AD_____

Award Number: DAMD17-00-2-0010

TITLE: Disaster Relief and Emergency Medical Services Project (DREAMS): Digital Emergency Medical Services and the Detection and Remediation of Chemical Threat Agents

PRINCIPAL INVESTIGATOR: Richard E. Ewing, Ph.D.
Larry Flournoy
Jim Wall
Jim Wild
Richard Crooks
Frank Raushel
Francois Gabbai
Ryland Young

CONTRACTING ORGANIZATION: The Texas A&M University System
College Station, Texas 77843-3000

REPORT DATE: October 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 2002	Annual (1 Oct 01 - 30 Sep 02)

4. TITLE AND SUBTITLE
Disaster Relief and Emergency Medical Services Project (DREAMS): Digital Emergency Medical Services and the Detection and Remediation of Chemical Threat Agents

5. FUNDING NUMBERS
DAMD17-00-2-0010

6. AUTHOR(S):
Richard E. Ewing, Ph.D., Larry Flournoy, Frank Raushel, Jim Wall, Francois Gabbai, Jim Wild, Ryland Young, Richard Crooks

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The Texas A&M University System
College Station, Texas 77843-3000

E-Mail: ewing@vprmail.tamu.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
Original contains color plates: All DTIC reproductions will be in black and white.

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

The Disaster Relief and Emergency Medical Services (DREAMS™) project is a consortium of scientists, medical professionals, and engineers from The Texas A&M University System and the University of Texas Health Science Center at Houston. The goal of DREAMS is to improve the diagnosis and treatment of critically ill or injured soldiers in the field by expediting their access to medical experts at trauma centers or field hospitals.

14. SUBJECT TERMS	15. NUMBER OF PAGES
telemedicine, chemical and biological warfare defense	34
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

INTRODUCTION

The Disaster Relief and Emergency Medical Services (DREAMS™) project has established a consortium of molecular research scientists, emergency medical professionals, and informational sciences engineers from The Texas A&M University System in College Station and the University of Texas Health Science Center at Houston. The primary goal of the DREAMS project is to improve the real-time diagnosis and treatment of critically ill or injured soldiers in the field by expediting the access of "first responders" to medical experts at trauma centers or field hospitals and providing accurate detection of the presence of Chemical and biological Threat Agents. Texas A&M University System research scientists and engineers are working on two components of the DREAMS program:

1. Texas A&M University System (TAMUS) Digital EMS, and
2. Detection and Remediation of Chemical Threat Agents.

Texas A&M University System Digital EMS

Texas A&M Digital EMS is the DREAMS component that allows trauma specialists to treat patients more quickly by providing the "virtual" presence of a physician on the battlefield or at the emergency scene. Digital EMS focuses on integrating existing and "state-of–the-art" developing technologies into the DREAMS Interact ambulance. These include multiple leading-edge telecommunications technologies, especially video processing, wireless communications, and innovate uses of COTS digital signal processing. The Digital EMS ambulance phase one prototype connects emergency medical personnel on the scene with trauma specialists in distant hospitals. This allows physicians to monitor patients using real-time video and vital signs data from a suite of advanced digital medical monitoring equipment normally only available in a clinic or hospital.

Detection and Remediation of Chemical Threat Agents

The component of DREAMS dedicated to the detection and remediation of chemical and biological threat agents that is critical to the welfare of the soldier in the field and citizens at home. One of the newly emerging concerns regarding military and civilian disaster response scenarios is the involvement of chemical or biological weapons of mass destruction, which might range from noxious chemical fumigants to neurotoxic, chemical warfare agents, or from infective biological spores and viruses or their biologically-active toxins. Texas A&M scientists are developing genetically engineered enzymes that recognize and decontaminate a host of chemical and biological threat agents, and methods to integrate these new materials into detection and decontamination systems.

BODY

The Texas A&M University System Digital EMS

Research Accomplishments

Task 1: Development of Site Specific Test Plans

The project team has further developed a communications suite that includes specialized software and modifications to the ambulance. Communications tests were conducted in Liberty County, TX using the customized coded software and documented the level of throughput, latency and jitter in cellular data channels throughout the county. The development team has completed a custom Linux-based terminal server that accommodates the desired number of simultaneous cellular connections. This was necessary to overcome a limitation found with the Xyplex terminal server originally specified for the hospital side infrastructure. The new system implements the fixed site ICM functionality capable of multiplexing data packets that need to be delivered from the fixed hospital site to one of the systems on the ambulance.

