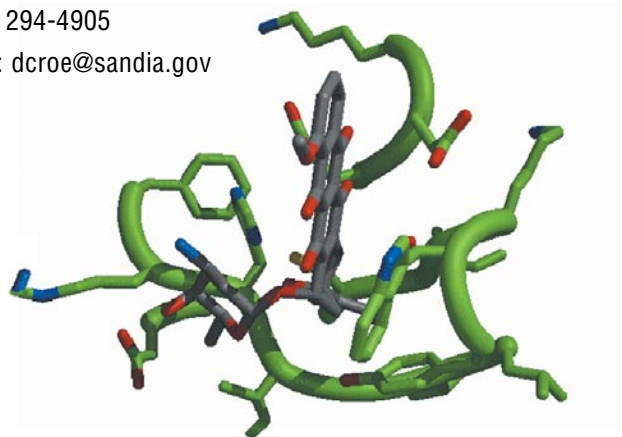
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computing capabilities at Sandia to add more realistic physics to our calculations. Our current focus is in biodefense, where we are applying these calculations to identify small molecule ligands that bind and recognize known biowarfare agents, and can be used in affinity-based detection systems.

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Small molecular (gray) bound to tetanus toxin (green), identified using docking calculations.

Computational Docking

Sandia has an active effort in computational docking calculations. These calculations simulate the interactions of biological molecules at the atomistic level and provide the fundamental basis for understanding important biological processes such as how a protein binds to its receptor, how an antibody interacts with an antigen, or how a drug molecule interacts with its target protein. Docking can further be used to predict which molecules will bind to a target biological molecule. For example in the pharmaceutical industry, docking calculations are used to identify new potential drugs to bind (and block) a particular target macromolecule. We are developing new docking tools that take advantage of the massively parallel



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BIO MEDICAL

Assistive technologies

Sandia is teaming with assistive device companies to help develop technical solutions to problems experienced by mobility-impaired people. Two such devices help wheelchair users prevent skin damage and spinal problems. Sandia applies its expertise in design, materials, batteries, actuators, sensors, reliability, safety, manufacturing, and



The Generic Total Contact Seat, which can be retrofitted to any wheelchair, adjusts automatically to prevent potentially deadly pressure sores.

software to help the industrial partners produce safe and effective products at reasonable prices.

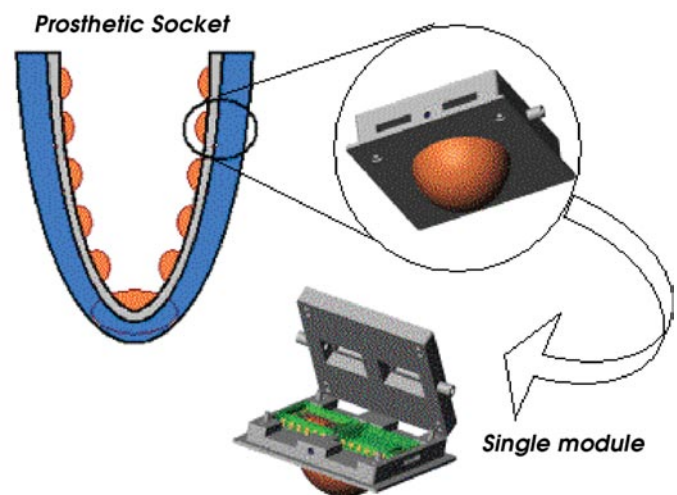
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Prostheses

Sandia is developing an above knee prosthetic socket that automatically conforms throughout the day to the varying



The Dynamic Socket uses a sleeve insert that contains several MEMS based bladders. The array of bladders distributes pressure over amputee residual limb.

size and shape of the residual limb. This will be achieved by using Micro-Electro-Mechanical System (MEMS) valves to measure the pressure and deliver fluid through channels to vary the bladder's volume. The automated socket will contain its own power unit (minimal consumption), controller, and allow integration with standard lower limb systems. The socket will sense and respond to redistribution of fluids of the residual limb and changes in pressure that occur during the gait cycle.

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Screening Mammography

Sandia has developed image analysis and pattern recognition software tools to aid radiologists in screening mammograms for cancer. A clinical trial has demonstrated that the software increases the odds of spotting certain cancers without increasing the risk of unnecessary biopsy or follow-up. The pattern recognition algorithms are based on the Sandia-invented concept of "dense feature maps," and are generally applicable to voluminous, noisy, and highly correlated data.

