10.0 DESCRIPTION OF FACILITIES AND RESOURCES

10.1 Argonne National Laboratory

Laboratory equipment available for the proposed project includes centrifuges, speed vacs, spectrophotometers, columns for chromatography, five 20-place isoelectric focusing units for use with tube gels (used for the carrier ampholyte method), a 12-place immobilized pH gradient electrophoresis unit, four boxes for casting slab gels, three 20-place tanks for running slab gels (denaturing or nondenaturing), a set of computer-controlled stepping pumps for casting slab gels under the direction of a computer program developed at Argonne, centrifuges, and low temperature freezers. Computer equipment and software associated with the acquisition, analysis, and management of 2DE data include a ProXPRESS Proteomic Imaging system and the Progenesis software for analysis of 2DE patterns, along with three PCs with Windows 2000 operating systems (one used as a dedicated World Wide Web server). ORACLE software running on both VAXstation and PC platforms is already in use.

10.2 Brookhaven National Laboratory

STEM1 (built at BNL, operational since 10/77)

Cold field emission, 40 keV, fixed beam current
Cs=1 mm, spot size 2.5Å dia. (single atoms on carbon)
Cold stage -160º C, vacuum transfer (cold or warm) to STEM air lock from external freeze dryer
Efficient bright field & dark field annular detectors (0-15, 15-40 & 40-250 mR acceptance)
Digital control and data acquisition
High reliability, 50 user projects

STEM3 (built at BNL, operational since 12/95)

Cold field emission, 70 keV, variable beam current
Cs=1 mm, spot size 2Å dia. theoretical (3-5Å actual)
Cold stage LN2, vacuum transfer (cold or warm) to STEM air lock from external freeze dryer or from STEM1
Efficient bright field & dark field annular detectors (0-15 & 40-250 mR acceptance)
Single channel electron energy loss spectrometer (0-40 mR effective acceptance)
Digital control and data acquisition

10.3 Oak Ridge National Laboratory

Protein Facilities
Laboratory space consists of four adjoining rooms, 600 sq. ft. each, in Building 4500S with adjacent office space and a workstation room. All labs are equipped with fume hoods and refrigerator/freezers; 2 triple-door refrigerated chromatography cabinets are available. Equipment and facilities to support microbiology include a 4 ft. laminar flow hood, an electroporator for both bacterial and yeast cells, temperature-controlled rotory shakers for multiple bacterial cultures (with lighting available for culture of phototrophs), plate incubators, and a bench scale fermentor. For molecular and biochemical work the lab contains several PCR thermocyclers with simultaneous capacity for 5 microtiter plates, a Qiagen 9600 DNA template robot, a Hydra 96 channel pipettor, a multipurpose pipetting robot, a DNA hydroshearing apparatus, 3 ABI377 DNA sequencers, UV/visible spectrophotometer, automated microtiter plate washer, plate reader/spectrophotometer, several electrophoresis power supplies and electrophoresis equipment, sonicator, French pressure cells, refrigerated high-speed centrifuges, a preparative ultracentrifuge, and both -20˚ and -80˚ C freezers for storing enzymes and bacterial cells. Computer controlled chromatographic equipment in the laboratory includes a refrigerated Pharmacia FPLC system with in-line UV monitor and conductivity meter, and an extensive selection of ion exchange, affinity, hydrophobic interaction, and gel filtration columns. The laboratory also contains several computers including a

Silicon Graphics O_2 for molecular modeling. Peptide HPLC separations equipment and a computer controlled spectrofluorometer are accessible.