The project team continually meets with Liberty County EMS to develop and review operation protocols. Development of test protocol documents continues.

Task 2: Digital Ambulance Systems Evaluation, Development and Integration

TAMUS personnel continue to improve the hardware and software components specified by the digital ambulance system functional requirements. During the fall of 2002 researchers began development of Version 2 of DEMS software that will be integrated for the Liberty County deployment next year. User guide documentation and training plan documentation are currently under development.

The research team continues to refine the client/server communications system managed by the Intelligent Communications Manager (ICM) routing system and bandwidth allocator. During the fourth quarter a new version of the ICM was developed using the built-in Linux kernel packet capture (libcap) library. Using this library allows for implementation of client/server communications transparency on the private network over which the DREAMS applications operate. With the new system, clients and servers executing on the remote or

fixed site can establish communication links over the ICM multiplexed network without the need for implementing ICM specific middleware routing functionality.

Hardware enhancements are covered under Task 5.

Task 3: Hospital-Side Systems Evaluation, Development and Integration with Digital Ambulance

Project personnel designed and implemented a DEMS hardware/software test bed at the DREAMS office located at the Institute of Biosciences and Technology in Houston. . The test bed is used for software/hardware evaluation and testing of DEMS components during the development and deployment phases established by the program. Testing and evaluation results are injected into the development cycle of the system at regular intervals during the implementation phase. TAMUS staff updates and maintain the test bed functionality to match the evolution of the DREAMS project.
The Deployable Telemedicine System (DTS) uses the test bed to support its evolution cycle.

Task 4: Develop Communications Infrastructure to Support Hospital-Side and Remote-Site Communications Systems

The radio and satellite equipment required to support communications to and from remote ambulances and DTS test sites has been defined. This equipment will initially support Ku band digital data and video. The system has been designed to be used for Ka band communications with the addition of amplifiers and splitters in the future. The equipment will be installed on the roof of the Institute for Biosciences and Technology (IBT) in the Texas Medical Center in order to minimize the impact on the Hermann emergency facilities operations which are the principal support center for LifeFlight, the busiest air ambulance service in the world. Contractors will install the antenna and supporting electronics in early 2003. DREAMS members continue to meet with Hermann Hospital to refine requirements and commit resources that establishes a private fiber network backbone between the IBT and Hermann.

Remote site work has focused on investigating new third generation (3G) cell phone technology to replace the digital TDMA cell phones currently being used on the ambulance. Emphasis is placed on evaluating Verizon and SPRINT 3G services. This new technology is expected to increase the capacity of each deployed cell phone to approximately 40 kbits per second. This is approximately a 10-fold increase in the throughput of each phone over existing

systems. Operational decisions by the telecomm providers may limit the performance improvements. Tests will have to be conducted to determine actual results.
The team is in the process of testing a mobile IMMARSAT satellite uplink for possible integration in future system versions.

Task 5: Integrate New Technologies into the Existing System Architecture; Enhance System Functionality and Support Upgrades

A new set of installation scripts and procedures was developed to streamline the software installation process for distribution and deployment of DEMS software. These scripts and procedures will first be tested upon the distribution of DEMS software to both the physician's training module at IBT in Houston and the functional testing facility in the Blocker Building at Texas A&M University.

An automated start-up module has been developed. The new module implements the capability to allow the different DEMS servers to register and function within the scope of the Microsoft services facility. In this manner, the Microsoft services API and Windows services configuration program can access the startup and finalization of different servers. As the system registers each DEMS server, they can then be started and stopped automatically by the DEMS startup script during system boot up. Paramedics will now be able to turn off the ambulance engine and electrical systems without the need to first shutdown the Digital EMS computers. Additionally, a 10-minute cycle time has been programmed that will prevent the system from shutting down when the ambulance is turned off during short periods.

New physician and paramedic prototype interfaces have been designed that include all of the functionality and requirements needed to support the Liberty County protocols, procedures and run record information. The School of Health Information Sciences, University of Texas Health Science Center, has evaluated the new interfaces for computer human interaction issues including usability and functional completeness.