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Sandia's microvalve technology is uniquely able to route extraordinarily small fluid volumes in a wide variety of microfluidic systems, including those with harsh solvents, high pressures, and high electrokinetic fields. Taken together, these technologies allow for sophisticated control of fluid flow in an unparalleled variety of chemical analysis, chemical synthesis, and fluidic microactuation systems.

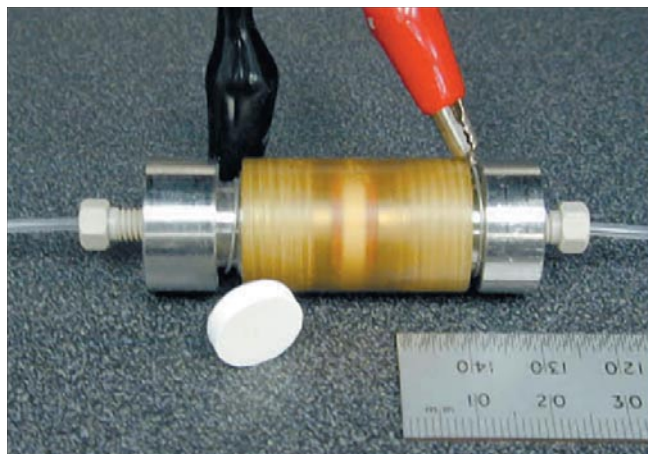
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Microvalves and Pumps

Sandia is a leader in novel techniques for pumping and routing fluids on microfluidic chips. Sandia's electrokinetic



Electrokinetic pump: voltages applied across porous matrices allow generation of extremely high pressures for microfluidic analysis or actuation.

pumps have no moving parts and can generate pressures exceeding 9,000 pounds per square inch in microchannels. These pressures have been used for high-pressure chromatography or for actuating microscale devices.



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BIO SENSING

Bio Sensing

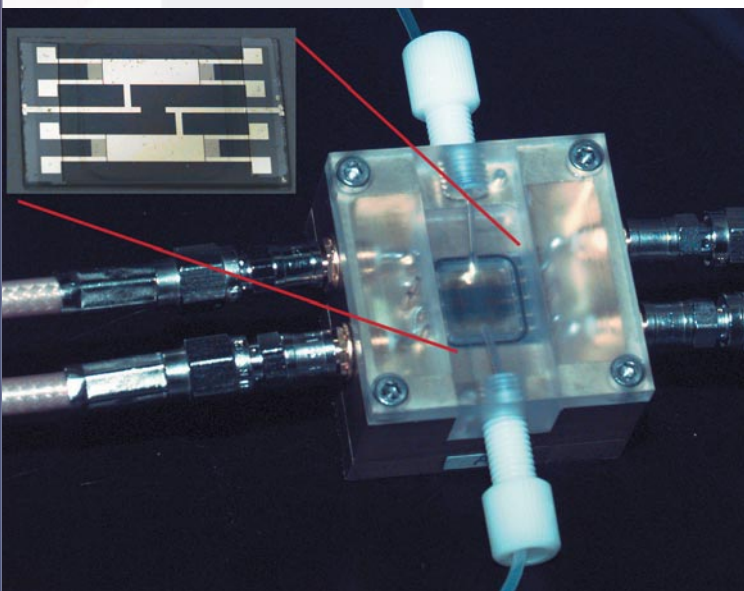
Sandia offers tremendous sensor expertise, including the capability to integrate biology with novel microtransducers, microelectronics and MEMS to yield innovative biological microsystems. As an example, by designing biomimetic transducers that mimic the molecular machinery of the cell, we can make highly "flexible" biological and chemical microsensors. Our research is working to combine chemistry, materials science, cell biology, genomics, advanced engineering and systems analysis for rapid identification of toxins, pathogens, viruses, and DNA. Current biosensor technologies include electrochemical, optical, and acoustic

transducer systems as a basis for the design of immuno-based and DNA sensors. In addition to developing sensor systems, we design new tools and instrumentation to detect and manipulate single molecules and to address cell membrane signaling.

