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Medical Applications and Biophysical Research Division
Office of Biological and Environmental Research
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Preface

The Medical Applications and Biophysical Research Division of the Office of Biological and Environmental Research has three major areas of responsibility: *Medical Applications* (primarily nuclear medicine), *Biophysical Research* (structural molecular biology, genome instrumentation and analytical chemistry), and the *Environmental Management Science Program*, which is funded by the Department's Office of Environmental Management. A brief statement of the scope of these programs follows this preface.

This summary book provides information on research supported in these program areas during Fiscal Years 1996 and 1997. The research summaries and other information in the main section of this publication are the most current ones available to the division, as provided by the principal investigator.

These two years saw many changes, beginning with the renaming of the Office from the Office of Health and Environmental Research. The new name is consistent with the long-standing budgetary classification of the Office's programs as the Biological and Environmental Research Program, but does not signify a change in the scope or priorities of the Office. Several new user facilities and experimental stations for structural molecular biology were completed at the Department's four synchrotron light sources, greatly increasing the ability of these facilities to meet the growing demand for access to them. A program in computational structural biology, emphasizing inverse protein folding, was initated. The redirection of the analytical chemistry instrumentation (measurement science) program was completed and planning began for the redirection and recompetition of the genome instrumentation research program.

In the medical applications component the early clinical trials of boron neutron capture therapy were expanded. Significant advances in the medical imaging research program were reported, for applications ranging widely from addiction to illicit drugs to cancer therapy to cardiology. The radioisotope development component was discontinued as a separate subprogram due to budgetary constraints although proposals for specific projects in this area continue to be supported. The division is engaged in planning the redirection of the nuclear medicine program to reflect advances in genomics, structural biology and other fields.

The Environmental Management Science Program (EMSP) was initiated at the direction of Congress in 1996 to support basic research directed at overcoming major obstacles to the cleanup of the nuclear weapons complex. The budget for the EMSP is in the Office of Science and Risk Policy in the Office of Environmental Management (EM), but the program is jointly managed by that office and the Office of Energy Research (ER). The Division is responsible for the largest single element of the EMSP, analytical chemistry and instrumentation, and, from the start of fiscal year 1997 has had the lead responsibility in ER for management of the overall program.

We hope that this publication will provide guidance to scientists considering submitting research proposals for financial support from this division. The research programs and their scopes are continually evaluated and adjusted taking into account the advances in new technology and basic research. Thus, scientists are encouraged to contact the appropriate staff of this division to determine the most current program priority and scope before submitting a proposal. Initiatives are generally announced through program notices in the *Federal Register*. These notices and other useful information are available on the World Wide Web at:

http://www.er.doe.gov/production/ober/mab/MABRD_top.html

Comments on this publication and suggestions to make it more effective and useful are welcome. We plan to publish it annually and will also post updates of this information on our home page on a regular basis.

This volume is largely the result of the efforts of Sharon Betson and Matesh Varma. Their substantial contributions are acknowledged with gratitude.

Roland F. Hirsch, Acting Director
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Program Scope

The Medical Applications and Biophysical Research Division supports and manages research in several distinct areas of science and technology. The projects described in this book are grouped by the main budgetary areas: General Life Sciences, Medical Applications and Measurement Science, Environmental Management Science Program, and the Small Business Innovation Research Program. The research funded by this division complements that of the other two divisions in the Office of Biological and Environmental Research (OBER): Health Effects and Life Sciences Research, and Environmental Sciences. Most of the OBER programs are planned and administered jointly by the staff of two or all three of the divisions.

The research summaries in this publication cover projects for which the staff of this division has the primary responsibility. To gain a comprehensive understanding of major programs supported by OBER, the reader is encouraged to consult similar publications of the other two divisions and the OBER site on the World Wide Web.

Structural Biology

Facilities Development: This component of the structural biology program supports development of new experimental stations and associated laboratory facilities at the synchrotron light sources, neutron beam facilities, and high field nuclear magnetic resonance and mass spectrometry instrumentation built and operated by the Department of Energy.

Facilities Operation: Another component of the structural biology program of the Office of Biological and Environmental Research is the support of user facilities for studies in structural biology. These user facilities are available to academic, industrial, and government laboratory researchers and are dedicated to crystallographic and spectroscopic studies of the structure of biological macromolecules, as well as small-angle scattering and x-ray microscopy in biological systems. User stations are also supported at neutron beam facilities.

Databases: The objective of this subprogram is to develop and maintain the database of three-dimensional structures of biological macromolecules, such as proteins and nucleic acids, done jointly with other government agencies.

Instrumentation Research: The objective of this research component of the structural biology program is the improvement of instrumentation available for studies in structural biology. New and advanced detectors, technologies and methodologies are developed and refined to increase the effectiveness of biological research.

Computational Structural Biology: This subprogram supports research in the development of sequence alignment methods, threading techniques, scoring functions, homology modeling, and algorithms for improving capability of protein structure prediction from amino acid sequence (inverse protein folding). To do this, research relating to the development of new computational strategies and use of existing software for graphics and visualization for exploiting structure databases for fold recognition problems is also supported.

Biological Research: The structural biology program supports research for determining the three-dimensional structures of important proteins, nucleic acids, and other biological materials using the full suite of structural biology techniques. This information provides the basis for understanding the relationship between structure and biological function of molecules. This subprogram is administered by the Health Effects and Life Sciences Division.

Genome Instrumentation Research

The Division manages research into high-speed sequencing technologies and into automation of all stages of chromosome mapping and sequencing for greater efficiency and cost effectiveness. Research areas include sequencing using single molecule detection, highly multiplexed capillary gel electrophoresis systems, electrophoresis in liquid media without gels, mass spectrometric analysis of sequencing fragments, and application of robotics to the entire sequencing process. This program will be redirected during 1998.

Measurement Science

The measurement science program funds research into instrumentation for analytical chemistry. It supports the OBER missions in the environmental and life sciences with new measurement technology. Advanced techniques for monitoring air, solution and solid chemical composition employing spectroscopic approaches are included, with particular emphasis on fundamental research on sensors for miniaturized, automated and remote analysis of contamination and waste for chemical and radiation hazards. This program supports four projects in the Environmental Management/Energy Research Pilot Collaborative Research Program. Similarly, spectroscopic interrogation of cells and the molecular consitiuents of living systems are supported.

Medical Applications

The Medical Applications program fosters multidisciplinary research to develop beneficial applications of nuclear and other energy-related technologies for medical diagnosis and treatment of human diseases. There are four major components of this program: 1) Radiopharmaceuticals, 2) Molecular Nuclear Medicine, 3) Instrumentation and, 4) Boron Neutron Capture Therapy. Another component of this program, Radioisotope Development, has been phased out; however, projects funded in FY 96–97 are included. The technology developed under the medical applications program provides for the non-invasive detection and localization of biochemical dysfunction associated with diseases, the quantitative measurement of organ function, and the selective treatment of cancer with internal radiation therapy. It is anticipated that this program will be redirected during 1999.

Molecular Nuclear Medicine: This component of the program seeks new approaches to imaging molecular biology *in vivo* for detecting pathological states in relationship to certain associated biochemical defects and for providing the ultimate specific targets for *in vivo* diagnosis and treatment of the disease. This involves research on *in vivo* biochemical reactions. With the applications of the advances made in the human genome program; structural biology and genetic engineering, innovative radiolabeled molecular, metabolic and genetic markers are being developed as receptor binding radiotracers and as substrates for transporters and enzymes. Research topics include studies on receptor populations, receptor signaling in cell communication, and gene expression in normal and disease states. Particular emphasis is placed on studies of neuroreceptors; neurotransmitters and enzymes regulating brain function, tumor receptors and tumor receptor targeting, and myocardial function.

Radiopharmaceuticals: This component of the program supports radiochemistry, organic and organometallic synthesis, molecular modeling, genetic engineering and computational chemistry for preparing labeled precursors of desired organic structure and biological properties. Specifically, it includes radiolabeling of precursor molecules with radioisotopes of appropriate half-lives and energy properties suitable for clinical investigations and potential applications. A vast array of molecules including medicinal agents, proteins, nucleic acids, and steroids are being prepared, radiolabeled and screened in laboratory animals for their efficacy and specificity. Those radiopharmaceuticals that reveal optimal properties are chosen for preclinical evaluation.

Instrumentation: The Instrumentation program supports the development of new technologies for

radioiostope imaging: 1) new three-dimensional imaging techniques with improved resolution, 2) new methods for merging different imaging capabilities (Positron Emission Tomography, Single Photon Emission Computed Tomography, Magnetic Resonance Imaging, and Magnetoencephalography) for superposition of structure and various functional aspects, 3) the application of new techniques for solving complex diagnostic problems such as evaluating the neurochemical status of the brain in patients with neurodegenerative diseases and substance abuse, and for improving the management of disease, such as in cancer treatment planning and monitoring the course of therapy, and 4) the application of molecular nuclear medicine techniques for imaging of gene expression and the course of gene therapy. The program also supports a group of centers for research into the medical applications of lasers.

Boron Neutron Capture Therapy: This program supports research for developing BNCT for clinical use. This includes research and development in: 1) neutron sources from reactors and accelerators; 2) boron-carrying compounds such as amino acids, porphyrins, nucleosides, amines, lipoproteins, and liposomes; 3) preclinical studies on pharmacokinetics and biodistribution of compounds, radiation dosimetry and biology in cell systems, and small and large animal models; 4) Phase I/II clinical trials to assess boron-compound biodistribution for treatment planning, and to test safety and effectiveness of boron compounds, neutrons, and their interaction in compliance with regulatory obligations.

Clinical Feasibility: The objective of this program is to investigate radioisotopes, radiopharmaceuticals, and monoclonal antibody fragments through animal screening for clinical potential and feasibility in humans.

Environmental Management Science Program

The Division manages the analytical chemistry and instrumentation component of the Environmental Management Science Program (EMSP). This component supports basic research for characterizing and monitoring contaminants at the nuclear weapons complex cleanup sites. Some of the research topics include biomarkers, metal-ion sensing devices, micro sensors, optical/electrochemical sensors, atomic and mass spectrometry, Nuclear Magnetic Resonance spectrometry, mass spectrometry and Laser Induced Breakdown Spectroscopy. This research will lead to development of advanced characterization and monitoring methods for use in waste management and environmental restoration at DOE sites.

Small Business Innovation Research Program(SBIR)

Objectives of this program include increasing private sector commercialization of technology developed through DOE-supported research and development, stimulating technological innovation in the private sector, strengthening the role of small business in meeting Federal research and development needs, and improving the return on investment from Federally funded research for economic and social benefits to the Nation. In this program the division also manages innovative research involving nuclear medicine technologies to facilitate and advance the current state of diagnosis and treatment of human disorders.

General Life Sciences:

Genome Instrumentation Research

1. Quantitation in Electrophoresis

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We have developed novel separation, detection, and imaging techniques for real-time monitoring in capillary electrophoresis. These techniques will be used to increase substantially the speed, throughput, reliability, and sensitivity in DNA sequencing applications in highly multiplexed capillaries. We estimate that it should be possible eventually to achieve a raw sequencing rate of 40 million bases per day in one instrument based on the standard Sanger protocol. Next, we plan to tackle other aspects of the mapping-sequencing process. Three distinct goals can be identified: 1) Miniaturization and integration of the fluorescence-labeled Sanger reaction to fit the multiple-capillary electrophoresis system. 2) Development of a high-speed, high-throughput analysis system for intermediate-sized DNA based on a simplified version of the sequencing system already demonstrated in our laboratory. 3) To collaborate actively with the DOE Joint Genome Institute to validate the high-speed, high-throughput sequencing technology developed by us. The basic expertise needed to achieve each of the three goals already exists in our laboratory, so it is a natural progression for us to tackle the next set of bottlenecks for speeding up DNA sequencing, viz., sample preparation and handling prior to electrophoresis of the Sanger fragments.

2. Advanced Detectors for Mass Spectrometry

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Major advances in the Human Genome Project would arise if DNA molecules could be analyzed mass spectrometrically. The present study assesses systematically the factors which affect the separation and detection of large molecules, evaluates potential alternative detection methods and applies newly developed mass spectrometer systems to DNA sizing and sequencing. This work is directed towards the solution of practical problems relevant to increasing the speed and efficiency of DNA sequencing for the Human Genome Project using mass spectrometry. We focus this study primarily on the problems associated with ion detectors used to detect large molecules in time-of-flight systems. Electrospray (ES) and matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometry (MS) are our current analytical systems of choice but each suffers from specific deficiencies that limit their usefulness for analyzing DNA. By developing improved ion detection methods, we plan to provide improved mass spectrometry capabilities that help to speed up the mapping and sequencing efforts already under way in LBNL's Human Genome Center. The successful implementation of mass spectrometry for large biological molecules requires that large molecules are ionized and vaporized intact, dispersed according to mass/charge ratio (m/z) and detected in a manner which provides mass data. Specific aims of the project include: 1) Improve ion detection capability in MALDI-TOF-MS by developing detectors that do not rely on secondary ion formation. 2) Develop a charge detector for measuring

the charge on electrospray ions of DNA. 3) Apply these new detection schemes to the analysis of DNA in the production environment of LBNL's Human Genome Center to aid mapping and sequencing efforts.

3. DNA Base Sequencing by Single Molecule Detection

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We are developing a technique to determine the sequence of bases in large fragments of DNA. Our goal is a sequencing rate of 100 to 1000 bases/second on DNA strands approaching 40 kb in length. The ideas presented represent the combined effort of a multidisciplinary team composed of physicists, physical chemists, cellular and molecular biologists, and organic chemists. A large fragment of DNA, approximately 40 kb in length, will be labeled with base identifying fluorescent tags and suspended in the flow stream of a flow cytometer capable of single molecule detection. The tagged bases will be cleaved sequentially from the single fragment and identified by laser-induced fluorescence as the liberated tag/base passes through the laser beam. We have made considerable progress and anticipate a demonstration of our sequencing approach on a small fragment of DNA using one or two color tags this year. As a "spin-off" of our sensitive fluorescence detection capability, we have demonstrated flow cytometric sizing of DNA fragments in a ~0.1 pg sample of a restriction digest of lambda DNA in a few minutes with sizing accuracy better than 98%.

3a. DNA Sequencing Using a Hybrid Microchip Capillary Electrophoretic Instrument

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The goal of this project is the development of high throughput DNA sequencing by capillary electrophoresis using replaceable polymer solutions. Current advances have included the ability to sequence over 1100 bases per run in less than one hour with high accuracy. Highly purified linear polyacrylamide with a molecular mass of 9M Daltons, as well as column operation at 60°C is used for this purpose. Secondly, sample clean-up protocols have yielded high reproducibility and rugged operation. Thirdly, new software has been developed yielding over 100 more bases per run at an equivalent accuracy of base calling. Currently, a rugged, 96-capillary array is being implemented using no moving parts. The sample clean-up procedure is incorporated into a robotic workstation. The goal is to achieve 1000 bases/column run time of one hour or less in routine fashion.

4. Multilabel SERS Gene Probes for DNA Sequencing

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The use of stable clone resources containing large human DNA insets has opened new possibilities to contig building for the Human Genome Project. The objective is to apply the surface-enhanced Raman scattering (SERS) multi-label technique for use in DNA mapping and bacterial artificial chromosomes (BAC) colony hybridization. The technology is based on a system that will integrate several concepts including:

1) multi-label SERS detection, 2) spectral multiplex mapping, and 3) BAC colony hybridization. Emphasis is on detection techniques that minimize the time, expense and variability of preparing samples by combining the BAC mapping approach with SERS "label multiplex" detection. Multiple DNA samples can be simultaneously prepared by automated devices. With this device, multiple samples can be separated and directly analyzed using multiple SERS labels simultaneously. This feasibility study is aimed at demonstrating the detection of two CDNA probes simultaneously in a single BAC hybridization. An interdisciplinary approach to the proposed studies will be pursued throughout this research project, involving a collaboration between Oak Ridge National Laboratory and the California Institute of Technology.

5. Laser Desorption Mass Spectrometry for Fast Human Genome Sequencing

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A new approach to DNA sequencing and analysis by a novel laser desorption mass spectrometer is under development. This approach can eliminate the need of gel electrophoresis, radioactive tagging, and chromophore tagging. It could speed up the sequence to a few minutes instead of hours or days. Different approaches to improve mass resolution and detection sensitivity have been pursued. Laser desorption mass spectrometry (LDMS) has also been used for different sequencing approaches. Special features include the capability of using matrix-assisted laser desorption/ionization (MALDI) for sequencing DNA with DNA ladders and direct mass spectrometry DNA sequencing without DNA ladders. LDMS can also be used for environemntal and medical applications in addition to human genome sequencing.

The major achievements in this program are listed in the follows. 1) Successful demonstration of LDMS to detect large DNA fragments with the size up to 500 base pairs. 2) Successful measurements of DNAs produced from polymerase chain reaction (PCR) and enzyme digestions. 3) Sequencing DNA fragment with size up to 100 nucleotides for ss-DNA and 200 bp for ds-DNA. Since the sequencing speed is much faster by LDMS compared to gel method, LDMS can become a major sequencing method if better mass resolution can be achieved for large DNA fragments. 4) Detection of base deletion and point mutation of cystic fibrosis disease with clinical samples by LDMS. It indicated that LDMS can emerge as an imporant new biotechnology for clinic diagnosis. 5) Develop matrix-assisted laser desorption/ionization/-fragmentation (MALDIF) for direct DNA sequencing without the need of DNA ladders. MALDIF is very valuable for sequencing primers and DNA probes.

6. Ultrasensitive Fluorescence Detection of DNA

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Our goal is to develop sensitive, rapid and highly parallel methods for DNA sequencing and analysis. To achieve rapid high throughput electrophoretic analysis and sequencing of nucleic acid samples we first developed the method of capillary array electrophoresis (CAE). Current effort in this area is directed toward the development of a 1000 capillary array scanner and sequencing system. We have developed energy transfer (ET) fluorescent primers that provide up to a 20-fold increase in fluorescence intensity compared to conventional primers in DNA sequencing and fragment sizing applications. ET primers and CAE are currently being used in pilot sequencing of portions of the genome of the cyanobacterium Anabaena and in a variety of health care diagnostic fragment sizing applications. Capillary array electrophoresis chips have been developed that can perform very high speed analyses of up to 96 DNA sequencing or fragment sizing samples on a single micro-plate. We have also developed integrated electrochemical detectors on our CE chips that permit high sensitivity detection of neurotransmitters and nucleic acids. Finally, techniques have been developed for performing single molecule fluorescence burst detection of ds-DNA fragments separated on CE chips. Single molecule methods should be useful in health care diagnostics and for pathogen detection.

7. Manipulation of DNA in Non-uniform Oscillating Electric Fields

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The project develops micro-fabricated structures that trap DNA by dipole forces. The traps combine static and periodic fields to allow precise control over the position of DNA molecules. The DNA-dipole traps are explored in a variety of applications: 1) One-step isolation of DNA from small aliquots of cell lysates; 2) The construction of arrays of electronic test tubes in which small but concentrated amounts of DNA are subjected to chemical reactions; 3) Size separation of DNA by combining DNA traps with fluid flow and/or DC currents; 4) Alignment of a cohort of DNA molecules for loading into an electrophoresis channel or other micro-fabricated analysis devices; 5) The construction of a "DNA flow cytometer" in which the fluorescence of individual DNA molecules is determined as they are moved through a measurement point by charge and dipole forces. The induced-dipole traps will provide new tools for manipulating DNA. Methods for trapping DNA will be helpful in automating and miniaturizing sample handling technologies for the genome project. DNA-dipole traps may lead to a new generation of analytical DNA instruments that combine micro-electronics and micro-sample handling.

7a. High Speed DNA Sequence Analysis by Matrix-Assisted Laser Desorption Mass Spectrometry

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The primary focus of this project is the continued development of the technique of Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry as a method for the rapid and accurate separation and detection of DNA ladders produced in the enzymatic sequencing reactions. Overlaying the four individual base-specific sequencing ladders yields the DNA sequence. Three major advantages of this approach over conventional electrophoretic separations are a) speed—milliseconds to seconds per sample, as opposed to hours for electrophoresis; b) elimination of gels from the sequencing process; and c) size is determined on the basis of absolute masses, in contrast to electrophoretic mobilities which are sensitive to modulation by secondary structure and solution conditions. This absolute nature of the results will greatly increase the robustness of DNA sequencing, eliminating artifacts such as gel "compressions" from the sequencing process.

8. The Development of Electrospray Ionization-Mass Spectrometry for DNA Sequencing and Characterization

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This project is aimed at the development of high speed DNA characterization methods based upon electrospray ionization (ESI) and advanced mass spectrometric approaches. Major emphasis is on sequencing methods involving the analysis of mixtures of DNA fragments (e.g., such as formed using cycle sequencing/Sanger methods). New approaches to sample clean-up and high resolution, high sensitivity and expanded dynamic range analyses using ESI-Fourier transform ion cyclotron resonance (FTICR) mass spectrometry have been demonstrated, and are being implemented and refined to provide a significant extension of read length. We have previously shown that large multiply charged individual ions of either single- or double-stranded DNA segments can be produced by ESI and their mass measured with very high accuracy by FTICR, and further efforts aim at developing practical mass measurement capabilities for much larger DNA segments. We are also exploiting non-destructive nature of FTICR for recovery of mass-selected DNA

segments, following high resolution FTICR analysis and separation (i.e., high resolution sorting), for subsequent cloning or PCR. A major emphasis of future efforts is to realize the ultra-high sensitivity feasible with FTICR so as to enable measurements of DNA samples where amplification methods are either not feasible or desired (i.e., for modified or unnatural DNA's). Successful development of these approaches will greatly reduce the cost and enhance the speed and accuracy of DNA sequencingand have broad application in structural biology, biomedical research, and other applications in the post-genomic era, as well as provide unique capabilities for ultra-sensitive DNA characterization.

General Life Sciences:

Structural Biology

Facility Development:

9. Biological and Inorganic X-Ray Spectroscopy

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Over the past two decades, the brightness of synchrotron radiation sources has increased by about eight orders of magnitude. Our group is developing new x-ray spectroscopic instruments and methods to exploit these amazing sources. We use these tools to learn more about the molecular and electronic structure of biological and inorganic materials. Soft x-ray spectroscopy allows one to observe the 2p->3d absorption edges of important first transition metals such as Mn, Fe, Ni and Cu. The fine structure in x-ray absorption and magnetic circular dichroism (XMCD) spectra contains information about metal oxidation states, spin states, and the degree of electron delocalization. We are developing instrumentation to make this spectroscopy easier on dilute metals in biological samples. We use this instrumentation in collaboration with biochemists around the country to study the electronic structure of Mn in photosynthesis, Fe and Ni in hydrogen evolution, and Cu in oxygen reduction. High resolution x-ray fluorescence spectroscopy is another valuable probe of electronic structure. We have developed a crystal spectrometer array which enables Kb (3p->1s) emission spectroscopy on dilute metalloproteins. Using this device, we are mapping the combinations of Mn oxidation states involved photosynthetic oxygen evolution, as well as the status of Fe and Ni throughout the hydrogenase catalytic cycle. Our spectrometer also permits 'site-selective' EXAFS spectroscopy, and we are developing this technique for biological, inorganic, and environmental science communities. An undulator beamline for this program is under construction at the Advanced Light Source and should commence operations in 1998.

10. Neutron Studies

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Neutron scattering is an ideal technique for the analysis of biological structures since it permits the elucidation of structural details not attainable by other techniques. Neutron scattering is often preferable to X-ray scattering because the sample's constituents present a higher contrast to neutrons, thereby localizing the hydrogen atoms. Replacement of H_2O with D_2O changes the contrast and reveals the localization of hydrogen exchange regions, thus facilitating the identification of structural landmarks in chemical terms. Neutron diffraction techniques are being used to clarify the structure and function of proteins, protein cornplexes and membranes. Studies are now being made to use spallation neutrons for structural biology studies and to develop plans to build instruments to use such pulsed neutron sources.

11. Structural Molecular Biology Beam Line 9 Project

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A beamline dedicated for structural molecular biology research, beam line 9, has been designed and built at SSRL in a project that started in 1993. It is based on a 16-pole, 2-Tesla permanent magnet wiggler insertion device, which provides a wide fan of ultra-high flux radiation. This fan is divided to provide beam for three experimental stations: BL9-1 for high-intensity, mostly single wavelength protein crystallography studies (3.0 mRad, side station), BL9-2, for Laue diffraction, MAD phasing and fast monochromatic protein crystallography applications, (2.0 mRad, end station) and BL9-3 for biological x-ray absorption spectroscopy experiments (2.5 mRad, side station). For BL9-1, the first optical element is a vertically focusing 1m long Rh-coated water-cooled silicon mirror followed by a horizontally focusing side-cooled silicon crystal monochromator. An experimental table can be swung in the horizontal plane to enable a limited range of x-ray wavelengths. The beam line is optimized for use at the Se K-absorption edge and has been running in user mode since Spring, 1997. For BL9-2, the first optical element is a vertically focusing 1m long Rh-coated water-cooled silicon mirror followed by a double crystal monochromator system with two pairs of crystals that allows switching from one to the other without entering the monochromator enclosure. A refocusing mirror follows the monochromator producing a ~1:1 vertical, and ~2:1 demagnified horizontal image of the source at the experiment. The station can also be used in white light and wide bandpass (only mirror(s) present) modes. The BL9-3 optics are very similar to those of BL9-2, but are limited to monochromatic beam use. BL9-3 has been optimized for focused capabilities in the ~4.5-23 keV range.Dedicated detectors, cryostats, optical rails, crystal mounting including flash-cooling and Xe derivatization equipment, combined x-ray absorption and crystallography equipment is/will be available. Dedicated data acquisition, data analysis and graphics display computers, software, and a high-speed network are also available. First beam was obtained in BL9-3 in June 1997, with general users to be accommodated in 1998, and BL9-2 saw first beam in late 1997.

12. X-ray Microimaging by Diffractive Techniques

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To develop techniques to examine thick (up to 10 micron) frozen hydrated biological specimens at 50 nm resolution or better. Develop techniques for labeling and for chemical identification via micro-XANES spectroscopy. A cryo version of the Scanning Transmission X-ray Microscope is being commissioned. It is designed to minimize radiation damage to the specimen. The XIA soft X-ray undulator beamline is being upgraded to provide higher spectral resolution and separate tuneability of the two branches. A cryogenic version of the holography apparatus is being commissioned as well. The user program based on the Scanning Transmission X-ray Microscope (STXM) provides opportunities for collaborators and other groups to exploit the techniques available and to develop them further.

13. Structural Biology Facilities Development

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One of the key questions in modern biology is that of coupling molecular structure and biological function, i.e., how is structure related to function. To be able to address such a question means that the overall scientific community needs to have access to a wide range of user facilities that can provide state-of-the-art methods for the determination of biological structures. High field liquid state NMR and mass

spectroscopy are examples of such methods. These methods complement more standard methods such as X-ray crystallography. Within the Environmental Molecular Sciences Laboratory we have world class examples of both capabilities. We have the highest field (11.5 Tesla) Fourier Transform Ion Cyclotron Resonance (FTICR) mass spectrometer in the world and the highest field high resolution NMR spectrometer in the DOE system. In the next fiscal year this 750 MHZ NMR spectrometer will be joined by the highest field NMR spectrometer in the world, a 900 MHZ spectrometer (with a 21.1 Tesla Oxford magnet). The FTICR provides incredible sensitivity with high mass resolution. The current demonstrated mass resolution of the FTICR is approximately 1,000,000 to 1. The 750 MHZ spectrometer is equipped with all of the capabilities to provide liquid state NMR structures. Currently, the spectrometer is a 4 channel Varian Unity plus. This same capability will be associated with the 900 MHZ system. This complement of spectrometers will provide the scientific user base with unparalleled capabilities to address biological structure questions in the 21 century.

Facility Operation:

14. The Structural Biology Center

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The Structural Biology Center (SBC) at Argonne National Laboratory has been established to build, operate, and develop synchrotron beamlines for protein crystallography. The bending magnet and undulator beamlines on Sector 19 of the Advanced Photon Source (APS) have measured flux densities of 2.8×10^{13} x-rays/s/mm^2 AND 3.6×10^{15} x-rays/s/mm^2, respectively, by far the nation's most powerful x-ray sources for structural biology. These beamlines deliver x rays onto extremely small (50 micron) crystal samples, with very low (0.1 degree) angular divergence, permitting crystallographers to study structures of complex molecular systems using very small samples. Diffraction from these sample crystals is recorded on large, fast, efficient area detectors and processed on high-performance, integrated computing systems with optimized software designed specifically for the SBC. Every part of the SBC facility—x-ray optics, detectors, computer systems, software, and laboratory/office space—has been specifically designed to make the best possible use of its powerful x-ray sources.

15. Membrane Structure by Neutron Diffraction

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Neutron diffraction is a very useful technique for structural studies of partially ordered systems, such as stacked biological membranes or lipid-water systems with reconstituted proteins, ion-channels, drugs or other molecules. Neutron diffraction helps in determining the phases of Bragg reflections, reveals the distribution of water within the bilayer, and locates any deuterium label with a precision that can approach atomic resolution. Studies of this type can be used to 1) understand the equilibrium position and binding with the receptor of a drug molecule, 2) determine the membrane conformation of physiologically significant peptides such as those that mediate fusion or have antibiotic properties, 3) obtain structural information about the transmembrane segments of proteins that form ion channels, and 4) understand the fundamental processes of membrane transport and assembly. High reflectivity thin-film multilayer monochromators are being developed to enhance the neutron flux on a sample by matching the characteristics of the beam with the experiment, and by focusing neutrons. Installing such devices on the neutron spectrometers will increase the signal-to-background ratio and allow a greater number of higher resolution studies to be done on the spectrometers.

16. Protein Crystallography by Synchrotron Radiation

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X-radiation produced by the National Synchrotron Light Source at BNL is a powerful tool for investigating biomolecular structures. Synchrotron radiation extends the use of x-ray diffraction methods by providing a much higher intensity beam, with adjustable wavelengths, than is available with conventional sources. The brighter beam allows collection of data at higher speeds and with higher resolution. An area diffractometer for crystallography, which immediately digitizes diffraction patterns, has been in use at beam line X12-C of the National Synchrotron Light Source during the last several years. The tunable synchrotron beam allows measurements with this instrument of data for multi-wavelength anomalous diffraction phasing. Because much work is required to make the instrument convenient and efficient to use, we are involved in a program to develop methods and software. We have a substantial program of collaboration and service to outside users. Significant effort is put into the training of visitors in synchrotron science and in the principles and practice of operation of the facility. Our staff undertake crystallographic studies and collaborate in preparation of crystals for new structural investigations.

17. Synchrotron Ultraviolet User Facility

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Ultraviolet radiation from the National Synchrotron Light Source is used to probe the structure and dynamics of biological molecules using circular dichroism, magnetic circular dichroism, and time-resolved fluorescence spectroscopy. Circular dichroism experiments, which provide information on the conformation of proteins, nucleic acids, and polysaccharides, focus on wavelengths from 120 to 200 nm, a region of the spectrum where conventional light sources are less intense than the synchrotron. Magnetic circular dichroism gives information about the electronic structure of biologically important molecules in the same spectral domain. Fluorescence experiments use the broad spectrum, time structure and brightness of the synchrotron source to elucidate the structure, dynamics, and excited state reactions of nucleic acids, proteins and their components. This project supports the operation, maintenance, and continuing development of the circular dichroism, magnetic circular dichroism, and fluorescence experiments on the vacuum ultraviolet ring of the National Synchrotron Light Source at BNL. These facilities are used by scientists from BNL, universities, and other research institutions.

18. Neutron Small-angle Spectrometer for the Study of Biological Structures

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This program in neutron scattering supports the efficient operation of a small-angle spectrometer and the continuing development of scattering instrumentation and methodology to benefit a long-standing multi-disciplinary user program. The fully operational spectrometer on beam line H9B at the BNL High Flux Beam Reactor exploits the intense long-wavelength spectrum from the cold neutron

facility to determine biomolecular structures as well as for applications in the materials and polymer sciences. To promote the user program, considerable effort is put into support—from experimental planning to data analysis—and into training investigators from universities, and industrial research institutions, as well as those from BNL. Increasingly, structural investigations by neutron scattering will be coordinated with complementary studies at other BNL facilities, primarily the Biology Scanning Transmission Electron Microscope, and the small-angle x-ray scattering beam line at the National Synchrotron Light Source. In addition, exploratory work on neutron fiber diffraction will be pursued, with the goal of providing a new and unique capability to extend neutron scattering analysis to oriented fibrous systems, such as muscle, filamentous bacteriophages, and chromatin. Instrument development will continue by planning and implementing upgrades towards expanding the first-order-resolution of the spectrometer beyond 1,000 Å.

19. Study of Biological Structures by Neutron Diffraction

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The macromolecular crystallography beam line at the High Flux Beam Reactor was rebuilt to incorporate three multi-wire area detectors. This new system was thoroughly tested and currently is servicing the macromolecular neutron crystallography user program. It is expected that data collection times will decrease by nearly a factor of three, thereby increasing throughput on the beam line. A diffraction study on fully deuterated myoglobin was completed, demonstrating that perdeuteration improves signal-to-noise by nearly a factor of four. Laue diffraction patterns, using white radiation on beam line HIB and neutron imaging plates, were collected on a myoglobin crystal. The data were reduced and found to be of a quality approaching monochromatic data. Work continues developing white radiation techniques to increase the throughput of experiments.

20. STEM operation and development

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The Brookhaven Scanning Transmission Electron Microscope (STEM) Facility is recognized as a leader in advancing the state-of-the-art in biological electron microscopy. To maintain this position, our instrumentation must be upgraded constantly. It is most desirable to carry out these improvements without compromising the high productivity of our outside user program. A new STEM demonstrated preliminary operation last year. It employs electron energy loss spectroscopy for chemical analysis of biological samples. Previous studies with this technique have been limited by radiation damage, and have yielded little of the information potentially available. Alignment and averaging of low dose images should allow us to attain the signal-to-noise ratio of high dose conditions, but without damage. This technique complements our present specific labeling schemes and should permit a wide variety of studies, such as determining the sites of phosphorylation on proteins, and localizing RNA in relation to the proteins in ribosomes. Mapping of other elements, such as boron and fluorine, should be possible, also.

21. The Macromolecular Crystallography Facility at the Advanced Light Source

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X-ray crystallography is the primary method used to determine the three-dimensional structure of biological molecules. In recent years the advantages of synchrotron radiation as a source for x rays has been demonstrated in the structure determination of several biologically-important molecules, due to its brightness, high flux, and tunability. These factors allow for data collection from microcrystals, crystals which diffract weakly or have large unit cells, and to

use of multiwavelength phasing methods. This structural information has then been use to explain the biological processes which these biomolecules mediate and to understand associated disease processes to a greater extent. The Macromolecular Crystallography Facility (MCF) at the Advanced Light Source (ALS) is a national user facility for biological crystallography offering bright x rays in the wavelength range of 0.9–4.0 Angstroms. A high field, multipole wiggler in straight section 5.0 of the ALS is the source for up to three beamlines. The first of these beamlines is optimized for multiwavelength anomalous diffraction (MAD) experiments featuring a vertically collimating mirror, double crystal monochromator, and a toroidal mirror into an end station with a CCD-based array detector, kappa-geometry goniometer,and low temperature system. The control system and data acquisition capabilities are linked with data processing and archiving computers over a fast ethernet switch with overall systems compututational resources accessible across the distributed network. These features allow for a high throughput of accurate data that can be collected and processed. The initial diffraction patterns from protein crystals were collected on this beamline on September, 1997. The second beamline is under development featuring an asymmetrically-cut, curved-crystal monochromator covering a range from 0.95 -1.6 Angstroms, and will be used primarily for monochromatic crystallography experiments. A massively-parallel pixel detector is under develop with other LBNL and UC-San Diego scientists and engineers. This detector will offer photon-counting capabilities with very large dynamic range and small point spread function making it ideal for MAD measurements. Its ability to collect data in the millisecond time range will allow for applications in time-resolved structural biology to be performed. The MCF participating research team includes Lawrence Berkeley National Laboratory, Amgen, Roche Biosciences, University of California -Berkeley, and Lawrence Livermore National Laboratory, who sponsor the program in addition to the support from OBER/DOE.