A market survey was performed to evaluate ruggedized 2D barcode scanners and appropriate formats for integration into the DREAMS ambulance. The PDF 417 format was chosen for implementation of the patient medical information. This format allows for storage of approximately 1,800 ASCII or 1,100 binary characters per symbol. The Symbol LS6800 barcode scanner was chosen for integration into the system. This scanner has the ability to read both 1D barcodes (currently used for scanning medications) and 2D barcodes for eventual implementation of patient medical information. A method for

encoding medical information is currently being developed for allowing large medical data sets to be included within the 1,800-character limit of the PDF symbol.

A market survey was performed on digital signature pads to be used for signing "Refusal to Treat/Refusal to Transport" forms when patients are seen by the ambulance paramedics. The Interlink ePad was chosen as the unit for integration into the system.

In order to support multiple remote (mobile or fixed) stations, a new Interface module was developed to provide the additional functionality on the physician side required to manage multiple simultaneous data streams. This effort required several new functional enhancements to the system, including: a new Java interface *MultiStation* system for supporting multiple remote data source abstract methods, video system support for multiple cameras across multiple video sources, a Remote Run Record Server that now takes a unique ID used to identify the source of the run records at the physician side. New compression libraries are currently being evaluated that increase compression in the compression performance to allow for faster streaming of the video across the wireless network.

Task 6: Initiate Field Testing of Digital EMS System(s)

Staged runs of the Digital EMS system were originally slated to begin in Spring 2002, but, due to the limitations imposed by 10 USC 980, DREAMS project staff had to postpone all testing involving any patients whether real or healthy normal volunteers.

The passage and signing of key legislation on 28 December 2001 should enable UT and TAMUS to file for exceptions to 10 USC 980, which will in turn allow for the testing of the Digital EMS system with actual patients. DREAMS researchers have been in regular contact with TATRC and USAMRMC personnel on order to develop a strategy for gaining an exception. Thus far no procedures have been defined by TATRC for filing an exception. Meanwhile, researchers continue to plan and develop systems for use in the initial field-testing of the system, which will involve volunteers acting as patients.

Task 7: Design and Develop Initial Prototype of Digital EM System for Military Use

Research personnel work with TATRC personnel to coordinate a concept development team in order to develop the next generation military ambulance through a continual series of meetings. The initial planning meeting was held February 2002 with subsequent meetings held in June 2002, August 2002 and October 2002. A project plan was developed to address issues regarding requirements, informatics, specifications, communications evaluation and training.

Researchers have developed preliminary designs that utilize the limited space inside the HMMWV patient compartment. All modifications for the HMMWV enhancements are being designed to be installable in the field and should not require a depot return. Two new form and fit computer systems have been designed for the box. Functional versions of these are now under construction for operational testing in the vehicle. A new vibration and shock isolation rack for the computing equipment was designed and is being manufactured.

A special Auregen AC power generator has been preliminarily designed for the vehicle. The HMMWV must be shipped to Auregen for approximately two weeks to complete the design and installation of the generator. Originally, delays at Auregen hindered this task, but now the limitation is a failure in the high-pressure fuel injection system of the HMMWV. It took almost four months of numerous civilian and military mechanical tests and examinations to determine the root cause of the problem. Arrangements have been made to replace the fuel pump in late November. It is hoped that the vehicle can be sent to Auregen in early January.

A COTS miniature color camera system has been modified to include infrared and white light illumination capabilities. This camera system is waterproof and is used in the same manner as the existing moveable lights in the HMMWV. The medic can remove the camera from the socket holder and point the camera and light source at any point of the patient body on either the upper or lower berth of the ambulance. The camera power and video cables are contained in a self-retracting, spiraled cord that prevents entanglement with the patient or litter. One camera system is to be installed on each side of the vehicle.

Individual patient upper-body observation cameras have been selected and tested. These are the same cameras and lighting system as the movable system with a different focal length lens selected. Initial cabling and connectivity topology has been determined for proposed camera, power and computers. This topology will not be validated until final assembly.

The Marines have requested two additional cameras to be used to study the ergonometrics of the medic's motions in the vehicle. These cameras have been designed and are under construction. They will be recorded separately from the retransmitted video of the medical operations.

The mapping system has been enhanced to allow for CADRG map products to be parsed, filtered and displayed on the mapping window within the Digital EMS system. Functionality to allow for panning and zooming has been included with the new mapping application. The new system allows for military iconology to be displayed as different layers on top of the NIMA products.