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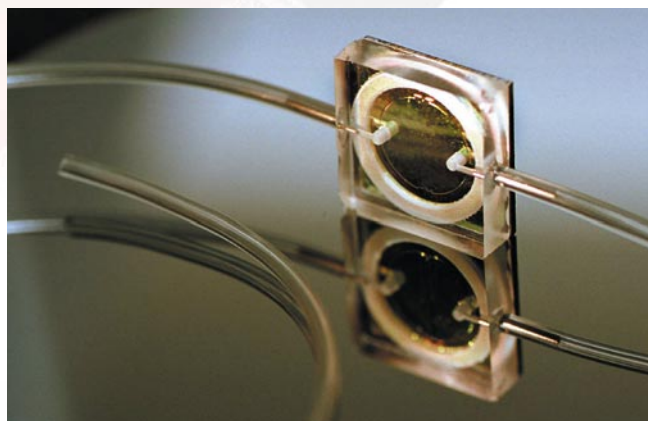


A miniaturized biosensor consisting of a shear horizontal surface acoustic wave sensor coated with a molecular recognition layer, applicable to BW agent detection and medical diagnostics.





DETECTION & ANALYTICAL SYSTEMS



Sandia's biocavity microlaser. The nanolaser, and microfluidic channels, also developed at Sandia, are too small to see without a microscope.

Biocavity Laser

Sandia has created a biological microcavity where biological cells form part of a semiconductor laser and impress cell information on the laser's optical output. Specifically, the spectral emission of the laser is very sensitive to the protein content of the cell. The mode pattern is also sensitive to the protein distribution in the cell. Using both sets of information, the device is able to distinguish cancerous cells from normal cells. It is anticipated that the bio-cavity laser will be used for rapid and relatively noninvasive medical diagnostics.

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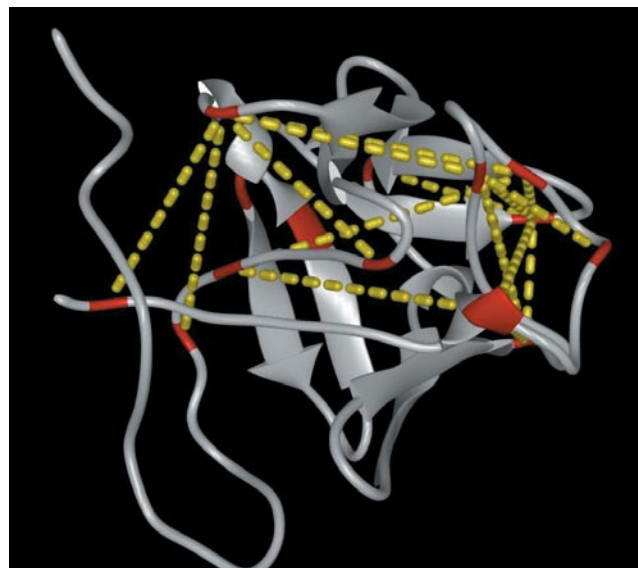
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Biomolecular Materials & Interfaces

Biological science has entered a new post-genomic phase in which the focus is to determine the structures and

functions of proteins, the DNA-encoded "workhorses" of the cell. Sandia is developing a unique technology, called MS3D, for probing the structures of membrane-bound proteins that are responsible for cellular signaling. The technology is a synthesis of state-of-the-art protein crosslinking, proteolysis, mass spectrometry and modeling approaches. The MS3D approach has the potential to rapidly probe the structures of membrane



Application of MS3D to the soluble protein target FGF-3 resulted in 18 crosslink derived distance constraints (in yellow). These distance constraints, used in conjunction with modeling approaches, were sufficient to build a model of the FGF-2 3D structure.

proteins and derive information about their function. Such information can be used to determine important information about biological processes with strong ties to national security such as biotoxin binding and action.

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Early Disease Detection

The threat of biological terrorism has introduced an entirely new aspect to weapons of mass destruction. Assessment of human exposure currently relies on pathogen replication or host responses using tests that can take from days to weeks. A collaborative DARPA-funded project between Sandia and the University of New Mexico Department of Pathology is developing methods to rapidly detect the onset of infection. Infrared data, combined with state-of-the-art multivariate analysis tools, are being used to quickly delineate cells presenting an infection-like response from healthy cells.