Additional adjacent laboratory space is available to this project for sample preparation in Bldg 4500S. One room is a 300 sq. ft. room housing a liquid nitrogen freezer for cell storage, a – 80^0 C chest freezer, a Beckman ultracentrifuge, a Sorvall mid-speed centrifuge, and an ice machine. A second room, 600 sq. ft., is equipped for cell culture and protein chemistry work. Equipment includes four CO_2 incubators, two laminar flow hoods, microscopes, centrifuges, a gamma scintillation counter, gel electrophoresis equipment, ELISA capabilities, a certified, monitored radioactive materials hood, and a chemical hood for immunohistochemistry. A third room, 600 sq. ft., is set up for molecular biology. Equipment includes several incubators for growth of bacterial and insect culture at various temperatures, a hybridization oven, Waters dual pump HPLC with gradient controller and several columns, gel electrophoresis equipment for agarose and sequencing gels, a Packard Instantimager and a Cyclone phosphorimager. Shared facilities are available for flow cytometry, cell sorting, and DNA sequencing. Offices for staff, students, and post-doctoral associates are adjacent to these laboratories.

Mass Spectrometry

The Organic and Biological Mass Spectrometry Group entirely occupies a roughly 6000 ft^2 building housing five instrumentation laboratories and a prep lab with adjacent offices (ORNL Building 5510), and also occupies two laboratories in the adjacent building (5510A). Relevant equipment includes Thermoquest LCQ Deca ion trap mass spectrometer systems (electrospray, nano-electrospray, and atmospheric presssure chemical ionization sources) equipped with an LC Packings Ultimate HPLC, FAMOS autosampler, and SEQUEST software for protein identification through database searching; a ThermoFinnigan Proteomex workstation (integrated LC/MS system), to be purchased in FY02 with institutional funds; two PerSeptive Biosystems matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) mass spectrometers with delayed extraction capabilities, one (Voyager DE) with linear geometry, and one (Voyager ELITE) with reflecting geometry and post-source decay capabilities, and a Symbiot MALDI sample preparation robot; a 9.4-Tesla IonSpec Fourier transform ion cyclotron resonance (FTICR) instrument equipped with an electrospray ion source; a Finnigan 6-Tesla FTICR with a nitrogen laser for MALDI. Other laboratories in the group contain a number of custom quadrupole ion trap mass spectrometers equipped for electrospray, laser photoionization, atmospheric pressure chemical ionization, LC/MS, and for ion/ion reactions and heated bath gas studies; PE-Sciex API 165 single quadrupole and PE-Sciex API 365 triple quadrupole mass spectrometers are equipped for electrospray, nano-electrospray, atmospheric pressure chemical ionization and atmospheric pressure photoionization; and a Finnigan Voyager GC-MS. A separate laboratory for sample preparation is available, and includes hoods, -20°C and -80°C freezers, a refrigerator, balances, a UV-visible absorbance spectrophotometer, chemical storage, and a Millipore deionized water facility. Other equipment in 5510 includes a SpeedVac for sample concentration, Genomic Solutions 2D gel electrophoresis system, Savant SpeedGel gel dryer, HPLC and FPLC systems, a capillary electrophoresis system, cooled and room-temperature centrifuges, water baths, a PCR machine. Personal computers with Internet access are available to all investigators for instrument control, data analysis, word processing, etc.

Chemical Synthesis

Two (600 ft^2) synthetic laboratories are equipped with all the equipment necessary for large and small scale synthesis of organic compounds and polymers: chromatography (ion exchange, size exclusion, reverse and normal phase, column, TLC, HPLC, capillary GC with FID etc.); distillation equipment; appropriate glassware (Schlenk-type) and glovebox for handling water and/or air sensitive reagent; glassware for microscale synthesis; high vacuum line; constant temperature circulating bath; Parr hydrogenator; freezer for storage of reagents at -25°C, centrifuges, and Speed Vac (Savant). Major characterization equipment includes: Bruker MSL 400 MHz (9.4 T) liquid/solid multinuclear NMR, Waters analytical/semiprep HPLC/GPC with 600 E gradient mixer, 717 auotsampler, 410 refractive index and 996 photodiode array detector, Agilent 1100 HPLC with 1100 multiwavelength UV-Vis detector, Agilent HPLC-MS with EI and CI, Agilent GC-MS (2) with EI and CI, Agilent capillary column GC's (4) with a variety of detectors (FID, TCD, ECD, etc.), Cary 4 UV-VIS spectrometer with specular and diffuse reflectance capability, FT-IR with diffuse reflectance capability, Spex Fluorolog emission spectrometer for solutions, slurries, and solid surfaces, and TA, Inc. 2010 DSC and 2050 TGA-MS (Balzers ThermoStar MS) for the thermal analysis of polymers and other materials including evolved gas analysis.