Task 8: Participate in Military Tests, Concept Evaluations and Exercises

Researchers and Liberty County EMS personnel participated in a user evaluation of the Combat Trauma Patient Simulator (CTPS) to determine if CTPS can be used to support the development of a medical treatment system or as a support component for training.

Activity now centers on the planning and implementation of a Marine Corp exercise in California slated for the 2nd quarter 2003. The Marine Corp has requested a demonstration on the Deployable Telemedicine System (DTS) and Physicians Workstation. This demonstration is scheduled for mid November 2002.

Task 9: Evaluate and pursue opportunities to develop and implement the Digital EMS system in disaster response scenarios

Researchers began designing a deployable telemedicine system (DTS) based on the Digital EMS capabilities but intended for forward deployment in fixed locations for military and civilian use. The system was designed as a static implementation of the Digital EMS system with significant emphasis on packaging, ruggedization and ease of deployment. Requirements were completed and researchers contracted for its manufacture. The system was successfully demonstrated at the ATA Annual Meeting in Los Angeles, CA in June 2002. A new version of DTS is under development that conforms to two man carry limitations, has expanded power options and occupies less volume and weight.

Texas A&M University representatives met with representatives of Loma Linda Medical Center to explore the feasibility of the utilization of DREAMS developments and a starting point for the Loma Linda Research grant.

Task 10: Work with University of Texas Health Science Center to Enhance Current Technologies within the Digital EMS Vehicle and Associated Hospital Systems

Working with UTHSC, TAMUS developed a series of use cases for the digital ambulance to use in refining system requirements, and in testing the functionality of the systems. Additional cases have been defined and the previous ones reviewed.

Researchers from both entities continue to evaluate new medical technology and portable equipment.

Task 11: Enhance the Existing Digital EMS System to Accommodate Additional Functionality

During the past year, researchers have modified the system to accommodate additional EKG waveforms and modified the user interface to reflect this new functionality.

The JAVA programming language is the primary development environment for the Digital EMS. Due to poor printing support available, considerable effort was placed in creating the run record to print in a forms orientation in lieu of on-screen graphical view.

A new terminal server was built to support the 8 cell phone connections coming from the remote vehicle. The new system implements the fixed site ICM functionality for multiplexing data packets that need to be delivered from the fixed site to one of the systems on the ambulance.

Task 12: Integrate New Technologies for Inclusion in the Digital EMS Vehicle to Support Additional Medical Functionality for Trauma Care at Remote and Hospital Sites

Researchers have integrated a new telestration system that will allow a physician to draw on the video window through the use of an Intous drawing pad. The system has the capability to draw geometric patterns in multiple colors and perform screen wipes using the eraser sensor on the stylus. Additional enhancements to the system are: A barcode scanner for recording the type, time and dosage of drugs utilized in the patient treatment process, and a digital signature pad for documentation of refusal of transport and/or treatment.

TAMUS personnel continue to coordinate with UTHSC personnel to test and evaluate capabilities that will identify additional technologies and functionality for integration into the system.

Task 13: Integrate new US Army, NASA and DARPA Technologies Such as Medic-CAM and WARP into the Existing System to Enhance System Functionality

Preliminary testing was performed on the Personnel Information Carrier (PIC) system as a possible solution to store patient medical history. Work was focused on testing the viability of the PIC modules for integration into the DEMS systems and its ability to store and retrieve information sources within its circuitry. Limitations of the system to work under a Windows NT kernel environment created an apparent incompatibility with the DEMS system specifications.

The LSTAT system was also investigated for possible integration into the vehicle. TAMUS and TATRC Personnel continue work on the possible of providing one for inclusion into the DREAMS Digital EMS.

Task 14: Enhance Existing Infrastructure for Supporting a Network of Multiple Digital EMS Vehicles and Hospital Systems in an Integrated Environment

TAMU personnel worked with UTHSC personnel to develop specifications for a large format up/down link for Ku band communications. This is detailed in section 4 above.

In an effort to manage vehicle calls arriving at the physician-side, a new physician-side notification system was developed. The system consists of a software/hardware alarm tied to one of the serial ports within the physician's computer that sounds an alarm whenever a new vehicle call is received or an existing session requires attention.

