**Nanotechnology Research for Energy Conservation, and Biological
and Environmental Applications**

Final Report

for Period June 1, 2002 – December 31, 2003

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March 31, 2004

Prepared for

The U.S. Department Of Energy
Award No. DE-FG02-02ER63378

Award Information

Award Number	DE-FG02-02ER63378
Title of Research	Nanotechnology Research for Energy Conservation, and Biological and Environmental Applications
Principal Investigator	Dr. Charles J. O'Connor
Organization	University of New Orleans

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Narrative. This report summarizes the contributions to this project of the participating researchers at the corresponding institutions.

Advanced Materials Research Institute, University of New Orleans – Dr. Charles J. O'Connor:
“Characterization of Cell Wall Interactions and Cell Membrane Damage Processes Using Mass Spectrometry and Molecular/Nanoparticle Probes”

Project 1 – *Hydrophobic Component in Noncovalent Binding of Fusion Peptides to Lipids as Observed by Electrospray Mass Spectrometry* – Dr. Richard Cole and co-workers used electrospray mass spectrometry (ES-MS) to study noncovalent associations between lipids and a fusion peptide. While the electrostatic component of noncovalent binding is known to be strengthened upon passage from solution into the gas phase, any hydrophobic component will be weakened as the final solvent molecules depart. Nonetheless, in this work, strong evidence was compiled to demonstrate the importance of the initial hydrophobic interaction to the observation of lipid-peptide binding by ES-MS. To establish that the association is hydrophobically driven, binding was shown to: a) be disrupted by addition of organic solvents, b) exhibit a temporal component, and c) be strengthened by an initial increase in ionic strength. Memory of the solution-phase hydrophobic interaction is thus preserved in the gas phase, and the possibility to employ ES-MS for the study of such hydrophobic associations was demonstrated. These findings regarding lipid-peptide binding have repercussions for the study of interactions of amphiphilic molecules in a variety of biological systems.

Project 2 – *Characterization of Membrane Damage Processes Using Molecular Probes* – Dr. Matthew Tarr and co-workers used fluorescent probes that partition to different regions of membranes to successfully develop a technique for measuring the penetration of hydroxyl radical into hydrophobic membrane environments. They focused on the study of hydroxyl radical mediated oxidation of cell membrane compounds, mainly focusing on phospholipids. The results of this study have provided a useful technique for measuring the ability of reactive species to penetrate into membranes. The technique, although only applied to hydroxyl radical in this study, could be easily modified for use with singlet oxygen, hydroperoxyl radical, or other reactive oxygen species of importance in biological systems. In addition, studies of protein oxidation can be conducted based on the methods developed in this project. Proteins embedded in a membrane will have altered reactivity depending on their accessibility to reactive oxygen species. Through additional studies, methods for determining this accessibility with protein probes can be developed.

Institute for Micromanufacturing, Louisiana Tech University – Dr. Kody Varahramyan:
“Nanodevices, Nanomaterials, and Nanoscale Structural Characterization for Bioenvironmental Studies”

Project 1 – *Protein and Drug Encapsulation* – Dr. Yuri Lvov and co-workers focused their efforts on the development of methods stemming from the layer-by-layer assembly technique for encapsulation of proteins and drugs, especially those that can be used in sensor applications. Three approaches were taken:

(1) the first approach was a development of our previous results on pH-induced opening of pores in preformed nanoshells due to charge disbalance in polyanion/polycation multilayers for encapsulation of glucose oxidase, myoglobin and hemoglobin: (2) the second and (3) third approaches involved encapsulation/assembly of materials prior to assembly of the polyelectrolyte shells to increase efficiency and stability of loading. As a part of our efforts to create a new protein microcarrier, we demonstrated polyion capsule loading with glucose oxidase, hemoglobin and myoglobin, and analyzed some features of the loading process. In particular, the concentration of proteins inside the capsules, the ratio between the mass of the encapsulated proteins and the mass of the polymer shell, an estimation of the mass of proteins which are bound to polycation/polyanion walls, and enzymatic activity of encased glucose oxidase were studied.