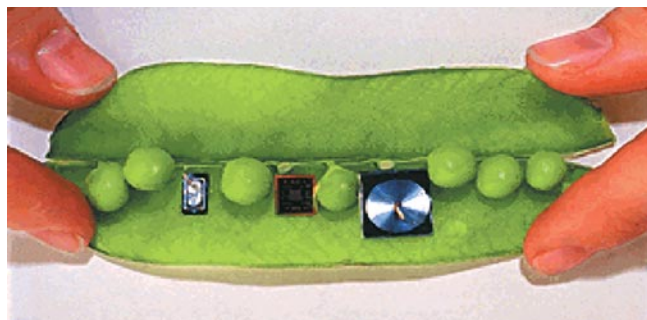
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Micro Total Analysis Systems

MicroChemLab™ technology is targeted at the growing and increasingly important class of problems requiring rapid detection and identification of minute quantities of chemical compounds in the presence of large quantities of



Sandia's biocavity microlaser. The nanolaser, and microfluidic channels, also developed at Sandia, are too small to see without a microscope.

background materials. Towards this goal, Sandia has miniaturized general-purpose gas and liquid chemical separations systems. The analytical methods needed to use these systems for the identification of a broad range of chemical agents are in development and prototype

instruments are entering field trials. To date, gas phase analysis has principally focused on volatile small molecules, with most work directed at analysis of chemical warfare agents and toxic industrial chemicals. Liquid analysis separation of other types of biomolecules including protein toxins and bioregulators has been demonstrated. Methods to detect pathogen signatures are currently under development. Chemical analysis with these systems is fast, very sensitive, and selective.

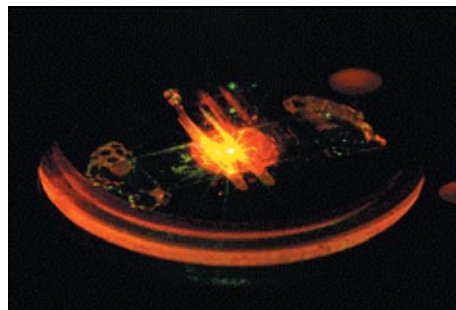
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DNA Detection Sample Processing Technologies

Basic building blocks can be fabricated using the standard SUMMiT technology. Sandia can provide technology for



This vertical cavity surface emitting laser (VCSEL) device can produce information about the state of millions of cells in a few minutes.

value-added biochip production, including advanced printing and scanning, analysis software, optics, and other aspects of biochip instrumentation. Sandia's superior microelectronics capability can help integrate any system.

Sandia researchers, with industry partner Cielo, Inc., recently developed the first 1.3-micron electrically pumped vertical cavity surface emitting laser (VCSEL) grown on gallium arsenide. This particular device will be used in a data communication capacity. In the biotech area, Sandia



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is currently working with a number of partners, including a gene sequencing project with Harvard University and the development of a next-generation gene chip microarray scanner with the University of New Mexico Cancer Center.

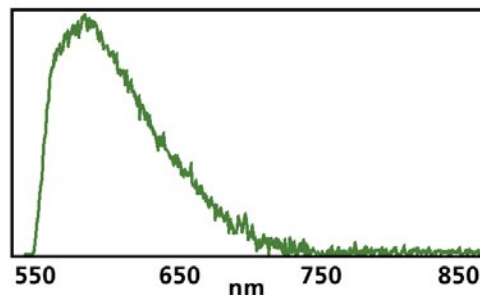
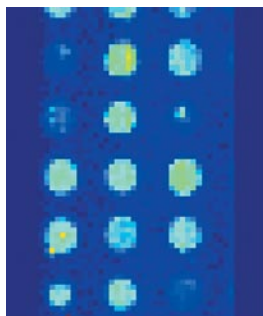
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Hyperspectral DNA Microarray Scanner

One of the more powerful technologies in biology today is the DNA microarray. While DNA microarrays have advanced the field of genomics at an amazing rate, the instrumentation available for scanning these fluorescent microarrays has limitations. We have developed a hyperspectral DNA microarray scanner that, in conjunction with Sandia's multivariate data analysis methods, can extract more useful information from DNA microarrays. Our scanner collects the entire spectrum at each pixel, allowing us to identify and quantitatively map the concentrations of all sources of fluorescence emission. The additional information our scanner collects has several advantages over current scanners: increased sensitivity, lower detection limits, more accurate background removal, discovery and removal of impurity contributions, and the ability to monitor multiple fluorophores simultaneously.