Task 15: Develop and Test a Prototype Digital EMS Vehicle in Diverse Urban and Rural Technologies for Evaluation and Performance Analysis of Integrated Digital Technologies

TAMUS personnel continue to work toward the initial Liberty County deployment. Anticipated time frame for deployment is second quarter 2003. Continued problems in delivery of the vehicle are due to the manufacture of a

new Ford chassis and delays in manufacturing of the ambulance box being mounted on the chassis.

Due to delays in obtaining an exception to 10 USC 980 regarding human subjects testing work has been limited to engineering analysis.

Task 16: Develop Methodologies for Using New Local, State and National Network Infrastructures for Providing the Digital EMS Vehicle with High Speed Terrestrial Connectivity to the Hospital Node

Members of the DREAMS development group have been instrumental in defining and establishing a State of Texas educational support network. This network will allow rural and urban medical support services to use the backbone at reduced to no direct cost. This network backbone is a work in progress but sections of it are available to the DREAMS project now.

Task 17: Publish Findings an Results in Appropriate Conference Proceedings and Journals and Demonstrate Capabilities of the Digital EMS Ambulance

The Digital EMS System was demonstrated and/or the project was presented at the following meetings and conferences:

- Texas EMS Conference, Austin, TX, November 19-21, 2001
- Digital EMS demonstration for IPT, February 2002
- ATA Annual Meeting, Los Angeles, CA, June 2002

The Texas A&M University System Detection and Remediation of Chemical and Biological Threat Agents Program

Research Accomplishments

The Detection and Remediation Working Group of the DREAMS project has expanded to include additional chemical and biochemical strategies for the detection and decontamination of chemical and biological toxins that might be BCW threat agents. This group involves six research teams of scientists at Texas A&M University who are oriented to the development of new chemical and biochemical technologies that will permit the sensitive and discriminating on-site detection and remediation of chemical contamination associated with

chemical threat agents. In addition, the program has expanded to include enzyme systems that are capable of similar development of new technologies for the detection and remediation of biological threat agents. In particular, this program now involves the collaboration of six research groups, which already have extensive experience with the detection and detoxification of chemical neurotoxins and pesticides.

Ongoing research studies in the laboratories of Drs. Richard M. Crooks (Chemistry, TAMU), Frank Raushel (Chemistry/ Bio-chemistry, TAMU), Francois Gabbai (Chemistry, TAMU), and James Wild (Biochemistry/ Genetics) are specifically addressing the recognition and destruction of G-type and V-type chemical warfare agents. The research teams of Alexsandr Simonian (Biochemistry and Chemical Engineering), Jim Wild, Richard Crooks and Francois Gabbai have developed prototype detectors or both chemical and enzyme-based biosensors for the detection of chemical toxins, which include organophosphate neurotoxins including agricultural pesticides as well as chemical warfare agents.

In addition, the ongoing genetic engineering of enzymes capable of hydrolyzing organo-phosphate neurotoxins has demonstrated the ability to modify existing enzymes to enhance the degradation of chemical warfare toward both type-G and type-V organophosphate nerve agents. We have utilized the new genomic technologies in a successful gene discovery program that has resulted in expanded opportunity for technology transfer into ongoing detection and decontamination applications. We have demonstrated that it is possible to utilize these enzymes in a variety of applications, which should be able to protect and decontaminate patients and medical personnel, sites, and equipment. This includes the incorporation of enzyme into fire-fighting foams, fogging aerosols, surface paints, surgical towelletes, and protective foams. There is further suggestion that enzymes may be introduced into OP-intoxicated animals as free-enzyme, encapsulated enzyme formulations, or as red blood cell decorations for prophylactic treatments.

Following is a summary, by task, of the research activities of The DREAMS Detection and Remediation of Chemical Threat Agents subproject.

Task 1: Determine the Catalytic Limits for the Existing Organophosphate (OP) Hydrolases

We have developed a large collection of native and mutant enzymes whose kinetic characteristics are being evaluated for their ability to detoxify an extensive library of OP-neurotoxins with a full range of molecular constituents attached to the phosphate core including OP-phosphotriesters (P-O bonds),

phosphonothioates (P-S bonds, V-type agents), phosphonofluoridates (P-F bonds, G-type agents), and Tabun (P-CN bonds).