Support Services
A full service glass shop is available for fabrication of glass materials, and state-of-the-art machine and electronic shops are available.

Bioinformatics and Computing
The PI and Co-PIs have direct access to a well-established computing environment at Life Sciences Division of ORNL, including

Hardware:
- 20 DEC Alpha cluster
- 2 4-way SMP Sun servers
- 3 4-CPU Pentium LINUX servers
- 20+ workstations and PC's
- 2 storage filers, each with more than 100GB

Software:
- ORACLE-8 – database manager
- GIST (Genomic Integrated Supercomputing Toolkit). A large-scale framework for biological applications, which provides transparent desktop access to supercomputing resources.
- A wide array of life sciences software and databases, e.g., GCG, BLAST, PDB, ARB, ProDom, ClusterW, etc.

In addition, the PI and Co-PIs have access to the state-of-the-art resources for high-performance computational science and computing science research in the Computational Sciences Division of ORNL. The primary computational resources currently include a 184-node, 724-processor IBM RS/6000 SP and a 64-node, 256-processor Compaq AlphaServer SC, each with over two terabytes (TB) of system-wide disk storage. Additional systems include a 16-node, 64-processor AlphaServer SC test system, a 32-processor SGI Origin for visualization, and various support servers. Center-wide storage is available in the form of the Distributed File System (DFS) and the High-Performance Storage System (HPSS), with 360 TB of archival storage. Internally, our systems are connected with gigabit Ethernet. The high-performance computing resource will allow us to carry out large-scale tests for our developments.

Fermentation facilities
ORNL staff have experience with a wide variety of microbial cultures atr all scales including photosynthetic microbes (cyanobacteria *Nostoc* sp., photoplankton *Emiliania huxleyi*, etc.) as well as aerobic or anaerobic bacteria including recombinants. Photobiosystems range from incubators to 1-l bubble columns. The Bioprocessing Research and Development Center (BRDC) was established in 1989 to support ORNL's efforts in bioprocessing, with members in its multidisciplinary team holding degrees in Chemical Engineering, Chemistry, and Microbiology. Equipment resources include: 500L and 70L New Brunswick fermenters for pilot scale bioprocessing operations, bench scale (1-10L) chemostats, a Sharples Continuous centrifuge for large scale cell harvesting, cross-flow ultrafiltration unit, a laminar flow hood for microbial transfers, microscopes, and a walk-in cold room at 4°C. Microbiological and genetic manipulation equipment includes electroporator, electrophoresis, climatic chambers, shakers, thermocyclers, microbiological transfer hoods, autoclave, and freezers.There are also a lyophilizer for sample dehydration, centrifuges, Karl-Fischer titrator and sonicators. Analytical equipment for sample analysis includes: integrated gas chromatographs and HPLC for fermentation metabolite analysis and a Spectramax 96-well plate reader.

10.4 Pacific Northwest National Laboratory

Mass Spectrometry
This research will utilize the advanced instrumentation available in the Environmental Molecular Sciences Laboratory (EMSL) at PNNL, and will include the world's highest magnetic field (based around a 11.4 tesla