22. Structural Molecular Biology Program at SSRL

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This program involves synchrotron radiation-based research and technology developments in structural molecular biology (SMB) that focus on protein crystallography, x-ray small angle scattering diffraction, and x-ray absorption spectroscopy. The goal of this program is to provide increased access and support for SMB users of the SPEAR storage ring at SSRL, to develop new instrumentation for SMB research using synchrotron radiation, to develop advanced computing algorithms for data analysis and graphic display, and to operate a new SMB beam line (BL 9). The program operates in strong synergy with a NIH/NCRR-funded BTP Biotechnology Resource. Protein crystallographic facilities include a focused 8-pole wiggler station (BL7-1) with a 30-cm MAR-Research imaging plate detector, a focused bending magnet beam line MAD station (BL1-5AD) with a 2X2 CCD array Quantum 4 detector, and a high-intensity station on the new focused 16-pole/2T wiggler beam line 9 (BL9-1) with a fast MAR345 imaging plate detector. The beam line 9 end station (BL9-2), dedicated for Laue diffraction, MAD phasing and fast monochromatic protein crystallography studies, is being commissioned. Facilities for on-site data reduction and analysis including advanced computer graphics are available at each station. Small-angle scattering/diffraction is performed on a semi-dedicated focused 8-pole wiggler station, typically using a multilayer monochromator. The camera system includes a variable beam path length, two PSD detectors, and a quadrant detector. A Hamamatsu CCD detector will soon be available. A computer-controlled stopped-flow apparatus and a jet-mixer are available for time-resolved measurements. Specialized instrumentation is provided for low-angle protein crystal diffraction (600-15 Å) with special emphasis for virus protein applications. For x-ray absorption spectroscopy is provided three 13-element Ge solid state detector arrays and associated electronics, three liquid He cryostats for studies down to 4 K, and other specialized equipment.

Stations available include a dedicated station (BL7-3, 8-pole wiggler) as well as on time-shared stations (5 wiggler, one bending magnet). Specialized software is provided for data acquisition and for on-line data analysis at each station, and is also available for user distribution. A new dedicated, focused 16-pole wiggler side station is being commissioned (BL9-3) that will have a 30-element Ge detector array, LHE cryostat, and specialized equipment for single crystal XAS measurements. Supporting facilities include well-instrumented sample preparation laboratories and coldrooms.

Databases:

23. Macromolecular Structure Database: The enhanced Brookhaven Protein Data Bank

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The Protein Data Bank archival database of three-dimensional structures of biological macromolecules is a resource in transition. As this data has grown rapidly over the past few years, access to the structural information in Protein Data Bank has become important to a broader community. New approaches are needed to meet the challenges of maintaining the quality and currency of the database, enhancing its utility, and containing costs. In fulfilling its mission to provide the international science community with access to knowledge on three-dimensional structures of proteins, nucleic acids, viruses, and carbohydrates, the Protein Data Bank is actively expanding its role as a center for coordination, development, and implementation of new database management, deposition, manipulation, and analysis tools that will be required if the rapid accumulation of three-dimensional structural information is to be utilized effectively. The Protein Data Bank has formulated a strategy to ensure that the database remains current, releasing annotated, validated data immediately following complete deposition, while satisfying the need of its user community for better data access and manipulation. Four core activities of the Protein Data Bank are delineated: a) Data submission, processing, and validation; b) User access and data distribution; c) Maintenance of the Protein Data Bank archive; and, d) Core research and development.

24. A Comprehensive Database of Nucleic Acid Structures

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The overall goal of this project is to create new validation and analyses methods for nucleic acid containing crystals. The first project is being done in collaboration with Professor Janet Thornton, University CollegeLondon (UCL). The principal focus of this project is to extend existing analyses methods that have been developed for protein-protein and protein-ligand interactions to protein-nucleic acid interactions. The second project is in collaboration with Shoshana Wodak, Free University of Brussels (ULB). In this project, the volume-based quality assessment methods and methods for validating the structure factors are being applied to nucleic acid structures.

Instrumentation Research:

25. Detector Development for Structural Biology

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Electronic area detectors for x rays permit rapid acquisition, manipulation, and storage of diffraction data from protein crystal samples. Currently, detectors based on the charge coupled device (CCD) are the most promising medium for recording x-ray diffraction data at synchrotrons, although it is necessary to continue to investigate other new technologies for this application. We have designed and built CCD area detectors for protein crystallography at synchrotron beamlines, with support from this grant. In particular, a detector system for Sector 19 beamlines (19ID and 19BM) of the APS was designed (actual fabrication was supported through the construction project itself). The first detector, the APS-1, has been extensively tested on 19ID and satisfies all design specifications. Numerous diffraction tests with crystals also indicate that this instrument performs as designed, and is an outstanding detector for synchrotron beamline applications.

26. Biophysical Instrumentation Research

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The development of detector and signal processing techniques for scattering and crystallography experiments which use x-ray synchrotron radiation and thermal neutrons in biological studies. Emphasis is on research concerning position sensitive detectors with high precision and capabilities of extremely high counting rates and millisecond dynamic studies. Approach: The fundamental processes of photon and particle absorption in gas, liquid and solids are investigated both experimentally and theoretically. Comprehensive analysis is made of noise, signal formation, radiation damage, background susceptibility and position encoding techniques. User feedback provides ongoing improvements to the devices. Increased emphasis will be placed on silicon pixel detectors for x-rays. Accomplishments: Two-dimensional neutron detectors with the highest known accuracy and long-term position stability have been provided for biology experiments using neutron scattering. Two-dimensional x-ray detectors with unsurpassed position resolution and counting rate capability have been produced for synchrotron facilities and universities. A multi-element silicon pixel detector has been developed. Expected Accomplishments: Improved position sensitive detection methods will be developed for neutrons and x-rays by further advances in proven technologies and investigations of new ones such as silicon drift and pixel detectors. Detectors for neutron spallation sources, and very high rate detectors for biological x-ray microscopy, are being developed.

27. Transcription in Eukaryotes

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This project is to achieve atomic resolution in biological structure determination by Atomic Force Microscopy. Our present procedures for measuring the mass and geometry of initiation structures by scanning transmission electron microscopy and conventional electron microscopy continue to contribute important information complementary to biochemistry, and will be essential for validating the results of atomic force microscopy. A new class of atomic force microscopy tips, of oxide-sharpened single-crystal silicon, can be made with a diameter as small as 1–2 nm, and should yield atomic resolution in measurements of protein surfaces when used in a new microscope design having carefully controlled contact force. Turning to biological applications, yeast chromosome sequences where DNA replication begins

are targeted by a multiprotein origin recognition complex. The complex, and associated proteins, are being studied by scanning transmission electron microscopy and electron microscopy for static structure and structural changes on initiation of replication. Human tumor suppressor protein p53 is studied similarly for binding at specific sequences to activate genes that control the cell cycle or initiate programmed cell death, and also at sites of DNA damage. These protein regulators of replication and transcription will be the subject of our initial high-resolution atomic force microscopy experiments.

28. Development and Applications of Photosensitive Device Systems to Studies of Biological and Organic Materials

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Develop x-ray detector instruments and techniques for biostructural and materials applications, especially at the synchrotron radiation sources. X-ray detectors are being developed based on phosphor screens coupled to CCDs via fiber-optics and on all-silicon, custom fabricated arrays of silicon pixels each with its own signal conditioning electronics. Software and calibration procedures are also being developed. CCD detectors developed by the group are now delivering significant biostructural data at storage ring sources. Preliminary designs of small-scale Pixel Array Detectors have been fabricated and tested.

29. ALS Soft X-ray Microscopy

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The long term goal of this project is to develop x-ray microscope techniques, including dose-fractionated microtomography and fluorescent labeling methods, to obtain unique information about biological structures at the subcellular level. In particular, methods are being developed to take advantage of the very high flux rates now available from third generation soft x-rays sources such as the Advanced Light Source (ALS) at the Lawrence Berkeley National Laboratory. It is only in the last few years that the complimentary development of high resolution zone plate lenses and intense, tunable, monochromatic x-ray sources has made possible the construction of x-ray microscopes with high resolving power. In this context, both scanning and imaging microscopes have been produced that are capable of better than 50 nm resolution, with improvements expected to reach below 20 nm. This is 4–10 times better than the resolution of the best visible light microscopes. However, to take advantage of resources like the ALS, specific techniques must be developed which are unique to soft x-rays. In this context, we have focused on the development of x-ray excitable luminescent probes for high resolution labeling, and microtomography techniques for 3D reconstruction. To date, our principal experimental focus has been on the development of radiation resistant luminescent probes based on lanthanide fluors. We have produced antibody, avidin, and biotinylated polychelate probes which have demonstrated sufficient brightness and radiation stability to form the basis of fluorescent labels for high resolution x-ray microscopy. In addition, we have done theoretical work that has demonstrated the feasibility of soft x-ray microtomography. We have shown, using computer simulations, that high resolution three dimensional reconstructions can be obtained by dividing the dose needed for a single projection into multiple low dose exposures in a tomographic set.

30. Semiconductor X-ray Detectors for Synchrotron Applications

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Development of semiconductor materials, semiconductor detectors, and detector components for improved detectors for synchrotron x-ray fluorescence and diffraction applications. The specific aims of this project are: 1) Develop a multi-element silicon detector capable of very low noise performance for a wide variety of synchrotron x-ray fluorescence experiments. This detector requires cooling only to -25C with a single-stage thermoelectric cooler, has an energy resolution of <200 EV FWHM (in conjunction with the associated readout electronics), has excellent spectral performance over the range 2-30 KEV, and contains hundreds of individual elements for high count rate performance. 2) Develop thin window contacts for Si(Li) and high resistivity Si detectors for reduced attenuation of low energy x-rays and for improved spectral performance in the <3 KEV regime. 3) Develop the crystal growth techniques for growing very high purity GaAs epilayers for high efficiency, room temperature x-ray detector arrays. 4) Develop or utilize new transistors for very low noise performance x-ray spectrometers. This project focuses on the development of new semiconductor detectors specifically designed for synchrotron applications, in particular for low noise x-ray fluorescence spectroscopy and diffraction experiments such as are encountered in the field of structural biology. A primary goal is the realization of specialized spectrometers capable of exploiting the unique properties of synchrotrons. Our approach combines existing expertise in semiconductor device fabrication, x-ray analytical measurements and synchrotron radiation experimentation. Current work focuses on the development of semiconductor detectors and detector systems optimized for high count rates and very good energy resolution. The development of multi-element silicon array detectors is now well under way. These silicon detectors will initially be targeted for high-count-rate fluorescence spectroscopy experiments, such as x-ray absorption spectroscopy and extended x-ray absorption fine structure absorption spectroscopy.

31. Structural Biology of the Sequestration and Transport of Heavy Metal Toxins

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This project, funded primarily by the Department of Energy's Environmental Management Science Program, involves basic research in structural biology and biochemistry, investigating the properties of proteins and other chemical substances that could be used to tie up, remove, or detoxify hazardous substances in the environment. This award provides support for a significant upgrade of the principal instrument used to determine the structures of the proteins responsible for the bioremediation of mercury and other heavy metals. NMR spectroscopy is being used to determine the structures of proteins related to merP and merT of the bacterial mercury detoxification system. The membrane protein merT presents substantial technical challenges that can be overcome by performing the experiments in a very high field NMR spectrometer. The support from this award will also enable us to obtain a high field magnet to up-grade the spectrometer used in these studies.

32. Ultra-High Field NMR

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The scope of this work is to test current experimental nuclear magnetic resonance (NMR) methods at high fields and to explore novel applications of labeling patterns using stable isotopes. NMR is a powerful tool for studying the structure and dynamics of biomolecules and advances in magnetic field strength

bring the promise of enabling NMR studies of DNA, RNA, and protein molecules of increased molecular weight. However, the currently used experiments will eventually fail as resonance linewidths increase with increasing molecular weight. Furthermore, problems with spin diffusion and cross-relaxation limit studies of DNA structure and dynamics. In order to address the problems of cross-relaxation in DNA, we pursued eliminating strongly coupled geminal proton pairs by stereoselective deuteration. The work was carried out in collaboration with the scientists in the Stable Isotope Resource at Los Alamos National Laboratory who were responsible for synthesis of specially labeled DNA. The major success of this project was in illustrating the significant benefits that stereo-selective deuteration in DNA had both on eliminating detrimental transverse cross relaxation and in significant sharpening of linewidths (and therefore sensitivity enhancements) due to reduced dipolar coupling. New experiments were developed to take advantage of improved spectral quality. Labeling patterns are continuing to be optimized with the goal of enabling the most reliable and accurate determination of DNA structures.

Computational Structural Biology:

33. Computer Simulation of Enzyme Reactions

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Using methods such as time-resolved x-ray crystallography structures of metastable intermediaries along enzyme reaction pathways can be determined. Such data are necessary but insufficient to characterize the mechanisms and energetic of enzyme reactions. Complete atomic mechanisms can only be determined using first-principle analysis based on quantum and statistical mechanics combined with structural information for enzymes and that reaction with substrates. Specific aims proposed in his project are: 1) Develop the capability to determine minimum energy and free energy surfaces and pathways for enzyme reactions. This will be accomplished by using a hybrid, semi-empirical, quantum mechanical and classical molecular mechanics (QM/MM) method together with free-energy perturbation and molecular dynamics techniques; 2) Demonstrate the utility of these methods by applying them to the reaction catalyzed by the enzyme malae dehydrogenase (MDH), which is used as a model system to understand the details of proton and hydride transfer reactions in dehydrgenase enzymes. Free energy transition states of native and site-directed mutants of MDH will be calculated and used to determine the relative transition-state-binding free energies of native and mutant enzymes. These calculated relative free energies will be compared with corresponding experimental values derived from enzyme kinetic data; 3) Implement an approach to improve the performance of the semiempirical quantum mechanics method for specific reaction systems through the generation of optimized parameters that are based on high level *ab initio* calculations and appropriate experimental data; 4) Implement all methods to run on massively parallel, multiple instruction and multiple data (MIMD) computers. The computational techniques developed in this project will provide a general tool for the determination of atomic structures and energetics along enzyme reaction pathways. Such methods will complement and enhance time-resolved crystallographic structural data and provide the means to predict the effects of site-specific mutagenesis experiments, which can be used to design enzymes with novel catalytic properties.

34. Homologous Modeling of Proteins using Empirical Free Energy Functions

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The objective of this proposal is to modify the traditional target functions used in homology protein modeling in order to account for the free energy of desolvation and conformational entropy. In preliminary studies the empirical free energy functions as implemented in the simple atomic solvation parameter (ASP) and the atomic contact energies (ACE) have been

applied to ligand-receptor complex formation and free energies of unfolding of staphylococcal nuclease. This proposal will extend the empirical functions to include only repulsive Van der Waals potentials and novel continuous contact function that provide a differentiable solvation term. The new free energy functions will be rigorously tested by unconstrained local minimization to see if the appropriate secondary structures are maintained and in a test to see if the new energy functions will help to produce correct backbone conformations from novel proteins. The free energy functions will be applied to the following aspects of protein structure prediction: 1) homology modeling algorithms (a. predicting side chain conformations, b. loop closure and c. simultaneous loop closure with and without bound ligands), 2) homologous modeling of biomolecular complexes (a. rigid body docking, and b. flexible ligand docking to homologues of the known receptor) and 3) free energy for core discrimination in threading, in this application the free energy functions will be applied to a model based on simplified representation of the side chains.

35. Protein Structure and Function Prediction from Physical Chemical Principles and Database Analysis

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This proposal has plans to work in two distinct areas: 1) Protein structure prediction; and 2) Prediction of function given a 3-D structure. There are a number of common threads between the two areas. First, they both involve the use of similar physical chemical methods and principles. Second, an underlying goal is the exploitation of the vast amount of genomic data currently becoming available in sequence and structure databases. Third, there will be an increasing degree of the interplay between the two areas in that even low structure prediction will help deduce function, while functional information may be used to verify the validity of a structural model. Specific aims include: a) The development of new methods to associate amino acid sequences with 3-D folds; and b) The construction of databases of the surface properties of proteins with the goal of predicting protein function given a 3-D structure.

36. Post Doctoral Fellowships in Computational Molecular Biology

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Kevin Atteson, *University of Pennsylvania*
J. Regan, *California Institue of Technology*
M. Pellegrini, *UC, Los Angeles*
T. P, Westcott, *UC, San Diego*
Boris Fain, *Stanford University*
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Early on it was also recognized by OBER that outstanding scientists trained in mathematics and computer science will be needed with experience in molecular biology to make a significant contribution to the objective of the CSB program. An available pool of talent with this mix of expertise and experience in the country was very limited. This was confirmed by a recent article in *Science* magazine. To address this issue, OBER initiated a joint postdoctoral fellowship program with the Sloan Foundation in Fiscal Year 1996. This joint program will support ten fellowships per year. In the first year, OBER is supporting three and Sloan is supporting seven fellows. In the second year, OBER will support four and Sloan will support six fellows. Finally, in the third year, OBER and Sloan each will support five fellows. The purpose of these fellowships is to catalyze career transitions into computational molecular biology from mathematics, computer science, chemistry, physics, and related fields. In particular, emphasis is placed on candidates who have outstanding credentials in above-mentioned fields and wish to bring these backgrounds to bear upon computational molecular research questions. Candidates already firmly rooted in computational biology and proposing to continue pursuit of research undertaken for their Ph.D.s, are normally not considered of high priority for these fellowships.

37. A Thermodynamic Approach for Determining the NMR Structure of Flexible Molecules Based on Free Energy Calculations

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The biological activity of peptides and proteins is determined, to a large extent, by their most stable 3-D structures. Therefore, elucidating the latter by experimental/theoretical methods is of great importance, in particular for drug design. The only experimental method that can provide biomolecular structures in solution is multidimensional NMR. This methodology is well established for globular proteins, which exist as well defined 3-D structures. On the other hand, peptides prevail in solution as ensembles of interconverting conformations, and elucidating their dynamic structure from NMR data is always difficult and sometimes impossible. Whether this task is feasible or not depends on the chemical composition and molecular architecture. If the peptide is small and linear, it also depends on the ability of the environment to stabilize a few dominant, relatively compact conformations. This is necessarily so since the spectroscopic parameters which carry the requested information depend on the interatomic distance r as r^{-6}. It is proposed to attack this problem with a set of theoretical tools based on thermodynamic first principles, integrated into a complete methodology. The following design has been conceived, based on the above mentioned rationale and on preliminary results. 1) Modeling—potential energy function including solvent effects implicitly will be used. Such models are relatively simple and therefore still computationally feasible; the proposed methodology is expected to help assess their validity. 2) Conformational dearth—three new methods have been developed. One is geared toward searching for the lowest energy structures and the other is aimed at identifying the lowest harmonic free energy regions. The third method evaluates the range of energies above the global energy minimum that contribute significantly to the partition function. These methods will be further developed and applied to models of solvated peptides. 3) Populations of microstates—each of the low energy structures obtained by the conformational search becomes a "seed" for Monte Carlo (MC) simulations. The corresponding samples define microstates, which are distinct regions around the "seed" each spanning a relatively large range of conformations. Their free energies are calculated from the MC samples using the local states method, which unlike other techniques, can handle any chain flexibility. 4) Microstate spectroscopic parameters are calculated from the respective MC samples, and the overall parameters are obtained as their averages, weighted by the microstate populations, and compared with their experimental counterparts. It is planned to apply the method to [LEU5]-Enkephalin, Dermenkephalin, linear Deltrophin, and cyclic Dermorphin analogues.

38. Statistical Modeling and Analysis of Proteins Involved in RNA editing, Alternative Reading of the Genetic Code and Protein Splicing

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Subtle residue patterns common to multiple sequences often reflect similar underlying structures and/or biological properties. Identification, modeling and analysis of the core elements of a family of related sequences likely to be determinants of their folding, structure and function are important both for understanding the properties of and relationships between molecules, and for providing guidance for further experimental and theoretical studies. The increase in sequence data lends added impetus to the need for computational means to detect such features both in a set of proteins known to be related and in novel proteins. The latter ability can suggest new or additional functionality for a protein and thus assist in elucidating and characterizing its biological properties. Hidden Markov Models, HMMs, is a statistical method designed to address the issues of identifying the core elements of related sequences and in detecting weak, but significant homologs that may be present in

sequence databases. The aim of the proposed research is gaining insights into three biological processes that contribute additional layers of complexity to a central dogma of molecular biology. As a consequence of RNA editing, alternative reading(s) of the genetic code and protein splicing, it may not be possible to infer MRNA sequence from DNA sequence and protein sequence from MRNA sequence. The goal of the research is identifying and characterizing protein families involved in these processes in terms of their sequences, structures, functions and evolutionary relationships. This involves creating and employing specific and sensitive HMMs to determine the core elements of each family and to detect potential new family members. Results should serve to direct experimental work on understanding the biological activities of these families and can be useful for inverse protein folding studies, protein design experiments and as components of programs aimed at parsing DNA from genome project.

39. Development and Application of Neural Net Based Protein Inverse Folding Potentials

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There has been considerable interest recently in developing and applying knowledge-based, empirical potential functions for protein, originating with the pairwise contact potential of Miyazawa and Jernigan, and more recent extensions. A major interest in such knowledge-based potentials is their use in homology modeling methods aimed at identifying the fold of sequences which structures have not been experimentally determined. This project proposes to develop more accurate potentials and also suggests new and novel applications closely coupled with experiments. More accurate potentials are needed because present-day potentials cannot consistently pair a native fold with its native sequence if sequence inserts and deletes representing loops are allowed. One hypothesis is that this occurs because the analytical techniques used to develop present knowledge-based potentials have consisted of relatively unsophisticated, statistically based approaches based on simple frequency counting of amino acid pairs. Important physical effects such as packing and steric hindrance are, therefore, lost. It is proposed to extend the accuracy of knowledge-based protein potentials by using statistical and pattern recognition techniques such as neural networks, which are capable of incorporating high order interactions and are very efficient at "mining" data. A careful comparison of the accuracy of the proposed approach with other approaches will be made to identify dominant physical effects contributing to increased accuracy. More accurate potentials developed in this program will be widely available to other researches via the internet.

40. Sequence Structure Relationships: Quantifying Residue Fitness Through Phylogenetic Analysis of Homologous Protein Sequence with Known Structure

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The principal objective is to develop new insights into sequence-structure relationships by analyzing multiple sequence alignments and corresponding protein structures, making use of rigorous statistical techniques to correct for evolutionary effects. Specific aims include correlating amino acid usage at each position of a protein alignment with its structural properties including secondary structure, solvent exposure, hydrogen bonding, and binding motifs, and likewise for pairs of positions taking phylogenetic effects into account. A new method is used for determining amino acid usage, which infers the evolutionary history at each site of the aligned sequences. The method is mathematically well-founded, using maximum-likelihood estimation of parameters in a model of amino acid replacement along branches of an evolutionary tree. By properly interpreting substitution events along evolutionary branches of all lengths, this method gives more accurate results than other methods, such as sequence weights can achieve from a given amount of data. This method is especially advantageous for detecting correlated mutations (covariation) in pairs of sites.

This investigation of sequence-structure correlation will lead to valuable information for predicting protein structures, identifying functional similarities in distantly related sequences, and improving models of protein evolution.

41. Combined Approach to the Inverse Protein Folding Problem

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This project proposes to greatly improve the sensitivity of the detection of a remote relationship between a trial sequence and a known 3-D structure as well as the accuracy of the final sequence-structure alignment by pursuing the following ideas: 1) Sensitivity of each pairwise sequence-structure comparison can be improved through the direct use of physical energy and detailed all-atom models; 2) Remote similarity between two proteins can be recognized by building a chain of pairwise sequence-sequence and sequence-structure comparisons between intermediate homologues called Multilink Recognition; and 3) Trial sequence can be aligned with (thread onto) the remotely related structure or structures via one or several chains of pairwise alignments between intermediate homologues—a process called Multilink Alignment. The outcome of this effort will be a comprehensive methodology comprising fold recognition, alignment/threading, building of the 3-D model, and testing and visualization of structural defects of the model. These tools will be provided to the scientific community through the World Wide Web site.

42. Computational Methods for Protein Structure Inferences

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This project proposes to develop key technologies for the computational determination of protein structure from sequence. These methods will improve our ability to predict the folded state of new protein sequences. In task one, a series of new computationally efficient algorithms will be developed for evaluating whether a protein sequence matches any folds in the protein structure database. These new global optimization algorithms for threading consider both variable gaps and residue pair interactions in polynomial time. The improvements in threading efficiency will allow realistic treatment of threading energy terms. In task two, a database of energy minimized protein structures will be developed from which new tables for sidechain pair interactions, hydrophobicity, and local conformational energies for threading will be derived. These will provide more accurate estimates of threading energy terms. Improved systems for defining threading templates and deriving alignment gap penalties will be developed. AI-based protein structure classifiers trained by machine learning will be used to assign protein sequences, with low homology to any sequence in the structure database to structural classes, and provide information and constraints for threading. In task three, additional techniques based on structure database-derived statistical information will be developed to improve partially correct threading results, to explore the possibility of additional secondary structure elements, and to improve/extend the target conformation obtained from one or several threadings. This technology will add flexibility and a wide range to present threading methods. The computational tools and libraries will be placed on-line for use by others.

43. Structure/Function Studies of Proteins Using Linear Scaling Quantum Mechanical Methodologies

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This research proposal has four major goals. First, to develop and fully test a linear scaling semiempirical divide and conquer (SD&C) approach that will allow to carry out quantum mechanical (QM) calculations on systems containing thousands of atoms. Second, to

combine the SD&C method with continuum electrostatic models in order to determine how solvent and counterions affect the electrostatic properties of proteins. Third, to use experimental and theoretical structural and energetic information to develop a new semiempirical parameter set exclusively for proteins, and fourth, application of these methods to proteins in order to better understand the electrostatic properties of proteins. The successful completion of these goals should provide knowledge to address fundamental issues regarding how quantum mechanical effects e.g. charge polarization, many-body effects etc. influence protein structure and dynamics.

44. Integration of Structural and Sequence Information for Homology-Based Modeling of Proteins

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The rapid growth of sequence and structure databases promises to make useful 3-D models of most proteins available through homology based modeling. This proposal focuses on the early stages of homology-based modeling, the selection of template structures, and mapping of the sequence onto the template. Specifically, it is proposed to improve on the current methods of homology-based modeling by: 1) Implementing a finite mixture model formalism for 3-D profiles; 2) Incorporating sequence data into structural profiles using the finite mixture model formalism; and 3) Validating 3-D profiles as templates for homology-based modeling, and implementing improved tools for the analysis of sequence/structure mapping.

45. From Atoms to Cells: Multi-Scale Computational Integration of Sequence Structure and Assembly

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This project will develop a computational environment for the molecular biology community that will advance understanding of biological function by integrating structural data from experimental sources that span the scale from atoms to cellular components. Using computational, computer graphics and database approaches this project will develop methods to produce, manipulate and analyze atom-based models of supramolecular assemblies by combining atomic-resolution data from genetic sequencing, X-ray crystallography and NMR spectroscopy with lower resolution data from electron microscopy, small angle scattering and other emerging ultrastructural techniques. Such models will ultimately give us the capability to decipher the genetic code for molecular recognition and assembly. Thus combined with rapid progress in genomic mapping of organisms lead to a general chemical description of biological processes.

46. Towards Large-Scale Automatic Modeling of Protein Structure From Sequence

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The protein folding problem remains one of the most important unsolved problems in structural biology because the solution would facilitate the design of effective drugs for diagnosis and treatment of human diseases. Understanding the mechanisms of protein folding and its simulation can help in the design of proteins that may have novel applications in such important tasks as environmental clean-up. The

principal investigator proposes to exploit the dramatic increase in the number of known DNA sequences for understanding the protein folding mechanisms. The proposed work is essential if we are to bridge the sequence/structure gap and maximize the value of sequence information in biology, pharmacology, and environmental sciences. Following are the specific aims proposed: 1) Improve the structural alignment method to the point where it can be used to find similar folds with high accuracy, high sensitivity, and reliability; 2) Extend the structural alignment method to multiple structures and to form multi-sequence templates. These templates will be automatically annotated with structural information to aid manual and automatic alignments; 3) Improve threading algorithms to be reliably convergent using iterative dynamic programming and energy functions that have been found to discriminate incorrect folds. Widespread use of threading for lattice models and the convergence of iterative dynamic programming in structural alignment indicate that this can be achieved. 4) Improve homology modeling by testing automatic methods for adding side chains and missing main-chain segments. Extend methods for rapid energy minimization to predict main chain shifts, which are poorly handled by existing methods. 5) Model large families of sequences by focusing on the globins as an example of all-alpha proteins and the antibody superfamily as an example of all-beta proteins. First known structures will be used to model sequences then the modeling will be extended to all sequences. 6) Apply development and advances in these methods to modeling projects in biological and environmental sciences. The first project is homology/mutant modeling of the universal TATA-box binding protein. The second more speculative project is to redesign the myoglobin heme cavity to scavenge environmental toxins.

47. Assigning Genome Sequences to 3D Protein Folds

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Our objectives are: 1) To develop and improve computational methods for assigning three-dimensional folds to genome sequences. The goal is to be able to proceed from knowledge of sequence to knowledge of structure and function. 2) To apply these methods to learn about structure and function of proteins encoded by genomes. 3) To set up a WEB-based server for automatic assignment of 3D folds to protein sequences. 4) To determine from genome sequences those proteins whose folds are not yet known, and to direct the attention of structural biologists interested in structure determination to these folds, as being novel and particularly informative. 5) To develop methods of determining from genome sequences pairs of proteins that interact with each other. This goal is of great importance to exploiting information inherent in genome sequence, because interacting proteins are at the basis of metabolism, signal transduction, and biological structure.

48. Computation of Electrostatic and Van der Walls Interaction in Full Six Degrees of Freedom Enumeration

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This proposal addresses the problem of determining the docking arrangement between two macromolecules through the use of fundamental physical potentials rather than employing the approach of using empirically derived potentials. The principal investigator proposes extension of his rapid Fourier transform-based evaluation of potentials to permit determination of the energy of interaction between two macromolecules on a full 6-dimensional grid involving the position and orientation of the second molecule. In cases where the free energy surface for the docking process may be regarded as funnel shaped, the PIs also propose calculation of a partition function and its use in calculating the trajectory of the molecule to be docked that is moving molecule, along the reaction coordinate. The model systems to which these techniques are applied include the interaction between plastocyanin and cytochrome f, between cytochrome c and cytochrome oxidase and the association of the catalytic and regulatory subunits of cyclic AMP dependent protein kinase.

49. Conscription of Proteins for New Functionality

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The long range goal is to develop a computational protocol to guide the engineering of modified proteins that exhibit new functionality. Ancillary goals are to develop computational approaches for prediction of function from unknown reading frames and the determination of mechanistic information for proteins for which only sequence information is available. Specific aims are: 1) To enhance the ability to identify distant relationships among protein sequences by developing "function-based" screening routines for sequence database search output using chemical information; 2) To describe structural relationships among members of a superfamily at both the sequence and tertiary structural level and to correlate those relationships with functional similarities and differences among superfamily members; 3) To develop strategies for determining a chemically relevant 3-D scaffold that can be used to identify both conserved structural features and determinants of specificity across the superfamily members; and 4) To integrate the computational methodologies described above into friendly graphical user interfaces that will be generally useful for a wide range of problems in biology and chemistry.

50. Infrastructure for Collaborative Protein Structure Prediction

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It is critical to have reliable ways of distinguishing a correct structure from an incorrect one. Many groups have developed potential functions for assessing the correctness of a predicted conformation. At present there is no mechanism by which these functions may be extensively tested and the results compared. Thus, we do not know how near to solved this problem is, or where further effort can most effectively be directed. In order to develop truly practical structure prediction methods this situation must change. The overall objective of this project is to put in place an infrastructure that will allow researchers to focus their efforts so as to provide a synergistic leap in our abilities to predict structure. Specific aims are: 1) To provide mechanisms to test and evolve potentials and search methods; 2) To provide mechanisms for developing common coding platforms; 3) To provide communication systems that will allow full sharing of the results of the project with the prediction community, participation in directing the project, and effective discussion of the scientific significance of the results; and 4) To provide an ongoing mechanism for the objective testing of complete prediction methods.

51. Expert System for Analysis of Multiple Aligned Sequences and 3-D Structure Prediction of Proteins

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The project will build an expert system for modeling and predicting the 3-D structure of proteins. This system will include current knowledge of the fundamental basis of protein folding, as well as computer-generated rules for secondary and tertiary structures, based on neural network algorithms. The knowledge will be formulated as rules coded in a flexible command language of an already existing program. All secondary structure prediction methods are limited by the dependency of the propensities for the regular secondary structures of the individual amino acids on their context. Therefore, some segments of regular secondary structures are determined from constraints of the proteins overall global fold. Proposed methods will predict secondary structures by applying iterative feedback loops with self-correcting distance geometrical methods. It is also proposed to test whether this method can successfully predict the correct packing of helices and beta strands in a set of representative proteins. Modeling tools developed in this program will be used for: a) Screening large

numbers of mutants of pyruvate kinases and predict 3-D structures; and b) Helping in the design of phage display libraries that are used to select peptides which bind with high affinities to drug receptors.

52. Bayesian Sampling Algorithms for Protein Structure Prediction Using Multiple Sequences

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The proposal aims to improve protein structure prediction using rigorous Bayesian methods and Markov chain Monte Carlo algorithms in two ways: 1) Combining structural information and multiple sequence information; and 2) Using a Gibbs sampler approach it is proposed to develop an algorithm that simultaneously predicts secondary structure and some features of tertiary structure. This group has applied Gibbs sampler approach to bacterial outer membrane protein (as a test case) to show that the technique can extend homology modeling to subtly related proteins using multiple sequences.

Biological Research:

53. Structure and Function of the Photosynthetic Reaction Center by Site-Specific Mutagensis

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The objective of this project is to use site-specific mutagenesis to establish structure/function relationships in the photosynthetic reaction center (RC), one of a very few transmembrane proteins for which a structure has been determined to atomic resolution. The study of this system is based on the structure of the reaction center that was determined at Argonne and is a collaboration with M. Schiffer (Protein Structure and Conformation). It includes spectroscopic characterization of mutant RCs, performed in collaboration with other investigators, including those in the Photosynthesis Group in the Argonne Chemistry Division. This study will enable us to determine how proteins and cofactors assemble to form a functional complex, and how a protein environment can modulate the chemical properties of the small molecules that are intrinsically associated with it. Many of the protein structure/function relationships that we will establish in the photosynthetic reaction center will be applicable to membrane proteins in general. Determining the conformational elements that lead to electron transfer, proton transfer, and protein folding should provide a model for understanding the structural basis for these pathways in other systems that are not nearly as amenable to this type of study. These investigations will increase our understanding of fundamental processes in photosynthetic energy conversion. Most importantly, they will also show us basic principles by which structure determines function in proteins. These concepts are fundamental for protein engineering and *de novo* protein design.

54. Structural Studies of Macromolecular Assemblies

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Recognition of biological macromolecules and their interaction and assembly into larger supermacromolecular structures are at the heart of many important processes in molecular and cellular biology. For example, macromolecular assembly occurs in protein biosynthesis; in the recognition of receptors by protein hormones; in the folding of proteins; and in the recognition of, and binding to, nucleic acids by

proteins which regulate the expression of genetic information. We are studying interactions of molecular chaperones of the hsp60 class. Because the crystals of macromolecular assemblies are usually small and fragile and have large unit cell dimensions, they diffract weakly. Furthermore, these crystals have large, complex structures, and their structure determination is experimentally demanding. These studies will take advantage of the synchrotron radiation beamlines at the Advanced Photon Source at Argonne. The techniques being used include protein crystallography, x-ray diffraction, high performance liquid chromatography, and electrophoresis.

55. Protein Structure and Conformation

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A combination of biophysical, biochemical, genetic, and computational approaches is necessary to elucidate structure/function relationships of biological macromolecules. This program combines these approaches to address specific questions relating protein structure with function. This group studies the three-dimensional atomic structures of immunoglobulins, dehydrogenase enzymes, and the photosynthetic reaction center. Specifically, structure/function analysis in the program comprises the study of electron and proton transfer in the photosynthetic reaction center; self-assembly of antibody light chains using mutagenesis and computer modeling; and the reaction mechanisms of dehydrogenase enzymes using computer simulations. The approach of this program will be enhanced in future years through access to unique Argonne facilities, including high-performance parallel computers and the beamlines of the Structural Biology Center at the Advanced Photon Source.