Project 2 – Development of Self-Assembling Electrical Circuits and Energy-Conserving DNA Nanowires – Dr. Don Haynie and co-workers focused their efforts on ascertaining the feasibility of using DNA to fabricate nanometer-scale wires that are at once reliably conductive, robust, mechanically stable, inexpensive, and self-assembling. Such small conductors are expected to be useful for miniaturizing circuits and reducing energy consumption by electronic devices. Specific objectives were to: (1) synthesize a double-stranded DNA module of random sequence and 180 base pairs; (2) design sites at each end of the module for recognition by a restriction enzyme that cleaves double-stranded DNA asymmetrically; (3) join any desired number of “cut” modules into longer pieces of DNA by self-annealing and ligation; (4) begin the metallization process by heating the double-stranded DNA template in the presence of 6 different oligonucleotides, each 30 bases in length, each complementary to a different region of the module, each modified with a thiol group, and each joined to a gold nanoparticle 5 nm in diameter; (5) visualize the hybrid structure by TEM; and (6) use an AC supply to align DNA strands. The first three of these objectives have been achieved. Significant findings from the work conducted indicate that the asymmetrical restriction site method, proposed by Qun Gu, works.

Project 3 – Structural Characterization of Eukaryotic Signal Transduction Proteins Involved in the Energy-Dispersive Processes of Cell Migration and Cell Attachment – Dr. Don Haynie and co-workers focused their efforts in determining the molecular basis of cell adhesion and cell migration. Detailed knowledge of such complex biological processes will surely be useful for medicine, but it could also serve as a model for nanotechnology development. Specific objectives are to: (1) design and synthesize genes corresponding to the four known domains of human tensin, a large, cytoskeleton-associated signaling protein; (2) clone the genes into an expression vector for large-scale production of the corresponding gene products; (3) purify the domains by column chromatography and characterize them using various biophysical techniques, *e.g.* circular dichroism spectroscopy (instrument purchased with funds from AFOSR) and differential scanning calorimetry (instrument purchased with funds from BoR and AFOSR); and (4) complement the experimental work with computational analysis, *viz.* homology modeling of the three-dimensional structure of the domains, analysis of the energetics and structure of domain-ligand interaction, *etc.* (software purchased with funds from AFOSR). Based on the work conducted, all 4 domains were designed and 1 was synthesized. No significant findings were realized.

Center for Advanced Microstructures and Devices, Louisiana State University – Dr. Josef Hormes: “X-ray Absorption Spectroscopy for the Investigation of Biomolecular Recognition and of Heavy Metal Interactions with Biological Systems” – In this part of the project, Dr. Josef Hormes and co-workers focused their efforts on the application of synchrotron radiation based X-ray techniques, *i.e.* EXAFS (Extended X-ray Absorption Fine Structure) and XANES (X-Ray Absorption Fine Structure) spectroscopy to environmental problems. Experiments were carried out in three different projects:

Project 1 – Free Radical Oxidation of Cell Membrane Analogs (Dr. Josef Hormes and co-workers collaboration with Dr. Matthew Tarr, Chemistry-UNO) – The primary focus of this project was the study of the interaction of free radicals with biological materials, such as cell membranes. The results of this

project demonstrated that synchrotron-based X-ray Absorption Spectroscopy as a powerful, element specific technique, can be used to investigate the chemical environment of both P and substituted S in phospholipid membranes, and that it is possible to measure XANES spectra of the important low Z elements such as P and S at low concentration levels also in biological systems.

Project 2 – *Hg in Ground Water from Landfills* (Dr. Josef Hormes and co-workers in collaboration with Prof. Don Haynie, IFM-Louisiana Tech) – The focus of this project was to investigate the use of X-ray fluorescence spectra to examine ground water samples for Hg contamination. The results and found that concentrations of Hg in the low ppm level in ground water samples are not sufficient to carry out X-ray absorption fluorescence spectra studies without “enrichment” of the samples to obtain higher concentrations.

Project 3 – *Chromium Uptake in Nasturtium officinale (Watercress)* (Dr. Josef Hormes and co-workers at CAMD) - The focus of this project was to evaluate the use of X-ray absorption fluorescence spectra to investigate the uptake and chemical modifications of Chromium uptake by the fast growing plant watercress (Nasturtium officinale), which could be used in bioremediation. XANES spectra revealed the uptake of Chromium by plants fertilized with Cr containing solutions, as compared to those plants which were fertilized with solutions containing no Cr. The data did not demonstrate speciation of Cr, which is reduced from valency +6 to valency +3, a valency in which Cr is not very soluble.