The figure shows the spectrum and concentration map of an impurity emission discovered with the hyperspectral scanner on a commercial DNA microarray. This impurity emission appears as signal with current scanners and gives rise to unreliable data from about 1/3 of the genes.

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DRUG DISCOVERY

Microfluidic Injector

Silicon drop ejector technology involves the delivery of very precisely metered, small volumes of liquid from a fluid



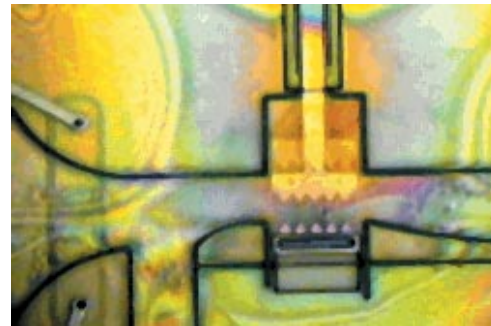
Two picoliter drops are ejected at 10 m/s and 1 KHz using an electrostatic drop ejector fabricated in Sandia's surface micromachining MEMS technology.

manifold (which mixes or splits off various fluid lines) to a target substrate. Sandia has taken this science a step further by developing an extremely small MEMS ejector that can produce patterns of drops of 2–10 microns in diameter. The device that positions the MEMS drop ejector can place small amounts of material very precisely onto a substrate with an accuracy of within 1 micron. Such precise patterns of minute amounts of material could be the basis of organic electrical circuits, which are key to the development of very small processors for micro applications. Significant interest in the drop ejector capability has been expressed by several groups, including the chem.-lab-on-a-chip group, to deposit adsorbing coatings onto specific locations in sensors (e.g., bio-chemical weapons detecting sensors).

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BioMEMS

Silicon microdevices are micromachines capable of interacting at the cellular level. Sandia is breaking new ground in developing these cell-altering devices. Prototype devices offer the possibility of considerable mechanical intervention at the cellular level because of the parallel



SANDIA's MICROTEETH bite in a channel that is 20 microns wide.

operation potential. Microneedles could potentially rapidly inject DNA, RNA, or proteins (including drug molecules) into living cells at precise points.

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Transdermal Drug Delivery

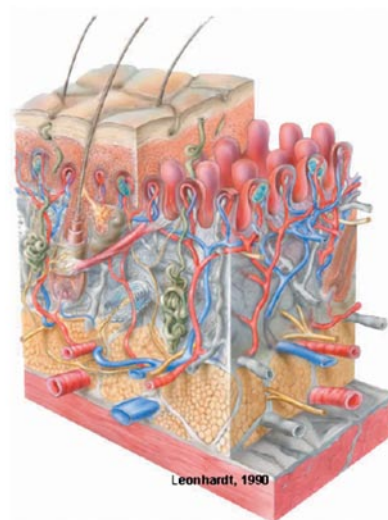
Sandia is developing mass-transfer models to better understand drug-delivery processes and chemical transport through the skin. Constitutive relations and simulation

methods developed for chemical transport in porous geologic media may be applicable to the heterogeneous features and layers of the skin, providing for improved modeling capabilities in an arena dominated by empirical studies. We hope to develop these models as a means to improve drug-delivery devices such as transdermal patches and to improve risk assessments of dermal exposures to toxic chemicals.

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Anatomy of human skin. (Leonhardt, 1990)



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FACILITIES

Biomaterials Laboratory

Sandia's Biomaterials Laboratory possesses equipment to perform molecular biology and biochemistry research, including genomic DNA, RNA, and plasmid isolation from many sources (e.g., bacteria, viruses, and eukaryotic tissues and cells). DNA sequences can be cloned into a variety of organisms, permitting the manipulation and modification of DNA and protein sequences, structure, and function. Cloned DNA sequences can be genetically engineered using reverse transcription, the polymerase chain reaction, and site-directed mutagenesis. Native and recombinant proteins also can be expressed, purified, characterized, and functionalized in this laboratory.