superconducting magnet) high performance FTICR (with two complete mass spectrometer vacuum systems) as well as the 3.5, 7 and two Bruker 9.4 tesla FTICR instruments, the first of which is being configured as a prototype for conducting high throughput proteomic measurements. Other mass spectrometers available include six ThermoFinnigan LCQ ion trap mass spectrometers that are interfaced with automated high pressure (10,000 psi) capillary LC systems for high efficiency separations of biological mixtures, and which have been integrated into a high throughput protein characterization environment and data management system. Also available are: two triple quadrupole mass spectrometers (ThermoFinnigan MAT TSQ 7000 and PE Sciex API 3000) and two triple quadrupole time-of-flight high sensitivity hybrid mass spectrometer (Sciex QSTAR and Micromass Q-TOF 2). Added features of each instrument include the (PNNL developed) electrodynamic ion funnel, unique devices for external ion accumulation with the FTICR instruments, and ultra-high sensitivity ESI sources developed at PNNL and installed on all instruments. The laboratory space devoted to these and their ancillary capabilities amounts to >5000 square ft. and includes a 1500 sq. ft. FTICR lab, a 1200 sq. ft. prototype high throughput laboratory, a 1500 sq. ft. lab for other mass spectrometers, and two 600 sq. ft. labs for the development of microscale separations and sample clean-up, ion source and vacuum system hardware development, peptide and protein separations, capillary LC, CE, cell growth, and wet chemistry. All spectrometers are on a common computer network with direct high speed access to the EMSL data storage and archive facility, a multi-terabyte storage system that allows large numbers of data files to be archived indefinitely.

The PNNL laboratories available for this research include the full range of facilities for cell culture, sample preparation, separations (CE, CIEF, SEC, etc.), storage, chemical synthesis and derivatization. Resources include a large-scale roller bottle culture facilities, and other necessary equipment, such as ultracentrifuges and cell fractionation apparatus. Other available resources include cold rooms, sequencing facilities, phosphorimagers, densitometers, bioreactors for large-scale cell culture, flow cytometers, culture hoods, incubators, microscopes, gamma counters, scintillation counters, lyophilizers, spectrophotometers, spectrofluorometers, luminometers, and PCR thermo-cyclers, autoclaves, shakers, centrifuges, etc. Common laboratory space has DNA sequencers, high-throughput data workstations and all necessary equipment for molecular and cellular biology.

Additionally, an Environmental Molecular Sciences Collaboratory (EMSC) has been developed in the EMSL at PNNL to allow geographically separated researchers to work cooperatively with EMSL staff even more effectively, using the facility's unique resources in combination with those at the researchers' home institutions. EMSC capabilities include a complete multi-platform environment that supports a broad range of research tasks, real-time access to data, software, research instruments, and expertise via the Internet. Electronic Laboratory Notebook: A secure, extensible, web based electronic laboratory notebooks allow sharing of information across a distributed research group.

Bioinformatics and Computing

The computational and bioinformatics work at PNNL will be carried out at PNNL in the William R. Wiley Environmental Molecular Sciences Laboratory. The DOE maintains and operates this user facility for both EMSL staff and external users, providing a robust research environment for the conduct of this proposed research.

From its inception in the early 1990's, the Environmental Molecular Sciences Laboratory (EMSL) has been a major contributor to the evolution in computational science. The Molecular Science Computing Facility (MSCF)m within EMSL is equipped with a large MPP computer, an experimental MPP system, a data archive system, and a graphics and visualization laboratory. The MSCF is a resource for the chemistry research community with a focus on environmental problems for access to advanced computing facilities and highly efficient computer codes in support of their research. Numerous computational studies in support of experimental projects in environmental chemistry as well as new theoretical developments are carried out as part of this program. Significant efforts are also devoted to the further development of new and more accurate computational models needed to attack the many complex problems of environmental sciences.

Funding from OBER supports operation of the MSCF in EMSL. The MSCF contains hardware and software. The hardware consists of a high-performance, massively parallel (MPP) IBM SP supercomputer with 512 processors, 256 gigabytes of memory, and 6 terabytes of disk space with 250 gigaflops of peak performance; a 64 processor next generation SMP parallel computer system from IBM; a graphics and visualization laboratory with

the first version of IBM's Scalable Graphics Engine, which connects directly to the switch of an MPP computer; and a scientific database system with 20 terabytes of storage and the Scientific Data Management software. We are presently involved in obtaining an upgrade to the MSCF hardware.