A better understanding of the structure of the active center of the enzyme has been developed through comparison of the X-ray crystallographic structures of existing genetic variants of OPH (e.g. H257L, a first generation mutation and H254G/H257W/L303T a third generation mutation both which have enhanced Soman hydrolysis rates). OPH and most of its genetic mutants have two metal molecules per monomer, but some of the mutants seem to have a single metal while some others may have three. This unexpected and exciting observation suggests a novel flexibility in the catalytic heart of the enzymes.

Detailed CW agent hydrolysis studies have been performed in collaboration with Dr. Douglas Cerasoli at USAMRICD (Edgewood, Maryland) with the native enzyme, first generation, and select second-generation enzymes against the V-agent neurotoxins (V-X and V-G) which has demonstrated a twenty- to fifty-fold increase in hydrolysis rates (V-G Kcat = 214 molecules/sec) over the native levels. These levels of activity are quite high and should be functional for V-G detoxification studies under most expectations.

Task 2: Investigation of Mutations of Individual Residues and Creation of Rational Combinations

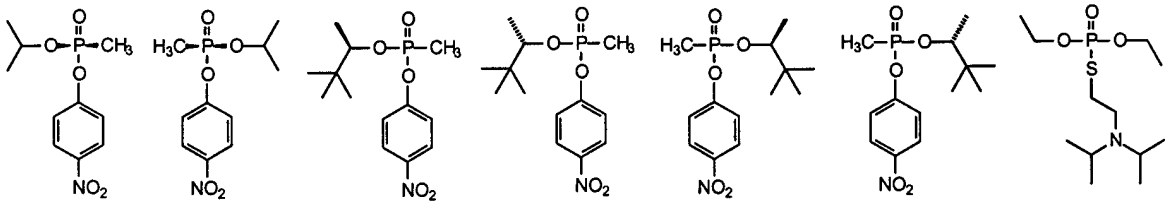
Individual amino acids have been developed to rationally change in their chemical nature in an attempt to modify the nature of the catalytic activity of the enzyme. Enzymes with enhanced rates of detoxification of both V-agents and G-agents have been identified, particularly substitutions of H254 and H257. In addition, variations of L303X from a mutant library have been shown to provide independent improvement of Soman isomer hydrolysis. It is clear that there is a significant structural flexibility to dramatically improve the catalytic ability of OPH as demonstrated by existing and new genetically engineered enzymes.

Task 3: Investigate Combinatorial Mutagenesis of Amino Acids Affecting the Active Site of the Enzyme

To identify unpredictable structural changes that could lead to the enhancement of V-agent destruction, a novel combinatorial, mutagenic approach will be used to create and select semi-random mutations in the enzyme. A collection of new genetically engineered enzymes has been prepared and screening for enhanced hydrolysis has begun with several regional mutational collections such as the L303X library.

We have completed the successful chemical and enzymatic synthesis of reactive analogs of the chiral forms of sarin and soman as well as a new analog of the nerve agent VX. We have used these analogs to explore the substrate specificity of the two most active native enzymes for the catalytic destruction of organophosphate nerve agents: phosphotriesterase (PTE) and organophosphorus acid anhydrolase (OPAA). These compounds work quite well and both enzymes were found to be able to hydrolyze these compounds catalytically. In each case the native enzyme was found to hydrolyze one enantiomer at a faster rate than the other. This information will be quite useful in the design and identification of mutant enzymes that are optimized for the most toxic forms of the organophosphate nerve agents such as GB and GD.

It is clear that the full catalytic potential of PTE with the CW-agents has not been fully exploited. However, significant improvements in the catalytic properties of PTE can be engineered into the protein through focused and global mutagenic procedures. Our approach has been to construct small combinatorial libraries of mutants that are modified at specific residues near the active site of PTE. These libraries are then rapidly screened for improvements in the ability to hydrolyze a series of chiral substrate analogs of the nerve agents GB, GD, and VX (see *Scheme 1* below). Those mutants with improved substrate profiles are then tested in a close collaboration with Dr. Joseph DeFrank and Steve Harvey at Edgewood. Our initial approach has been to concentrate on the most toxic chiral forms of GD.