56. 3-D Structural Studies of Biomacro-Molecules with Unusual Properties

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X-ray crystallographic methods are being used to study the three-dimensional structure-function relationship of biological macromolecules with unusual biological properties. We will focus our studies on acetylcholinesterase and proteins related to it, as well as on enzymes involved in DNA repair. Acetylcholinesterase is a very rapidly acting enzyme, as it terminates transmission of nerve impulses at cholinergic synapses by hydrolysis of the neurotransmitter, acetylcholine. As symptoms of diseases which involve a depletion of acetylcholine levels might be alleviated by controlled inhibition of acetylcholinesterase, e.g. the use of anticholinesterase drugs for therapy of Alzheimer's disease, we determined the structure of more than a dozen different complexes of acetylcholinesterase with inhibitors including tacrine, other potential anti-Alzheimer drugs, a complex with a transition state analog, and the three-fingered snake neurotoxin fasciculin-II. In parallel, we are using computer simulations to explore the possibility that conformational changes in the enzymes structure may be related to its rapid action. These studies use x-ray crystallography, high-performance computer graphics, solution-spectroscopic techniques, site-directed mutagenesis, and several theoretical methods.

57. Structural Studies on Proteins by X-ray Diffraction

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Crystallographic methods are being used to determine the three-dimensional structures of selected proteins in two categories: classical proteins that interact with DNA, and antigens relevant to disease. For the first

category, our purpose is to determine how specific features of DNA are recognized, and to understand how protein function is regulated. Detailed knowledge of structure-function relationships at the molecular level may lead to rational design of proteins with altered specificities or functions that may have applications in biotechnology. Antigenic proteins from *Borrelia burgdorferi* (the Lyme disease spirochete) and other organisms also are under investigation to determine how the protein folds and to define structural parameters for use in rational vaccine design. Some of our projects complement ongoing Human Genome Project research in the Biology Department; all use BNL's unique x-ray and neutron crystallography facilities.

58. Ultraviolet Photobiology and Spectroscopy of Nucleic Acids

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Ultraviolet and visible radiation from the sun affects living organisms in ways that are both harmful and beneficial. Realistic evaluation of the biological hazards of these radiations requires an understanding of their multiple, sometimes antagonistic effects. This project studies the damage inflicted upon DNA irradiated both *in vitro* and *in vivo* and relates specific types of molecular damage to cell death, mutation, cancer induction, and loss of agricultural productivity. We are particularly concerned with developing new systems for monitoring DNA damage based on quantitative gel electrophoresis. We pioneered the use of electronic imaging systems to record the distribution of DNA in electrophoretic gels, and developed analytical procedures for analyzing the resulting images. Our imaging systems and analytical methods are used to quantify the damage done to the DNA of human skin, plants, and other organisms irradiated in situ, and the subsequent repair of this damage. Ultraviolet radiation also is used to probe the solution structure of biologically important macromolecules. We use both conventional ultraviolet sources and the unique properties of the ultraviolet generated by the National Synchrotron Light Source to study nucleic acids and proteins using circular dichroism and fluorescence spectroscopy.

59. Studies of Biological Structures by STEM

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The Brookhaven Scanning Transmission Electron Microscopy (STEM) Facility is unique in the United States and has an extensive user/collaborator program in heavy atom labeling and quantitative, low-dose imaging of biological molecules. Currently 43 projects are active in the following areas: virus structure and assembly; cell organelles; Alzheimer's filaments; intermediate filaments; specific and non-specific DNA/protein interactions; DNA replication; nucleic acid transport, and many others. The ability of STEM to image unstained molecules means that heavy atoms can be used for tagging specific sites. Gold clusters containing 11 and 67 heavy atoms were developed as monofunctional reagents which can be attached to protein side chains or to intermediate linkers, such as antibody fragments. These clusters are being used in studies of DNA replication complexes, proteasomes, virus particles, chaperonins, ribosomes, blood proteins, and enzyme complexes. STEM quantitative imaging permits detailed interpretation of structures by measurement of local mass. A frequent application is determination of the number of subunits in a complex by measuring total mass (accuracy 0.5-5%) mass per unit length or mass per unit area. Radial density profiles can be computed for particles with cylindrical or spherical symmetry. Embedding the specimen in negative stains of different density permits higher spatial resolution and determination of the projected density. STEM is operated as a users' facility, open to outside scientists through the assistance of a National Institutes of Health Grant.

60. Molecular Structures of Membrane Transport Systems

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The goal of our research project is to obtain the molecular structures of selected membrane channel proteins through the use of electron and/or x-ray crystallographic methods. The selected proteins are representative members of superfamilies which have been demonstrated to be of medical and physiological importance. Naturally low levels of many of these proteins in their native environments require that these proteins be over-expressed in order to obtain amounts sufficient for crystallographic studies. Our research work therefore involves the cloning of the desired genes and their over-expression, the purification and crystallization of these proteins as well as crystallographic structure determination. The selected superfamilies are the ATP-binding cassette (ABC) superfamily and the potassium channel superfamily. In the past year, we have focused our research effort in the large scale production and crystallization of purified maltose transporter, a member of an ABC transporter superfamily, and the cloning and overexpression of *E. coli* potassium channels. The ABC superfamily is involved in many diverse functions such as cell division and DNA repair; medically relevant members include, for example, the multi-drug resistance protein responsible for the transport of cancer drugs out of cells and the cystic fibrosis transmembrane conductance regulator which is a chloride ion channel and where defects in this protein result in cystic fibrosis. We have developed a large-scale purification method for the maltose transporter. Several milligram quantities of purified maltose transporter can be routinely produced. We have also obtained initial crystals of this protein and are currently refining our crystallization protocol. Potassium channels play a crucial role in the functions of a wide variety of cells. In electrically excitable cells such as neurons and muscle cells, voltage-gated (Kv) potassium channels repolarize the membrane, thereby regulating the time course of an action potential for muscle contraction as well as for neuronal excitability. Kv potassium channels are also important in the function of non-excitable cells such as immune cells and those found in endocrine and exocrine glands. For example, KV1.3 potassium channel is thought to play an important role in the modulation of T-cell activation by regulating the T-cell membrane potential; blockers of this channel, including high affinity scorpion toxins, suppress T-cell activation. We have successfully expressed a member of the superfamily, the *E. coli* potassium channel, using an over-expression system that we have constructed. We have also been able to purify the expressed protein in milligram quantities and have begun to conduct crystallization trials necessary for subsequent crystallographic structure determination. The long range objective of this research is obtain the molecular structure of these selected membrane proteins so that it will be possible to develop a thorough understanding of their functional activities at the molecular level. Membrane channel proteins play a crucial role in the interactions between a cell's internal machinery and its neighboring cells and environment. These interactions are major factors affecting cell development, differentiation and transformation. Knowledge of the molecular mechanisms of these proteins can be expected to make a major contribution to our understanding of how transmembrane communication is regulated. This may provide us in understanding the molecular basis for diseases such as diabetes, cardiac arrhythmias and sudden death syndrome; this, in turn, will be useful in providing information critical to future drug design.

61. Structure and Mechanism of Catalytic RNA

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The goal of this project is to determine the three-dimensional structures of functional catalytic RNAs in order to understand the mechanisms of catalysis and facilitate design of novel RNA enzymes. Initially, we focused on the STRSV (satellite Tobacco Ring Spot Virus) "hammerhead" catalytic RNA by X-ray crystallographic methods. In order to retain catalytic activity, but slow the rate of cleavage enough

to allow crystallization, we selected a mutant hammerhead sequence in which a guanosine involved in a G-A non-canonical base pair is mutated to adenosine. This mutant RNA self-cleaved with an estimated half-life of one day at 37° C, at least 100X slower than the native. Thus, it was possible to purify and crystallize this functional RNA before a significant fraction of the molecules had been cleaved. After development of synthesis, purification and crystallization techniques, we achieved large (>0.5 MM) diffraction-quality crystals of this RNA. X-ray diffraction data for STRSV catalytic RNA have been collected to 2.4Å resolution from crystals grown in the presence of SM^{+3} and to 2.8Å resolution from crystals containing no samarium. We are exploring a variety of approaches including molecular replacement (MR), isomorphous replacement (IR), and multiwavelength anomalous diffraction (MAD) to solve the three-dimensional structure of this catalytically active RNA molecule. A two-fold non-crystallographic symmetry axis and the cell volume indicate the presence of two RNA molecules per unique crystallographic unit. Using models based on similar, but non-functional, hammerhead catalytic RNA crystal structures, we are applying molecular replacement to determine the three-dimensional structure of our molecule. Although we have been able to locate the orientation of the major helix, we have not yet been successful in positioning the complete model or in using phases based on the partial model to extract the remaining structure. Thus, we suspect there are substantial differences in this structure compared to previously determined inactive hammerhead crystal structures. We have conducted numerous heavy atom soaking experiments to obtain isomorphous derivatives. Diffraction datasets were collected on 6 potential derivatives from the samarium crystals and 10 potential derivatives from the crystals grown without samarium. Many of these datasets show significant differences compared to the native data and retain isomorphous cell dimensions. We are attempting to locate and refine heavy atom positions for phasing. Synchrotron data were collected at multiple wavelengths on an iridium-soaked crystal for MAD phasing, but no localized metal binding sites were observed for this derivative. We are currently pursuing each of these approaches, as well as covalent attachment of heavy atoms in order to ultimately determine this structure. We have recently initiated two collaborations aimed at the structure solution of other types of catalytic RNA. Dr. Arnold Hampel is preparing large quantities of a "hairpin" catalytic RNA for crystallographic studies at LBNL. This RNA uses a completely different mechanism for its cleavage reaction. Our first crystallization studies of the hairpin RNA will be of a mutant which cleaves only in the presence of cobalt hexammine. Together with Dr. Norman Pace, we are studying the RNA component of RNAse P, which processes tRNA and has intrinsic catalytic activity. While purification of an intact, active RNA is in progress, we are currently attempting crystallization of oligomers corresponding to the tRNA CCA-binding site and the highly conserved magnesium binding domain required for the cleavage reaction. In summary, structural characterization of these diverse ribozymes will provide a basis for understanding RNA mediated endonucleolytic cleavage and provide insight into RNA catalysis in general.

62. Scattering Techniques for Structural Biology

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We will develop and use scattering capabilities for the study of biomolecular structures. To make full use of the genome sequence data as it becomes available, it will be important to understand how biomolecules interact to translate DNA sequences into the working units, i.e. the proteins, of a healthy individual and how proteins then interact to carry out function. NMR spectroscopy and crystallographic techniques have yielded high resolution structural data on the subset of biomolecules amenable to study by these techniques. X-ray and neutron scattering techniques are ideally suited to complement these data with information on the interactions and conformational flexibility of biomolecules. Importantly, neutron scattering can be used with deuterium labeling and contrast variation to study the individual components of biomolecular assemblies in solution. Highlights from our past years work include: the first structural data on a key calcium regulatory protein complexed with a functional enzyme giving insights into the enzyme activation mechanism; new structural data on protein kinases and the structural transitions required for cyclic

nucleotide-dependent kinase activation; new structural data on a DNA/protein complex from the hyperthermophile *Sulfolobus acidocaldarius* giving insight into how the protein helps stabilize the DNA so the organism can survive at high temperatures.

63. Structural Biology

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One purpose of this research program is the study of macromolecular structures by single crystal X-ray diffraction complemented by the techniques of small-angle scattering (neutron and X-ray) and neutron crystallography. Macromolecular crystallography research encompasses six broad programs; structural studies of macromolecular components in genetic material (this research program eventually will provide the near-atomic resolution information which is of fundamental importance to understanding the structures of DNA and chromatin as well as the controlling role of nucleosomes in modulating gene expression, retroviral host integration, and DNA replication), structural elucidation of mouse gene products that serve as "mouse models of human disease" (Fumarylacetoacetate hydrolase, an enzyme linked to human hereditary tyrosinemia type I.), the structural basis of stability of extremozymes (enzymes from extremophiles having applications in bioremediation and bioprocessing), nerve and growth factor structures (epidermal growth factor), arthropod venom structures, and collaboration with NASA in microgravity crystallization research. The application of neutron science to structural biology is being advanced through instrumentation planning and design in conjunction with thermal and cold neutron upgrades to the High Flux Isotope Reactor (HFIR), and planning for the National Spallation Neutron Source (NSNS). The HFIR thermal neutron diffractometer will provide high resolution atomic structures of proteins and small molecule pharmaceuticals. To achieve this result, a novel prototype position-sensitive neutron detector will be developed for the instrument. A cold neutron diffractometer will be designed to study very large macromolecular complexes. To make use of the increased flux and improved instrumentation, general methods will be developed for the growth of the very large protein crystals required in neutron crystallography.

64. Structural Studies of Chromatin and Chromosomes

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This grant is directed to understanding the structural and functional relationships of histone modifications and other variables such as HMG proteins etc. in the chromatin and chromosomes of somatic and sperm cells. X-ray and neutron scatter will be used to study the effect of histone acetylation on the structures of nucleosomes and chromatin. Atomic force microscopy will be used to study the effect of histone acetylation, addition of very lysine rich histones (H1/H5), and HMG proteins on the structures of nucleosomes and chromatin. We will also study histone-DNA contacts in nucleosomes *in vivo* by zero length DNA-protein crosslinking. To understand the structure of sperm cells and genome organization in human sperm nuclei, we will use atomic force microscopy and fluorescence *in situ* hybridization (FISH) to study genome architecture and higher-order chromatin structures.

65. Structure and Stability of Nucleic Acids

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The specific goals of the project are: a) To determine atomic-resolution structures, thermodynamics and kinetics for RNA molecules. b) To predict how RNA molecules fold into their biologically active forms, in particular to predict their tertiary folding. c) To understand how the structure of an RNA affects its

function in transcription, translation and as a ribozyme. The main method used is 2D and 3D NMR with isotope enrichment (^{13}C and ^{15}N) to determine atomic-resolution structures of nucleic acid molecules. We are applying the newest high resolution NMR methods to molecules of 30 to 60 nucleotides in length. Larger molecules may be feasible if only smaller subunits are isotopically labeled, or if deuterium labeling is used to provide windows of proton-containing sections of the molecule to study.

66. Determinants of Membrane Protein Structure

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Our objectives are: 1) To create libraries of amino acid sequence changes that a) do not affect the activity of Diacylglycerol Kinase (DGK), b) alter the stability of DGK and c) destroy the ability of DGK to function. These mutants will identify amino acid residues in the DGK sequence that are key determinants of its structure. 2) Solve the crystal structure of DGK to determine the structural role played by the critical amino acid residues identified in objective 1. 3) Measure the stabilities of mutants identified in objective 1, so that the strengths of interactions in the protein can be quantitatively assessed.

67. Protein structure by nuclear magnetic resonance spectroscopy

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The lab determines the solution structures of biologically significant proteins and protein complexes using 'state of the art' nuclear magnetic resonance (NMR) spectroscopic methods. Particular emphasis is placed on proteins involved in the repair, recombination and transcription of genetic material. We will continue our structural studies of the integrase from the conjugative transposon Tn916. During FY96-97 we successfully determined the solution structure of the DNA binding domain of the integrase protein. Future research will focus on the catalytic subunit. We will also refine our structural model of the DNA binding domain and investigate how it interacts with DNA. Finally, we will attempt to solve the solution structures of proteins involved in a variety of processes, including: the disease ataxia-telangiectasia, DNA repair and DNA transcription.

Medical Applications:

Radioisotope Development

68. Radionuclide and Radiopharmaceutical Research at BLIP and HFBR

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This program is devoted to radionuclide and radiopharmaceutical research leading to new, more effective, diagnostic and therapeutic agents. The major purpose of this program is to carry out research on new BLIP- and HFBR- produced radioisotopes and to use these products in the design, development, and evaluation of new and more specific radiopharmaceuticals for both diagnostic and therapeutic applications. The research includes collaborative projects with onsite and offsite DOE-supported programs. Several unique BNL facilities, e.g., the Brookhaven Linac Isotope Producer (BLIP), the High Flux Beam Reactor (HFBR), the Brookhaven Medical Research Reactor (BMRR), cyclotrons, high-level hot cells, and laboratory animal and clinical facilities are used. Our long-range objectives are to: A) identify and develop new radionuclides for specific research applications by carrying out studies on nuclear cross-sections, targetry, radiochemical and radioanalytical procedures, and generator development; B) provide, when possible, certain commercially unavailable radionuclides for other DOE-supported research, and C) develop and test new radiopharmaceuticals, which provide better diagnostic and therapeutic approaches and aid in the quantitative assessment of *in-vivo* biochemistry and function. Specific goals for FY 1997-1998 are as follows: i) Develop optimum methods of production and processing chemistry, for ^{47}Sc, ^{55}Co, ^{117m}Sn, and ^{195m}Pt. To a limited extent, address outside requests for research isotopes; ii) Develop labeling chemistry and test ^{47}Sc and ^{55}Co labeled antibodies BRE-3 and CC-49, and a somatostatin-receptor-positive decapeptide; iii) Continue development of rigid bifunctional chelating agents, in particular mono- and bis-cyclohexyl DOTA and TETA, and tethered bis-CDTA; iv) Provide scientific and technical support for the manufacture (outside BNL) of $^{117m}Sn(^{4+})$DTPA and assist with Phase III clinical trials of this agent.

69. RFQ 3He Linac for PET Isotope Production

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In 1995, Fermilab and SAIC formed a collaboration with partners from the University of Washington and the Biomedical Research Foundation of Northwest Louisiana to explore an innovative approach to the production of radioisotopes. The accelerator system that is being developed accelerates He-3 to 10.5 MeV and then delivers this beam to the target to produce the short lived radioisotopes of interest to the PET community (F-18, O-15, N-13, C-11). Research is being conducted to investigate the contribution this promising approach can make to the clinical and research PET.

70. Biomedical Radioisotope Development

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This project conducts research at ORNL in medical radioisotope technology development which complements the radiopharmaceutical research efforts at ORNL and in other DOE programs. This effort is directed toward measurement of necessary nuclear data and optimization of the nuclear parameters for preparation of radionuclides, development of innovative radiochemical and radioisotope processing procedures, and development of radionuclide generator systems. In addition to these macroscopic studies, methods for attachment of radioisotopes and targeting to cellular components for therapy of cancer and virus-infected cells are being explored. An important resource for this research is the ORNL High Flux Reactor (HFIR) which has the highest steady state thermal neutron flux and most versatile sample irradiation facilities available in any reactor in the U.S. Activities include assessment of the best routes for preparation, evaluation and optimization of processing technology, and development of radionuclide generator systems which provide through decay of HFIR-prepared radioisotopes, daughter radioisotopes of biomedical interest.

71. Commercial Exploitation of Electron Beam Sterilization of Infectious Hospital Waste

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The University of Miami (UM) proposes to demonstrate the feasibility of using electron beam irradiation to disinfect hospital waste and also to render the waste unrecognizable by shredding. Electron beam irradiation of medical waste in order to disinfect it to safe levels is emerging as an attractive treatment alternative prior to disposal as a nonhazardous material. The process is capable of any level of disinfection up to full sterilization by controlling the dose received by the waste material. Other advantages include nominal electric power requirements, no steam needed, no residual heat in the treated waste, and no harmful emissions. Disadvantages include high capital costs and the need for material processing for size reduction and to render the waste unrecognizable. Estimated costs of the electron beam process range from $0.15 to $0.40 a pound. These costs compare favorably with the current Miami cost for hauling waste to a commercial incinerator of $0.25 a pound. The technical and economic feasibility of the process has been supported to date primarily through engineering studies. However, in order for a new technology to be adopted over more traditional approaches to disinfecting hospital waste it must be demonstrated in a real environment under large scale conditions. Such a demonstration allows the engineering and development of working systems that can be realistically evaluated by the hospital community. The purpose of this proposal is to implement such a demonstration. The work will be performed in close collaboration with the staff of Jackson memorial hospital, which is the second largest hospital in the nation. In March 1997 installation of the electron accelerator and associated equipment was completed at the Jackson memorial hospital. The present proposal requests fund for conducting the following five tasks: 1) Operational testing of electron beam medical waste system to determine treatment efficiency and reliability, 2) Dosimetry studies utilizing surrogate red bag waste material, 3) Inactivation of indicator microorganisms in surrogate and actual red bag waste, 4) Processing medical waste (red bag) to render material unrecognizable and, 5) Evaluate the use of pulpers in conjunction with electron beam irradiation for treatment of medical waste.

[72. Intentionally Omitted]

Medical Applications:

Radiopharmaceuticals

73. Physiological Imaging

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To investigate physiological and neurochemical mechanisms underlying human brain function and the effects of psychoactive drugs on these processes with special emphasis on the investigation of the role that dopamine has on drug reinforcement and addiction. We have more recently started an investigation of the effects of normal aging on the human brain dopamine system and its functional significance. A secondary objective is to identify clinical applications for PET in oncology and to determine if they can be translated to SPECT. We will use the new 4 Tesla MRI to assess the interaction of abused drugs with specific cerebral circuits as well as the effects of age on these processes. Until now most of the work has involved PET methodology; with SPECT and MRI now available at BNL, we will be able to assess comparability between imaging modalities and to investigate the functional role of the neurochemical processes with PET and SPECT.

74. Radiotracer Chemistry and Neuroimaging

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The Brookhaven PET Program is a core element of the Brookhaven Center for Imaging and Neuroscience. The program had its origins in basic research in chemistry. A unifying central theme is radiotracer chemistry and research related to the advancement of PET as a scientific tool in studies of the living human and animal body with special emphasis on problems in the neurosciences. PET studies are supported and guided by a number of basic neuroscience methodologies including microdialysis in freely moving animals. Parallel research efforts in radiotracer chemistry include the development of targetry systems for the production of medically important nuclides such as Fluorine-18, Iodine-123 and Iodine-124; a new effort on the development of targetry for use with low energy, high beam current accelerators; the implementation of new microsynthetic technologies; and novel approaches to image analysis and the kinetic analysis of PET data.

75. Positron Tomographic Imaging of Tumors using Monoclonal Antibodies and Peptides

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Basic investigations of the molecular biology of tumors have led to the generation of proteins and peptides which might be exploitable as carriers for the selective delivery of radionuclides to malignant cell populations. The appeal of cell-specific approaches for tumor diagnosis and therapy is exemplified by the intense effort attempting to develop radiolabeled monoclonal antibodies (MABs) for these applications. Labeled peptides specifically reactive with receptors present on human tumor cells represent a similar strategy for achieving selective targeting. The long-term objective of this research is to use positron emission tomography (PET) to enhance the clinical utility of radiolabeled MABs and peptides. The ability to accurately quantify labeled MAB and peptide distribution in realistic animal models and eventually, humans, is important for several reasons. Quantification of tracer dynamics by PET could be used to increase the effectiveness of radioimmunotherapy treatment planning and also to determine the feasibility of using labeled peptides for therapy. The capability of more reliably quantifying tissue activities *in vivo* also could facilitate evaluation of strategies for enhancing tumor uptake such as the up-regulation of antigen or receptor concentration with biological response modifiers, or modification of tumor hemodynamics using hyperthermia. In this research project, we propose to apply promising radiohalogenation methodologies developed in our laboratory for labeling MABs with ^{124}I. In addition, we shall adapt these strategies for labeling peptides with ^{18}F and ^{124}I, an application where maintaining specificity and stability after radiolabeling will be an even greater challenge. Our work plan will focus on analogues of "-melanocyte stimulating hormone (MSH), a peptide which has been shown to bind specifically to receptors present on melanomas. "-MSH is of particular interest because cyclic and D-amino acid substituted analogues are available with enhanced stability and prolonged biological potency in vitro. Thus, we can investigate the effects of these alterations in peptide structure on in vivo behavior.

76. Treatment of Neoplastic Meningitis with [I-131/I-125]IUdR

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The objective of the proposed research is to establish the potential of 5-iodo-2'-deoxyuridine [IUDR] radiolabeled with ^{125}I, an Auger electron emitter, or ^{131}I, a beta emitter, in the radiotherapy of neoplastic meningitis. To this end, experiments have been designed to examine the therapeutic merits of DNA-incorporated ^{125}IUDR and ^{131}IUDR in nude rats bearing human neoplastic meningitis within their spine after the intrathecal administration of radiolabeled IUDR. Experiments will be carried out to determine a) the dose required to ensure that tumor cells in S phase incorporate a lethal quantity of radioactivity, b) the length of drug infusion time required to kill the entire growth fraction of the cancer, and c) the optimal time interval for scheduling sequential treatment courses to kill tumor cells subsequently recruited into cycling. The maximum tolerable dose of therapeutic agent that can be administered with acceptable side effects will be assessed in nontumor-bearing animals. The therapeutic efficacy of ^{125}IUDR will then be determined and compared with that of ^{131}IUDR. In addition, the therapeutic effects of radiolabeled IUDR and methotrexate [which enhances IUDR uptake and modulates the cell-cycle parameters] will be assessed. The approaches described

in this application should provide an opportunity for (a) the selective killing of dividing cancerous cells, thereby leading to a prolongation in the survival of animals with neoplastic meningitis, and (b) monitoring tumor response to treatment [onset of paralysis, animal survival, and scintigraphic detection using ^{123}IUDR]. We expect that these preclinical studies will form the basis for the clinical trials that are being planned in our institution. These studies will assess the therapeutic efficacy of radiolabeled IUDR in patients with neoplastic meningitis that are concurrently undergoing methotrexate treatment.

77. Development of Dopamine Receptor Radiopharmaceuticals for the Study of Neurological and Psychiatric Disorders

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Dopamine is a neurotransmitter (a molecule that transmits/relays messages from one part of the brain to another) which is responsible for several aspects of normal human behavior. There are various proteins in the brain that interact with dopamine. These interactions are necessary for normal human functions such as mood, emotion, movement etc. Interruptions, either in the availability of dopamine or in the interaction of dopamine with the proteins can result in anomalous behavior and various diseases. These can range from movement disorders such as Parkinson's disease, Tourette syndrome, tardive dyskinesia, to substance abuse and to the more complex neuropsychiatric illnesses such as depression, autism and schizophrenia. Our objective is to develop radiotracers (or markers) for dopamine and the proteins it interacts with so that the mechanisms by which the interactions occur can be followed in the brain using imaging methodologies such as positron emission tomography (PET) which will eventually be extended to human studies. These studies will enable us to understand the role of dopamine in normal and abnormal human behavior. Proteins, like the Dopamine D-2 receptors exist in two states, a high affinity state and a low affinity state. The high affinity state of the dopamine D-2 receptor is the functional state of the receptor. In pathophysiological disorders involving the dopaminergic system there could potentially be an increase in the population of high affinity states without a change in receptor density, resulting in a dysfunction of the receptor system. Therefore, development of radiotracers for the measurement of the high affinity state of the dopamine D-2 receptors will be appropriate for the *in vivo* assessment of potential anomalies in the function of the dopamine D-2 receptors. In order to successfully develop such tracers for purposes of *in vivo* imaging, the concept of silent agonist (defined as ligands that bind preferentially to the high affinity state of the receptor without eliciting a cellular response) is introduced. In this project, radiotracers based on silent agonists that will allow the *in vivo* evaluation of high-affinity states of dopamine D-2 receptors using PET are proposed.

78. Experimental Medicine Development of Radionuclides

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The goals of this program are to develop the chemical methods for the introduction of radioisotopes into pharmaceuticals and biologically active compounds, and to evaluate the potential efficacy of these radiolabeled compounds to study physiological processes and mechanisms in both normal and diseased states. This program also complements continuing research efforts in quantitative *in vivo* physiological measurements using PET and SPECT as well as the development of new

detection systems and image reconstruction software. Our approach is to use readily available generator produced (^{68}Ga, 82Rb, ^{122}I) and cyclotron produced (^{18}F, ^{11}C, ^{13}N, ^{123}I) isotopes as labels for novel radiotracers. These isotopes possess desirable properties which allow one to easily incorporate them into biologically active compounds. We intend to attach these isotopes onto markers of metabolic and cellular function. The strength of our program lies in the chemistry of the short-lived positron emitting isotopes (^{18}F, ^{11}C, ^{13}N and ^{15}O), which are produced by our RDS-III cyclotron, housed in the Biomedical Isotope Facility at LBNL. The setting of this cyclotron has enabled us to more readily develop labeling strategies for incorporation of ^{18}F and ^{11}C into new radiotracers. We are developing new target systems for the cyclotron (^{64}Cu, ^{76}Br, ^{124}I) to enhance our isotope production capabilities and increase our options for new tracer development. We are also advancing automated radiochemistry with the design and fabrication of an electrophilic fluorination chemistry unit for use with ^{18}F-fluorine gas and automation of the ^{122}Xe/^{122}I generator. We have a strong radiopharmaceutical chemistry development program which is not only synthesizing new radiolabeled tracers for neurologic, oncologic and cardiologic applications but also developing the strategies and techniques to label these tracers efficiently and in high yield. Neurochemistry imaging goals are served by receptor-based tracers for the a2-adrenergic (RS-15385-fluoropropyl), steroidal (fluoroestrogens), sigma (iodo- and fluoro-benzylpiperadine analogs), mitochondrial (Complex I, iodo- and fluoro-rotenones) and vesamicol receptor (fluorobenzyl-trozamicol) systems as well as the metabolic tracers for the glucose (fluorodeoxyglucose), and dopaminergic (fluoro-meta-tyrosine) pathways. Oncology applications include synthesis and evaluation of radiotracers for the epidermal growth factor receptor (iodo- and fluoro- quinazolines; breast tumors), steroid receptors (fluoroestrogens; breast tumors), tumor specific proteins (labeled DNA aptamers; breast tumors) and BNCT (labeled boron containing compounds to evaluate distribution characteristics; brain tumors). Cardiac imaging radioparmaceutical development includes fluoro- and iodo- rotenones (mitochondrial markers) and ^{122}I labeled quarternary amines as flow agents.

79. Labeling Chemistries for Arsenic Radiopharmaceuticals

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From 1985 through 1992 OHER supported the development of a Se-72/As-72 PET generator in the Medical Radioisotope Research Program at Los Alamos. Support was also provided through the early 1990's for the development of radiolabeling methods for arsenic radiopharmaceuticals for PET (As-72) and therapy (As-76). We also developed some strategies for the covalent labeling of molecules with radioarsenic for pharmaceutical applications. Under a program funded through DOE Office of Non-Proliferation and National Security, we have established a Cooperative Research and Development Agreement with Technology Commercialization International (TCI), a U. S. company specializing in the commercialization of technologies that can mutually benefit the economies of former Soviet countries and U. S. interests. Specifically the company is negotiating with LANL/DOE to license the patented generator technology. Under this agreement a collaboration has been established between the LANL Isotope Program and the Isotope Production Group at the Institute for Nuclear Research in Troitsk, Russia to develop reliable methods of routine production of the SE-72 parent to support a commercial generator system. We have successfully demonstrated labeling of molecules with arsenic radionuclides adapting the Meyer method of nucleophilic substitution. Specifically we synthesized radiolabeled arsonoacetic acid and arsonopropanediol using this approach. We have also generated evidence that it is possible to label primary amines existing in peptides with radioarsenic using a carbodiimide-mediated coupling reaction. This is

significant since many pharmacologically active molecules are proteins or polypeptides. Through the process of systematic reduction in the size of natural peptides, it has become apparent that certain portions of the peptides are more important than others in terms of retaining biological activity. Identification of these active regions allows chemists to synthesize analogs for endogenous peptides that contain the smallest sequence possible without compromising the biological activity of the peptide. Since it is possible to label peptides with radioarsenic, numerous radiopharmaceuticals could be envisioned for both imaging and therapy. The support for this project will be used to further the development of such pharmaceuticals.

80. MAB for Study of Metastases

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The central objective of this project is to develop efficient methods for radioisotope therapy for carcinomas and test these methods in rodent model systems. Specific aims: 1) Develop monoclonal antibodies (MABs) which bind to tumor vasculature. 2) Identify peptides which accumulate specifically in rat tracheal carcinomas grown at different anatomical sites. 3) Test the efficiency of targeting agents as CHX-DPTA conjugates with ^{111}In and ^{213}Bi. 4) Determine the extent of cellular damage to tumor cells, vascular cells, and adjacent normal tissue induced by ^{213}Bi alpha particles. The major goal of this project is to identify monoclonal antibodies or peptides which bind selectively to tumor vascular endothelia, to be used for targeted radioisotope therapy. This includes vasculature of tumors grown at different anatomical sites since the ultimate challenge of radioimmunotherapy is destruction of metastatic foci. Both hybridoma and phage peptide display library technologies are used. It is now possible to target a large fraction of the injected dose to specific sites in minutes. Different radioisotopes can now be considered for use in radioimmunotherapy. A short half-life, high LET radionucleotide should induce cellular damage in a range limited to 6–10 cell diameters. We have targeted the alpha emitter, ^{213}Bi, to the lumenal membrane of Balb/c lung vasculature using MABs. Assessment of vascular cells, adjacent epithelium, and surrounding tumor cells allows evaluation of alpha irradiation damage *in vivo*. It is anticipated that vascular targeting of alpha emitting isotopes for cancer therapy will be effective through destruction of tumor blood supply as well as direct toxicity to tumor cells.

81. Nuclear Medicine and Biomedical Radioisotope Technology

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This program involves the design, development, and preclinical testing of new tissue-specific radiopharmaceuticals for use in nuclear medicine. The objectives are to develop new and improved radiopharmaceuticals for use in nuclear medicine procedures for the early detection, diagnosis and therapy. In addition, new agents are evaluated in collaborative programs in conjunction with universities, research clinics, and other research institutions through the Medical Cooperative Programs for further preclinical study and clinical evaluation. Agents are supplied from ORNL as radiochemicals not approved for human use, and it is the responsibility of the collaborating institutions to obtain the required approval for human testing. In conjunction with this effort is the development of radionuclide processing techniques and radionuclide generator systems, which is supported by a complementary project. The approach for this research involves an interdisciplinary research team focussed on the development of new radiopharm-

aceuticals with key technical expertise in the areas of synthetic organic chemistry, medicinal chemistry, biochemistry and radiochemistry. The development of new agents involves: 1) The design of new agents and development of new radiolabeling methods, 2) preclinical testing of new radiopharmaceutical agents in in vitro models and laboratory animals, and 3) distribution of new agents for both preclinical testing and clinical evaluation through the Medical Cooperative Programs. Following development and implementation of synthetic strategies, new compounds are systematically evaluated using animal models *(in vivo)* and/or cell systems *(in vitro)* to identify the most promising candidates for further study.

82. Receptor-Targeted Metalloradiopharmaceuticals

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The goal of this project is development of copper-labeled radiopharmaceuticals designed to exhibit affinity for the estrogen receptor. As diagnostic agents these compounds would be useful for detection of estrogen-receptor-positive breast tumors, and for planning and monitoring antihormonal chemotherapeutic approaches to control such lesions. These agents radiolabeled with copper-64 or copper-67 might also be useful at high doses for receptor-mediated delivery of radiation therapy. Specifically, it is hypothesized that square-planar copper(II) complexes of tetradentate chelating ligands can be derivatized to impart affinity for the estrogen receptor. Copper(II) complexes are synthesized that have been designed to mimic the three-dimensional molecular structure of the steroidal and non-steroidal estrogens, estradiol and diethylstilbestrol. These metal complexes, and the corresponding free tetradentate ligands, are then screened *in vitro* to determine their relative binding affinity for the estrogen receptor. Compounds exhibiting significant receptor affinity will be radiolabeled with high-specific-activity copper-64 and/or copper-67. The receptor-targeting ability of these radiopharmaceuticals is then be evaluated *in vivo* through radiotracer biodistribution studies using rodents that present a known estrogen-receptor positive target tissue.

83. Improving Cancer Treatment With Cyclotron Produced Radionuclides

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Our goal is to improve the scientific basis for tumor diagnosis, treatment and treatment follow-up based on the use of cyclotron produced radiotracers in oncology. The grant includes 3 interactive components: Radiochemistry /Cyclotron; Pharmacology; and Immunology. The radiochemistry group seeks to develop innovative cyclotron targetry, radiopharmaceuticals, and radiolabeled antibodies, which are then used to assess important unanswered questions in tumor pharmacology and immunology. Examples include selected positron emitting radionuclides, such as I-124, Y-86 and Ga-66; I-124, I-123, I-131 labeled iododeoxyuridine, C-11 Colchicine, and antimetabolites, like C-11 methotrexate; and radiolabeled antibodies, 3F8, MI95, A33, and MRK16 for application in the pharmacology and immunology projects. The pharmacology program studies tumor resistance to chemotherapy, particularly the phenomenon of multidrug resistance and the relationship between tumor uptake and retention and the tumor response for anti-metabolite drugs. The immunology program studies the physiology of antibody localization at the tissue level as the basis for novel approaches to

improving tumor localization. Also, a number of new tracers have been developed under this grant for gene imaging. These include i-131, i-124 and i-125 FIAU, fluoroiodoarabinosyluridine, a substrate for the herpes type thymidine kinase. Dosimetric studies for radioimmunotherapy are being studied to give greater insight into the physiology of tumor localization and dosimetry. *In vivo* therapy with ^{124}I, ^{125}I and ^{131}I iododeoxyuridine is being explored for targeted therapy in animal tumors.