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Scanning Probe Laboratory

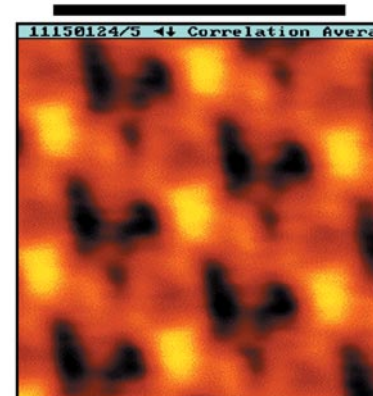
Sandia has extensive facilities for the structural characterization of biomaterials. We use a variety of scanning probe techniques such as atomic force microscopy (AFM). AFM allows us to obtain high-resolution images (shown here) of proteins that reside in membranes. The technique consists of scanning a very sharp tip (radius <10 nm) with very low forces over the biomaterials surface. The low forces prevent distortion of the soft proteins and membranes. By acquiring the images

in a fluid cell, we also allow the materials to assume their natural shape and to retain their functionality. We are also using AFM to study the forces between proteins and specific receptor sites that we synthesize and place in model membranes composed of lipid bilayers. This is accomplished by adhering the protein to the AFM tip and bringing it into the vicinity of the receptor site. Finally, we can use an AFM tip to unfold proteins that are immobilized on a surface or in a membrane. By unfolding the protein, we obtain valuable structural information that complements the high-resolution images.

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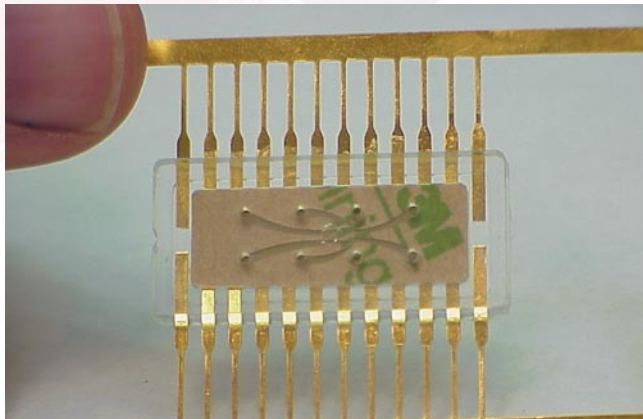


Using a very sharp probe (radius <10 nm) that scans over samples with very low forces, we obtain topographic images of biomaterials. In the image shown here, we reveal the crystalline array of membrane proteins.





PACKAGING



Electro Microfluidic Dual In-Line Package, bottom view.

Electro Microfluidic Packaging

Microfluidics is experiencing explosive growth in new product developments. Already there are many commercial applications for electro-microfluidic devices such as chemical sensors, biological sensors, and drop ejectors for both printing and chemical analysis. As the number of surface micromachined microfluidic devices increases, manufacturing efficiency and integration of microfluidics with electronics will become important. In order to realize applications for these devices, an efficient method for packaging microfluidic devices is needed. Sandia developed the Electro Microfluidic Dual In-Line Package (EMDIP) in response to this need. EMDIP is an inexpensive packaging method for silicon based electro-microfluidic devices. This package is durable, modular, easy to handle, and easy to install. In addition, this patent-pending technology allows for electrical connections on

the top side of the silicon devices and fluidic connections on the bottom side as part of the assembly process.

EMDIP has many other beneficial features:

- It is inexpensive and can be manufactured through an automated process.
- It can be adopted as a standard.
- It includes a great selection of package materials for better fluid compatibility. This technology has been built and tested. Currently, there is no standardized package for silicon based electro-microfluidic devices available in the marketplace.

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PROTEOMICS

Membrane Protein System and Structural Proteomics

In the area of Mass Spectrometry for Protein Structure Function Analysis, Sandia has established protein expression, protein chemistry, and separations and mass spectrometry methods for studying membrane protein structure/function relationships and protein/protein interactions. Membrane systems are of particular importance in cell signaling and pathogenesis, but they present severe challenges to existing structure/function approaches. We are able to express proteins, reconstitute them in proteoliposomes, expose them to a variety of commercial and proprietary cross linkers and reagents and a range of proteolysis protocols, and analyze resulting peptide signatures with LC/MS methods. The resulting data allow three-dimensional distances between residues to be derived in different functional states of the protein. These methods are used to study the mechanism of actions of toxins and key signaling proteins, with applications in the areas of bioterrorism countermeasures, pharmaceutical development, biotechnology, and biosensors.