Scheme 1: **Structures of Chiral Analogs of Sarin, Soman, and VX**

Task 4: Introduce the Best Currently Existing Enzymes into Existing Biosensor Detectors

Enzyme-based biosensors will be constructed to utilize an array of genetically modified enzymes which will allow discrimination between chemical warfare agents and other chemical neurotoxins.
Construction of a new type of an optical, fluorimetric OPH-based chemical sensor utilizing existing enzymes has been constructed. A two-channel microfluidic system has been developed to provide a new, highly sensitive

method for the detection of electroactive compounds such as those generated by the hydrolysis of OP-neurotoxins

Task 5: Select and Purify Mutants with Specifically Desired Catalytic Characteristics

Recombinant proteins will be screened and selectively purified. Their ability to detoxify G-agent and V-agent surrogates will be determined by the use of novel micro-total screening systems which are expected to allow for the optical screening of thousands of recombinant enzymes. The on-going task of screening of semi-random mutation libraries continues; however, there are no significant new activities.

Task 6: Introduce the New Genetically Engineered Proteins into New Biological/Chemical Sensors

Technical Milestones 4 and 5 (above) will come together to produce a new generation of discriminating detectors that are designed for stability and environmental tolerance as well as chemical agent sensitivity. There are no introductions of new enzymes into new sensor elements although there are new developments in both new proteins and new sensor elements.

Multiple enzyme biosensors have been shown to be very effective in discriminating between various classes of chemical neurotoxins. Currently we need to find an appropriate transducer design and technology, to develop the high-sensitive device operated on principles of direct and discriminative detection of OP neurotoxins. The best sensitivity was obtained with a fluorescence detection approach, therefore, we are now looking to develop a small portable device based on that principle. The main obstacle to use that instrument in our design is that an excitation source of "Analyte 2000" is a laser diode with a wavelength of 635 nm, whereas an excitation maximum for SNAFL-1 dye occurs at 510 nm. To overcome that problem we investigated another pH-dependent dye – naphofluorescein carboxylic acid (CNF), produced by Molecular Probe, Inc. The sensitivity of that dye is about the same as the sensitivity of SNAFL-1, but the excitation wavelength is 610 nm, which is appropriate to use in "Analyte 2000".

The direct determination of enzyme-catalyzed neurotoxin hydrolysis was shown to be able to be provided by the self-referencing, pH-sensitive dye SNAFL.1, whose emission spectrum changes at ? = 550 (excitation maximum at 510 nm.) in response to pH. Using spectrofluorimetry and paraoxon as a model organophosphate, paraoxon concentrations as low as 4 x 1 0-7 M was

detected. Having in the mind a development of small portable device, we have found an appropriate industrial device, "Analyte 2000", developed by Research International. The "Analyte 2000" is a 4-channel, single wavelength fluorometer optimized for performing evanescent-wave fluoro-assays, that was developed in conjunction with the Naval Research Laboratory for biomolecules detection. "Analyte 2000" is patented solid-state fluorometer system based on a careful integration of optics, electronics, and software, that can monitor the progress of enzymatic reactions on exposed optical waveguide surfaces using fluorescent-tagged reagents.

The new fiber-optic-based biosensor will use co-immobilized molecules of OPH and fluorescent dye CNF as a recognition and reporter elements. The interaction of substrate with OPH will produce protons. Direct neurotoxin detection is thus possible via measurement of the pH change associated with enzyme activity. When the molecule of interest will bound, it will generate a pH-dependant fluorescent signal that permits real-time remote detection.

Task 7: Introduce Genetically Designed Enzymes into Decontamination Applications

Researchers continue to identify decontamination applications compatible with genetically designed enzymes. A new aerosol, fogging technology for the decontamination of chemical warfare agents is being developed in collaboration between Texas A&M University and Pacific Northwest National Laboratory in Richland, Washington. This new technology is designed to integrate three already developed components: A consortium of well known enzymes capable of the rapid and complete detoxification of CW Agents (including DFP, Sarin, Soman, VX, and R-VX) as well as many neurotoxic agricultural pesticides; an ultrasonic fog-generator which produces a stable "microdroplet fog" of 2-5 microns and fog-quality carrier solution, which is non-toxic and fully bio-compatible. Each of these components has been extensively studied independently, and preliminary results have demonstrated that the combination provides an excellent possibility for the development of an acceptable new decontamination technology that would work for people as well as materials.

The new class of enzymes developed in Task 4 will be introduced into a variety of decontamination studies, including the formation of chemical decontamination wipes, in the formulation of chemical decontamination solutions, in enzyme-based protection filters, in bioreactors, etc. agent sensitivity. The native enzyme and selected genetically-engineered variants of OPH introduced into various paint formulations were shown to effectively hydrolyze field-grade Soman in a NATO PG-31 decontamination trial held in

Cazaux, France in September 2002. (Directed in collaboration with Reactive Surfaces, LTD., Austin, Texas.)