84. Radioimmunotoxin Therapy of Experimental Human Gastrointestinal Cancer

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Recombinant fusion toxins consisting of cytokines conjugated to toxins may be useful for therapy of solid tumors. Radiolabeled fusion toxins would be capable of killing tumor cells by two different mechanisms, inhibition of protein synthesis and DNA damage. The addition of the radionuclide to the fusion toxin would allow for killing of cells within a tumor to which the fusion toxin does not bind or internalize through the cross-fire effect produced by the emitted radiation. The objective of this research project is to synthesize and purify a new class of compounds known as radiolabeled fusion toxins, in which both toxin and radionuclide tags are contained on the same growth factor (murine interleukin-4, MIL-4), that are to be tested for binding reactivity, protein synthesis inhibition and cytotoxicity in *in vitro* studies with mouse and human colon cancer cell lines and for *in vivo* binding and therapy in a syngeneic colon cancer model and in athymic nude mice bearing human colon cancer xenografts. The fusion toxin consisting of MIL-4 linked to recombinant diphtheria toxin (DT_{390}) lacking the receptor-binding domain of native DT was labeled with ^{125}I by the iodogen procedure and by the use of meta-[^{125}I]iodophenyl-N-hydroxysuccinimide (^{125}I-mIP). A recombinant adenovirus expressing MIL-4 receptor under control of the cytomegalovirus promoter (AdmIL-4r) was used to induce mIL-4r in human colon (LS174T) and mouse colon (MC-38) cancer cells, which are MIL-4r (-). Following infection with various multiplicities of infection of AdmIL-4r, cells were tested for binding of ^{125}I-DT_{390}-MIL-4 or ^{125}I-MIP-DT_{390}-MIL-4. Mouse P815 mastocytoma cells were used as a MIL-4r (+) control. The fusion toxins bound to cells induced to express MIL-4r, but not to uninfected colon cancer cells, which was blocked by excess unlabeled anti-MIL-4r antibody, demonstrating the specificity of binding. Cell proliferation assays were used to determine whether the cytotoxicity of the fusion toxin was retained following radiolabeling. Similar growth inhibition was observed in AdmIL-4r infected colon cancer cells and uninfected P815 cells. These results indicate that the radiolabeling did not destroy the binding and cytotoxic activities of the DT_{390}-MIL-4 fusion toxin. Experiments are ongoing to determine if radiolabeled DT_{390}-MIL-4 produces greater tumor growth inhibition than unlabeled fusion toxin in solid tumors induced to express MIL-4r.

85. Cyclotron & Chemical Sciences

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The major objectives of this project are: a) design of new [^{18}F]F_2 target systems and b) synthesis of ^{18}F-labeled radiopharmaceuticals for probing the dopaminergic system in health and disease states. Fluorine-18 labeled F_2 is one of the most important reagents for the preparation of positron emitting radiopharmaceuticals. Over the years, cyclotron production of [^{18}F]F_2 has been reported from various

laboratories. In all these studies only a very narrow and limited scope of the production techniques have been addressed. Recently we have embarked on the most extensive investigation of the production of this important radiofluorinating agent. The following are the specific aims in this regard: 1) An in-depth examination of various nuclear reactions for the preparation of electrophilic fluorinating agents; 2) Conclusive identification and quantitation of ^{18}F species generated in all the above nuclear reactions; 3) Investigation of different metals for the construction of target bodies; and (4) Utilization of the ^{18}F species from these target systems in radiochemical synthesis.

86. Developmental Neurobiology

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To determine the role of neurotrophic factors in neuronal development and functional plasticity following lesion, cerebral hemispherectomy (hemidecortication) is performed in children with intractable seizures. Neuroanatomical changes following hemidecortication may be responsible for functional recovery following surgery. This project is designed to determine the role of neurotrophic factors in this recovery. We have previously demonstrated that hemidecortication results in region- and age-specific changes in the expression of one neurotrophic factor, transforming growth factor alpha (TGFA) in the rodent brain. The specific aims are: 1) To determine the functional role of TGFa and its receptor, the epidermal growth factor receptor (EGF-R) in normal brain development using the following approaches: A. Determine the developmental appearance of TGFA and EGF-R in the brain. B. Determine the cell types that express EGF-R. C. Determine the effects of TGFa on these identified cell types in vitro. D. Assess the deficits in brains of mice that do not express TGFA or EGF-R. 2) To determine whether TGFA expression is important for neuroanatomical plasticity that occurs following hemidecortication using the following approaches: A. Determine the extent of plasticity of the corticostriatal system following hemidecortication using neuroanatomical tracing techniques B. Determine the extent of corticostriatal plasticity in TGFA knockout mice, or mice administered exogenous TGFA following hemidecortication.

87. Molecular Neuropharmacology

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The project focuses on positron emission tomography (PET) imaging of brain plasticity changes throughout development, after transplantation strategies in Parkinson's disease models and after cocaine or methamphetamine exposure. Following subprojects are pursued: a) Metabolic Maturation. This project studies post-natal brain development in monkeys. FDG-PET studies are used to document normative metabolic brain development from 2 weeks to 48 weeks in vervet monkeys. In addition, the ongoing project has shown significant differences between environmentally enriched and deprived animals with FDG-PET, b) Intracerebral transplantation in MPTP-Treated Monkeys. This characterizes biochemical and behavioral effects of intervention strategies in the MPTP-monkey model of Parkinsonism with FDOPA-PET and behavioral measures. Effects of growth factors in conjunction with fetal cells are being tested. Autologous fibroblasts and stem cells that are genetically modified to produce aromatic acid decarboxylase are being evaluated as local dopamine delivery systems upon exogenous administration of L-DOPA, and c) Effects of Cocaine and Methamphetamine. This project

characterizes the effects of the psychomotor stimulants, cocaine and methamphetamine, on the monkey striatal dopamine system with 6-[^{18}F]-fluoro-L-DOPA (FDOPA), [^{11}C]WIN 35,428 and PET. High- and low-dose-drug protocols are used to examine whether irreversible changes in striatal dopamine function results. We are developing ethological behavioral methods to quantify the full range of species-typical social behavior, including dominance relationships, aggression and affiliative interactions.

88. Development of Gamma- Emitting Receptor Binding Radiotracers for Imaging the Brain

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The use of high specific activity radiotracers to monitor biochemical changes in Alzheimer's disease (AD) may lead to an understanding of a temporal sequence of pathogenesis. The *sine qua non* for the diagnosis of AD is the pathologic demonstration of numerous anatomical changes such as cerebral cortical atrophy, neuronal loss, intraneuronal neurofibrillary tangle formation, and widespread deposition of extracellular beta-amyloid. These anatomical changes occur after biochemical and molecular changes have initiated the AD process. Indeed, there have now been a number of studies carried out on autopsy samples that indicate a regional biochemical change in subtype muscarinic acetylcholine receptor concentration as a common occurrence in patients with Alzheimer's disease. We have been involved in the development of radiopharmaceuticals for the muscarinic family of G protein-coupled receptors. We wish to take advantage of the dramatic improvements in current computer software and hardware to create molecular models of these receptors and their subclasses to assist in our development of selective radiopharmaceuticals of diagnostic use in positron emission tomography (PET) and single photon emission computed tomography (SPECT). We will incorporate aspects of homology, site-directed mutagenesis studies and structure-activity studies of specific lead compounds in the construction and refinement of our receptors models with a primary focus on the structure of the binding sites. Our goals are: 1) build putative muscarinic m2 and m5 subtype receptors by molecular modeling techniques. 2) perform quantitative structure-activity relationship (QSAR) analysis of 3D models of ligand-receptor complexes. 3) Build a knowledge database of quinuclidinyl benzilate (QNB) analogues for QSAR analysis in 3D. 4) Use 3D-QSAR techniques to predict activity of lead compounds from de novo designed ligands, and 5) Identify optimal new lead compounds for m2 and m5 receptors by integration of the results of molecular modeling studies with the chemical synthesis and pharmacological studies. The subcontract to George Washington University has a long term objective to develop blood-brain barrier permeable m2-selective receptor-binding radiotracers for PET and SPECT. The chemical synthesis goals are: 1) synthesize novel *in vivo* m2 selective fluoro- and iodo-derivatives of QNB analogues. 2) resolve diastereomeric mixtures of novel in vivo selective fluoro- and iodo-derivatives of QNB analogues. 3) Prepare precursors for radioiodinating and radiofluorinating if *in vitro/in vivo* tests are successful. The pharmacological goals are: 1) Determine the ability of nonradioactive QNB analogues to bind *in vitro* to the muscarinic receptor to hippocampus and cardiac tissue and to membranes from cloned cells containing a single subtype as well as cloned cells containing chimeric m2/m5 mixtures. 2) Determine the ability of the pure diastereomer nonradioactive form of QNB analogues to cross the blood brain barrier and bind selectively to the m2 subtype using *in vivo* competitive dissection studies if Ki values are below 5 nM 3) using competitive autoradio-graphic studies if Ki values are below 5 nM. 4) Determine ability of QNB analouges to cross blood brain barrier and bind selectively to the m2 subtype using *in vivo* direct autoradiographic studies.

89. Consideration of Beta and Electron Transport in Internal Dose Calculations

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This research program focuses on computational techniques for extending the MIRD schema to include explicit consideration of beta particle and electron transport. At present, the MIRD schema considers these particles to be "non-penetrating" with no allowance for particle transport. This assumption is generally valid for adult patients given the size of the organs of interest in relation to CSDA ranges. In pediatric patients, this assumptions may lead to considerable overestimates of the source organ dose. The project has focused on three broad areas. First, a revised mathematical model for the head and brain has been developed for each age of the Cristy & Eckerman phantom series. These models include internal regions such as the thalamus, cauduate nucleus, and ventricles needed to support the dosimetry of new and developing SPECT and PET neuroimaging agents. Second, an entire database of electron absorbed fractions was developed to compliment those for photons. Monte Carlo transport was performed with the EGS4 code system, the C&E phantom series, and a number of improvements to that series including a defined mucosal region for the GI tract, a prostate, a colon, an esophagus, a 3-region kidney, as well as the revised head. The third major thrust of the research has involved the development of a 3-D transport model for electron sources in both trabecular and cortical bone based upon the chord length distributions of F.W. Spiers. This work thus extends existing transport models in providing sitespecific skeletal s values for bone and marrow seeking radionuclides which account for delta-ray production, bremsstrahlung, and particle deflection due to elastic scattering.

90. Radiolabeled Androgens and Progestins as Imaging Agents for Tumors of the Prostate and Breast

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Many tumors of the breast and prostate contain receptors for progestins and androgens, respectively, and the presence and levels of these receptors can usually be correlated with the endocrine responsiveness of the cancer. These receptors also provide a potential mechanism whereby suitable radiolabeled progestins or androgens could be selectively concentrated in a manner that would permit imaging of the tumor, thus providing an assessment of the stage of the cancer and its potential for endocrine responsiveness, and diagnostic and prognostic information of importance in the selection of the most favorable treatment regimen. These receptors should be particularly useful targets for imaging in patients who are on endocrine therapy. In this project, we are proposing to prepare progestins and androgens, labeled with the single photon emitters technetium-99m and rhenium-186 and the positronemitting radionuclide fluorine-18. In both cases, ligands will be selected to have very high affinity for the respective receptor, low affinity for blood and non-specific binders and to be reasonably resistant to metabolism: a) Structural template analysis and molecular graphics will be used to design N2S2 complexes of a bisbidentate or tetradent form whose structure mimic that off a steroid. b) Cyclopentadienyl tricarbonyl organometallic units will be incorporated into steroid conjugates designed to have high receptor binding affinity, and methods will be developed to prepare these in TC-99m and RE-186 labeled form at high specific activity. c) F-18 labeled progestins in a promising 16A, 17A-ketal series will be prepared, and an alkene halofluorination method developed to

produce 6-fluoro-6-dehydro 9b, 10a-retroprogestins with promising uptake characteristics. d) Halofluorination will be used to explore 11b-fluoroandrogens that have high receptor affinity and are resistant to metabolic defluorination. these compounds will be evaluated *in vitro* by receptor binding assays and lipophilicity measurement, and tissue distribution studies with the radiolabeled progestins will be done in estrogen-primed rats using the uterus as a target, and with the radioandrogens in estrogen-treated rats using the prostate as a target. Ultimately, in collaborative studies, these radiopharmaceuticals are to be used with SPECT or PET to image the receptor-positive tumors.

91. Advancing PET Science for New Measures of Brain Function

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This research project advances the analytical power of quantitative positron emission tomography (PET) so as to reveal in the living, but failing, human brain those flaws in complex neurochemical interrelationships which are crucial to degenerative cerebral disorders such as Alzheimer's and Parkinson's diseases. New strategies are introduced for more comprehensive exploration of neurochemical anatomy in the living human brain by 1) basing molecular design of potential tracers on considerations of regional as well as fine target structure, 2) making possible nearly concomitant kinetic measures of multiple tracers which are individually targeted and, 3) introducing correlational analysis schemes optimized to dissect meaningful biological insights from these new data complexities. This project should introduce a richer picture of living brain function in degenerative disease which is expected to impact directly the design and development of therapeutic and preventive drugs, to monitor objectively gene therapy efficacy, and to allow an earlier identification of those patients who are most like to benefit from therapy or preventive measures.

92. Novel Strategies for the Formulation of New Site-Directed Diagnostic and Therapeutic Radiopharmaceuticals

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The principle objectives are to investigate the potential of using hydroxymethylphosphine (HMP)-based chelate frameworks for labeling receptor-avid peptides with Tc-99m(Re-188) peptides, that target specific receptors on colon cancer cells, as potential radiopharmaceuticals. New colon cancer specific peptide derivatives will be synthesized by SPPS techniques for conjugation with Tc-99m(Re-188) specific BFCAs. Effects of conjugation and metallation on *in vitro* binding affinities of the peptides with CACO-2 cells will be determined. The Tc-99m(Re-188) labeled agents with high binding affinities will be evaluated in tumor-bearing nude mice to determine their ability to selectively localize in human colon cancer xenografts. Significant progress has been made in synthesizing multidentate HMP liands that form stable and well-defined TC-99M and RE chelates in high yields. For example, a tetradentate P(2)S(2) ligand (i.e., a bis HMP-bis thia ligand) selectively forms the trans-dioxo- Tc-99m(V)-p(2)s(2) and trans-dioxo Re(V)-p(2)s(2) complexes at low ligand concentrations. These are the first multidentate HMP ligands to be reported in the literature. Efforts are now being directed to forming P(2)S(2)-conjugated peptides for Tc-99m labeling. Synthesis of receptor-avid peptides with high binding affinities are progressing, however, only one radiolabeled

analogue has been made. Preliminary biodistribution studies demonstrate tumor uptake, however, other peptide analogues must be made to improve *in vitro* binding affinities and biolocalization properties.

93. Boron and Tin in Nuclear Medicine: The Development of Reactive Solid-State Reagents for use In PET and SPECT

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We propose to develop new solid-state organometallic reagents to be used in the synthesis of radiopharmaceuticals containing fluorine-18 and iodine-123 for use in positron emission tomography and single photon computed tomography. We propose to develop reactive solid-phase, organometallic reagents based on inorganic (alumina, silica, etc.) and organic (polystyrene, polysaccharied, etc.) matrices which will be modified by attachment of both organoborane and organotin reagents. The utility of the reagents will be evaluated by synthesizing neuroactive agents containing short-lived isotopes designed to evaluate intraneuronal effects as well as amyloid diseases such as Alzheimer's disease and type II diabetes mellitus. We have successfully synthesized an iodine-123 labeled Congo Red derivative and used it to evaluate amyloid deposition in cyclic neutropenic dogs which are subject to extensive amyloid deposition. The agent has also been evaluated in hamsters with amyloidosis. The results have been encouraging and we are planning to initiate human clinical trials in 1997.

94. Labeling of Receptor Ligands and Other Compounds with Halogen

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This proposal is a continuation of work on the development of radiolabeled ligands for steroid hormone receptors. The work is part of a collaboration with the research group of Professor John Katzenellenbogen at the University of Illinois. Work focuses on the development and assessment of receptor ligands labeled with fluorine-18, carbon-11 and technetium-99m. New ligands for the estrogen receptor labeled with fluorine-18, carbon-11 and technetium-99m will be synthesized and evaluated. Ligands designed for technetium and rhenium developed by our collaborators will be radiolabeled and evaluated. A final area of research will be the preclinical and preliminary clinical evaluation of fluorine-18 labeled ligands for the androgen and progestin receptors. binding affinities to hormone binding globulin (SHBG) that may be beneficial for both the target uptake and decreased metabolism of ligands for the estrogen and androgen systems. We are studying ligands with varying affinities to SHBG in several animal models to further evaluate this hypothesis.

95. Preparation of Radiopharmaceuticals Labeled with Metal Radionuclides

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The current proposal plans to continue to investigate these general areas. Specific emphasis in the upcoming funding period will be placed on the development and application of radiopharmaceuticals labeled with metal radionuclides and copper. An automated system for the production of various copper radionuclides will be developed. New chelates and targeting systems will be synthesized and investigated. These systems will be, in general, applicable to copper, but in certain instances, to radionuclides of indium and gallium. We are evaluating copper-64 in animal models as an agent for radioimmunotherapy. This area of work has the potential to provide a new isotope for therapy that can be produced on all biomedical cyclotrons. Other studies being carried out are the development of new peptide radiopharmaceuticals that can be labeled with copper and gallium. We are continuing our work on the metabolism of copper labeled proteins and peptides. Cu-64 labeled antibodies have been found to cure tumors in animal models. Large quantities up to 1 curie of copper-60 and copper-64 have been produced. New ligands for copper, indium and gallium are being synthesized and evaluated.

Medical Applications:

Instrumentation

95a. A Center of Excellence for Laser Applications in Medicine

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The objective is the continued development of a university hospital-based center for laser medical research and clinical application. This will be accomplished in the combined setting of a leading teaching hospital, the University of California Irvine Medical Center (UCIMC), and its major outpatient laser surgicenter and research facility, the Beckman Laser Institute and Medical Clinic (BLIMC). This multidisciplinary program spans basic science and clinical boundaries with the ultimate goal of translating research and technical knowledge into new diagnostic and therapeutic modalities. Interdisciplinary teams will achieve this through: 1) generation of basic biophysical data/observations on photon interaction with cells and tissues; 2) characterization of appropriate laser parameters (wavelength, fluence, etc.) to produce specific molecular and structural alterations in cells and tissues; 3) interfacing of lasers with optical and computer devices to perform specific cellular and tissue manipulations/analyses; 4) development of new or improved integrated laser systems for clinical diagnosis, analysis, and treatment; 5) transfer of these technologies to the private sector; 6) training of clinicians, nurses, and technicians with respect to the devices and modalities referenced above; and 7) advanced education of students (undergraduate, graduate, and post-graduate) related to the above activities. We propose to accomplish the above objectives in key programmatic areas: *Dermatology*: 1) determination of optimal treatment parameters in conjunction with controlled cooling for the clinical management of port wine stain birthmarks and other hypervascular dermatologic pathologies (a collaboration with Lawrence Livermore National Laboratory); and 2) development of optical Doppler tomography for high resolution tomographic imaging of blood flow in human skin. *Otolaryngology*: Development of a prototype feedback control system for laser-assisted cartilage reshaping in: reconstructive surgery of head and neck congenital defects, joint replacement, and burn treatment. *Gynecology*: The use of photosensitive drugs, in combination with integrated laser and fiber optic delivery systems, are being developed for: a) treatment of dysfunctional uterine bleeding; b) treatment of precancerous cervical disease; and c) diagnosis of ovarian cancer. *Breast Cancer*: A non-invasive fiber optic system is being developed for the early detection and characterization of breast cancer. *Wound Healing*: This project employs photodynamic targeting of macrophages to modulate the wound healing process. *Ophthalmology*: 1) use of ultraviolet (excimer) and infrared (Free Electron Laser at Los Alamos National Laboratory) lasers for refractive surgery; and 2) use of photodynamic therapy for virus-induced retinitis in AIDS patients. *Pulmonary Medicine*: The use of lasers to increase the effectiveness of lung volume reduction surgery will be evaluated. *Dissemination*: The extensive BLIMC dissemination program will provide: 1) clinical training; 2) student education; and 3) technology transfer of new technologies and medical therapies. The existing corporate relations/technology transfer program will be expanded to facilitate the movement of ideas and preliminary research into commercial products. This is a key element in the realization of the DOE program goals and will be expanded through a jointly-funded "Photonics Incubator" program with the U.S. Department of Commerce.

96. Scintillation Materials for Medical Applications

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This phase is a continuation of our work on the basic physics and improvement of performance of scintillators, primarily used in Positron Emission Tomography. The next three years will be devoted to a full exploitation of results obtained earlier. Dominant among these is the importance and understanding of energy transfer which determines the overall efficiency of a scintillator. Good models of scintillation processes now exist but must be refined, proved and applied to the development of yet better materials. To this end emphasis will be placed on the importance of transport properties (photoconductivity, scintillation under applied fields) and the role of carrier traps as evidenced by thermoluminescence. A continuing effort will be devoted to find alternatives to lutetium containing compounds.

97. High-Field Magnetic Resonance Imaging

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The Brookhaven Center for Imaging and Neuroscience is dedicated to basic and biomedical research, and to integrating data from positron emission tomography (PET), magnetic resonance imaging (MRI), and single photon emission computed tomography (SPECT), in order to investigate the synergistic uses of multiple imaging modalities in studies of the human and animal brain, as well as other organs. The Imaging Center is being built upon the Brookhaven PET Program, expanding it to include two other imaging modalities, MRI and SPECT. The fully integrated Center was developed in stages. A recent stage was the establishment of High-Field MRI Laboratory. Construction of a new building for this Laboratory has just been completed. It is located across the street from the PET Laboratory. An MRI instrument that utilizes a superconductor magnet with a field strength of 4 Tesla, the largest used for humans, was commissioned in FY 1996. This is a cutting-edge instrument for activation studies, for in vivo spectroscopy, and for further developing relaxographic imaging, which was originated in this MRI group.

98. Synchrotron Medical Research Facility

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Provide support personnel and supplies for the X17 superconducting wiggler and cryogenic facility during operation periods dedicated to medical research. The research is coronary angiography, computed tomography, radiotherapy, bronchography, and mammmography. The wiggler operates 25% of the time for medical and biomedical research programs on the X17 beamline. It has been operated now for many years in support of these programs. The medical facility will continue to operate throughout this coming year with this support.

99. Multiple Energy Computed Tomography for Neuroradiology with Monochromatic X-ray from the NSLS

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The goal is to establish the performance of a monochromatic computed tomography system, and to apply the system to certain clinical research programs, including assessment of the composition of atherosclerotic plaques in the carotid artery, using the system in the dual-photon absorptiometry mode to separate cholesterol/lipid, fibrous, and calcified tissues. A monochromatic computed tomography system, Multiple Energy Computed Tomography (MECT), is being developed at the X17B2 superconducting wiggler beamline, National Synchrotron Light Source, for imaging the human head and neck. It employs a horizontal fan beam and a subject's chair rotating about a vertical axis. MECT's narrow 0.2% energy bandwidth eliminates beam-hardening artifacts. Experimental results indicate that, compared to conventional CT (CCT) for the same spatial resolution, subject absorbed dose, and slice height, MECT produces images with less noise and larger image contrast resolution. Also, with the beam tuned above the iodine K-edge, contrast is 20-fold larger for MECT compared to CCT of the same mean beam energy. Mect also would allow efficient implementation of the dual-photon absorptiometry (DPA) method that gives separate images of the low-Z- and the intermediate-Z-element tissues. Presently, MECT uses a two-crystal monochromator with flat Si-111 Laue crystals, and a CDW04-photodiode linear array detector. Completing the clinical MECT system requires constructing two new monochromator sections with bent Laue crystals, and the patient's chair. Clinical research will include DPA imaging of atherosclerotic plaques in the carotid artery to distinguish between the tissues—lipid/cholesterol, collagenous, and calcified.

100. SPECT Assay of Radiolabeled Monoclonal Antibodies

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The long term goal is to improve the capability of single photon emission computed tomography (SPECT) to quantify radiolabeled monoclonal antibody (MAB) biodistribution. The principal objective of this project includes the development of ultra high resolution parallel hole and pinhole collimation for SPECT quantification of therapeutic-level, intratumorally administered I-131-labeled Mabs. Our research has focused on the development of ultra-high resolution, high energy parallel hole and pinhole collimation, and reconstruction algorithms, for SPECT imaging of I-131-labeled Mabs. We have accomplished: 1) An ultra-high resolution parallel collimator insert that can be mounted on one head of our SPECT system and can be indexed about an appropriate axis has been designed and built. This collimator is being evaluated using experimentally acquired scans of line sources and phantoms. 2) An ultra-high resolution SPECT pinhole collimator has been designed for imaging I-131. This collimator is currently being built to our specifications. 3) Initial studies of the effect of the pinhole insert material on I-131 tumor imaging has been performed. In particular, the potential use of lead and tungsten pinhole inserts for high resolution SPECT imaging of intratumor activity was investigated using experimental point source measurements and photon transport simulations. 4) Filtered backprojection and maximum-likelihood expectation-maximization algorithms for SPECT pinhole imaging has been substantially enhanced.

101. Positron 3D Imaging Instrument

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The primary objective of this project is the development of advanced detector concepts for the imaging of radio-labeled tracers in humans and animals with substantial improvements in spatial and temporal resolution. TASK I: To overcome the limitations in spatial resolution and maximum event rates that conventional PET tomographs have, we are developing a detector module consisting of a group of small, high density LSO (Lu2SiO5:Ce) scintillation crystals coupled on one end to a square phototube for timing information and coupled on the opposite end to an array of silicon photodiodes for position information. This included development of noise photodiode arrays and custom integrated charge amplifiers that can provide the signal-to-noise necessary to measure the depth of interaction in the crystal to correct the off-axis radial blurring caused by parallax error. The immediate goal is a 43,000-crystal tomograph for imaging the brain with <2 mm resolution in 3D. We are collaborating with a commercial U.S. positron tomograph manufacturer who will supply LSO crystals for detector modules of our design. TASK II: To overcome the limitations in photoelectric stopping power, speed, and luminosity of existing scintillators for PET, we are developing new scintillators by i) synthesizing and measuring a large number of pure and doped heavy atom compounds to find those exhibiting fast fluorescence, ii) measuring the scintillation properties of optical crystals of promising compounds, iii) investigating scintillation mechanisms through the use of synchrotron radiation, and iv) using existing quantum chemistry computer programs to guide the search. TASK III: To reduce the number of breast biopsies, we are developing a compact PET tomograph and a compact gamma camera for the non-invasive clinical identification of x-ray mammography false positives.

102. Biomagnetism Research

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Construct and test full-head biomagnetometer for the human brain.

103. DOE Center of Excellence in Medical Laser Applications

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An engineering network of collaborating medical laser laboratories are developing laser and optical technologies for medical diagnosis and therapy and are translating the engineering into medical centers in Portland OR, Houston TX, and Galveston TX. The Center includes the Oregon Medical Laser Center (Providence St. Vincent Medical Center, Oregon Health Sciences University, and Oregon Graduate Institute), the University of Texas-Austin, Texas A&M University, Rice University, and the University Texas Medical Branch-Galveston. New members are the University of Oregon, Eugene OR, and Case Western Reserve University with translation to the University Hospitals of Cleveland. DIAGNOSTICS include reflectance, fluorescence, Raman IR, laser photoacoustics, optical coherence tomography, and several new video techniques for spectroscopy and imaging. THERAPIES include photocoagulation therapy, laser welding, pulsed laser ablation, and light-activated chemotherapy of cancer (photodynamic therapy, or PDT). Medical applications reaching the clinic include optical monitoring of hyperbilirubinemia in newborns, fluorescence detection of cervical dysplasia, laser thrombolysis of blood clots in heart attack and brain stroke, photothermal coagulation of benign prostate hyperplasia, and PDT for both veterinary and

human cancer. New technologies include laser optoacoustic imaging of breast tumors and hemorrhage in head trauma and brain stroke, quality control monitoring of dosimetry during PDT for esophageal and lung cancer, polarization video reflectometry of skin cancer, laser welding of artificial tissue replacements, and feedback control of laser welding. Find us at: http://ee.ogi.edu/omlc/doe.

104. Center of Excellence–Microlaser Microscope

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This grant has two major components: a central project using vertical cavity surface emitting lasers (VCSELS) to make a confocal microscope "on a chip", and a group of some 20 associated projects in real-time medical imaging and diagnostics that share much of the expertise and experience of the microlaser microscope but extend that into other fields of biomedical endeavor. The associated projects are funded outside the grant, using the Center for initiation of new directions and for expertise in microscopy and optical diagnostics. The microlaser microscope has demonstrated confocal imaging using the VCSEL array as both source and detector (which makes it inherently confocal), and has explored electronic and optical parameters of VCSELS in this application. It is now ready for incorporation in a commercial microscope. Some of the associated projects are: Tandem Scanning Ophthalmoscope: a hand-held confocal ophthalmoscope using no moving parts. Video-rate CSLM: a confocal microscope for imaging at a cellular level as deep as 500 μm into living tissue. Calibrator for confocal microscopy: A calibration phantom for confocal microscopy. Stabilized imaging for the scanning laser ophthalmoscope: a means to remove motion artifacts from confocal images of the retina (or other tissues). Multiply scattered light tomography: an extension of VCSEL technology to deep imaging of the retina in a confocally indirect mode. Eye movements in visually handicapped: use of a confocal scanning laser ophthalmoscope to observe learning and coping strategies. Measurement of RPE Lipofuscin: confocal techniques for diagnostics of an indicator for age-related macular degeneration. Spatially resolved refractometer: a device to measure to wavefront error caused by imperfect visual optics. Training: graduate and post-doctoral students, about 8 per year, are trained in the Center laboratories.

105. Instrumentation Sciences

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To investigate and develop the physical and methodological aspects of applying radiation measurement technology to nuclear medicine imaging techniques, particularly Positron Emission Tomography (PET). Current basic instrument projects include. 1) A benchtop version of a beta imaging intraoperative probe to assist the surgeon in finding all tumors and tumor remnants, which have been labeled by a beta emitting, tumor seeking radiopharmaceuticals, has been designed, built and tested, and is in the process of being modified for *in vivo* applications. 2) A gamma imaging probe, designed for ^{99m}Tc labeled, tumor seeking radiopharmaceuticals, is in the preliminary design and testing phase. 3) A small scintillation camera for imaging tumor seeking radiopharmaceuticals is under development with the goal of having the device placed in the mammography section of the hospital to use for immediate confirmation of suspicious findings in the mammogram. Current PET projects are: 1) Development of 3-D data collection, data correction and system calibration techniques in a manner appropriate for the new high resolution, long axial field-of-view PET systems. 2) Implementation of 3-D iterative reconstruction algorithms in 3-D Whole Body PET and evaluation of their properties. The basic physics and instrumentation of each project are

initiated with DOE funding, and currently, NCI and California Breast Research Program funds have been sought and received for the continued development of these projects.

106. Biomathematical & Computational Sciences:

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This project uses mathematical, computer, and engineering techniques to improve the value of positron emission tomography (PET) and nuclear medicine images for extracting reliable functional biological information that improves early diagnosis of diseases and monitors their treatment efficacy. Emphasis is in two major areas: 1) modeling of tracer behavior based on biological information to help the estimation of kinetic parameters from tracer kinetics measured with PET, and 2) image and signal processing/reconstruction to improve the data quality of PET and nuclear medicine studies. Active projects include a) investigation of the kinetics of FMT, a new F Dopa analog developed by the chemistry section, for its potential use to study the pre-synaptic dopaminergic system, b) modeling of tumor Ga-68 EDTA kinetics to study the BBB permeability change due to the infusion of a bradykinin analog, RMP7, used by neurosurgeons to help the delivery of anti-tumor drugs, c) development of factor analysis methods to provide a non-invasive method for getting the blood tracer time course from a dynamic cardiac PET study, d) improvement of PET-to-PET and PET-to-MRI image registration techniques to increase their reliability and usability, e) study of iterative algorithms for convergence improvement of tomographic image reconstruction, and f) performance characterization of a new axial averaging method (based on elastic image mapping) to improve the image signal-to-noise ratio of images from new PET scanners.

107. Oncology

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Develop, characterize, and validate new methods for whole body imaging of tumor metabolism and determine the clinical significance of these images. Specific Aims: 1) In breast carcinoma, what is the sensitivity and specificity of whole body FDG PET imaging in the identification of primary and metastatic lesions. 2) In renal cell carcinoma, what is the relationship between 18-fluoro-2-deoxyglucose (FDG) and the presence of viable tumor cells. Using patient survival as the gold standard of measure. The use of whole body PET for staging of residual tumor masses has been traditionally been performed with x-ray computed tomography. The specific project addresses the accuracy of whole body PET in the staging of renal cell carcinoma patients receiving immunotherapy. 3) In prostate carcinoma patients, quantitative and whole body FDG images were performed to determine the effect of suramin therapy on *in vivo* cellular metabolism. This project allows the testing of a variety of quantitative methods for the measurement of tumor uptake and therapy induced changes since the biochemical assay of prostate specific antigen is a fairly reliable reference tumor burden marker. Since these studies are acquired in both the dynamic mode and whole body mode, both data sets will be used for correlation to clinical tumor activity.

108. Positron Ring System using Anger-Type Detectors

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Build high performance 3D imaging brain PET scanner-measure and evaluate performance characteristics.

109. Center of Excellence in Laser Medicine

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This multidisciplinary program, at Massachusetts General Hospital (MGH), focuses on research and development in the area of medical applications of lasers and light. Clinical trials are not supported by this grant, but the work is aimed at producing cost-effective health care solutions. The grant supports 1) research fellowships, 2) prototype device engineering, and 3) management of the MGH Laser Center. The MGH Laser Center conducts scientific symposia designed to transfer the results of the sponsored research to industry and government representatives, medical-laser safety courses, and produces a regular newsletter describing the results of the DOE-sponsored research. Each year, this program supports 3 to 5 MD or Ph.D. research fellows, chosen by competition, who must also receive matching support from a hospital department (e.g., gynecology, urology, vascular surgery, etc.). The research is focused upon 3 areas: (1) novel laser technology, (2) optical diagnostics, (3) photosensitization for treatment of both cancer and non-cancer disease.

[110. Intentionally Omitted]

Medical Applications:

Clinical Feasibility

111. Use of SPECT in Physiological Imaging

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This research is performed in conjunction with work described in project 35.

112. Advanced Nuclear Medicine and Nuclear Magnetic Resonance Technologies for Investigating Mechanisms of Aging, Atherosclerosis, Heart Disease, Mental Disorders and Cancer

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This research project, carried out within the Center for Functional Imaging at the Lawrence Berkeley National Laboratory, is involved in developing advanced positron emission tomography (PET), single photon emission computed tomography (SPECT), and nuclear magnetic resonance imaging (MRI) systems with capabilities beyond those currently envisioned for commercial implementation. The purpose of this research is to apply new technologies to the study of atherosclerosis, heart disease, aging, neurological and psychiatric diseases, and cancer. The joint approach of new technologies and experimental physiology is applied to medical science problems by a team of physicists, chemists, mathematicians, computer scientists, physiologists, and research physicians devoted to development of quantitative methods of experimental medical science. Autoradiography, and tracer studies in animals are also used, in addition to the noninvasive methods of nuclear medicine and MRI. There is a major emphasis on mathematical modeling and statistical analyses. Early results of this work included the first demonstration of the clinical usefulness of the Anger camera and the tomoscanner. More recently, quantitative reconstruction algorithms, new compartment modeling methods, and statistical models of dynamic PET have been developed. A unique 2.6 mm resolution PET instrument and a state of the art commercial PET scanner are used to conduct clinical studies in epilepsy, Alzheimer's disease, schizophrenia, coronary artery disease, brain tumors, breast tumors and prostate tumors. Three instrument development projects are supported by this research: a PET breast cancer imager, a 2 mm resolution PET, and a proposed 10 tesla whole body magnet. Proposed work includes deployment of the xenon-122/iodine-122 PET generator for practical brain and heart blood flow imaging, PET aptamer biodistribution studies, imaging studies of gene expression, and boron compound biodistribution studies for neutron capture therapy.