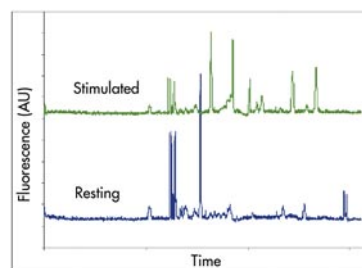
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Cellular Signaling

We are building on Sandia's strengths in micro-scale separation and detection techniques (μ Chem Lab™ Program) and applying these capabilities to the study of the molecular events associated with cell signaling. Such



Fingerprinting of Signaling Proteins: Capillary gel electrophoresis displays differences in the phosphoprotein populations from resting and stimulated immune cells.

molecular events include changes in the phosphorylation state of key proteins. We are employing a proteomics approach in which we selectively collect the phosphorylated proteins from cultured cells by affinity chromatographic techniques and subject them to a variety of sensitive and micro-scale separation techniques. We seek to use the separation patterns or “fingerprints” of phosphorylated proteins as a diagnostic tool for the state of the cells. A specific application for this work is the development of a rapid diagnostic tool for pathogen exposure, although the fingerprinting of phosphorylated protein levels has broader implications in the cell-signaling and biomedical communities.

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Microfabricated Integrated Protein Analysis Systems

Microfabricated systems are attracting significant attention in the areas of proteomics, national security, and health care because of their portability, speed of analysis, potential for multiplexing and high throughput, and ability to analyze

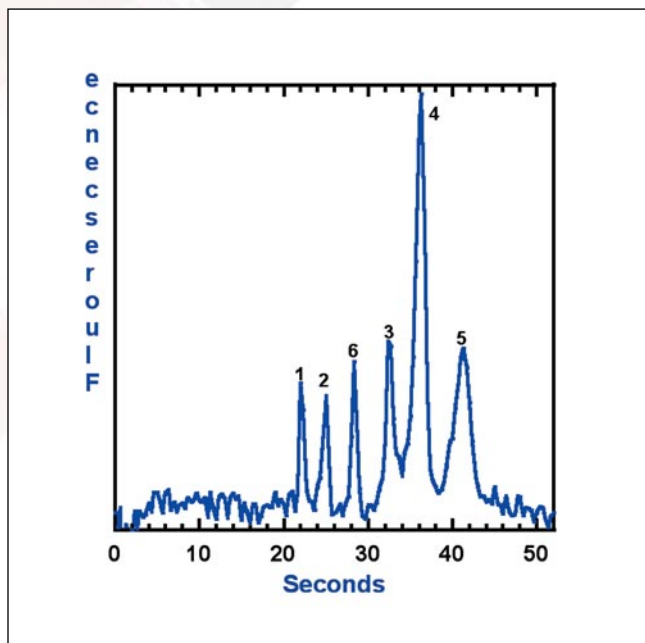


Fig 1: Separation of 6 bioactive peptides in a microchip in less than 45 seconds. (1) papain inhibitor, (2) proctolin, (3) Casein fragment 90-95, (4) Ile-angiotensin III, (5) angiotensin III, (6) Gly-Gly-Gly.

minute sample volumes. Sandia is developing integrated microchips for rapid separation and detection of minute levels of proteins and peptides with the goal of application in biotoxin detection and proteome analysis.

The integrated module will consist of three elements 1) a preconcentrator to concentrate proteins by 2–3 orders of magnitude, 2) a separation channel for fast and efficient separation; and 3) a microfluidic valve to selectively isolate proteins of interest for further analysis by another module. Modules can be combined to achieve multidimensional



A glass microchip for chromatography.

analysis. A number of on-chip approaches have been developed for separation of proteins and peptides including zone electrophoresis, gel electrophoresis, isoelectric focusing, and chromatography.