For software development, code testing, and simulation calculations, we already have access to the MSCF computational resources through the Computational Grand Challenge Project GC8 "Computational Studies in Molecular Geochemistry and Biogeochemistry". The planned upgrade of the massively parallel computer systems in the MSCF will provide a significant improvement of the computational resources, and will enable us to perform the large scale simulations proposed for this project.

Biological Laboratories
The molecular biology and biochemistry laboratories within the Life Sciences building consist of approximately 10,000 sq ft of newly remodeled space. Available equipment includes multiple FPLC systems, UV/visible spectrophotometers, 96-well format thermal cyclers, a Fluoromax-2 fluorimeter, light cyclers (Roche), Vistra Fluorimager, chemiluminescent imager (Lumiimager), two Spectramax scanning plate spectrophotometers and fluorimeters, a high-throughput luminometer (Luminoskan), low-speed, high-speed and ultracentrifuges, 2 shaking incubators as well as hybridization ovens, shakers and water baths. Other available resources include cold rooms, phosphorimagers, densitometers, bioreactors for large-scale cell culture, culture hoods, incubators, microscopes, gamma counters, scintillation counters, lyophilizers, autoclaves, shakers, centrifuges,etc. The common laboratory space has DNA sequencers, high-throughput data workstations and all necessary equipment for molecular and cellular biology. Core facilities include specialized microbial culturing facilities, confocal microscopy laboratories and a flow cytometry facility. In addition, a specialized proteomics production facility is being constructed in the Life Sciences II building. This consists of 4000 sq ft of laboratory space for protein purification and analysis, including ThermoFinnigan LCQ ion trap mass spectrometers interfaced with automated high pressure (10,000 psi) capillary LC systems and integrated into a high throughput environment and data management system. All spectrometers are on a common computer network with direct high speed access to the EMSL data storage and archive facility. Other resources include a specialized tissue culture area and the appropriate safety hoods, large-scale roller bottle culture facilities, and other necessary equipment, such as ultracentrifuges and cell fractionation apparatus.

10.5 Sandia National Laboratories (California)

The experimental work will be performed in laboratories at Sandia National Laboratories (California), namely the Chemical and Radiation Detection Laboratory (CRDL) and the Combustion Research Facility (CRF). The pertinent capabilities of both facilities are described below.

Experimental Resources
The CRDL has standard biochemistry, molecular biology and analytical/synthetic chemistry laboratories, with equipment such as freezers, chromatography cases, fermentors, centrifuges, Speedvac, fraction collectors, etc. that will be used for the proposed work. The CRDL houses a Finnigan-MAT (Now Thermoquest) LCQ electrospray ion trap mass spectrometer with a dedicated ultra-low-flow capillary HPLC system (Micro-Tech Scientific Inc. Ultra-Plus II), as well as facilities for fabricating microcapillaries packed with a variety of separation materials, and equipment for capillary electrophoresis, electrochromatography with optical absorbance and fluorescence detectors. Also present is a clean room and microfabrication facilities for chip-based microseparation devices. We will site an additional dedicated mass spectrometer, a MicroMass Q-Tof instrument in Spring 2002.

The CRF houses a Bruker 7.0 Tesla Fourier-Transform ion cyclotron resonance (FT/ICR) instrument with an Analytica electrospray interface. The instrument has extremely high mass resolution and sensitivity for protein and peptide analysis, as well as a wide m/z range that facilitates the analysis of large, poorly ionizable peptides. It is interfaced to a Hewlett Packard HP1100 HPLC system with optional diode array detection, allowing LC/MS analysis, or samples may be directly electrosprayed into the Analytica source. We anticipate having 1/3 to ½ of the FT/ICR instrument time available for this work, as well as access to a majority of the operating time of the LCQ and Q-Tof instruments.

Computational Resources

The computational work will be performed on a network of workstations (SGI Octanes, IBM RS6000, individual Linux workstations, and Linux clusters), or if needed, on massively parallel computers (ASCI Red, Cplant). We anticipate having unrestricted access to both the local network and the supercomputers on-site to perform data analysis and structure modeling.