113. Cardiovascular Sciences

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Development and validation of radiotracer kinetic approaches for the noninvasive study and quantitation

of blood flow, substrate metabolism, neuronal activity, membrane function and gene expression in the human myocardium and the application of these techniques in order to characterize the function of the normal human myocardium, to enhance our understanding of human cardiovascular diseases and to determine benefits and mechanisms of novel therapeutic strategies. Positron Emission Tomography affords the application of tracer kinetic principles for the noninvasive study and quantitation of regional functional processes in the human myocardium. It is thus possible to measure regional myocardial blood flow, oxidative metabolism and oxygen consumption, glucose utilization, fatty acid metabolism as well as regulatory systems such as pre- and post-synaptic neuronal control and, more recently, gene expression. These novel tools offer the opportunity for characterizing the human heart's function, alterations in cardiovascular disorders and their underlying mechanisms, responses to physiologic and pharmacologic challenges as well as the effects of therapeutic interventions. Because PET measures only the radioactivity concentrations in tissue and their changes over time, implementation of tracer kinetic principles is predicated on careful characterization of the relationship between the externally observed tissue tracer kinetics and the functional process to be studied. Most important accomplishments: 1) Simplification and validation of a tracer compartment model for C-11 acetate in normal human volunteers which demonstrates the possibility of simultaneous measurements of regional myocardial oxygen consumption and blood flow in absolute units. 2) Glucose utilization in ischemically injured myocardium in dogs can be augmented pharmacologically which enhances glucose oxidation. 3) Adrenergic stimulation of dysfunctional myocardium in patients with coronary artery disease causes a rather selective increase in regional glucose utilization suggesting superimposition of acute ischemia upon chronic hibernation and/or greater reliance on the more oxygen efficient glucose as energy fuel during enhanced mechanical demand in chronically compromised myocardium. 4) The amount of myocardium that is viable as demonstrated with PET in patients with severe coronary artery disease, poor cardiac function and heart failure predicts the magnitude of the improvement of congestive heart failure symptoms and the quality of life in response to surgical revascularization. 5) Imaging of blood flow and metabolism with PET in patients with endstage coronary artery disease considered for cardiac transplantation provides a rationale for surgical revascularization instead of transplantation. 6) Demonstration that measurements of myocardial blood flow in human volunteers are highly reproducible both, at rest and during pharmacologically induced hyperemia. 7) Elucidation of factors that account for the variable hyperemic responses of blood flow in the normal myocardium. 8) Demonstration of elevated resting blood flows and impaired coronary vasodilator reserve in patients with dilated cardiomyopathy. 9) In patients with Syndrome X or with clinical symptoms of coronary artery disease in the absence of angiographic coronary stenosis, myocardial flow reserve is normal. Administration of L-arginine as a precursor of endothelium derived relaxing factor (NO) failed to modify blood flows which argues against a defect residing at the level of the endothelium in these patients. 10) In parallel with our signal transduction experiments in the heart, we have investigated macrophage activation by bacterial lipopolysaccharide (LPS), which shares common mechanistic features and has important cardiovascular effects in sepsis and other pathophysiological conditions. 11) Ischemia and reperfusion lead to the rapid activation of ERK and JNK signaling cascades in the perfused heart. Activation of ERK was blocked by catalase, mimicked by hydrogen peroxide and calcium-insensitive whereas activation of JNK was not mediated by hydrogen peroxide and was calcium dependent. Stimulation of both ERK and JNK required protein kinase C activation. ATP depletion/repletion in cultured ventricular myocytes activated ERK and JNK with a similar time-course to the perfused heart.

114. Methodology for Evaluation of Diagnostic Performance

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Receiver Operating Characteristic (ROC) analysis is now recognized widely as the best way of measuring and specifying the performance of diagnostic procedures, because it is able to distinguish between actual differences in discrimination capacity, on one hand, and apparent differences that are due only to decision-threshold effects, on the other. Key methodological needs remain to be satisfied before ROC analysis can address all of the practically important situations that arise in diagnostic applications, however. This project employs signal detection theory and computer simulation to address several of those needs, by: 1) refining and continuing distribution of software for ROC curve fitting and for testing the statistical significance of differences between ROC curves; 2) developing and evaluating a new method for maximum-likelihood estimation of ROC curves from continuously-distributed data; 3) developing and evaluating a new ROC curve-fitting algorithm that provides more meaningful curve fits with small samples of cases; 4) developing and evaluating a new method for statistical comparison of two ROC curve estimates obtained from partially-paired data sets; 5) investigating the usefulness of "jackknifing" in reducing the bias of ROC curve estimates that are obtained from small samples of cases; and 6) developing an integrated software package for ROC data analysis.

Medical Applications:

Boron Neutron Capture Therapy

115. Brookhaven Medical Research Reactor Operations

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To provide neutrons for the Boron Neutron Capture Therapy (BNCT) program in support of clinical trials of BNCT. To operate the reactor in a safe manner and in accordance with all applicable regulations. Following tasks are either planned or completed: 1) 42 weeks of reactor availability are planned, and up to 15 patient irradiations. 2) Installation of a new secondary cooling water supply system. 3) Final design of the new epithermal shutter. 4) DOE safety review of the epithermal shutter.

116. Neutron Capture Therapy: Preclinical Research and Clinical Investigations

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The Medical Department at Brookhaven National Laboratory conducts a multidisciplinary research program in boron neutron capture therapy (BNCT) for the highly malignant brain tumor, glioblastoma multiforme. Using the amino acid analog p-boronophenylalanine (BPA) as the boron delivery agent, preclinical animal studies quantified the biological effectiveness of BPA-based BNCT relative to conventional photon radiation. Based on this work, an FDA-sanctioned Phase I/II clinical trial is underway using the epithermal neutron beam at the Brookhaven Medical Research Reactor. Two dose groups of 15 patients each have been completed; a third dose group is underway (seven patients as of January 1998). The median survival of patients in the first dose group (12.9 Gy-Eq to target volume) was better than that obtained with conventional therapy (14–18 months versus 10 months); one patient in this group is still alive at 23 months post-BNCT with no sign of tumor recurrence. In the second dose group, (18.9 Gy-Eq to target volume) the median survival was comparable to the first group and slightly better than standard treatment; five patients are still alive. Of the seven patients treated in the third dose group, (27.8 Gy-Eq to target volume) all are alive, only one has tumor recurrence, but the follow up period is too short to determine whether they will do better than the other dose groups. Most of these patients experience a better quality of life (BNCT is delivered in a single session lasting less than 1 hour) than patients in standard fractionated therapy that lasts six weeks or more. Plans are underway to further escalate the dose.

Animal studies will continue in order to further improve BPA-based BNCT and to address specific issues arising from the clinical experience such as the sensitivity of the oral mucosa to BNCT, the possibility of BNCT following photon therapy and the potential usefulness of fractionated BNCT. The BNCT program continues to evaluate new and improved boron delivery agents as well as the application of BNCT to other types of tumors. Screening of BPA for uptake in other human tumors identified lung tumors as a potential target for BNCT. Selective BPA accumulation was shown in cell culture and in nude mice with human lung tumor xenografts. A series of boronated porphyrin compounds have shown very favorable

distribution properties in tumor-bearing mice. High levels of boron in the tumor, clearance from blood and brain, and long-term retention in the tumor have been demonstrated. The effectiveness of these compounds will be evaluated in the rat brain tumor model.

117. Subcellular Boron Distribution in BNCT using Ion Microscopy

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Boron neutron capture therapy (BNCT) has the potential of damaging or killing individual tumor cells while sparing microscopically contiguous healthy tissues. This potential, however, is critically dependent upon the tumor selectivity of the boron delivery agent. Sophisticated analytical techniques are required for evaluating the efficacy of boron delivery agents at the cellular and subcellular level. Ion microscopy methodology has been developed in our laboratory with the principal objective to quantitatively image boron at the subcellular level in cell cultures and in sections of animal tissues relevant to BNCT. This objective emphasizes the unique capability of ion microscopy to isotopically image boron in biological samples and provides both fundamental and applied understanding of compound performance at the microscopic level. Ion microscopy is an imaging technique based on secondary ion mass spectrometry. The technique can be used to quantitatively localize any element or isotope from hydrogen to uranium with ppm to ppb sensitivity. Cell culture and tissue sections are prepared for ion microscopy analysis using rigorous cryogenic procedures to preserve the original chemical morphology of the specimens. A sample, under high vacuum, is bombarded with a primary ion beam. Secondary ions, ejected from the top few atomic layers of the sample, are accelerated into a double-focusing mass spectrometer. After energy and mass filtration, the secondary ions are projected onto a microchannel plate/phosphor screen assembly so that their final positions at the detector correspond to their original positions on the sample surface. The final output is recorded using a charge-coupled-device camera and ion images are produced showing the distribution of a specific element with a lateral resolution of 0.5 μm.

118. Carboranyl Oligonucleotides for Neutron Capture Therapy

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The primary aim is to synthesize boron-rich oligonucleotide analogues, which combine the advantages of both boron neutron capture therapy (BNCT) with antisense oligonucleotide therapy. These agents would then serve as a binary system for treatment of cancers. We have also completed the synthesis of novel compounds that could serve as lead agents for BNCT or as building blocks for oligomer synthesis. Oligonucleotides synthesized include analogs in which the internucleotide linkage has been modified with (o-carboran-1-yl) alkyl residues substituting the non-bridging oxygen atoms of phosphodiester moieties and oligomers containing one or more 5-(o-carboran-1-yl)-2'-deoxyuridine (CDU) units. The oligonucleotides synthesized have been characterized both chemically and biochemically. Studies on oligomer cellular uptake and egress in relevant cell culture systems have been initiated. A major obstacle of BNCT is low selectivity of known boron-containing compounds toward malignant tissue. Achieving high tumor-to-normal-tissue and tumor-to-blood drug ratios, as well as penetration into the tumor is essential. Approaches are under investigation to improve cellular uptake and targeting characteristics of the compounds. We have also initiated the synthesis of nucleic acid bases and nucleosides, including nucleosides with the unnatural L-configuration, and have begun to study their biological characteristics compared to the natural nucleosides. We are poised to investigate the biological effectiveness of some of these compounds in animal models for brain and prostate

malignancies using the reactor at Brookhaven National Laboratory. The newly synthesized compounds are being considered as building blocks for new classes of oligonucleotides. Oligomers will be rationally designed to determine their effects on protein expressed in a variety of human tumors, including gliomas.

119. Clinical Trials of Boron Neutron Capture Therapy at The Beth Israel Deaconess Medical Center and The Massachusetts Institute of Technology

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The Harvard/MIT BNCT research team comprises three principal areas of research effort: clinical trials, supporting technologies, biological studies, and fission converter beam development. In the two ongoing phase-I clinical trials five peripheral melanoma and ten brain tumor patients (nine GBM and one melanoma metastatic to the brain) have been irradiated in the M-67 epithermal beam at the MIT research reactor. Clinical results are encouraging although the studies are designed according to classical phase-I goals and are still in the midst of dose-escalation. Supporting technologies that have been developed include: MACNCTPLAN, a Monte Carlo based BNCT treatment planning software package developed for the Macintosh platform, soon to be made available in the public domain; BNCT macro- and microdosimetry; BPA fructose conversion methodology; neutron beam delivery, control, and computerized monitoring; patient support and immobilization systems; high-resolution autoradiography for quantitation of ^{10}B in tissue with subcellular resolution; and prompt-gamma neutron spectroscopy and inductively coupled plasma atomic emission spectroscopy for rapid and accurate ^{10}B quantitation in blood and bulk tissue. In the area of biological studies we are investigating biodistribution and pharmacokinetics of BPA-fructose in murine brain; measuring BPA-fructose uptake by colorectal carcinoma metastatic to rat liver; measuring BPA-fructose uptake by cultured human colon cancer cells growing directly on an alpha track detector; and microdosimetric analysis of ^{10}B in normal and cancer cells using a unique stereological approach developed in our laboratory. A complete radiation transport and engineering design has been completed of a high intensity and high quality fission converter based epithermal neutron beam. Funding has been obtained to construct such a facility in the MIT research reactor over the next two years (see project 124).

120. Idaho National Engineering Laboratory BNCT Program

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Development, deployment, and application of supporting technologies required by the DOE/U.S. BNCT Program. Specific fields of activity are: 1) boron chemistry, including tissue-boron analysis for all DOE BNCT grantees, 2) computational and experimental radiation dosimetry, treatment planning software, and treatment planning services to support the human glioma trials at Brookhaven National Laboratory, 3) reactor- and accelerator-based neutron source development to support the U.S. effort in BNCT. An integrated multidisciplinary team having internationally recognized expertise in the relevant scientific and engineering fields is in place at the INEL. Since the end of FY 1994, the INEL program has been focused on meeting DOE technological needs using our in-house capabilities in chemistry, physics, and engineering, with close collaboration with several University BNCT grantees who, prior to FY 1995 were funded through but who now are directly funded through the ER university grant system. These grantees bring additional expertise into the program in the fields of boron chemistry and radiobiology.

121. Accelerator-Based BNCT Clinical Trial

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Develop an accelerator-based BNCT facility at LBNL for clinical trials: 1) Design and fabricate an electrostatic quadrupole (ESQ) accelerator. 2) Develop a neutron-production target. 3) Design a neutron beam shaping assembly. 4) Prepare a treatment room and a treatment control system. 1) Accelerator design study (J. Kwan, C. Peters, L. Reginato, J. Staples, S. Yu): Our effort was aimed at producing a conceptual design for converting the ADAM Injector into an ESQ accelerator that is capable of delivering up 100 mA of proton beam at 2.5 MeV beam energy. It has four subprojects: a) ion source, b) poser supply , c) ESQ, and (d) extracted proton beam transport.

122. BNCT Treatment Planning Improvements

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Provide a near real-time capability for BNCT treatment planning with the MACNCTPLAN Monte Carlo code for use at New England Deaconess Hospital (NEDH) and MIT. The approach for FY 1996 was to provide for MACNCTPLAN multitasking on commercially available Pentium PCs using entirely freeware obtained from the Internet. For FY 1997 and subsequent years, enhancements and speedups of MACNCTPLAN for specific BNCT-types of treatment planning calculations are to be developed. In the one-fourth calendar year of FY 1996 available for work after the funding was received, the multitasking phase of the work was achieved. MACNCTPLAN was modified to use Fortran, Parallel Virtual Machine (PVM), and LINUX freeware, and was successfully multitasked on two PCs and several SUN workstations at LANL. Speedups scaled approximately with the number and capability of the processors as expected.

123. Accelerator-based neutron beams for neutron capture therapy

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The feasibility of accelerator-based neutron capture therapies is under investigation at MIT's Laboratory for Accelerator Beam Applications. Designed and developed under this DOE-funded project is a unique, high-current tandem electrostatic accelerator capable of generating proton or deuteron beams up to 4.1 MeV in energy. Completed in 1996, the accelerator has been used for a number of different experiments in the evaluation of the feasibility of accelerators for BNCT. The recent installation of a large-bore switching magnet (separately funded) has made available five separate beamlines on which various long- or short-term experiments are set up. Recent results include: the full characterization of neutron spectrum and yield from the Be(p,n)B reaction at proton energies between 3.0 and 4.0 MeV, design of epithermal neutron beams based on the Be(p,n)B reaction predicted to result in tumor dose rates of 13 RBE-cGy/min-mA, experimental mixed-field beam characterization of a number of beams based on deuteron bombardment of beryllium, the complete design and testing of a means for accelerator based fast neutron brachytherapy, the successful design and experimental testing of a target cooling configuration capable of removing power levels of approximately 30 kW.

124. High Intensity and High Quality Fission Converter Based Epithermal Neutron Beam for Neutron Capture Therapy

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An improved epithermal neutron irradiation facility is needed by the Boston/Cambridge based Beth Israel-Deaconess/MIT BNCT Group for current and future Phase II and Phase III clinical trials of boron neutron capture therapy (BNCT). It is proposed to construct a fission converter based epithermal beam which has high intensity, very low background, and produces high therapeutic ratios. The design has been carefully developed at MIT during the last few years. This facility would be installed at the 5 MW Research Reactor (MITR-II). The design utilizes proven technology and has a high degree of safety, exceeding the safety of the currently licensed MITR-II. If advanced clinical trials show that BNCT is a viable treatment modality, the proposed facility could provide the focal point for a regional BNCT treatment center and provide a resource for all the medical centers in the Boston and New England area. Patient irradiations will require only a few minutes, therefore, a high patient throughput could be achieved. The cost is reasonable compared to other major new cancer treatment facilities and compared to other currently proposed epithermal beam projects for neutron capture therapy. The facility would be superior to all existing epithermal beam facilities in the world, and would be superior or comparable to all other facilities which have been proposed. The project team has a proven track record in BNCT and in the construction and operation of major nuclear facilities which provides assurance that project goals can and will be achieved.

125. BNCT Real Time Dosimetry

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Design and construct a prototype realtime flux monitor for use in BNCT. The monitor will consist of small sections of neutron sensitive scintillating glass fibers connected to passive waveguides, so that all electrical components are far from the radiation field. Contact was made with clinicians at the Brookhaven National Laboratory to determine the design goals for the detector (size and number of probes etc.). Photomultiplier tubes are being compared to avalanche photodiodes as the optimum light detection method for the very high count rates expected. Selection of this component will determine the development of all data analysis electronics.

126. Pharmacokinetics and biodistribution of sodium borocaptate in patients with anaplastic astrocytoma/glioblastoma multiforme

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Boron neutron capture therapy is in theory an appealing method of treating neoplastic disease and is specifically suited for tumors of the central nervous system. In spite of a long history of clinical attempts at controlling malignant astrocytoma, definitive positive results have not yet been achieved. Tests of this concept in laboratory animal brain tumor models have consistently resulted in increased life survivals that are improving incrementally as knowledge is gained. The Ohio State University has an established multidisci-

plinary team of researchers who are committed to carrying BNCT to clinical application. This team consists of investigators in neurosurgery, radiation oncology, pathology, neuropathology, pharmacy and chemistry, and nuclear engineering. We intend to continue research in drug development, drug delivery systems, treatment response in tumor bearing animals, dosimetry planning, and neutron source development. Specific clinical activity applicable to BNCT at the OSU Comprehensive Cancer Center includes a program to test effectiveness of Blood Brain Barrier Disruption Chemotherapy for treatment of brain tumors. Phase I trials of BSH are also underway to help determine whether this compound may be useful for clinical trials. The primary goal of the clinical BNCT program is to establish a regional clinical treatment facility to provide BNCT as a management option to patients with CNS neoplastic disease at such time BNCT is shown to demonstrate a significant degree of efficacy. Specific Aims: 1) To continue laboratory research using the F98 experimental rat brain tumor model to establish the most efficacious boron delivery agent or combination of agents for BNCT. This research aims to establish methods of optimizing tumor uptake of boron as well as to test methods of improving survivals of tumor bearing animals after BNCT. Results of these studies are to be analyzed for possible clinical applications. 2) To complete the dose escalation phase I trial of BSH and document pharmacokinetic, biodistribution, and toxicity profiles of this compound. Biodistribution data are to provide a basis for dosimetry planning in order to determine if clinical treatment protocols are feasible and advisable. 3) To implement an international collaborative agreement with the Beijing Neurosurgical Institute to attain patient accrual necessary to complete phase I BSH studies. It is anticipated that this agreement will promote BNCT research in China and aid in furthering the goal of establishing BNCT as an international research and clinical investigative effort. 4) To closely monitor ongoing clinical trials at the Brookhaven National Laboratories, The Massachusetts Institute of Technology, as well as the treatment trials in Japan and Europe. Should significant improvements in patient outcome be achieved in these studies, the available resources at the Ohio State University will be utilized to establish a regional BNCT treatment facility.

127. An Accelerator Neutron Source for BNCT

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The overall goal is to develop an accelerator-based neutron source (ABNS) for Boron Neutron Capture Therapy (BNCT). Specifically, our goals are to design and confirm by measurement a target assembly and a moderator assembly which fulfill these design requirements: 1) they produce a neutron field with a quality as good as the neutron field quality for the Brookhaven Medical Research Reactor, 2) the proton current required to treat patients in reasonable times is technologically achievable, at reasonable cost, with good reliability, and with accelerator space requirements which can be met in a hospital, and 3) the target assembly removes the heat deposited by the proton beam in the target safely and reliably. The ABNS we are designing is based on an accelerated beam of 2.5 MeV protons bombarding a ^{7}Li target and thereby producing neutrons. These neutrons are then reduced to an appropriate range of energies for therapy using a moderator assembly. The Ohio University Van de Graaff accelerator is being used to test moderator assembly designs at beam currents of approximately 20 microamperes, a current about one thousandth that which would be necessary for human therapy of malignant brain tumors. This testing verifies the performance of the moderator assembly and the methods used to design it. The moderator assembly which is being tested consists of a heavy water moderator surrounded by a lithium carbonate reflector. A beam delimiter is downstream of the moderator and reflector regions of the moderator assembly. The beam delimiter is filled with lithium carbonate. The moderator, reflector and delimiter materials are encased in thin magnesium metal walls. The downstream face of the moderator assembly and the beam delimiter form the patient treatment port, which is lined with thin sheets of ^{6}Li enriched lithium carbonate in a silicon matrix.

128. Synthesis/Evaluation of Boron-Containing Nucleosides for BNCT

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The objective is to develop boron-containing compounds with the capacity for targeting brain tumors for treatment by BNCT. Specific aims: 1) To develop the methodology for synthesizing carborane containing nucleosides and analogues of deoxyuridine and thymidine in which the carbonyl group in the four position of the pyrimidine nucleus is replaced by a hyroxylboryl moiety. 2) To evaluate biochemically these boron-containing nucleosides with respect to human cytosolic thymidine kinase. 3) To correlate chemical structures with their physiochemical and biochemical parameters including the optimization of their hydrophilic/lipophilic properties to enhance tumor cell penetration and the velocity of phosphorylation. 4) To determine the *in vitro* uptake, persistence and subcellular distribution of these compounds in glioma cells. 5) To study the *in vivo* pharmacokinetics and tumor-localizing properties in tumor-bearing rodents of those appropriate nucleosides and to assess their therapeutic efficacy by BNCT in rat brain tumor models.

129. Boron-Containing Compounds for Liposome-Mediated Tumor Localization and Application in Neutron Capture Therapy

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The overall goal of this project is to identify superior boron compound candidates for the liposome-mediated delivery of boron to tumors for application in BNCT. This is accomplished through a) the identification and synthesis of appropriate hydrophilic and lipophilic boron agents; b) the incorporation of the candidate boron-containing compounds within small unilamellar liposomes; and c) the evaluation of liposomal preparations through boron biodistribution experiments in animal models. The successful application of BNCT for cancer requires the preferential concentration of significant quantities of the stable ^{10}B within tumors over normal healthy tissues. Small unilamellar liposomes have been shown to be a viable transport modality for the selective, intracellular delivery of boron-containing compounds to neoplastic tissues. Extensive animal studies have demonstrated that suitable liposomes containing the appropriate boron agent can deliver therapeutically useful concentrations of boron to tumors with the administration of relatively low injected doses. Previous investigations performed in this laboratory have identified the characteristics of boron agents that result in successful liposomal delivery. Based upon these prerequisites, new boron-containing species are designed, synthesized, and incorporated into liposomes for comparison with previously known boron compounds. The evaluation of candidate compounds for their suitability as liposomal agents is accomplished through biodistribution experiments in mice bearing EMT6 tumors (performed at Washington State University). Successful boron-containing liposomal formulations are usually characterized by relatively low injected doses of boron (10-30 mg B/kg body weight), high tumor uptake of boron (>30 ppm), and a favorable ratio of tumor boron/blood boron (3:1 or higher). Several new promising boron-containing compounds have been synthesized. Among these newly developed species, the $[B_{20}H_{17}OH_2]$ anion was most significant due to its high boron content, water solubility, and ability to form derivatives with a wide variety of organic substituents. For previously developed boron compounds encapsulated within liposomes, the most successful murine biodistribution results were obtained with water-soluble $Na_3B_{20}H_{17}NHCH_2CH_2NH_2$ (1) and lipophilic $KC_2B_9H_{11}CH_2CH_3$,(2) Sterically stabilized liposomes containing 1, at an injected dose of 19 mg B/kg body weight produced a maximum tumor boron concentration of 72 ppm at 48 h with a tumor/blood

boron ratio of 24. Similar results were obtained in murine biodistribution experiments with liposomes that incorporated both 1 and 2. A maximum tumor concentration of 71 ppm boron was observed at 30 h with an injected dose of 17 mg B/kg body weight. When the administered dose of these liposomes was doubled to 35 mg B/kg, the tumor boron concentration reached 151 ppm at 48 h with a tumor/blood ratio of 5.7. These results clearly demonstrate therapeutically useful boron delivery and the potential value of boron containing liposomes.

130. Molecular Medicine: Synthesis and *In-Vivo* Detection of Agents for use in Boron Neutron Capture Therapy

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The objectives are: a) to continue the development of boron MRI for use in BNCT patient selection; b) to continue the use of fluorine-18 labeled BPA for use in pretreatment planning for BNCT; c) to prepare unnatural amino acids containing B for use as BNCT therapeutic and imaging agents. MRI: We continue to develop boron MRI pulse sequences for use in *in-vivo* imaging of boron containing BNCT agents. The techniques are based on the sequences we originally developed but which we are now adapting to the more powerful MRI scanners currently available.

131. Boronated Amino Acids for Site Specific BNCT

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There is a great need for boron-containing metabolite analogs capable of selectively delivering high levels of boron to malignant cells for BNCT while clearing the plasma and normal surrounding tissues. The most successful boron-delivery metabolite analog to date, p-borono-L-phenylalanine, is an amino acid analog; investigation of more boron-containing amino acids is thus a logical and potentially viable approach. The major goal of this work is the application of sophisticated organic chemical techniques to prepare boronated amino acid analogs, peptide mimics and ligands for cell surface receptors. Enantiomers of carboranylalanine and its derivatives, as well as isomeric polyhedral carborane amino acids, are to be synthesized and evaluated for toxicity, stability and cellular uptake either alone or as replacements for natural amino acids in small peptides. Peptide hormones and neuropeptides have a high and specific affinity for their respective receptors, and a number of cancer cell lines are known to express unusually high levels of receptors for particular peptides. This provides an obvious opportunity to meet the need for specific delivery of boron to malignant cells using boron-containing peptide analogs as specific delivery vehicles.

132. Boron Neutron Capture Therapy Large Animal Model Studies Utilizing Epithermal Neutrons and Fast Neutrons

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The overall objective is to determine BNCT parameters for the initiation or modification of safe human clinical trials when they are warranted. Clinicians have proposed BNCT in the United States and Europe to treat malignant brain tumors utilizing an epithermal neutron beam to prevent the surgical reflection of the intervening tissues prior to irradiation. The biological effects of these epithermal neutron beams have been largely unstudied. Large animal models were utilized to study the epithermal neutron beam to arrive at acute and late tissue effects at an acceptable whole body radiation level. Large animal models have been used to study the Brookhaven Medical Research Reactor

epithermal beam and the epithermal beam at Petten, The Netherlands. We collaborate on the large animal studies that are underway in Finland. In addition to the epithermal neutron irradiations, we have begun preliminary studies into a BNCT boost of fast neutron irradiation. Large animal models are required to provide sufficient thermalization of the beam at depth, to allow the study of the acute and late normal tissue reactions. These studies have two main components: 1) modifications to brain tumor irradiation with epithermal neutrons using dogs with spontaneous brain tumors, and 2) normal tissue tolerance of the thorax, primarily of the cardiopulmonary systems utilizing BNCT boost to fast neutron irradiation. This study utilizes purpose-bred laboratory dogs and dogs with spontaneous lung tumors. Additional studies in the laboratory include pharmacokinetic studies of emerging boron compounds. These compounds are first screened in *in vitro* systems and then, if warranted, in transplantable tumors in rodents. The few compounds that successfully show benefits over current compounds, when produced in sufficient quantities, will be studied in dogs with spontaneous tumors.

133. Microdosimetry for Boron Neutron Capture Therapy

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The objective is to design and construct small volume tissue-equivalent proportional counters for the dosimetry and microdosimetry of high intensity thermal and epithermal neutron beams used in boron neutron capture therapy (BNCT), and of modified fast neutron beams designed for boron neutron capture enhanced fast neutron therapy (BNCEFNT); and to develop analytical methods for estimating the biological effectiveness of the absorbed dose in BNCT and BNCEFNT based on the measured microdosimetric spectra.

Medical Applications:

Molecular Nuclear Medicine

134. Radioimmunotargeting With Modified Streptavidin-Biotin

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Encouraging results have been obtained in the *in vivo* use of tetrameric (strept)avidin and biotin for tumor localization with radioisotopes. The detection of tumors has been demonstrated in numerous trials in animals as well as in at least two patient studies, and consideration is now being given to the use of this approach for cancer therapy. Nevertheless, many difficulties to radioimmunotargeting with (strept)avidin and biotin remain to be overcome. This project is an interdisciplinary program that utilizes the tools of molecular modeling, molecular biology and nuclear medicine to enhance the effectiveness of radioimmunotargeting for diagnosis and therapy of cancer. Biotin-conjugated monoclonal antibodies are used to select a target tumor by conventional methods. Then radionuclides are delivered to these target antibodies by an entirely novel approach employing metallothionein fusion proteins modified by techniques of molecular biology to improve the pharmacokinetics of the label for this application. The novel feature of this project is that the streptavidin-metallothionein fusions are engineered using molecular modeling methods to enhance their performance in *in vivo* mouse model systems. Smaller constructs such as dimers or monomers rather than the tetramer imposed by the native structure of streptavidin greatly diminish background activity levels in blood and in normal tissue due to the rapid clearance of these small constructs through the kidneys. The use of metallothionein permits the stable labeling of the constructs with ^{99m}Tc for diagnosis and with radiorhenium (i.e., ^{188}Re or ^{186}Re) for therapy. Tighter molecular structures will diminish proteolysis of the fusion proteins *in vivo*. The ultimate goal is to redesign streptavidin and metallothionein completely to make a much smaller vehicle that still retains the desirable properties of tight, specific binding to biotin and heavy metal ions. The advantages of working with genetically engineered materials as radionuclide delivery vehicles are their uniformity, homogeneity and the ease at producing new reagents with potentially enhanced performance.

135. Radiolabeled DNA Aptamers for Cancer Targeting

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Aptamers are DNA or RNA oligonucleotides which form secondary and tertiary structures that bind with high affinity and selectivity to a target molecule. Aptamers with particular binding properties are selected by sequential affinity chromatography from an enormous pool of random oligonucleotides. Although the utility of the aptamer approach in drug development has been demonstrated, its use in radiopharmaceuticals has not. Here, we propose to develop aptamers against carcinoembryonic antigen (CEA) for use as potential tumor imaging agents. Initially, this study will focus on using phosphorothiolate DNA aptamer libraries and native phosphodiester DNA libraries. Phosporothiolate DNA aptamers should be especially useful as radiopharmaceuticals

because of their *in vivo* stability. Eventually, it should be possible to apply these same methods to RNAs and modified DNAs such as peptide nucleic acids (PNA). The molecules in these libraries will contain a 60 base random sequence plus constant regions on each end to allow for polymerase chain reaction (PCR) amplification and transcription. Aptamer libraries will be subjected to several cycles of CEA-affinity capture and PCR amplification. The consensus sequences of the CEA binding aptamers will be determined and used to design a reduced complexity aptamer library. Sequential rounds of CEA-affinity capture with increasing reduced complexity libraries should allow for an increasing enrichment for high affinity aptamers. Individual aptamers showing selective binding at high affinities will be sequenced, cloned and further characterized. For instance, tumor binding properties *in vitro* and *in vivo* in nude mice implanted with a CEA-expressing tumor, will be determined using aptamers radiolabeled with imageable radionuclides (^{111}In and ^{99m}Tc). Binding will be compared to that obtained using a labeled anti-CEA antibody.

136. Cocaine Analogs for PET Studies of Synaptic Activity

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The actions of some drugs of abuse such as cocaine and some therapeutic drugs such as methylphenidate (Ritalin) stem from their abilities to inhibit clearance of neurotransmitters from the synaptic cleft. Increased dopamine in parts of the brain causes the "high" experienced by cocain abusers. Our goal is to develop methodology for examining local brain concentrations of dopamine and other neurotransmitters, using positron emission tomography (PET) and single photon emission computed tomography (SPECT), based on competition of neurotransmitters with radioligands of the dopamine transporter and other binding sites. This will lend depth to the results of nuclear imaging studies by providing a perspective on the molecular mechanisms underlying normal and abnormal synaptic biology. Increases in neurotransmitter levels decrease the uptake of suitable radioligands, whereas decreases increase radioligand uptake. Although techniques such as microdialysis and electrophysiology can be used to assess synaptic activity in animal models, these are not applicable to individual human patients. The importance of noninvasive *in vivo* radioligand binding technologies such as PET is that they can be applied to humans, and therefore provide a bridge between experimental and preclinical studies, and medical practice. Monitoring neurotransmitter concentrations will help the evaluation of neuropsychiatric disorders and of drug treatments.

137. Detection and Assessment Using Positron Emission Tomography of Defects in Myocardial Fatty Acid Utilization in Cardiomyopathy

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The heart is dependent on the preferential utilization of fatty acids to provide energy for contractile function. Thus, genetic or acquired defects in which components of the fatty acid oxidation pathway are impaired are particularly devastating. During the initial grant period, we successfully developed and implemented a mathematical approach using positron emission tomography (PET) by which myocardial fatty acid metabolism could be delineated in patients with both inherited as well as acquired cardiomyopathy. We were able to demonstrate that the severity of the cardiomyopathy was related to the intensity of the fatty acid defect. Using the mathematical model developed, we further demonstrated that the subcellular loci of the defect could be differentiated in patients with different etiologies of their metabolic dysfunction. The intensity of the abnormality was an important prognostic indicator of the severity of the

contractile dysfunction and, in adults, for the need for transplantation or for sudden cardiac death. The goals of this project are to continue development of the approach for detection of abnormalities of cardiac fatty acid metabolism in patients with inherited or acquired cardiomyopathy and to use PET for defining the severity of the cardiac manifestations and correlate these results with history, functional assessments, disease progression and tissue assays. There is reason to believe that defects in the handling of fatty acid may be important progenitors of contractile dysfunction. In the laboratory we have demonstrated that amelioration of the build up of long-chain fatty acyl intermediates that accompany certain cardiomyopathies was associated with recovery of contractile dysfunction. Thus, if patients with cardiomyopathy can be identified who have alterations in myocardial fatty acid metabolism that is contributing to their contractile dysfunction, it may lead to novel therapies for treatment of heart failure including the use of substrate switching by administration of exogenous substrate, by pharmacological manipulations, and ultimately through gene-replacement therapy. The approach developed should also aid in defining the efficacy of such therapeutic interventions. We will continue to focus initially on patients with defects in metabolism of long-chain fatty acids (long-chain and very long-chain acyl-CoA dehydrogenase deficiency, carnitine palmitoyltransferase deficiency, and long-chain 3-hydroxy acyl-CoA dehydrogenase deficiency). But since these are relatively rare disorders, we plan to expand our observations to both pediatric and adult patients with mitochondrial myopathy as well as those with acquired, idiopathic cardiomyopathy. Patients will be evaluated tomographically to define the rate of oxidation of 1-^{11}C-palmitate (a long-chain fatty acid) compared with that of 1-^{11}C-acetate (which we have shown reflects overall mitochondrial oxidative flux). Studies of fibroblasts and of myocardial biopsies in patients going on to cardiac transplantation will permit correlation of the defects observed noninvasively with PET. The proposed research should improve our understanding of the biochemical abnormalities that underlie inherited and acquired cardiomyopathy and aid in the development of the objective evaluation of the efficacy of therapeutic interventions. In addition, it should help to delineate risks in those for whom no anticipatory diagnostic procedure presently exists. Thus, it offers promise of improving health care through the novel applications of nuclear medicine.