We have recently demonstrated rapid (6 peptides in less than 45 seconds) and high-efficiency (up to 600,000 plates/m) chromatographic separations in microchips using photopatterned porous polymer monoliths (Fig 1). The chips being used are fabricated in glass and fused silica and UV light-initiated polymerization is used for patterning of polymer in channels for optimal design of multi-functional

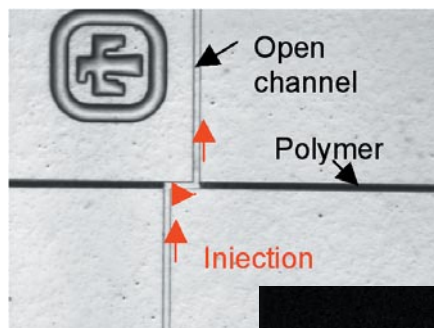
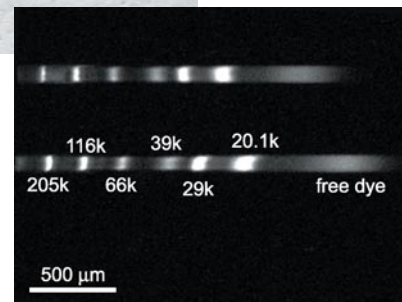


Figure 2

Fig 3: SDS-PAGE separation of six proteins molecular weight markers (20.1-205 kD) in a microchip in 25 seconds using photopolymerized polyacrylamide.



and multi-dimensional analysis systems (Fig 2). Efforts are also under way to miniaturize 2-D gel electrophoresis, the most common method of analyzing complex protein mixtures such as cellular protein content to a microchip format where proteins are first separated by isoelectric focusing (IEF) and then by capillary gel electrophoresis (CGE) in orthogonal parallel channels. We have developed chip-based IEF and CGE (Fig 3) using very short channels (<5 mm) that require less than 30 seconds to perform separation of proteins.

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Modeling of 3D Structures of Membrane-Associated Complexes

Sandia/CA has an active research program for modeling the 3D structures of membrane-associated complexes by adapting a technique called MS3D to specifically address integral membrane proteins. MS3D uses distance constraint information derived from intramolecular crosslinking, proteolysis, and mass spectrometry experiments to construct a 3D model of a protein structure. We plan to use MS3D to derive distance constraints within and between proteins in membrane-associated complexes. These distances are integrated with theoretical information, such as helical packing preferences and genomic information, to produce model structures that are consistent with experimental data. The IBIG Grand Challenge and other related LDRD programs at Sandia are currently validating MS3D on a well-characterized integral membrane protein of known structure, bacteriorhodopsin, and are mapping protein-protein interactions in the light-activated rhodopsin-transducin complex.

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Prion Detection and Discrimination by Multi-Spectral Ultraviolet Fluorescence

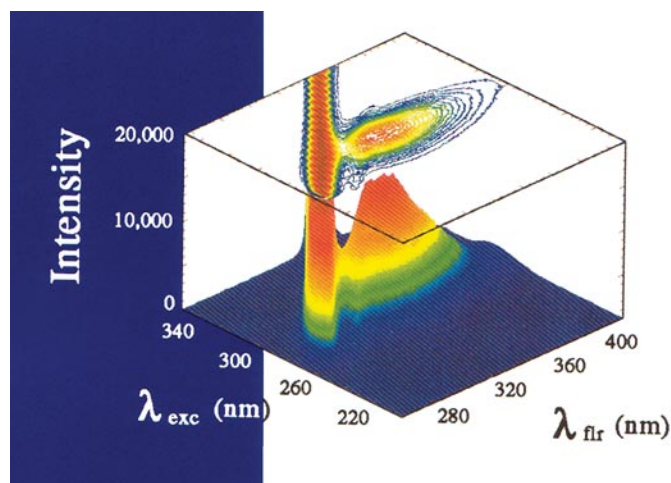
Sandia has been evaluating the use of multi-spectral UV fluorescence as a means of detecting and distinguishing between different forms of PrP^{Sc}, the protein associated with spongiform encephalopathies such as scrapie and BSE (Mad Cow disease). Spectroscopic measurements of fluorescence from PrP^{Sc} purified from 263K scrapie strain infected hamsters and ME7 scrapie strain infected mice were made. The spectral signatures from the protein and calculations of the fluorescence cross section indicate that

detection and discrimination are feasible by this method. This technique has the potential for rapid, specific, and direct detection and discrimination of the protein at extremely low concentrations.

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Multispectral ultraviolet fluorescence signature of E. coli suspended in phosphate buffered saline solution.