138. Recombinant Anti-Tenascin Antibody Constructs

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The development of hybridoma technology some twenty years ago rekindled interest in exploiting the specificity of the antigen-antibody interaction to target radioactivity to tumor for diagnostic and therapeutic applications. Despite widespread efforts, radiolabeled monoclonal antibodies (MABs) have not had a significant impact on the clinical management of cancer patients. Our approach for increasing the utility of labeled MABs employs DNA recombinant technology to generate genetically engineered MAB-based constructs and evaluate their potential utility for oncologic nuclear medicine. The central hypothesis of this proposal that the general framework of these molecules will be a critical factor in determining their utility with radionuclides and that the ideal molecular form will also depend on the nature of the radionuclide, labeling method and route of administration. Our work plan focuses on MAB-based constructs reactive with tenascin, a polymorphic extracellular matrix glycoprotein found in gliomas, neuroblastomas, melanomas, as well as prostate and breast carcinomas. Variable regions will be derived from murine 81C6 MAB, which binds to the alternatively spliced fibronectin type III domain 12 of the tenascin molecule. We have obtained promising therapeutic responses with ^{131}I-labeled 81C6 in patients with cystic gliomas, surgically-created glioma resection cavities and neoplastic meningitis. This research proposal seeks to identify the optimal antibody-based constructs for use with radionuclides. Experiments will utilize radioiodine nuclides, the alpha-emitter ^{211}At and the positron emitter ^{18}F in order to exploit novel radiohalogenation strategies

developed in our laboratory. The first goal will be to select a construct for use with longer half-life nuclides, such as ^{131}I, which can be best exploited in applications where slow blood clearance and limited tumor penetration are not major problems. For this task, chimeric MABs with constant regions from the four different human IgG classes will be constructed, as will molecules with domain deletions engineered to alter pharmacokinetics. Our preliminary data suggest that use of the IgG_2 constant region offers significant advantages for radionuclide applications. We also will investigate sFv monomers and dimers because their rapid normal tissue clearance and tumor penetration should be compatible with shorter half-life nuclides such as ^{18}F and ^{211}At.

139. New Labeled Cocaine Analogues For *in vivo* study of the Dopamine Transporter

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Emission tomographic imaging of the dopamine transporter sites in man depends upon the development of high specific activity radioligands which show very high selectivity and affinity for the dopamine transporter site with very low nonspecific binding. The proposed research is focused on the synthesis and pharmacological characterization of fluorine-18, iodine-123 and technetium-99m labeled ligands for the potential in vivo imaging of the dopamine transporter sites in patients with neurodegenerative diseases and cocaine users. The research involves five phases: 1) the development of synthetic methods for the preparation of new fluorine-18, iodine-123 and technetium-99m labeled 3ss-(p-substituted-phenyl)tropane-2ss-carboxylic acid esters, 2) characterization of the *in vivo* and *in vitro* pharmacological properties of the series of analogs, 3) *in vivo* imaging of cerebral dopamine transporter sites in primates with the best radiolabeled analogs using PET and SPECT, 4) *in vivo* imaging using PET with the fluoroethyltropane analog FECNT to determine test-retest reliability of measuring dopamine transporter site density in monkeys, and 5) *in vivo* imaging of both the best new PET and SPECT ligands in a non-human primate model of Parkinson's disease.

140. CDR-grafted MAb with Sitespecific Labeling Capability

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Antibody fragments are recognized as important agents for imaging and therapeutic applications. In order to preserve the ability of antibody fragments to bind to their target antigen, methods must be developed to link effector ligands, such as radionuclides or agents that cannot otherwise be engineered in an antibody, in a manner that will protect the antigen binding site. We have used molecular engineering techniques to insert specific peptide sequences (Asn-X-Ser/Thr tripeptide acceptor sequence) in an antibody molecule that will induce glycosylation at these sites. These glycosylation sites can then be modified by standard chemical methods to provide a specific linkage site on the antibody molecule. The glycosylation sites can be tailored specifically to domains of an antibody molecule preserved in bivalent and monovalent antibody fragments. In this project a humanized antibody to the CD22 antigen (found on non-Hodgkin's lymphoma) was engineered to produce single glycosylation sites in several regions of the CH1 and Vk domains of the antibody molecule. Several glycosylation variants were developed, but only two were selected based on their more extensive carbohydrate substitution and the orientation of the carbohydrate. The carbohydrate structure of these two variants was delineated and conjugation studies suggested that the immunoreactivity of fragments made from these variants is retained at a higher substitution level than the non-glycosylated parent fragment. Studies are underway to determine

their loading capacity for the radiometal chelating agent DOTA and to determine if these agents will improve anti-tumor responses in a non-Hodgkin's lymphoma xenograft model. We also developed a specific peptide that can be coupled to the carbohydrates and quantitatively radiolabeled with ^{99m}Tc for imaging applications. Preliminary data are very encouraging that this combination will provide the first fully humanized antibody fragment, technetium-labeling agent for human use. In addition to these engineered agents, we also constructed a fragment of the humanized anti-CD22 antibody that contained the human IgG3 hinge region. This region is rich in cysteine residues (11 compared to 2–3 in human IgG1), which could provide additional opportunities for site-specific conjugation to these SH2 residues. Thus, these molecular engineering projects are designed to produce both bivalent and monovalent antibody fragments that can be used in tumor imaging and therapy applications. To date, we have provided proof of principle that these new constructs can be made, not only in this antibody but one other anti-carcinoembryonic antigen construct, and we are now exploring their targeting in animal models.

141. Imaging of Apolipoprotein E-binding Receptors *In-Vivo*

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We have postulated that high effects on atherogenic lipoprotein metabolism of a peptide consisting of a dimeric repeat of the receptor-binding domain of apolipoprotein apoE. Previous studies utilizing a similar apoE-related peptide have demonstrated a high affinity for cell surface receptors for apoE. More recently, this peptide has been modified so as to enhance its binding to plasma lipoproteins, particularly very low density lipoproteins (VLDL), the metabolic precursors of atherogenic lipoprotein remnants and LDL. Preliminary studies using this peptide have indicated that it can promote a substantial reduction in plasma cholesterol levels in a mouse model in which hypercholesterolemia has been induced by targeted inactivation of the apoE gene. The following specific aims are designed to investigate the kinetic behavior, tissue distribution, and therapeutic potential of this peptide using animal models for human disorders of atherogenic lipoprotein metabolism.

142. Novel Approaches to Cancer Targeting Using Epitope-binding Peptides That Mimic Monoclonal Antibodies

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In recent years, several new methods for screening vast repititors of peptide sequences for receptor/epitope interaction have been developed. These techniques include both molecular biological techniques and soild phase peptide synthesis approaches. We plan to use these approaches to develop radiolabeled peptides for imaging breast cancer by ligand receptor-interaction with the 185 kDa glycoprotein product of the c-erb-2 oncogene. In 15–20% of patients with breast cancer, there is overexpression of the c-erb-2 185 kDa glycoprotein (p185erbB2). The low level of expression of this protein in normal tissues makes the c-erb-2 gene a selective tumor-specific target for receptor mediated tumor detection and therapy. We plan to use combinatorial chemistry approach to develop radiolabeled peptides for imaging breast cancer by developing peptide based radiopharmaceuticals that will selectively target the p185erbB2. Our preliminary investigations using both a synthetic combinatorial peptide library and a phage display peptide library have resulted in several peptide leads which bind p185erbB2 or inhibit the binding of anti-p185erbB2 monoclonal antibody (ICR12) to its anti-idiotype Mab (ICR101). In this application, we plan to screen random peptide libraries for p185erbB2 binding sequences using two approaches: 1) Positional

scanning-synthetic combinatorial peptide libraries (PS-SCLs) of all possible hexapeptide and decapeptide sequences. These libraries will be screened for inhibition of binding of p185erbB2 specific monoclonal antibodies (ICR12, ICR51, ICR52, ICR53, ICR54, ICR55 and CEB2J) to isolated p185erbB2, linear determinants of p185erbB2 and in the case of ICR12 to anti-idiotype MAb ICR101. 2) A recently developed molecular biological technique using phage display libraries. With this approach, each phage or phagemid particle contains the genetic information for one unique random peptide. Individual varions selected for phage binding to the external domain of p185erbB2 (using p185erbB2 expressing cells and/or immobilized p185erbB2), will be clone purified, their specificity of binding confirmed, and their nucleotide sequence determined to establish the specific random peptide expressed on their surface. The best peptides will be subjected to microsequencing. The most promising candidates will be synthesized in large quantities, radiolabeled with ^{99m}Tc and ^{18}F and biodistribution and imaging studies (single photon and PET) will be performed. Quantitative autoradiographic measurements and tissue fractionation techniques will be used to formulate a kinetic model for the *in vivo* tissue and cellular distribution of radiolabeled peptides in tumors and normal tissues. In conjunction with PET imaging, this model will be used to obtain a detailed quantitative understanding of the kinetics of peptide receptor interaction. The successful completion of this proposal should lead to several contributions to the fight against breast cancer, including: 1) Improved diagnostics, 2) Novel biological marker for c-erbB2 expression *in vivo* and *in vitro*, 3) Targeting vectors for therapy (e.g., radionuclides, chemotherapeutics, biologicals, toxins,etc.) and 4) Quantification of oncogene expression *in vivo* by PET.

143. Pharmokinetics of Genetically Engineered Antibody Forms using Positron Emisson Tomography

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The objective of this grant is to develop and validate methods which will lead to improved knowledge regarding the *in vivo* behavior of genetically engineered antibodies and antibody forms, that are useful for targeted therapy. The approach being taken is to employ the quantitative power of positron emission tomography for high resolution quantitative imaging in animals and man. The physics of imaging of the positron emitter I-124, which was begun under other DOE grant, is now being extended to the radiolabeling and *in vivo* characterization of the genetically engineered monoclonal antibodies A33, M 195 as well as other genetically engineered antibody forms. Methods for labeling of these antibodies with Y-86, a positron emitter, are also being pursued, as a initial step in the development of "2 step" approaches that will be applicable to radionuclide therapy. In addition, the application of Y-86 as a method for determining the dosimetry of Y-90 is ongoing.

144. Single Chain Fv Constructs of Anti-ganglioside G_{D2} Antibodies for Radio-imaging and Radioimmunotherapy

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T-lymphocytes are ideal targeting vehicles because 1) they are naturally equipped with trafficking

capabilities, 2) they can undergo clonal expansion when they come in contact with antigen if given the appropriate mitogenic signals, 3) they release cytokines which recruit other inflammatory/immune cells, 4) they can initiate other arms of immunity at the tumor site and 5) they are capable of being engineered with powerful cytotoxins or enzymes. Both clonal expansion and recruitment of T-cells can greatly magnify targeted delivery, improving the therapeutic index of the intended diagnostic or treatment modality. Although lymphokine activated killer cells (LAK) and tumor infiltrating lymphocytes (TIL) have been tested in tumor targeting, the clonal frequency of tumor-specific lymphocytes is generally very low and <0.016% of administered lymphocytes arrive at the tumor sites, accounting for the limited efficacy and generalized toxicity of LAK/TIL adoptive immunotherapy. In addition, defects in T-cell signal transduction prevalent among lymphocytes from cancer patients further compromise their utility. Until the specific delivery of these cells is optimized, the potential of cell targeting cannot be explored. Quantitative analysis and trace-labeling of human lymphocytes for homing studies to human tumors *in vivo* have been limited by the unavailability of cell-labeling techniques. Issues unique to cellular targeting include specificity of labeling of cell subpopulations (CD4+ vs CD8+ T-cells vs NK cells), clonal expansion of antigen-specific cells at the tumor site, and the pharmacokinetics of tumor-avid (versus tumor-nonspecific) lymphocytes. Using scFv of different affinities (5FII and 3G6) and specificity (NS.7) we propose to optimize the quantitative delivery of transduced T-lymphocytes to the tumor site. Using highly efficient retroviral gene transfer methods, scFv and Herpes Simplex Virus Thymidine kinase (HSV1-tk) can be permanently transduced into lymphoid cells enriched to homogeneity and after fluorescent activated cell sorting. The radioiodinelabeled 5-iodo-2'-fluoro-2'deoxyl-β-D-arabinofuranosyluracil (FIAU) substrate is metabolized by the enzyme HSV1-tk whereby cells become radiolabeled. HSV1-tk thus functions in several capacities: as a marker gene and a suicide gene (in the presence of ganciclovir) for transduced T-lymphocytes. The targeting of HSV1-tk carried in lymphocytes to tumor sites can be assayed by *in vivo* uptake of ^{131}I (tissue counting, quantitative autoradiography, and gamma imaging). With ^{124}I, precise dynamic measurements can be made over time using quantitative positron emission tomography (PET). Cellular delivery, multiclonal expansion, and recruitment may also be quantified. We want to build on our clinical experience of targeting to G_{D2}, an antigen highly expressed on human neuroblastoma, melanoma, small cell lung cancer, brain tumors and sarcomas, novel strategies of genetically tagging cells with marker genes and labeling with radioactive substrates, as well as our nuclear medicine quantitation capabilities. Together with the active clinical monoclonal antibody program and an on-site cyclotron facility at Memorial Sloan-Kettering Cancer Center, we hope to maximize the potentials of molecular nuclear medicine in antibody-based strategies and to facilitate their translation into clinical applications.

145. Radiopharmaceutical and Gene Therapy Program

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We have formed a Molecular Nuclear Medicine Research Group for the study of radiolabeled peptides combined with molecular biological approaches to gene transfer, which together can be used to treat local/regional and metastatic cancer. The goal of this Molecular Nuclear Medicine Research Group is to provide an efficient mechanism for preclinical in vitro and animal model studies of radiopharmaceuticals and gene transfer with an emphasis on determining the therapeutic effect of such treatment strategies. Among the limitations of radioimmunotherapy, low levels of intratumoral antigen expression and restricted tumor penetration hinders successful targeting of radiolabeled antibodies. We thus sought to address these problems employing a novel approach whereby gene transfer methods would be employed to induce tumor cells to express a new membrane-associated receptor with high affinity for a radiolabeled peptide to thus improve tumor uptake and therapeutic efficacy. Somatostatin receptor 2 (SSTR2) was selected for study

due to its high expression in a number of human tumors but low expression in normal tissues and the availability of peptide analogues with high binding affinity to SSTR2. A replication-incompetent adenovirus coding for SSTR2 under control of the cytomegalovirus virus promoter (ADCMVSSTR2) was produced. Infection of SKOV3.ip1 human ovarian tumor cells and A427 human non-small lung cancer cells *in vitro* with ADCMVSSTR2 for two days resulted in the induction of SSTR2 as evidenced by binding of ^{125}I-somatostatin and ^{111}In-octreotide to membrane preparations of these cells. The binding was inhibited by an excess of unlabeled somatostatin and the level of binding increased as the multiplicity of infection increased. A biodistribution study was carried out with ^{125}I-somatostatin and ^{111}In-octreotide in athymic nude mice bearing i.p. xenografts of SKOV3.ip1 human ovarian carcinoma cells transduced *in situ* with ADCMVSSTR2 or control adenovirus coding for murine gastrin releasing peptide receptor (ADCMVGRPR) as a control. Nude mice were injected i.p. with 2 X 10^7 SKOV3.ip1 cells. At 5 days the mice were injected i.p. with 1 X 10^9 plaque forming units of ADCMVSSTR2 followed by an i.p. injection of ^{125}I-somatostatin or ^{111}In-octreotide at day 7 and sacrificed after 4 h or 24 h. The mice transfected with ADCMVSSTR2 and injected with ^{111}In-octreotide showed 59.7 ± 18.7% ID/g in the tumor at 4 h after injection which decreased to 18.1 ± 6.2% ID/g at 24 h after injection. By contrast, ^{125}I-somatostatin showed only 3.1 ± 1.2% ID/g in ADCMVSSTR2 transfected tumors at 4 h after injection. Other normal tissues showed much lower concentrations of ^{111}In-octreotide. These results indicate the selective transduction of human ovarian cancer xenografts with SSTR2 which resulted in high tumor uptake of ^{111}In-octreotide following regional administration. We have labeled octreotide with ^{64}Cu and shown binding to ADCMVSSTR2 infected SKOV3.IP1 cell membrane preparations, and plan to investigate therapy in this ovarian cancer model with ^{64}Cu-labeled octreotide. The coupling of gene transfer technology and radioligand therapy represents a new paradigm for cancer gene therapy.

146. Biochemical Sciences

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Our program on the development of biological probes is focused on the design, synthesis and validation of positron-emitting and fluorescent labeled probes for enzyme localization and function, presynaptic neurotransmission, postsynaptic receptor interactions and gene expression. Our investigations have three general aims: 1) Development of biological probes (i.e. radiolabeled fluorinated amino acids, presynaptic dopamine transporter ligand probes) to establish the structural requirements for probing central dopaminergic mechanisms with positron emission tomography (PET). 2) The design of PET reporter probes in the development of PET procedures to quantitate gene expression. Probes for glucose transporters (GLUT-1), dopamine-D2 receptors, aromatic amino acid decarboxylase and thymidine kinase are currently being investigated. 3) The synthesis of fluorescent dyes whose intramolecular rotational relaxation is solvent polarity and viscosity dependent for use with visible fluorescence spectroscopy to establish the biochemistry and kinetics of pathological processes in tissue.

147. Receptor-DNA Binding to Target Auger Electron Radiation

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To assess the potential for steroid receptor-directed therapy using Auger electron-emitting estrogens by elucidating the molecular and cellular effects of the electron radiation on DNA cells and tumors, estrogens bearing the Auger electron emitting nuclides I-123 and

I-125 have been synthesized. The specific activities, binding to estrogen receptors (ER) and ER-positive cells and tumors are studied, along with unlabeled estrogen competetive uptake and radiotoxicity. Parallel studies in cells assess chromosomal damage and DNA strand breaks to correlate such damage with radiotoxicity. Molecular damage to DNA containing estrogen response elements (ERE) in isolated oligonucleotides will attempt to identify the molecular specificity of the radiation. Finally, effects on ER-positive cancer cells and tumors implanted in nude mice will assess the therapeutic potential of this approach to cancer therapy. Auger electron-emitting nuclides have been attached to estrogens, which have been shown to be of the high specific activity needed. Steroidal and non-steroidal estrogens have been compared to identify the best ligand in terms of uptake and prolonged retention in ER positive tissues *in vivo*. I-123-estrogens have been shown to effect cold estrogen-inhibitable, dose-dependent radiotoxicity of ER+ but not ER- cells in culture, DNA strand breaks and chromosome aberrations. The mean lethal dose corresponds to about 100 decays per cell and ~ 1 aberration per cell. Auger electron-emitting estrogens effect dose-dpendent radiolysis of oligonucleotides containing estrogen response elements (ERE) under conditions which do not cause detectible damage to the estrogen receptor protein itself. Cells treated with nM Auger electron emitting estrogen demonstrate at least 100-fold reduction in tumor formation in the nude mouse.

148. Molecular mechanisms of enhanced [^{18}F] fluorodeoxyglucose uptake in ischemically injured myocardium

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Clinical PET studies with the glucose analog ^{18}F-fluorodeoxyglucose (FDG) have identified regions of ischemic but viable myocardium which are characterized by increased FDG uptake when compared to either normal or irreversibly injured myocardium. These and other studies have suggested that increased metabolism of exogenous glucose plays a cardioprotective role during myocardial ischemia. Recently, the structures of the proteins responsible for myocardial glucose uptake, the facilitative glucose transporters (GLUTs), and the enzymes which catalyze the phosphorylation of exogenous glucose, the hexokinases have been identified. Thus the reagants are now available to determine the molecular basis for the increase in FDG uptake during myocardial ischemia. Our specific hypothesis is that myocardial cells regulate FDG and glucose uptake by modulation of plasma membrane GLUT expression and cytosolic hexokinase levels, and that an increase in expression of these proteins is responsible for the augmented glucose uptake and metabolism demonstrated by ischemic myocardium. We propose that there is chronic induction of GLUT and hexokinase protein levels in repetitively ischemic myocardium, leading to chronically elevated glucose metabolism which plays a cardioprotective role during ischemic exacerbations. Finally, we propose that this adaptive response is altered in diabetic myocardium, thereby increasing the risk of permanent myocardial injury during ischemia. The aims of this project are: 1) To determine the relative expression of myocardial glucose transporters in normal and ischemic dog myocardium, and to correlate this expression to glucose uptake and phosphorylation as determined by ^{18}F-fluorodeoxyglucose (FDG) uptake. 2) To determine the activity and the relative levels of the major cardiac hexokinases (HKs) in normal and repetitively ischemic dog myocardium, and to correlate these values to glucose uptake and phosphorylation as determined by FDG uptake. 3) To determine GLUT4, GLUTI, HK I and HK II levels, HK activity, FDG uptake and phosphorlyation in repetitively ischemic and non-ischemic myocardium from diabetic dogs, and to compare these findings to those from normal animals. 4) To determine, through the use of quantitative reverse transcription polymerase chain reaction (RT-PCR) techniques, the relative GLUT and HK mRNA levels from chronically ischemic and non-ischemic regions of humans with coronary heart disease and to determine, through semi-quantitative immunohistochemical techniques, the expression of GLUT and HK proteins. These findings will

then be correlated to regional glucose uptake and phosphorylation as determined by FDG PET studies. Our have shown that GLUT4 is translocated to the sarcolemma of ischemic cardiomyocytes, that GLUTI gene expression is upregulated by longer (6 hr) ischemia in both ischemic and non-ischemic areas of hearts with regional ischemia. We have also noted tendencies toward increased GLUT4 and GLUTI MRNA expression in ischemic regions from human hearts as well. Because GLUTI expression is increased in non-ischemic regions, we felt that this may be a protective response to prevent future ischmia-induced cell death. We have now confirmed that GLUTI overexpression, prior to hypoxia, can prevent cell death, largely through inhibiting apoptosis. GLUTI overexpression prevents activation of the stress-activated protein kinase cascade which plays an important role in potentiating apoptosis in hypoxia and ischemia. Investigation of the manner in which enhanced glucose transporter expression specifically modulates both stress-activated protein kinase and apoptotic caspase cascades are now ongoing.

149. Radiolabeled Peptide-Based Melanoma and Breast Carcinoma Imaging and Therapeutic Agents

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This proposal outlines a research effort to design and characterize radiolabeled tumor-avid peptides as potential melanoma and breast carcinoma diagnostic and therapeutic agents. The incidence rates of both melanoma and breast cancer are increasing. Early detection, through the development of highly sensitive and specific radiolabeled tumor probes, will potentially lead to more rapid therapeutic intervention and ultimately improve patient prognosis. It is also likely that tumor-selective deposition of radionuclides will result in novel malignant melanoma and breast cancer therapies. A multi-disciplinary approach is described which combines powerful new molecular biology technologies for the discovery of new tumor-avid peptides and novel radiochemistry techniques for direct incorporation of metallic radionuclides by peptides. Metallic radionuclides used in this research program, ^{99m}Tc, ^{186}Re, and ^{188}Re, are all readily available and have the physical decay and half-life properties compatible with radiopharmaceutical development. The hypothesis that is implicit in this work is that the direct coordination of metallic radionuclides by tumoravid peptides will yield new radiopharmaceuticals with high diagnostic sensitivities and potential therapeutic applications. The specific objectives designed to test our hypothesis are: 1) characterize the melanoma tumor targeting and biodistribution properties of ^{99m}Tc and $^{186/188}$Re labeled melanotropin peptide analogues in vivo, 2) evaluate the radiochemical stability and in vivo pharmacokinetics of ^{99m}Tc and $^{186/188}$Re labeled peptides which bind the breast carcinoma-associated T antigen, and 3) develop an efficient and economical method for the semi-automated synthesis of peptide-based radiopharmaceuticals on bench-top solid phase peptide synthesizers. The first specific aim will yield important information on how the sequence dependent properties (charge, hydrophobicity, chain length) of both cyclic and linear radiolabeled melanotropin peptide analogues influence tumor binding and penetration and radionuclide clearance from the body. Optimizing tumor specific radionuclide deposition and minimizing non-specific organ irradiation is crucial to the successful development of potential agents for melanoma imaging and treatment. The second aim will employ random peptide bacteriophage display libraries to identify new sequences with high affinities and specificities for tumor-associated antigens and receptors. Efforts in this area have led to the discovery of several peptides which bind to the breast carcinomaassociated T antigen in vitro. The breast tumor-avid peptides will be radiolabeled with ^{99m}Tc and $^{186/188}$Re and examined for radiochemical stabilities and their abilities to bind breast tumors induced in nude mouse animal models. Breast carcinoma selective radiolabeled peptides may improve the sensitivity of early tumor detection methods and have therapeutic potential. The last aim will apply solid phase peptide synthesis chemistries to the addition of preformed radionuclide complexes to

peptides during automated synthesis. Radiolabeling peptides during synthesis will allow the production of milligram or Ci amounts of each anti-tumor agent in a rapid and cost-effective manner. The automated coupling procedure will also decrease the radiation exposure of laboratory personnel during labeling process. The research described in the specific aims will contribute significantly to the goal of designing tumor specific radiolabeled peptides for melanoma and breast carcinoma diagnosis and treatment.

150. Genetically Engineered Multivalent Single Chain Antibody Constructs for Cancer Therapy

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Radiolabeled monoclonal antibodies (MABs) directed against tumor associated antigens have been used as radioimmunoimaging and radioimmunotherapeutic agents. Radiolabeled MABs localize in tumors as shown by external imaging techniques, and may also be useful for tumor therapy with the appropriate radionuclide. Virtually all radioimmunotherapy studies in patients with adenocarcinomas using intact MAB immunoglobulins have shown hematopoietic toxicity to be dose limiting. Investigators have been studying a number of ways to modify MABs to alter their pharmacology to obtain more favorable biodistribution and better tumor to normal tissue ratios. One way is to use MAB variable regions (Fv) to develop single chain Fv molecules (scFv), that contain only the variable regions of the heavy and light chains. Single chain Fv molecules, which have been further engineered to have 2 or 3 binding sites connected by peptide linkers should have a more favorable clearance rate in vivo than monovalent scFv. This should result in: 1) better penetration throughout a tumor mass than the intact IgG resulting in less dose heterogeneity; 2) a higher percentage of the injected dose per gram localized in the tumor as compared to monomeric scFvs; and 3) more favorable biodistribution and better tumor to normal tissue ratios than intact IgG. The hypothesis of this grant is that multivalent forms of single chain antibodies (scFv) can be generated that will have high avidity for the corresponding antigen and that these multivalent scFv molecules should have a more favorable *in vivo* biodistribution than the monovalent scFv. The specific aims are to: 1) Characterize monovalent and multivalent forms of MAB CC49 single chain Fv constructs before and after radiolabeling for purity and immunoreactivity. Evaluate the effects of different labeling methodologies on integrity and immunoreactivity; 2) Evaluate the pharmacology and biodistribution of radiolabeled monovalent and multivalent forms of MAB CC49 single chain Fv constructs in both normal and tumor bearing animals; and 3) Perform studies to determine the relative therapeutic efficacy, and normal tissue toxicity, of radiolabeled multivalent scFv constructs as compared to intact IgG and/or IgG fragments. Monomeric and dimeric scFv constructs of MAB CC49 have been generated purified, characterized, and tested *in vivo*. These studies have demonstrated the potential utility of these constructs for the localization and treatment of malignancies.

151. Receptor Specific Ligands for SPECT Imaging

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Selective radioactive ligands for in vitro binding and in vivo imaging of the somatostatin receptors (SSTR) will be developed. The radioactive-labeled (^{125}I) ligands are powerful tools for *in vitro* and *in vivo* characterization of the SSTR in cloned cells, tissues and animals. When labeled with ^{123}I, they are potentially useful for noninvasive *in vivo* imaging in conjunction with Single Photon Emission Computed Tomography (SPECT) in humans. This imaging technique may

provide useful information on the density, distribution and function, as well as the functional linkage, drug-ligand interaction of these somatostatin receptors in normal and disease states. After suitable radioiodinated ligands have been identified and characterized, they may be derivatized and labeled with ^{111}In and ^{99m}Tc for SPECT imaging.

152. PET Imaging of Protein Synthesis, Ribosomes and mRNA

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This grant supports the basic science studies necessary to develop radiopharmaceuticals that can be used for biomedical imaging in man by positron emission tomaography (PET). Our long term goal is to develop measurements for protein synthesis in tissues that can assess the extent of cancer treatment in tumors and normal tissue damage from therapy. We will also develop radiolabeled probes to investigate the feasibility of imaging specific genetic expression in tissue. Measurement of specific genetic expression could be useful for distinguishing tumor tissue from normal tissue in PET images.

153. Dimeric scFv Antibodies and Their Streptavidin/Biotin Conjugates in Cancer Imaging and Therapy

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The overall goal is to develop new reagents, through chemical and molecular engineering methods, that will improve delivery of radioactive materials (i.e. radionuclides) to tumors in patients. The approach being investigated for delivering the radionuclides to cancer cells in patients is called "pretargeting". In the pretargeting approach used, cancer cells are targeted with injected monoclonal antibodies (MABs) that have biotin conjugated to them. After allowing the biotinylated mAbs to localize on cancer cells and to clear from blood, a protein which binds with biotin, streptavidin, is administered. Again, the streptavidin is allowed to localize on cancer cells (by binding with biotinylated mAbs) and to clear from blood, then a radiolabeled biotin molecule is administered. Some of the radiolabeled biotin binds with streptavidin on cancer cells and the rest is cleared rapidly from the body by excretion through the renal system. There are a number of reasons for choosing this sequence of administration of reagents, but perhaps the most important is that the tumor localization and body clearance of the administered radionuclide is rapid. This results in good tumor localization while minimizing the radiation dose to non-target tissues providing favorable dosimetry for patient therapy. The use of three (or more) different reagents for tumor targeting of radionuclides makes these protocols complex, but it also provides more flexibility in optimizing the process (in relation to using directly labeled MABs). The focus of our initial research plan was to prepare dimeric (bivalent) MAB fragments for improved targeting of tumors. The MAB fragments, single chain Fv are produced by genetic engineering methods and are composed primarily of the cancer cell (antigen) binding region of a mAb. We have been successful in obtaining a scFv of a MAB reactive with renal cell carcinoma, A6H. However, this molecule has a high tendency to aggregate making it very insoluble and difficult to work with. We have changed the structure of the scFv protein by genetic engineering methods and have made it considerably more water soluble (a requirement for use in patients). We have also modified its structure to make it possible to prepare dimeric forms of the scFv for testing as tumor targeting agents. This work is continuing. Our unexpected difficulties with aggregation of the A6H scFv has made progress slow. However, our studies have uncovered other areas of research that are perhaps more important in obtaining improved tumor

targeting in patients. For example, in our studies (and from other investigations) it was found that the biotin molecules used had to be designed in a manner that would block the cleaving action of a serum enzyme, biotinidase. Thus, we conducted a study of chemical modifications of biotin derivatives and were successful in developing new reagents that are not susceptible to biotinidase cleavage. This is very important as biotinidase cleavage of biotin from radiolabeled biotin derivatives or biotinylated mAbs can result in loss of the specific cancer targeting capabilities of the reagents used. In our development of new biotin reagents, we also found that it was necessary to design in water solubilizing moieties because biotin molecules are very insoluble without them. In the studies conducted, we have prepared many new biotin derivatives that are by design water solubilized and biotinidase stabilized. The reagents prepared are used to biotinylate MABs (and other proteins), carry radionuclides such as radioiodine, and cross-link proteins such as mAb Fab′ and scFv′ (to make dimers). We have also studied the use of biotin dimers and trimers to increase the quantity of radionuclide that can be bound to cancer cells showing (for the first time) that biotin trimers possess the appropriate properties to do this. Further work will be conducted to demonstrate that increased tumor localization can be accomplished in an animal model. The molecular engineering efforts have also prepared new variants of streptavidin. Commercially available streptavidin for use in pretargeting is prepared by enzymatic digestion of streptavidin from bacterial sources. The enzyme digested streptavidin provides a "core" streptavidin protein, but this material can have different sized proteins due to the digestion process. We have a recombinant streptavidin that has a discrete size for use in our pretargeting studies. The genetic engineering of streptavidin allows for site-directed mutagenesis of the "wild type" amino acid sequence to provide streptavidin mutants which have altered properties. One of the streptavidin mutants prepared has a single amino acid change that allows site-specific conjugation of other molecules to it without affecting its biotin binding properties. We have shown that this streptavidin mutant targets biotinylated mAbs at tumors in an animal model in the same amount as the "wild type" streptavidin. This site-specific conjugation has allowed us to prepare new streptavidin reagents that may provide improvements in pretargeting. Additional studies will evaluate this. We have also found that, through chemical modification of streptavidin, its characteristic kidney localization can be eliminated. This has opened up the possibility of attaching (certain) radionuclides to the streptavidin for delivery to cancer cells. Our studies have provided new reagents for delivery of radionuclides to tumors. We are continuing to improve on the reagents developed and to evaluate the improvement that they offer.

154. Targeting Multidrug Resistance with Tc-Complexes

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The multidrug resistance P-glycoprotein, a 170 kDa plasma membrane protein encoded by the human multidrug resistance gene (MDR1), functions as an energy-dependent efflux pump of many of the most potent chemotherapeutic drugs in cancer treatment. Strategies designed to block expression or to circumvent this form of drug resistance are being actively sought by many academic and industrial laboratories in cancer research. Expression of MDR1 P-glycoprotein and related gene family members (such as the multidrug-resistance associated protein, MRP) also appear to have prognostic significance wherein high levels of gene expression are correlated with a poor clinical outcome. Our objective is to discover and develop radiopharmaceuticals as functional probes of P-glycoprotein transport activity. Functional assays with these gamma-emitting agents should provide new information about the functions of MDR1 P-glycoprotein and homologous transporters and their relationships to cell growth and chemoresistance in human tumors *in vivo*. Study of these compounds may also reveal mechanisms that regulate drug resistance in cells. Functional imaging of the MDR phenotype with these metal-complexes may also provide a novel tool to rapidly characterize clinically

relevant P-glycoprotein expression in human tumors *in vivo*, target reversal agents *in vivo,* and ultimately provide a means to direct patients to specific cancer therapies. We discovered that the gamma-emitting metallopharmaceutical, [^{99m}Tc]Sestamibi [hexakis (2-methoxyisobutylisonitrile) technetium-99m], a mitochondrial targeted lipophilic cation, is recognized as a transport substrate by the P-glycoprotein at tracer concentrations as low as picomolar. Because this agent is non-cyotoxic at tracer concentrations and has extremely low non-specific membrane partitioning, we have been able to develop sensitive quantitative assays to address basic biophysical questions related to the transport function of P-glycoprotein. Based on this observation, it is now feasible to specifically design and develop novel organometallic drugs of this class targeted to P-glycoprotein and related ATP-binding cassette transport proteins. One major effort in the laboratory is directed toward the development and characterization of novel arylisonitrile ligands of this class of cationic Tc(I) radiopharmaceuticals with improved properties for use as transport substrates of the MDR1 gene product. The broad ligand binding properties of P-glycoprotein also suggest that selected [^{99m}Tc]$N_2O_2P_2$ and [$^{67/68}$GA]N_4O_2 compounds could also be designed to mimic known MDR agents. Building on our experience with [^{99m}Tc]Sestamibi, new generations of lipophilic catonic complexes are under development which possess properties *in vitro* exceeding those of [^{99m}Tc]Sestamibi, thus the agents have the potential for improved patient imaging characteristics. We synthesize the novel agents and characterize them with biochemical, tracer flux, and P-glycoprotein photolabel inhibition studies with cultured cancer cell and membrane vesicle preparations. Overexpression of recombinant human MDR1 and the non-transporting MDR3 P-glycoproteins in a baculoviral expression system allow detailed structure-activity analysis of transport, binding, and drug-stimulated ATPase activity of novel metal complexes. Our quantitative [^{99m}Tc] Sestamibi transport assay is also used to explore the relationship of P-glycoprotein phosphorylation and transport function. Cytotoxicity modulation assays of metal complexes, quantitative biodistributions, tumor biodistribution studies in a nude mouse xenograft model *in vivo*, and single-photon scintigraphy are used to further evaluate and optimize P-glycoproteinmediated transport activity *in vitro* and *in vivo.*

Measurement Science:

155. Single-Cell Fast Repetition Rate (FRR) Fluorometer to Measure Phytoplankton Organic Carbon and Cell-Specific Carbon Fixation.

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Single Cell Fast Repetition Rate (SCFRR) fluorometry was developed by Zbigniew Kolber and Paul Falkowski at Brookhaven National Laboratory. This technique enables real-time measurements of photosynthetic parameters, including the functional absorption cross section, photochemical conversion efficiency, and rates of electron flow in single individual cells. These measurements can be used to assess cell-specific rates of carbon fixation and to characterize differences between photosynthetic performance of various phytoplankton taxa. Using a species-specific photosynthetic signature, SCFRR measurements can also be used to characterize phytoplankton species composition in the field. Phytoplankton photosynthetic rates display a high degree of spatial and temporal variability, which is largely related to nutrient distributions, temperature, and turbulence. Such variability may be due to direct response of the photosynthetic apparatus to physical and chemical forcings, or may represent changes in species composition or cell size. SCFRR measurements discriminate between these two cases, thereby increasing the understanding of how ocean physical processes affect photosynthetic response. SCFRR fluorometer was used in the Ocean Margin Program (OMP), a major DOE field effort conducted in the Middle Atlantic Bight and along the Western Atlantic continental margin. In this program the instrument characterized spatial and temporal dynamics of species-specific photosynthetic responses. The results revealed high degree of spatial variability in all photosynthetic parameters, related to the spatial and temporal distribution of cell size. The functional absorption cross section was inversely correlated with cell size, cellular chlorophyll, and irradiance, while photochemical energy conversion efficiency was primarily correlated with nutrients.

156. SIMS Technology For The Study Of Microsurface Chemistry

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The objective of this program is to research new concepts that enable the development of secondary ion mass spectrometry (SIMS) for mapping the chemical features of unmodified, heterogeneous mineral surfaces and elucidating the interactions of contaminants with these surfaces. These concepts are incorporated into SIMS instruments to establish their viability and to demonstrate their application in providing insight into the nature of chemical interactions at the mineral surface. The path by which to achieve this objective is to establish technologies that breach existing barriers caused by sample charging, highly variable sample size and topography, and limitations of the primary ion beam. Toward this end, two major advances have been made: the first is a novel and highly effective method for maintaining charge neutrality on large insulating samples termed "self-discharging sample charge compensation," and the second is a new set of ion optics for the primary ion gun capable of focusing ions from a polyatomic solid state ion emitter into a beam that can be zoomed

between 10 microns and 2mm. The solid state ion gun produces the polyatomic perrhenate anion at mass 250, which is exceptionally efficient for desorbing ions of chemicals adsorbed onto the surface of the material to be analyzed. These new technologies are now being incorporated into an imaging SIMS to meet the demands of imaging adsorbates from large mineral surfaces.

157. Subcell Imaging of Insult from Environmental Carcinogens

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This project is concerned with the development of two new laser-based technologies for the study of biological insult from environmental chemical carcinogens at the cellular and sub-cellular levels. One of the two technologies, referred to as hole burning imaging (HBI), can be viewed as an optical analog of magnetic resonance imaging with several important attributes for cellular analysis, including high sensitivity and spatial resolution (0.1 micron). Whereas magnetic resonance imaging is based on proton T1-relaxation times, HBI is based, in part, on T2*-pure optical dephasing times of suitable dye molecules bound selectively to different cell components. HBI would be applicable to the diagnosis of any type of cancer since it does not depend on the nature of the initial insults that lead to the cancerous cell. The laser-based instrumentation is simple and relatively inexpensive. The second technology involves the marriage of laser-induced fluorescence spectroscopy with capillary electrophoresis for on-line structural analysis of oligo- and polynucleotide-carcinogen adducts. This technology can distinguish between different stereochemistries and conformations of a chemically defined base-metabolite adduct (external vs. base-stacked vs. quasi-intercalated) in sequence defined oligo- and polynucleotides. Its utilization in fundamental studies with sequence defined oligonucleotides is important for the construction of DNA-carcinogen adduct maps, the relationship between adduct and mutation maps, and *in vivo* studies of DNA repair.

158. Photoinduced Nucleation: A New Technique for the Detection of Chemical Contaminants

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Using Photoinduced Nucleation to detect low and ultralow concentrations of airborne contaminants is a novel technology which will be very useful for characterizing and monitoring chemical contaminant vapors emanating from polluted soils and in ground waters, as well as in factory environments, homes, etc. When a supersaturated vapor containing low or even ultralow concentrations of a light absorbing chemical is illuminated with light of sufficient intensity and at a wavelength where it absorbs strongly, copious nucleation occurs. This nucleation is wavelength specific and can be used to detect and identify very low concentrations of the contaminant. To date, (using a flow diffusion cloud chamber and a 150 watt arc lamp), we have detected concentrations as small as 100 parts per trillion (of o-tolualdehyde in nitrogen). We were able to quantify this contaminant's concentration since the nucleation rate has a power law dependence on concentration. This new tool has additional valuable features: its response time is about a second, so it can be used as a real time monitor. It also can be used in conjunction with a gas chromatograph to separate the contaminants when several are present and to detect and identify each.

159. High Rate, Low Noise Silicon Array Spectrometer for Synchrotron X-ray Fluorescence Applications

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The objective of this project is the development of a high rate, low noise x-ray spectrometer for synchrotron x-ray fluorescence applications which is based on silicon detector arrays connected to application specific integrated circuits for signal processing. Experiments which are designed to fully exploit the capabilities of modern synchrotron sources require detectors that offer both good energy resolution and high count rate capability. Highly-segmented silicon detectors fabricated using photolithographic techniques offer the advantages of a high total count rate by distributing the flux over many detector elements and low noise due to the small capacitance of each element. Application specific integrated circuits for charge integration, amplification, and digitization facilitate an economic solution to the signal processing requirements of many parallel channels. Such a detector system has applications in a wide variety of synchrotron x-ray fluorescence experiments and can also provide linear position information for one-dimensional diffraction experiments. The specific aims are to: 1) fabricate monolithic silicon detector arrays with hundreds of elements for spectroscopic measurements of 2–20 keV x-rays; 2) develop an application specific integrated circuit which contains many channels of low noise, charge integrating preamplifiers followed by shaping amplifiers; 3) develop an application specific integrated circuit for the independent analog to digital conversion of many parallel channels; 4) build a detector system capable of handling a total count rate > 2 MHz with an energy resolution of ~ 200 ev FWHM for 6 keV photons.

160. Room Temperature Semiconductor Spectrometers for Field Application

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The objective is to develop a field portable gamma-ray and x-ray spectrometer to detect and quantify radioactive contaminants at remediation sites. In restoring contaminated sites at DOE facilities, advanced instruments are needed to characterize contaminants at remote locations. Of these, radiological contaminants can be detected through their gamma-ray and x-ray emissions. This method can be carried out directly on contaminated samples *in situ* or with minimal sample preparation, thus avoiding the need for time consuming and costly chemical analysis. To identify specific radioisotopes, detectors with good energy resolution are required. In the past, high resolution detectors based on cryogenic Ge and Si(Li) devices, which are expensive and not well suited for field deployment, have been used. Recent advances in technology at LBNL have made it possible to realize high resolution detectors that operate at room temperature. Our goal is to develop a portable spectrometer system based on these detectors for use in site remediation activities. Two types of detectors will be pursued: CdZnTe coplanar grid detectors for gamma ray detection and Si drift detectors for x-ray detection. Specific aims: 1) Understand the effects of crystal defects in CdZnTe on charge collection and develop criteria for material selection. 2) Improve energy resolution of room temperature CdZnTe gamma-ray detectors by mitigating the effects of charge trapping with the coplanar grid charge sensing technique. 3) Develop Si x-ray detectors based on a low-capacitance drift detector design, which minimizes the noise due to capacitance and reduces the noise contribution of the detector leakage currents. 4) Evaluate detectors in the laboratory to determine their performance and effectiveness in sensing the actinide radionuclides. 5) Integrate the Si and CdZnTe detectors into a field portable instrument. 6) Compare in the lab the per-

formance of the field-portable instrument with conventional systems in the characterization of actinides. 7) Evaluate the instrument under field conditions.

161. Near-Infrared Detection Methods for Complex Mixtures

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Develop fiber optic probe using near infrared light to determine water content, polyatomic ions, metals, and total organic carbon in mixed wastes stored at DOE facilities. We are compiling near infrared spectra of representative mixtures of the materials of interest and performing chemometric analysis on the spectra. We are developing prototype fiber optic sensors and studying their capabilities and suitability for use in mixed waste systems. We have identified suitable near infrared bands for measurement of water, total organic carbon, and inorganic ions that are often present in mixed wastes, and are performing chemometric analysis on the spectra to develop procedures to quantify the measurements. We are developing chelate coatings that will permit the measurement of metal ions by near infrared spectroscopy.

162. Multifrequency Phase/Modulation Flow Cytometer

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The objective of this project is to develop an advanced, multifrequency (harmonic), laser-based measurement system for quantifying multiple excited-state lifetimes by frequency-domain methods on fluorochrome labeled cells and subcellular components. Develop an advanced frequency-domain flow cytometer for measuring excited-state lifetimes by phase shift and amplitude demodulation methods at multiharmonic frequencies using a pulsed laser excitation source; 2) Characterize the instrument in terms of measurement sensitivity, precision, accuracy, and dynamic range; 3) Evaluate the instrument's ability to measure fluorescence lifetimes and separate signals from heterogeneous fluorescence emission(s) based on differences in lifetimes at multiharmonic frequencies; 4) Utilize this new technology to study the interaction of fluorescence probes with cells and subcellular components in the context of biological responses induced by radiation and chemicals associated with energy production and utilization; and 5) Improve and further advance the technology based on results of our initial biological studies.

163. New Multi-Tracer Methods in Biological Oceanographic Research

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The Radioisotope Research Group at Los Alamos National Laboratory uses its expertise and unique facilities to produce tracer radionuclides and to facilitate the application of the tracers in science and industry. One component of this work is the development of radioanalytical methods for application of tracers to the solution of practical problems. This activity has led to several opportunities for important collaborative work with researchers in fields not directly related to nuclear and radiochemistry. For several years we have worked with Dr. Mark Brzezinski of the University of California, Santa Barbara on a project that has resulted in materials and methods for the application of Si-32 as a radiotracer in biological oceanographic research. The tracer is used to measure silicate uptake by diatoms, siliceous phytoplankton that require silicate as a nutrient. Because of their sheer volume, the diatoms sequester significant amounts of atmospheric carbon dioxide, thus representing a major sink for this "greenhouse"

gas. By correlating the data from silicate-uptake studies with data from studies on the uptake of other essential nutrients, such as bicarbonate, inorganic nitrogen, and inorganic phosphorus, important information on the role of diatoms in the global climate is obtained. Current theories indicate that silicate may be the limiting nutrient in some important ocean bodies, and data from studies such as those supported by our methods will be used to test these theories. The availability of high specific activity Si-32 and the radioanalytical methods developed in the collaboration are therefore significant new tools in this scientific area. In this project we will develop methods to use both ^{14}C and ^{32}Si in radioanalytical measurements of simultaneous uptake of carbon and silicon by diatoms in single incubation experiments. In order to accomplish the dual tracer experiments the challenge will be to develop radioanalytical methods that permit the simultaneous quantitative measurement of three different beta-emissions from incubated samples. The ^{32}Si decays to ^{32}P, and since phosphate is also a nutrient for diatoms, both of these beta-emitters are present in incubated samples. The large difference in the half-life of the parent and daughter isotopes causes stock solutions to attain secular equilibrium where equal amounts of radioactivity from both parent and daughter isotopes are present. However, the uptake rates of the two elements are not the same, so the quantitative distinction of the two tracers is required in the radioanalytical method used to measure the separate uptakes. The measurement is complicated by the fact that the maximum beta energy of ^{14}C and ^{32}Si are similar enough to require deconvolution methods to quantify the two tracers. Methods will be developed using scintillation techniques and Cerenkov counting. The development of the triple label counting method would allow real-time or near real-time sample analysis and permit determination of phosphate uptake rates when desirable. Since the triple-label counting technique will ultimately be adapted for field oceanographic measurements, we will also develop methods based upon gas-flow proportional counting, and multiple foil methods adapted from the technique developed for the simultaneous measurement of non-equilibrium ^{32}Si and ^{32}P.

164. Nanometer Scale Imaging/Spectroscopy of Biosamples with Photon Tunneling

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Seek to use photon tunneling to image and spectroscopically analyze biological samples on the nanometer scale. Approach by variation of sample preparation methodologies and instrumentation in order to obtain resolution superior to that of conventional optics.

165. Optically-Coupled Microstructured Biosensors

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Research and develop biosensors which use optical signals to detect chemical compounds and with are constructed so as to take advantage of microstructured surfaces. Photon-absorbing and photon-coupling microstructures are used on optical fibers to enable the detection of medically or environmentally important compounds in liquids or air.

166. Biological and Environmental Microsensor Development

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The approach uses micromachined cantilevers that respond to stress or mass changes induced by adsorption, radiation, temperature, flow, viscosity and other environmental factors. Chemical and biochemical sensors utilize analyte-specific coatings. Electrochemistry is combined with cantilever electrodes by controlling electrical potential. Micro-analytical measurements are explored by observing calorimetry on cantilever surfaces. The temperature sensitivity of 10^{-6} K is being exploited for infrared radiation sensing. Other types of sensing such as nuclear radiation are being explored using this approach. Extension of this flexible concept into a universal detection paradigm is being pursued. This research will yield unique, microminiature techniques for monitoring. Mercury vapor could be sensed in the parts-per-trillion range, about 100 times better than commercial mercury sensors. Mass sensitivity of one picogram was demonstrated. Stress sensitivity may ultimately allow single molecule detection. The first infrared imaging using micromachined sensors was recently demonstrated.

167. Advanced Mass Spectrometry for the Molecular Characterization of Biological Insults

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The goal is to develop new mass spectrometry-based capabilities for the detection and identification of normal and modified biomolecules in cells. Novel gas phase processes are being examined to yield increased structural information from complex molecules, taking advantage of the trapped ion capabilities of Fourier transform ion cyclotron resonance (FTICR) mass spectrometry. Matrix-assisted laser desorption/-ionization (MALDI) and electrospray (ES) are being employed for the generation of singly-and multiply-charged ions, respectively, from peptides, proteins, oligonucleotides, and other biomolecules. Improvements in detection limits and manipulation of trace samples are being examined to allow detection of components in single cells. This includes examining new methods for sample handling and purification techniques with MALDI combined with time-of-flight mass spectrometry, developing new approaches for efficient introduction of ES ions into the FTICR, and improving ion manipulation and detection techniques. The research will result in new capabilities for the detailed structural identification of molecules in cells that are important in human health, energy production, environmental remediation and advanced biotechnology processes.

168. Monitoring Systems for Energy Related Pollutants

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The objective is to develop novel or improved monitoring technologies to measure chemical and biological indicators of human exposure to potentially harmful agents associated with energy-related technologies. The research focus is on the development of portable instruments, cost-effective screening tools, and advanced biochemical sensing technologies for measuring human exposure to key pollutants and related biological compounds in complex mixtures where current techniques are unavailable or inadequate. Research efforts are also devoted to the development of selective and sensitive detection methods for human biochemical responses to hazardous compounds. Examples of research topics in biological and chemical monitor development are: 1) surface-

enhanced Raman spectroscopy (SERS), 2) bioreceptor based sensors for *in vivo* measurements of biological samples, 3) fiberoptics monitors for *in situ* and remote sensing, 4) cost-effective indicators (such as DNA adducts) of human exposure and health effects.

169. Magnetic Resonance Microscopy of Mammalian Cells

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NMR microscopes will be developed, operating in large external magnetic fields, and capable of 1H magnetic resonance imaging (MRI) and 1H and 31P localized magnetic resonance spectroscopy (MRS) of live three dimensional cell clusters. Several probes will be manufactured, operating in external magnetic fields of 9.4, 11.7, 14.1, 17.6 and 22 Tesla. The probes will contain a flow-through tissue culture device in which a constant environment for the cell systems can be maintained for at least 48 hours. The different probes will be used to examine the performance of MRI and MRS at the various fields. For 1H MRI the intra-cellular water will be imaged. The maximum target voxel size is 6X10-17 m3 at the highest field, corresponding to a spatial resolution of 4 micrometer in all directions; the maximum target measuring time is one hour. ^{1}H and ^{31}P MRS will be used to measure metabolic concentrations in selected voxels in the cell cultures. The maximum target volumes for are $3x10^{-13}$ and $9x10^{-11}$ m^3, respectively; the maximum target measuring time is 4 hours. Calculations have been performed to predict the sensitivity of the MRI and MRS experiments in a quantitative way. These calculations have been used to develop and optimize the experiments, and to determine metabolite concentrations from the MRS spectra. At present it is possible to calculate the signal intensities within 20 -50% of its experimental value. Probes operating at 9.4 and 11.7 T without culture devices have been built and evaluated. First images of V79 tumor spheroids have been obtained. Also, other applications of MR microscopy are being explored in collaboration with experts within and outside PNNL. Examples of such applications are the determination of bone structures in excised mouse vertebrae, the evaluation of tree seeds of importance to the lumber company, and small-scale flow phenomena in porous media.

170. Automation of Radiochemical Analysis Flow Injection and Sequential Injection Methods

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The objective of this research is to develop new microscale automated techniques to separate and measure radionuclides. The primary focus will be on radiochemical analysis methods as they apply to te analysis of tank wastes, but the methodology will also be applicable to environmental samples. The radionuclide separations to be automated will be those fission products and actinides in tank waste that are important in waste characterization. New automated radiochemical analysis methods will be developed in an interdisciplinary research program of separation science and measurement science. Flow injection (FI) and sequential injection (SI) methodology will be used to automate sorbent extraction and chromatographic radiochemical separations processes and deliver the sample to the detector. Radiochemical separation chemistry will be investigated with regard to issues that must be addressed to successfully automate such methods for analytical purposes, including analyte recoveries, reproducibility, rigorous control of speciation, kinetics of on-line reaction chemistry, characterization of separation materials, and the effects of complex sample matrices. Novel methods will be developed for renewing separations materials on-line. Multiple detection will be used for the quantification of separated radionuclides. New radiation detecting flow cells will be designed specifically for use with FI/SI systems performing radiochemical separations. In the final phase, we will investigate integrating separation

and detection operations in a single compact unit. In preliminary work prior to the funding of this project, a new automated sequential injection analysis system for separation and analysis of SR-90 in tank waste samples was developed. This new system has served as the platform for investigating automated separations of actinides by sorbent extraction methods. Procedures have now been developed to separate a fraction containing gross actinides; to separate transuranic elements from uranium; to separate plutonium and americium, and to sequentially separate americium, plutonium, thorium, and uranium, all in automated format with on-line detection. These procedures were developed in conjunction with investigating on-line redox chemistry as it applies to radionuclides captured on a sorbent column.

171. Miniaturized Optical Based Sensors for Extreme Environments

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To capitalize on the rapid progress in opto-electronics and semiconductor microfabrication technologies to produce miniature optical spectrometers to enable the development of miniature optical sensors for *in situ* characterization of wastes in extreme environments. Specifically, we propose to develop a microspectrometer based on a novel correlation spectrometer coupled with high brightness, semiconductor LED sources and semiconductor detectors. By tuning the operating wavelength to match the spectral signatures of different chemical species the microspectrometer concept can be adapted to the detection of a wide variety of of organic and inorganic compounds. We have developed an algorithm which allows the calculation of dffraction grating profiles for which multiple wavelengths can be diffracted at the same angle. This algorithm can be used to define novel diffraction gratings that synthesize the spectra of molecules. Spectra of chemical species that are difficult or dangerous to handle or that exist for a very short period of time can be synthesized. Such a grating can then be used in a correlation spectrometer to provide a simple monitor for such species. Conventional correlation spectroscopy passes light through a gas volume to be sampled and then through a reference cell containing the target species. The spectrum of the reference species is modulated using pressure or a large electric field. If the spectral features from the reference cell overlap those from the sample volume, a modulated output occurs. If there is no overlap, then there is no modulation of the output. The reference cell can be replaced by the novel grating that generates the desired synthetic spectrum and the wavelength modulated by rocking the grating. Such correlation spectrometers will be simple, inexpensive monitors for wide array of dangerous chemical species. Diffraction gratings that synthesize infrared spectra of HF, toluene, MIBK, and kerosene have been fabricated using reactive ion etching of single crystal silicon wafers and a gold coating to form the reflection grating. The spectral content of the diffracted beam, measured for HF, reproduces the calculated spectrum with variations in relative line intensity that may be due to non-uniform illumination of the grating. A computer model, including atmospheric interferences, has been developed to simulate the performance of correlation spectroscopy and investigate optimal spectral ranges and expected sensitivities. We have found that gases can be detected at concentrations of < 1 - 10 ppb. A 2 stage/ 2 color LED has been grown where the Sb mole fraction of the active regions differs by 0.03 which allows emission from each stage to be observed. These two overlapping peaks increase the emission bandwidth of the LED. LED emission has been observed at 300K, 4 m with 10 W peak power.

172. Cadmium Zinc Telluride Gamma-Ray Imaging Spectrometer System for the *In Situ* Characterization of Underground Waste Tanks

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There is an expressed need for *in situ* characterization of the general and detailed composition of wastes contained in underground storage tanks to allow for the application of safe and efficient remediation activities. Current methods rely on extensive core sampling and laboratory analyses, which is an expensive and time-consuming process, as well as having the potential for exposure of personnel and creating secondary waste. This proposal focuses on a new tool for *in situ* characterization. The primary element of this tool is a cadmium zinc telluride (CZT) gamma-ray imaging spectrometer. The gamma-ray spectrometer allows for the identification of waste constituents by their unique radiological features, either through their intrinsic emissions, or through their excitation and subsequent emissions. The imaging aspect of the detector allows for the spatial mapping of the waste homogeneity by imaging liters of material with milliliter resolution. The fundamental enabling technology for this characterization tool is the CZT detector, as these semiconductor detectors are capable of operating in many difficult field applications: high temperatures, high radiation levels, and chemical harshness. The intended accomplishment of this project is to provide a new capability for the spatial characterization of the radiological and elemental constituents of wastes stored in underground tanks. The end product will be the components necessary to develop and implement an *in situ* waste characterization system.

172a. Aequorin as a Bioluminescent Indicator for Use in the Determination of Biomolecules in Single Cells

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The goal of our work is to develop new methods of analysis capable of determining chemical components of single cells. Specifically, methods are being developed for the quantitative determination of biomolecules in picoliter-volume vials by employing the bioluminescence of the photoprotein aequorin along with photon-counting detection. For example, using this approach avidin can be detected reproducibly at femtomole levels by taking advantage of its inhibitory effect on the bioluminescence signal generated by biotinylated recombinant aequorin. The picoliter vials are being fabricated by employing laser ablation techniques. This is the first time that binding assays are being performed in picoliter vials, which provides new opportunities for quantitative measurements in small volumes. Thus, this type of detection should be well suited for microanalysis, single cell analysis, and in high-throughput screening of biopharmaceuticals prepared by combinatorial methods, where the amount of sample is very small or limited.

173. A Fiber-Optic Profiler for Studying the Distribution and Transport of Organic Contaminants in Marine Sediments

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The major objective of this project is to develop a fiber optic profiler with the application of rapid assessment

and detailed organic geochemical studies of contaminated subsurface environments. This new instrument is designed specifically for real-time *in situ* measurements in the marine environment. Five specific objectives created to meet this overall goal are: 1) to develop a fiber optic profiler to measure fluorescence emission spectra and time-resolved fluorescence in seawater, sediment, and sediment porewater. 2) to relate the fluorescence observed in environmental samples to polycyclic aromatic hydrocarbon (PAH) concentrations. 3) to use the profiler in conjunction with a benthic flux chamber to measure fluxes of PAH out of contaminated sediments. 4) to obtain high resolution vertical and horizontal distributions of PAH in polluted sediments. 5) to further develop and integrate our instrument into a fully autonomous, submersible, *in situ* system for environmental studies and monitoring. Significant progress has been made in addressing all five objectives with four field efforts in San Diego Bay and Boston Harbor to provide environmental samples and *in situ* data. We have learned that benthic fluxes of PAH in Boston Harbor and San Diego Bay are significant compared to other sources, but are not the dominant source as simple models based on sediment concentrations would predict. Probe work is ongoing, but it appears that time-resolved fluorescence is a reasonable measure of dissolved pyrene concentrations and of total PAH in sediments. Finally, we are examining the sediment-water interface at high resolution (mm) in mesocosms to better understand the fate of PAH in the marine environment. A small, low-power, submerisble system is currently being designed and constructed.

Environmental Management Science Program:

Analytical Chemistry

This program is funded by the Office of Environmental Management of the Department of Energy and is co-administered by the Office of Energy Research. The projects in analytical chemistry are managed by the Medical Applications and Biophysical Research Division for the Office of Energy Research.

174. Sensors Using Molecular Recognition in Luminescent, Conductive Polymers

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This program integrates three individual, highly interactive projects that will use molecular recognition strategies to develop sensor technology based on luminescent, conductive polymers that contain sites for binding specific molecules or ions in the presence of related molecules or ions. Selective binding of a particular molecule or ion of interest to these polymers will result in a large change in their luminescence and/or conductivity, which can be used to quantitatively sense the presence of the bound molecules or ions. Molecular recognition sites for a variety of toxic metals and organics will be developed. These include transition metals, heavy metals such as lead, uranium, and plutonium, as well as toxic organics, such as chlorinated and nitrated aromatic molecules. Research problems that will be addressed include: 1) designing molecular recognition sites that are highly selective for the ions and/or molecules of interest in the presence of a large background of other chemical species, 2) finding ways to incorporate many different selective groups into a single polymer, 3) fabricating polymer films, strips, sheets, and coatings that can be applied to other materials, such as fiber optics and surfaces, 4) developing interfaces between the polymers and substrates that can be used to produce prototype arrays of many sensor elements for rapid multi-contaminant detection and quantitation, and 5) developing multiplexed data collection techniques to rapidly process the data obtained from many polymer sensors into a chemical profile of a waste stream or waste site in real time. Each project is designed to carry out an important aspect of sensor development that will be integrated into an overall effort to produce novel sensors for use in the rapid assessment of environmental remediation strategies.

174a. High Temperature Condensed Phase Mass Spectrometric Analysis

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Our current studies with temperature ion emitting materials have demonstrated a significant lack of methods for determining chemical species in condensed phase materials in general, and at elevated temperatures in particular. We have developed several new research techniques for approaching this issue of high temperature chemical speciation, but new instrumentation is needed in order to more completely

address the resarch needs. It is proposed to develop a new ultra high vacuum mass spectrometer system which combines the features of static and dynamic SIMS, surface ionization, and electron impact ionization of evaporating neutrals, in which miniaturized samples can be studied from room temperature up to 1500 C. All data will be collected from the same sample by rapidly interlacing the different ionization modes which will significantly expedite data correlation. This proposed instrument will enhance research efforts in understanding chemical reactions and materials at high temperatures. Ongoing research at the INEEL in ion emitter studies will greatly benefit from this work, but benefits across a wide range of other fields can also be expected. An issue of significant interest to DOE waste management is that of chemical stability of waste storage forms. These solid materials primarily glasses will be produced at high temperatures then cooled and put into storage. The possibility exists that in a geologic repository heating from radioactive decay, combined with limited cooling, could result in extended storage at elevated temperatures. The studies that can be conducted with the proposed instrument can be applied to a wide range of high temperature processes and materials, and will add a new and needed dimension to the study of waste storage glasses, providing insights on the mobility and chemical speciation of the hazardous components.

175. Improved Analytical Characterization of Solid Waste Forms by Fundamental Development of Laser Ablation Technology

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Chemical characterization is listed as the top priority need in every DOE EM major problem area (high-level waste tanks, contaminant plumes, D&D activities, spent nuclear fuel, mixed wastes, landfills, etc.). The DOE National Laboratories have invested millions of dollars in effort and equipment in order to utilize laser ablation technology to address chemical characterization needs within these problem areas. However, fundamental laser ablation processes are not being addressed in these studies, but rather specific applications of a complex, unknown technology. This research will address several fundamental issues, including energy coupling, mass removal, gas dynamics, and transport. Energy coupling is critical in that it governs the sensitivity and accuracy of constituent removal. Experiments will provide new knowledge on space charge effects, plasma screening, and plasma expansion. We plan to investigate calibration technologies that do not rely on "matched" standards. For the diverse mixed-waste samples from DOE environmental sites, standards will not exist and it will not be practical to fabricate them. Solid vapor entrainment and transport processes can significantly influence sensitivity and accuracy and will be investigated. The particle size distribution generated during laser ablation influences transport. Mechanisms responsible for particle generation will be addressed. Samples to be emphasized initially will include prototype vitrified waste glass and metal/metal-oxide systems. This fundamental work will support the efforts at all the National Laboratories investigating laser ablation technology for the management of DOE radioactive, hazardous chemical, and mixed waste.

176. Rapid Mass Spectrometric DNA Diagnostics for Assessing Microbial Community Activity

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The use of DNA-based procedures for the detection of biodegrading organisms or genes that code for pollutant-degrading enzymes constitutes a critical technology for following biochemical transformation and substantiating the impact of bioremediation. In previous studies, DNA-based technology has been demonstrated to be a sensitive technique for tracking

micro-organism activity at the molecular level. These procedures can be tuned to identify groups of organisms, specific organisms, and to detect signals that measure microbial community activity. This proposal describes the evaluation of a monitoring strategy that relies on the combined use of DNA diagnostics with mass spectrometry as the detection scheme. The intent of this work is a two-fold evaluation of: 1) the feasibility of replacing the use of gel separations for identifying polymerase chain reaction (PCR) products with a rapid and automatable form of electrospray mass spectrometry and 2) the use of matrix-assisted-laser-desorption-ionization mass spectrometry (MALDI-MS) as a tool to score oligonucleotide ligation assays (OLA). Mass spectrometry is an attractive detection alternative for PCR and OLA procedures because it is a sensitive analytical technique capable of providing high sample throughput performance and the introduction of samples into a mass spectrometer is automatable. Mass spectra are generated rapidly, in the order of minutes, thus eliminating a time bottleneck caused by time consuming gel electrophoresis separations. The adaptation of OLA for mass spectrometric analysis will cut costs by eliminating expensive reagents. The techniques are automatable which translates into a decreased human error rate when numerous samples are analyzed as a component of large scale bioremediation studies. The successful conclusion of the proposed work will be the development of a mass spectrometry capability for sizing PCR products and scoring OLA tests in formats that provide high throughput and automation for bioremediation studies.

177. Real-Time Broad Spectrum Characterization of Hazardous Mixed Waste by Membrane Introduction Mass Spectrometry

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It is proposed to expand the real-time monitoring capabilities of Membrane Introduction Mass Spectrometry (MIMS) to the problem of mixed hazardous wastes, with secondary emphasis on monitoring incinerator stack gases for both organics and toxic metals. The methodologies developed could also be applied to other environmental and effluent monitoring problems that require highly sensitive online detection of a wide variety of chemicals including both volatile and semi-volatile organic compounds and heavy metals. It is proposed to conduct research and development necessary to develop a MIMS methodology for broad range characterization of both organic and heavy metal contaminants in a variety of matrices and effluents. In the MIMS experiment a polymer membrane is the interface between the sample and the vacuum of the mass spectrometer. While polydimethylsilicone membranes have proven to be extremely suitable for real time VOC analysis, other analytes and matrices pose technically challenging problems in the normal MIMS configuration. We will explore several areas to address these problems: improved membrane selectivity for sample introduction; application of ion-molecule chemistry and advanced mass spectrometer operation to improve speciation of complex samples; development of techniques for extraction of soil-bound contaminants; and the pursuit of derivatization methods to make MIMS amenable to heavy metal analysis. This research will lead to real-time mass spectroscopic methods that can be applied to a variety of problems in environmental characterization.

178. High Fluence Neutron Source for Nondestructive Characterization of Nuclear Waste

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We propose to research the basic plasma physics necessary to develop a high fluence neutron source based on the inertial electrostatically confined (IEC)

plasma. An intense neutron source directly addresses the capability to characterize nuclear materials under difficult measurement conditions. Some of the applications for Environmental Management are the characterization of TRU wastes for WIPP (particularly remote handled [RH]), the measurements of residues prior to stabilization and disposal, the measurements of cemented or vitrified wastes, the measurement of spent nuclear fuel, and the measurement of high level wastes. Existing technology is insufficient to measure these contaminants because it addressed a substantially different technical problem: the measurement of very pure material. However, the present need is to develop measurement capability for highly impure, contaminated, and heterogeneous materials that are the wastes and residues of the production process. These measurement conditions demand measurement capabilities several orders of magnitude above existing ones. This neutron source could extend existing instrumentation sufficiently to meet these requirements. The benefit of this approach is that mature, neutron-based, nondestructive characterization methods could be used, but their capabilities would be increased by the same amount as the increase in neutron intensity. We propose to develop a source at the 10^{11} n/s level, with a cost, weight, and size comparable to 10^{8} n/s systems, for a three order of magnitude improvement.

179. The Development of Cavity Ringdown Spectroscopy as a Sensitive Continuous Emission Monitor for Metals

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We propose to conduct an innovative science-driven technology program to explore the viability of using Cavity Ringdown Spectroscopy (CRS) to monitor the remediation processes for hazardous and radioactive wastes. This is a technique capable of providing ultra-sensitive absorption measurements ($< 10^{-6}$ fractional absorption) in hostile environments using commercially available easy-to-use pulsed lasers. This project has three primary goals: 1) Determine the viability, through experiment and model validation, of marrying an exciting new science tool—Cavity Ringdown Spectroscopy—with standard analytical instruments, e.g., the ICP and graphite furnace. 2) Provide the first quantitative evaluation of cavity ringdown spectroscopy for trace analysis. 3) Make a significant and positive impact in support of the management and disposal of DOE radioactive, hazardous chemical, and mixed waste. Cavity ringdown spectroscopy is a measurement of the rate of absorption of a sample with a closed optical cavity rather than the standard measurement of the absorbed signal strength over a given signal path. The inherent high sensitivity stems from both the long effective sample pathlengths possible (in the tens of kilometers) and the relaxed constraints on the accuracy of the measurement of the cavity decay time ($\sim 1\%$ accuracy yields ppm absorbance detection capability).

180. Novel Miniature Spectrometer for Remote Chemical Detection

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This research will develop an entirely new class of chemical sensing technology that will enable qualitative and quantitative remote, real-time diagnostics of chemical species in hazardous gas, liquid, and semi-solid phases through a completely novel implementation of evanescent wave spectroscopy. The sensor design utilizes a small, solid block (< 1 cm^3) of ultra-high purity optical material that is fabricated into a regular, planar polygon with a convex facet to form a total-internal-reflection ring cavity. For light undergoing sustained circulation by total-internal-reflection inside the solid, the facets of the polygon act as extremely high-reflectivity ($\sim 99.9999\%$ in some

cases) mirrors, resulting in a relatively long and accurately measurable life time for an injected light pulse. Evanescent waves, which are generated by total-internal-reflection, are absorbed by matter in the vicinity of the cavity where the evanescent wave decays exponentially in space. The absorption spectrum is extracted by measuring the mean life time of an injected light pulse as a function of pulse carrier frequency. Errors associated with light source fluctuations, which typically limit the sensitivity of conventional absorption methods, are eliminated by this single pulse measurement, as in the gas-phase technique known as cavity ring-down spectroscopy. By locating the light source and detection system at a distance (e.g., 0.1 to 10 Km) through the use of fiber-optics, this new technology will permit remote, high-sensitivity, broadband chemical sensing with rugged, cost effective, miniature spectrometer. For the laboratory program the tasks include: 1) experiments that verify chemical sensitivity, 2) development of a fabrication strategy for ruggedly mounting the coupling prisms to the TIR-ring cavity, 3) design and fabrication of TIR-ring cavities that allow detection of chemical species in the near-and mid-infrared (IR) spectrum, 4) development of a fiber-optic interface to TIR-ring cavities, 5) characterization of the technology by using these devices to detect chemical species of importance to the EMSP mission, and 6) investigation of potential interferences, e.g., particulates, abrasives, inhomogeneities, temperature and density gradients.

181. Gamma Ray Imaging for Environmental Remediation

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We plan a three year research program to apply high resolution gamma-ray imaging technologies to environmental remediation of radioactive hazards. High resolution, positron-sensitive germanium detectors are being developed at the Naval Research Laboratory for space applications with support from the Office of Naval Research and the National Aeronautics and Space Administration. In the program, we will model the performance of these detectors for direct imaging of spent nuclear fuels and fissile materials and compton scatter imaging of large objects of arbitrary size, investigate fabrication of field-usable detectors, and demonstrate the performance of such a system using a small configuration of detectors.

182. Development of an *In-Situ* Microsensor for the Measurement of Chromium and Uranium in Groundwater at DOE Sites

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The goal of this program is to develop, optimize and deploy a silicon-based micromachined stripping analyzer for field monitoring trace levels of chromium and uranium. Such system will integrate the sample-handling steps and necessary chemical reactions (using a flow-injection operation) with the already proven adsorptive-stripping voltammetric operation on a small planar chip. Besides the drastic reduction in the size of the analytical system, such miniaturization should lead to increased speed, minimal reagent consumption and disposal, higher sensitivity and improved precision, and would thus revolutionize the way by which toxic metals are being monitored. In order to fully exploit this opportunity, it will be necessary to develop a fundamental understanding of the behavior of such scaled-down flow-injection stripping system. Considerations of proportionalities and similarity will thus be used for deriving theoretical expressions for the dependence of the response upon variables to be miniaturized. The new knowledge will serve as a useful guideline for the rational design of the system manifold, and through the optimization, characterization and field deployment of the micro-

machined analyzer. Overall, this research will create powerful and economical microsystems for *in situ* monitoring of metal contaminants in DOE sites, and will shed useful insights into the micromachining and behavior of miniaturized flow analyzers, in general.

183. Investigation of Techniques to Improve Continuous Air Monitors Under Conditions of High Dust Loading in Environmental Settings

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The proposed investigation is an-in-depth exploration of environmental influences that can cause degradation of the performance (sensitivity, alarm functionality, etc.) of Continuous Air Monitors (CAM's), such as the LANL/Canbara alpha-particle CAM, and a study of techniques to correct for this degradation. Such degradation is of ongoing importance, especially since concerns about health effects of environmental radioactivity are leading to tighter controls on atmospheric releases of manmade radioactive substances. Degrading factors to be studied include ambient aerosol particulate deposits on CAM filters and the interactions of radon progeny background, as well as plutonium or uranium, with such deposit structures. Making use of recently available time-lapse video microscopic technology, the formation of dendritic structures from aerosol loading will be studied on present LANL CAMs. In addition, we have recently received from the University of Lund a research prototype pulsed ionization chamber with large surface area. Adapting such chambers for innovative CAM use will be investigated. Their ability to monitor filters with areas five to ten times larger than in present CAMs might lead to a significant alleviation of the aerosol loading problem and improved sensitivity from increased air sampling flow rates. Findings from the project will be of significance for the design of CAM pre-separators, filter media and use, development of data analysis software, and other critical CAM design and operational issues. The outcome will be a more reliable, sensitive air monitoring instrument for environmental settings such as waste processing/disposal sites.

184. Measurement of Radon, Thoron, Isotopic Uranium and Thorium to Determine Occupational and Environmental Exposure and Risk at Fernald Feed Materials Production Center

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The proposed research at the Fernald Environmental Restoration Management Corporation site will provide radionuclide data, and realistic risk evaluation for isotopic radon, thorium, uranium and lead exposures. We have developed and tested a passive radon monitor with proven accuracy and precision that can be miniaturized to provide accurate personal exposure during remediation. These monitors will be used in area and personal exposure assessment. We have a novel aerosol particle sampler that can provide measurements of the particle size distribution over long time periods (days to weeks). This will be the first remediation to provide personal and area particle size distribution measurements. The aerosol particle size is the major determinant in lung dose variability. There was historic occupational and environmental exposure to Rn-222. There will be exposure to Ra-228, Ra-226, Rn-220, Rn-222, Th-228, Th-230, Th-232, and Pb-210 during remediation. There are two silos containing 4000 Ci of Ra-226 which will undergo some form of vitrification. There are eight waste pits containing radium, thorium and lead in particulate form with concentrations to 4000 pCi/g. These particulates will be airborne as material is resuspended during the excavation procedures. Our study will provide accurate personal exposure assessment for all of the hazardous procedures with data available throughout each

process so that rapid decisions concerning risk can be made. On site and remote soil, water and air samples will provide a full delineation of the environmental radionuclide contamination. A realistic carcinogenic risk assessment, based on these extensive environmental and occupational measurements, will be available throughout the restoration.

185. Novel Mass Spectrometry Mutation Screening for Contaminant Impact Analysis

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This project is to address the DNA mutation due to the exposure to contaminated media and to promote a better understanding of the relationship between exposure and health impact. The objective is to develop innovative mass spectrometry technology to achieve fast mutation screening from contaminated area and to reveal the linkage between gene mutation and contaminants. Mass spectrometry has the potential to achieve very fast speed sample analysis. However, the poor mass resolution and low detection efficiency for long DNAs limit the broad application for mutation screening. In this program, new innovative approaches for improving mass resolution and detection sensitivity will be pursued to achieve rapid DNA screening. Allele specific polymerase chain reaction (APCR) will be coupled with the proposed mass spectrometry for detecting DNA mutations. At completion of the technology development, the merit of this approach will be directly tested by analyzing the possible mutations in ras gene wildlife, such as fish, exposed to environmental genotoxic agents. Successful accomplishment of the proposed project will allow the genotoxic effect of hazardous waste to be routinely assessed directly at DNA level at an affordable cost. The specific approaches of this program are: 1) to develop innovative laser-induced acoustic desorption mass spectrometry and matrix-assisted laser desorption/ionization/fragmentation mass spectrometry for rapid DNA analysis, 2) to couple with APCR and competitive PCR for quantitative mutation analysis, 3) to develop new methodology to improve the detection efficiency of long DNAs, 4) to identify and sequence the specific exon of fish ras gene by reverse-transcript PCR, 5) to develop automated systems for rapid sample analysis, and 6) to demonstrate that pollutant-mediated mutations can be used as biological indicators for contaminant impact analysis.

186. Thermospray Mass Spectrometry Ionization Processes Fundamental Mechanisms for Speciation, Separation and Characterization of Organic Complexants in DOE Waste

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This project will develop and interpret positive and negative ion thermospray mass spectra of complexants and complexant products using liquid chromatography combined with themospray mass spectrometry, to understand the equilibria of organic complexants and their products in multi-component mixtures. The approach will be in two phases: 1) thermospray mass spectrometry for complexers and their products with metals (including radionuclides) will be investigated at ORNL in order to define the chemical species in aqueous media of high ionic strength. A parallel task at the University of Minnesota will study liquid chromatography on inert stationary phases to facilitate the introduction of individual chemical species or simplified mixtures into the mass spectrometer 2) the knowledge from the previous phase will be used to determine any unavailable equilibrium constants in order to evaluate the impact of complexants on waste treatment.

187. Microsensors for In-situ Chemical, Physical, and Radiological Characterization of Mixed Waste

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An innovative single-sensor platform approach is proposed that is based on recently discovered extreme sensitivity of microcantilever sensing using adsorption-induced forces. The objective of this research program is to gain a better understanding of the mechanism of adsorption-induced differential surface stress variation and to use this novel idea for developing sophisticated microsensor concepts for sensing in liquids. As a demonstration the project will develop: 1) chemically specific microsensors by coupling surface modification chemistry with molecular recognition agents for identification of inorganic species found in ground water, 2) coating techniques that will enable operation in corrosive, high ionic strength, and radioactive environment, and 3) microcantilevers for assessing local radioisotope content and speciation based on chemical sensing and radiation damage. This multipurpose sensor technology offers the potential to provide real-time *in-situ* characterization of the chemical, physical, and radiological properties of groundwater, contaminated soils and process streams. The advantage of this approach is that once the basic technique is developed, it can be the basis for a universal sensor platform for many DOE needs such as the chemical, physical, and radiological characterization of tank waste, monitoring of environmental cleanup, and detection of emitted gases from incinerators and waste vitrification plants.

188. Optically-Based Array Sensors for Selective *in situ* Analysis of Tank Waste

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Fundamental research will be directed toward developing an array of chemically selective sensors, based on highly selective molecular recognition agents and fluorescence techniques, coupled to fiber optics for the safe and cost effective *in situ* characterization of high level waste tanks. This multidiscipilinary approach to the design of sensors will be to immobilize agents for selective molecular recognition, chosen from solvent extraction process, in an organic polymer matrix that mimics the organic medium in an aqueous-nonaqueous extraction. In this manner the matrix will enhance both the separation and the achievement of chemical sensitivity. Principal investigators plan to utilize complexation of the Cs and Sr by derivatives of calix-crown ether ligands which contain a fluorophore that is designed to turn on the emission intensity upon metal ion complexation. By also using ligands that are selective for Na and K in separate sensor elements, a correction for the interference can be obtained. Proposed studies will also examine Cs selective ligands designed so that the heavy atom effect of Cs will promote formation of the lowest triplet excited state of a potential phosphorescent moiety. Surface adsorption is known to inhibit quenching of the phosphorescence, and we will investigate complexation induced phosphorescence of matrix embedded ligands as a sensing mechanism. An array of sensor sites , each with optimized selectivity for one of the components in the analyte, attached to an imaging fiber optic bundle will be used. Any deficiencies in selectivity, arising from cross reactivity for competing ions, will be overcome by using pattern recognition algorithms to deconvolute the array's response pattern.

189. Monitoring Genetic & Metabolic Potential for *In Situ* Bioremediation

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A number of DOE sites are contaminated with mixtures of dense non-aqueous phase liquids (DNAPLs) such as carbon tetrachloride, chloroform, perchloroethylene, and trichloroethylene. At many of these sites, *in situ* microbial bioremediation is an attractive strategy for cleanup, since it has the potential to degrade DNAPLs *in situ* without producing toxic byproducts. A rapid screening method to determine broad range metabolic and genetic potential for contaminant degradation would greatly reduce the cost and time involved in assessment for *in situ* bioremediation, as well as for monitoring ongoing bioremediation treatment. In this project, advanced mass-spectrometry-based methods will be developed at ORNL to screen for genetic and metabolic potential and for metabolic activity for both assessment and monitoring of in situ bioremediation of DNAPLs. The work builds on proof-of-principle experiments already completed in our group that demonstrated detection of relevant microbial PCR products by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry and detection of polar metabolites associated with bioremediation by electrospray mass spectrometry. This work will lay the foundation for development of a field-portable mass-spectrometry based technique for rapid assessment and monitoring of bioremediation processes on site.

190. Development of Advanced Electrochemical Emissions Spectroscopy for Monitoring Corrosion in Simulated DOE Liquid Waste

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The Department of Energy currently stores millions of gallons of high level liquid radioactive waste in underground, carbon steel-lined concrete tanks at the Hanford Reservation in the State of Washington and at the Savannah River Site in South Carolina. Because of the indefinite time of storage, general corrosion, even though it may occur only at low rate, must be considered as a principal threat to tank integrity. However, various forms of localized corrosion (pitting, crevice corrosion, and stress corrosion cracking) are known to occur on iron and carbon steel in alkaline environments, in general and on carbon steel in DOE liquid waste environments, in particular. Because localized corrosion often results in sudden and unexpected failures, knowledge of the conditions under which localized corrosion occurs is vital for assessing the possibility of failure and for assessing remaining life. In this program, we propose to use a variety of electrochemical techniques, but most notably Electrochemical Emission Spectroscopy (EES), to explore the fundamenatl aspects of the general and localized corrosion behaviors of iron and carbon steel in alkaline environments, including simulated DOE liquid waste. Our goal is to resolve important mechanistic issues using the most modern electrochemical and analytical techniques. These include the analysis of EES data in terms of non-linear dynamics methds; the use of Electrochemical Impedance Spectroscopy (EIS), rotating ring-disk voltametry, chronoamperometry, and steady-state (dc) polarization techniques to explore the passive state of iron in these media; the determination of passivity breakdown potentials and induction times, as well as the characterization of electrochemical emissions due to metastable pitting;

the analysis of electrochemical noise in the coupling current between a crack or a crevice and the external surfaces as a means of determining the mechanisms of these processes; and the further development of deterministic theories and models for predicting the evolution of corrosion damage. These studies are expected to resolve important mechanistic issues, including the origin of electrochemical emissions in general corrosion, the viability of current deterministic models and theories for describing the passive state and for predicting passivity breakdown and the nucleation of pitting, and the delineation of aodic dissolution and hydrogen-induced fracture as the mechanism of crack propogation in steels in highly alkaline solutions. Although the principal emphasis will be on the fundamental aspects of the subject, we will also assess the potential of techniques developed in this work to be used for *in situ,* continuous monitoring of DOE's storage tanks.

191. Development of Advanced *In Situ* Techniques for Chemistry Monitoring and Corrosion Mitigation in SCWO Environments

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We propose to develop chemical and corrosion sensors for use in high subcritical and supercritical aqueous environments, to improve their precision and reliability, and to use them to characterize the fundamental properties of supercritical aqueous solutions. A better understanding of phenomena in supercritical water will enable us to develop advanced, safe and emission-free waste treatment processes for the destruction of DOE Waste. Our work will emphasize the development of advanced reference electrodes, fabrication of pH sensors and redox sensors, as well as exploring electrochemical emission spectroscopy as a means of characterizing metal/water interactions (including corrosion). We will also define a practical pH scale for use with supercritical aqueous systems. This work will be carried out in cooperation with Idaho National Engineering & Environmental Laboratory (INEEL), and it will employ the bench scale reactor at INEEL to test the sensors, and to perform studies of aqueous chemistry and electrochemistry at supercritical temperatures in model waste streams. This proposal addresses the following areas of research: 1) Fundamental characterization of wastes and development of knowledge of chemical behavior of waste species. 2) Development of a fundamental chemical basis for waste treatment. 3) Development of novel monitoring and controlling waste treatment processes. Supercritical Waste Oxidation (SCWO) is now being actively developed as a means of destroying highly toxic organic waste (including physiological agents) and for reducing the volume of low-level nuclear waste. Very high conversion factors (typically greater than 99.99%), high throughputs, and closed cycle operation makes this process very promising for destroying a varity of DOE wastes. The major problem inhibiting the wide implementation of SCWO is the lack of fundamental knowledge about various chemical and physico-chemical processes in SCW and the excessively high corrosion rates of structural materials. The purpose of this proposal is to continue our work on the fundamental characterization of processes in SCW. In this follow-on work, we propose to further develop chemical and corrosion sensors, improve their precision and reliability, and use them for *in-situ* characterization of the fundamenatl properties of supercritical aqueous solutions and to model waste streams. The sensors will include: a pH sensor, reference electrode, redox sensors, and electrochemical noise sensors. We will place emphasis on the fundamental investigation of chemical and corrosion phenomenon in SCWO environments. Using the developed sensoring techniques, we will be able to carry out in-depth studies of the speciation and solubility phenomena in supercritical water and to obtain much-needed quantitative data for the modeling and development of SCWO processes. The development of these technologies should permit the more extensive and efficient use of relatively inexpensive reactor materials, and hence may result in a feasible and cost-effective SCWO process for destroying DOE wastes. In the proposed research, we will concentrate on fundamental studies of the

supercritical aqueous systems and on improving the precision and reliability of sensoring techniques, as well as on developing new sensing concepts for use in SCWO systems. The development of sensors for monitoring the chemical conditions in supercritical water will also enable us to characterize, in a fundamental manner, ionic equilibria in supercritical water, and to improve our understanding of the thermodynamics and kinetics of oxidation reactions. The use of pH sensor will permit us to develop a supercritical pH scale and obtain independent data on dissociation constants of a variety of electrolytes.

192. Design and Development of a New Hybrid Spectrochemical Sensor

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The required remediation of over 300 underground nuclear waste storage tanks at USDOE sites together with the associated needs to characterize and monitor the chemical compositions of the tanks themselves present a major scientific challenge. In addition to the previously identified chemical complexity of such storage materials, the added dimensions of limited tank access and harsh chemical and biological environment preclude the straightforward application of well-established laboratory-based chemical analysis techniques to this national problem. Beyond any solution to this immediate problem also lies a present and long-term need to monitor low-level subsurface contaminations associated with storage facilities. While an approach to removing high-level nuclear wastes has been identified (vitrification), the chemical analysis technology available in-hand to assist in this important task is inadequate. The general aim of the work is to design and implement a new sensor technology which offers the unprecedented levels of specificity needed for analysis of the complex mixtures found at USDOE sites nationwide. The new sensor concept proposed combines the elements of electrochemistry, spectroscopy and selective partitioning into a single device that provides three levels of selectivity. This type of sensor has many potential applications at DOE sites. As an example, the enhanced specificity embodied in this new sensor design is well-suited to the analytical problem posed by the addition of ferrocyanide to radioactive tank wastes at the Hanford Site. Since ferrocyanide/nitrate mixtures are potentially explosive in the dry state at elevated temperatures, a ferrocyanide remote sensor is needed for characterization of tank waste before and during the disposal process.

193. Ultrahigh Sensitivity Heavy Noble Gas Detectors for Long-Term Monitoring and Monitoring Air

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A University of Cincinnati/Argonne National Laboratory team will develop and demonstrate novel ultrahigh sensitivity noble gas (krypton, xenon, and radon) detectors for long-term monitoring of spent fuel and TRU waste, as well as for distinguishing background radon alpha particles from other alpha emissions in air monitors. A new technique for concentrating the heavy noble gases from air will be integrated with state-of-the-art radiation detector technology to provide sensitivities on the order of two orders of magnitude better than current technology. In addition, these detectors can be configured such that heavy noble gas concentration in air is monitored continuously and recorded in real-time and *in-situ*. This real-time data acquisition coupled with the ability to measure the beta particles and gamma rays emitted by krypton and xenon in coincidence mode will result in an enhanced ability both to use spectral information to detect and identify the different noble gas isotopes and to discriminate against all other signals. Finally, such an integrated concentration and detection system has the potential to provide low-cost and low-complexity detectors which would be ideally suited for long-term monitoring and fieldable air monitors. It is

proposed that a broad range of potential detectors and measurement techniques be identified and evaluated for use in DOE Environmental Management applications. Furthermore, at least two of the most promising techniques for detecting the heavy noble gas emissions will be developed experimentally. First, the concentrated radioactive gases can be mixed directly with standard proportional detector fill gases such that a 100% detection efficiency is realized when the mixture is passed through a proportional detector. Second, the concentrated gases can be used in a scintillator flow-cell geometry to achieve a similar detection efficiency. While both of these techniques provide the ability to distinguish alpha and beta particle interactions within the detector, a gamma ray spectroscopy detector can be used in coincidence mode with both techniques to further enhance background discrimination and species identification. Consequently, coincidence mode operation will also be demonstrated experimentally using both the proportional and the flow-cell detectors. If additional detectors and techniques are identified as promising, similar experimental development will be pursued for those systems. Finally, the developed detection systems will be evaluated and one or more systems identified, constructed, and demonstrated. This final demonstration of the technology will be conducted initially in the laboratory environment to establish operating characteristics, and subsequently will be conducted at a DOE EM site.

194. Miniature Nuclear Magnetic Resonance Spectrometer for *In Situ* and In-Process Analysis and Monitoring

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The objective of this research project is to develop a new analytical instrument based on the principal of nuclear magnetic resonance (NMR) for *in-situ*, in-field and in-process characterization and monitoring of various substances and chemical processes. The new instrument will be a highly miniaturized version of an NMR spectrometer and its development will involve application of the most recent advances in the fields of micromachining and microfabrication, permanent magnet materials and design, and microelectronics and signal processing. The proposed NMR spectrometer will be a hand-held unit weighing around 5–6 pounds and intended to perform measurements on liquid samples of micro- to nano-liter volumes. The resolution of the instrument is projected to be better than 0.1 ppm with sensitivities approaching 10 to 100 ppm (1 millimolar to 10 millimolar) for proton containing molecules. While initial developments will focus on applications of proton NMR, further developments will be aimed at other nuclei, such as F-19, P-31 and C-13. Applications of the miniature NMR system will include down hole monitoring of ground water pollutants and flow, real-time in-process monitoring of waste remediation activities, spatial composition analysis in chemical and waste storage tanks, in-field characterization of waste materials and many others.

195. Supramolecular Chemistry of Selective Anion Recognition for Anions of Environmental Relevance

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The supramolecular chemistry of selective anion recognition by synthetic poly ammonium macrocycles will be explored in a comprehensive, long term program designed to provide new solutions to problems critical to the environmental initiative of DOE. Highly shape- and charge- selective systems will be designed, synthesized, and examined for their capabilities in sequestering environmentally important anions. Phase I of this program will involve selected oxo anions: nitrate, phosphate, sulfate, chromate, and pertechnetate. Longer term initiatives will include expansion to other environmentally important anions as well as to other non-amine-based receptors. This

project will involve major basic research components in the selection of target macrocycles using three areas to assess suitability. These areas are x-ray crystallographic structural determinations, molecular dynamics simulations, and thermodynamic and kinetic studies of anion binding. The results from this component will be applied to environmental challenges including both sensing and separations of anions of interest. The results from this comprehensive program can potentially lead to superior systems for sensing and sequestration of anions of environmental importance, and therefore could have major impact on issues involving hazardous waste sources, including Hanford underground tanks, groundwater, and process waste waters.

196. Structural Biology of the Sequestration and Transport of Heavy Metal Toxins: NMR Structure Determination of Proteins Containing the -Cys-X-Y-Cys-Metal Binding Motif

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We propose to gain insight into the mechanisms of biological detoxification and the possibilities for ex vivo bioremediation by applying the methods of structural biology to proteins involved in detoxifying and binding heavy metals in bacteria humans. Nuclear Magnetic resonance (NMR) spectroscopy will be used to determine the structures and describe the dynamics of proteins containing the -Cys-X-Y-Cys- metal binding motif. These proteins are in two categories. Several 70 residue modules that are homologous to merP (periplasm), a metal binding protein of the bacterial mercury detoxification system will be investigated as well as merC, a membrane protein that has all of the necessary functions for binding and transporting heavy metals across cell membranes. Once the structures of these proteins are determined, it may be possible to re-engineer and place them in chemical or plant based devices for removal of a range of heavy metals from the environment, including mercury, cadmium and lead.

197. A Fundamental Study of Laser-Induced Breakdown Spectroscopy Using Fiber Optics for Remote Measurements of Trace Metals

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Improved technologies are required by DOE for characterization and monitoring for site clean-up and waste processing applications. Especially needed are field deployable methods and devices for real-time monitoring to reduce dependency on laboratory analyses which are costly and time consuming. Improved sensors are needed for on-site analyses to provide real-time analytical capabilities for screening level and/or decision-quality data. Matrices of interest to DOE are soils(or other solids), slurries, and aqueous and non-aqueous solutions. Laser-induced breakdown spectroscopy (LIBS) is a useful method for determining the elemental composition of solids. In the LIBS technique, a high-power pulsed laser is used to generate a plasma from the sample of interest. The elemental composition of the sample is accomplished by measuring the atomic emission from the atoms and ions in the plasma. LIBS shows great potential for measuring metal contaminants in soils and on particles (e.g., stack emissions) based on their atomic emission in laser-induced plasma. Another important application for this technique is the remote analysis of highly radioactive materials, such as the glasses produced by the Defense Waste Processing Facility. The use of fiber optics for both collection of the atomic emission and delivery of the laser to the sampling area could eliminate the need for sampling. In this work we will study the time-evolution of the LIBS emission for different matrices to better understand how to optimize the signal. Also, we will investigate the use of fiber optics for laser delivery and signal collection, and the influence of the geometry of the fiber optic launch and

collection probes to determine the effect on the signal-to-noise ratio (SNR). Finally, we will study the LIBS signal for different sample matrices as a function of excitation wavelength across a broad spectrum. A result of this study should be a determination of the optimal excitation and collection conditions and sampling times for metal contaminants in different matrices, and an understanding of the strengths and limitations of using fiber optics for LIBS sampling.

198. Novel Analytical Techniques Based on an Enhanced Electron Attachment Process

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Present analytical methodologies for the detection of chlorinated compounds important to DOE's environmental restoration program, such as DNAPLS (dense non-aqueous phase liquids such as carbon tetrachloride, trichloroethylene, perchloroethylene), polychlorinated biphenyls (PCB) and others, involve detection by negative-ion-based analytical techniques. These techniques exploit electron attachment to analyte molecules in their ground electronic states, and are limited to particular compounds with appropriate electron capture cross sections (> about 10^{-17} cm^{-2}). For example, PCB contamination is detected by analysis of mixtures of chlorinated homologs of these biphenyls. Homologs with lower numbers of chlorines do not efficiently attach thermal electrons and thus are not detected by either electron capture chromatographic detectors or by negative ion chemical ionization mass spectrometry. A basic research program recently conducted by the PI has shown that highly-excited electronic states of molecules have electron attachment cross sections of $> 10^{-12}$ cm^2, orders of magnitude larger compared to electron attachment cross sections for ground-state molecules. Furthermore, this enhanced electron attachment process appears to occur in a wide variety of molecules; in contrast, electron attachment to ground-state-molecules—which the present negative-ion-based analytical techniques rely on—is limited to certain classes of molecules. We propose three novel analytical techniques based on this enhanced negative-ion formation process. In one of the proposed techniques, the excited states of the (analyte) molecules are populated via laser excitation; the resulting negative ions are mass analyzed for identification. Preliminary results show that analytically useful fragment negative ions can be produced with this technique in molecules which do not produce any negative ions in conventional analytical methods. The other two proposed techniques utilize a specialized gas discharge for the formation of excited species (and low-energy electrons for attachment), and thus will provide a cost-effective method if successful; in one version the negative ions will be mass analyzed and in the other the decrease in electron density due to excited state attachment will be monitored. A plasma mixing scheme will be employed to excite the analyte molecules (they will not be directly subjected to the discharge) so that the excited states of the analyte molecules will not be destroyed by discharge. We plan to bring the laboratory studies to where they will be ready for field-study demonstrations at the end of the three-year period.

199. The Sonophysics and Sonochemistry of Liquid Waste Quantification and Remediation

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The legacy of waste left from the cold war has created an environment that requires unique and breakthrough technologies in order to solve some of DOE's waste problems. It has been demonstrated that acoustic cavitation—the birth, growth and violent death of bubbles—can lead to chemical bond cleavage, leaving initially toxic waste inert. We believe that through a deeper understanding of the underlying physics of cavitation, cavitation-induced chemical

activity will lead to safe, *in situ* methods of waste characterization and remediation. We propose a comprehensive and thorough investigation into the physics and chemistry associated with acoustic cavitation. There are two major aspects to this proposal: 1) Physical mechanism(s) of acoustic cavitation—we plan to design and undertake a series of experiments that will uncover the dominant mechanism (electrical, thermal, etc.) associated with chemical activity. 2) Waste characterization through sonoluminescence—we plan to undertake a systematic study to ascertain the potential for sonoluminescence to characterize and monitor alkali metals, mercury and other waste contaminants *in situ*. The goal of the proposed research is to develop as complete an understanding of acoustic cavitation as needed so that upscaling studies can be initiated for eventual use in waste management.

200. Construction of a Bending Magnet Beam Line at the APS for Environmental Studies

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To help satisfy the growing need for synchrotron radiation based environmental research, it is proposed to carry out the design and construction of a bending magnet (BM) beamline at the Advanced Photon Source (APS) by the Pacific Northwest Consortium-Collaborative Access Team (PNC-CAT). The line will be optimized for various forms of x-ray absorption spectroscopies (XAS). Environmental and cleanup issues are a major focus of the fundamental research to be performed on the BM beamline. The beamline will share the PNC-CAT experimental facilities to be fabricated for the neighboring Undulator A Insertion Device beamline to utilize the experimental techniques of x-ray absorption spectroscopy for both bulk and surface studies, with spatial and time resolution and elemental imaging , on toxic and radioactive samples. These capabilities have been cited in a recent DOE workshop report as most desirable for future environmental research.

201. Adsorption/Membrane Filtration as a Contaminant Concentration and Separation Process for Mixed Wastes and Tank Wastes

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The Hanford Reservation is the largest and perhaps most complex Superfund site in the U.S., containing numerous hazardous chemicals and medium- to long-lived radionuclides. Each category of waste presents a different set of scientific and technological challenges for characterization, handling, treatment, storage and disposal. This project is for research on novel approaches for separating colloidal matter, uranium, strontium, and cesium from the remaining constituents of the liquid wastes with which they are associated, and from one another. A wide variety of organic membranes, anion- and cation-exchange resins, other organic adsorbents, solvents and complexing agents have been developed for the treatment of complex, mixed wastes. Some of the adsorbents and ion-exchange resins are exceptionally selective and efficient, but they have serious drawbacks that impair their performance when used to treat radioactive wastes. Namely, they are susceptible to thermal, chemical (i.e., oxidative), and radiation induced degradation, and they are often very expensive. The regeneration of organic media with adsorbed or otherwise retained radionuclides often is incomplete, and, given their chemical instability, their safe disposal is an additional problem. The major focus of this project is the development of advanced inorganic processes to treat radionuclide-bearing wastes. Inorganic materials are generally much cheaper and less prone to deterioration in radioactive environments than organic materials, their long-term behavior is

easier to predict, and they can be used in vitrification processes. However, for inorganic materials to be useful in such applications, significant improvements are needed in their mechanical properties, adsorption and/or exchange capacity, and level of characterization. In this project, we explore new processes that employ relatively inexpensive and easy-to-use inorganic materials specifically designed to satisfy the needs of mixed nuclear waste treatment. Another major, novel aspect of this proposal is the combination of inorganic media with electrochemical methods to enhance, accelerate or create an additional useful control parameter in treatment process.

202. Particle Generation by Laser Ablation in Support of Chemical Analysis of High Level Mixed Waste from Plutonium Production Operations

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Methods for compositional analysis of fissile materials and radioactive/toxic wastes are being developed to support characterization prior to treatment and remediation. The need for rapid, real-time, on site characterization of waste at DOE sites has led to development of laser ablation-inductively coupled plasma mass spectrometry (LA/ICP-MS) systems for elemental and isotopic analysis at several locations, including Hanford, Los Alamos, and INEEL. These systems can provide qualitative or semi-quantitative analysis of certain sample types with minimal sample handling. Research into the fundamental physical processes of particle formation during laser ablation is required to provide basic understanding that will allow us to maximize the utility of these systems. There is no research currently underway that addresses the key scientific issues raised by application of laser ablation in the systems already installed at DOE sites. A major consideration is the presence of nonmetallic, crystalline and polycrystalline wide bandgap materials or oxide thin films, materials where we have established a breadth of expertise. We propose a collaborative project that combines fundamental studies of mechanisms to provide underlying scientific insight into the relevant parameters influencing production of particles, the properties of these particles, and relating directly to glove-box installed ICP-MS analysis of both simulants and actual radioactive and toxic waste. Our work focuses on 1) ablation mechanisms and 2) the effect of physical and chemical state of the sample (e.g., valance state, impurity concentration, particle morphology, defect concentration, and presence of liquids) on the character of the particles produced by laser ablation. Our goal is to provide physical and chemical insight and useful information for the analytic community to improve prototype instrumentation and analytic techniques for characterizing waste and similar materials. We examine the solid and condensed state properties of the sample, the coupling to the laser beam, and the dynamic electronic, physical, and chemical processes which ultimately generate the particles that are entrained and delivered to a distant ICP unit. This requires careful examination of the intermediate steps such as formation of submicron to micron sized particles. Our proposed research includes the following: a) The absorption mechanisms and the role of laser parameters (wavelength, pulse width, fluence) in coupling energy into the solid. b) Time resolved analysis of the formation of metal zone, the mechanical response (fracture, shock fronts), and physical ejection of material. c) Careful characterization of the chemical and physical nature of the ejected particles (using several spectroscopies and microscopies). d) The consequences of aggregation and vapor deposition on the particle. e) The role of sample morphology and physical state on particle formation and stoichiometry. f) The influence of liquids (e.g., water, lubricants, solvents) on particle formation and composition. g) The formation mechanisms and morphology and composition of laser produced particles including gas phase aggregation and chemistry. In general these studies directly support other methods where are used, e.g., production of plume fluorescence for spectral analysis, vaporization for direct mass analysis, as well as vaporization and particle formation for possible capture by various charged particle traps.

Small Business Innovation Research Program

203. A Dedicated PET Scanner for Imaging Gene Expression in Mice

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The ability to image and quantify gene expression *in vivo* will create an important and powerful tool for modern biology. Several techniques for imaging gene expression using positron emission tomography (PET) are being actively pursued and the preliminary data is very encouraging. Currently, all approaches are limited to PET scanners designed for clinical work. These scanners are both bulky and expensive with sufficient resolution for small animal work. The goal of this proposal will be to develop a small compact, ultra-high resolution PET scanner specifically optimized for imaging small animals. Such an instrument would have a target price in line with other laboratory equipment, making the available to a large number of biologists and chemist. For optimum sensitivity, and minimum cost, the detector ring needs to be only large enough for the animal under study. These requirements dictate very small crystals with depth encoding to maintain the resolution across the field of view. In Phase I, we designed, fabricated, and characterized a poswich detector block consisting of an 8x8 matrix of 2mm crystals. Each poswich crystal consists of a 7.5 mm crystal of LSO coupled to a 7.5 mm crystal of GSO. Block encoding was achieved through the use of a glass light guide. A intrinsic resolution of 1.3 mm was achieved. In Phase II, a full detector system optimized for imaging small animals will be designed, fabricated and tested. This detector will be incorporated into a gantry optimized for table top or laboratory bench. Characterization of the detector response in this embodiment will be necessary to allow the design of scatter, randoms, and attenuation corrections for optimal accuracy in imaging small animals. Commercial applications and other benefits as described by the awardee: The primary benefit of this work will be to provide a PET tomograph specifically optimized for use as a biological tool with small animal models. Such a system can provide the biologist with a tool capable of imaging small concentrations of radiotracers and following the biological distribution of the tracers as a function of time and obtain all related biological information.

204. Production of Intermediate-Lived Radionuclides for Biomedical Applications Using Small Cyclotrons

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A recent Institute of Medicine report recommended that the Department of Energy establish a National Biomedical Tracer Facility (NBTF) to provide a dedicated, reliable source for research radionuclides. It has since become apparent that an NBTF will not be built within the next decade. Consequently, other sources must be developed to fulfill the growing needs of researchers interested in the application of medium half-life research radionuclides. The overall objective is to develop cyclotron target systems for the high-yield production of several potentially useful radionuclides using existing small biomedical cyclotrons installed in hospitals and research centers nationwide. The radionuclides that will be studied in Phase I, gallium-66, bromine-76, bromine-77, yttrium-86, technetium-94, and iodine-124, have

important applications in radiopharmaceutical development, quantitative imaging, and radiotherapy. All can be produced in solid targets using 11-18 million electron volt proton beams from small biomedical cyclotrons. The goal of the Phase I program is to determine the feasibility of on-site production of these radionuclides in large quantities and with high radioisotopic purity and specific activity. Techniques for radionuclide separation and purification and recovery of the isotopically enriched target materials will also be investigated. In Phase II, targets and automated processing systems will be developed for those radionuclides that show promise for routine, high-yield production. Commercial applications and other benefits as described by the awardee: If successful this research will provide techniques to make available a series of important radionuclides to biomedical researchers throughout the United States. The development of high-yield targets and processing systems will allow these isotopes to be produced at major medical centers having on-site cyclotron facilities. Additionally, the relatively long half-lives of these isotopes make them suitable for distribution through regional centers that would serve as a "virtual NBTF."

205. Software Development for Real-Time Radiotracer Guidance of Breast Biopsy

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Innovative research using nuclear medicine technologies is necessary to advance noninvasive methods of diagnosing breast cancer. While detection of early breast cancer in women between the ages of 40 and 49 has been identified as a major societal issue by national health authorities, x-ray mammography can miss fully one quarter of cancers in patients of this age range. A possible adjunct to mammography would be highly specific radiotracer imaging, yet this imaging is limited by two critical factors: The current generation of detection hardware is not optimal for detecting the clinically important class of early small breast cancers, and clinical studies of such small cancers are hampered by the inability to compare radiotracer images of specific cases with correlative x-ray and biopsy results. In pilot clinical studies, a demonstration prototype device has been used capable of superimposing x-ray images of specific small cancers on images created with positron-emitting radiotracers. Image acquisition is performed on a conventional x-ray mammography platform that can provide biopsy correlations. In Phase I, algorithms will be integrated into a real-time display package, and limitations of the technique will be studied using positron emitting and single-photon emitting point sources. Phase II technical development will include increasing the field-of-view of the demonstration cameras. Phase II clinical trials will definitively assess medically relevant parameters such as sensitivity and specificity in the detection of early breast cancer, as well as the accuracy of the device in assisting biopsy. Commercial applications and other benefits as described by the awardee: The potential market for a reliable imaging device capable of direct breast biopsy is great, due to the high prevalence of breast cancer and the limitations of x-ray mammography.

206. Fast-Timing Silicon Drift Photodetectors for PET

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This project will develop a novel solid-state imaging device for positron emission imaging to eliminate the need for photomultiplier tubes and allow for integration of electronics with the detector. This improvement will lead to specialized, dedicated lower cost systems for Positron Emission Tomography (PET). To accomplish this, a new type of detector module will be developed that consists of arrays of lutetium orthosilicate scintillation crystals coupled to a new type of silicon photodetector array specifically designed to provide the excellent energy and timing resolution needed. In Phase I the detector structures will be designed, fabricated, and evaluated. They will also be optimized for low noise, high quantum efficiency, and

fast signal speed. In Phase II the optimized detector units incorporating specialized processing electronics will be developed and tested, and a prototype breast imager will be built and evaluated using realistic phantoms. Commercial applications and other benefits as described by the awardee: A dedicated imaging system employing two imaging planes has applications for use with specialized positron emitting radiopharmaceuticals for detection of breast cancer. Such a system would have the potential to be 20 times more sensitive than a full PET system and have large improvements in count rate and spatial resolution. This system would have a large market potential as a non-invasive diagnostic tool for breast cancer.

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Index of Investigators

Many of these projects have several co-investigators. This publication only gives the principal investigator for each project; it is hoped to include a more complete listing in the next edition.