Studies on the effectiveness of enzyme-decorated cotton towelletes have been completed in collaboration with LynnTech INC. of College Station, Texas. The towelletes have been shown very effective at decontaminating dead skin and hard surfaces. The studies are scheduled to move to phase II animal studies this fall.

Task 8: Develop New Enzyme Biocatalysts for Detection and Destruction of Chemical Warfare

There are four different types of enzymes (paraoxonase from animals, organophosphate hydrolase from bacteria, organophosphate anhydrolase from Altermonas, and DPFase from squid) that have been identified to be capable of detoxifying Chemical Warfare Agents. These enzymes have varying catalytic capabilities against different classes of chemical neurotoxins, pesticides, and herbicides. The studies involved in the characterization and development of new enzyme catalysts continues. We have cloned three variant forms of the human enzyme and are beginning the process of evaluating its enzymatic character. In addition, we have identified three new OPAA enzymes that have significant activity against G-agents and we have begun a cloning and expression procedures to isolate those genes. The bacterial species that have this unusual enzyme are aeromonas, hafnia and stenotrophomonas

Task 9: Develop a General Approach for High Throughput Screening of Mutant Enzymes for Remediation of Chemical and Biological Agents Using Arrays of Living Cells

Activities relating to this task were documented and published in two separate articles, the abstracts for which follow:

M. L. Amirpour; P. Ghosh; W. M. Lackowski; R. M. Crooks; M. V. Pishko "Mammalian Cell Cultures on Micropatterned Surfaces of Weak-Acid, Polyelectrolyte Hyperbranched Thin Films on Gold" *Anal. Chem.* 2001, *73*, 1560-1566.

Abstract: "A four-step soft lithographic process based on microcontact printing (μCP) of organic monolayers, hyperbranched polymer grafting, and subsequent polymer functionalization, results in polymer/*n*-alkanethiol patterns that direct the growth and migration of mammalian cells. The functional units on these surfaces are three-dimensional cell "corrals" that have walls 52±2 nm in height and lateral dimensions on the order of 60 μm. The corrals have

hydrophobic, methyl-terminated *n*-alkanethiol bottoms, which promote cell adhesion, and walls consisting of hydrophilic poly(acrylic acid)/poly(ethylene glycol) layered nanocomposites that inhibit cell growth. Cell viability studies indicate that cells remain viable on the patterned surfaces for up to 21 days, and fluorescence microscopy studies of stained cells demonstrate that cell growth and spreading does not occur outside of the corral boundaries. This simple, chemically flexible micro-patterning method provides spatial control over growth of IC-21 murine peritoneal macrophages, human umbilical vein endothelial cells, and murine hepatocytes."

P. Ghosh; W. M. Lackowski; R. M. Crooks "Two New Approaches for Patterning Polymer Films using Templates Prepared by Micro-Contact Printing" *Macromolecules* 2001, *34*, 1230-1236.

Abstract: "Two new methods for preparing micron-scale patterns of hyperbranched polymer films are reported. Both approaches rely on passivation of a reactive surface by micro-contact printing (μ-CP) followed by polymer grafting to unpassivated regions of the surface. The first method involves patterning of a hyperbranched polymer composite film containing poly(amidoamine) dendrimers and Gantrez (an active anhydride copolymer) onto Au. These structures are prepared by partial passivation of an Au surface with an *n*-hexadecanethiol self-assembled monolayer (SAM) using μ-CP, followed by multiple covalent grafting iterations of the dendrimer/Gantrez polymer film onto the unpassivated regions. The second patterning method involves partial passivation of an activated mercaptoundecanoic acid (MUA) SAM, followed by modification of the unpassivated SAM with a layered poly(acrylic acid)/polyethylene glycol (PAA/PEG) nanocomposite. The approach for fabricating these structures consists of formation of an activated MUA SAM, μ-CP of *n*-hexadecylamine ($C16NH_2$) to partially passivate the MUA SAM, and sequential covalent grafting of PAA and then PEG onto the unpassivated regions. Ellipsometry, Fourier transform infrared-external reflection spectroscopy (FTIR-ERS), optical microscopy, and tapping-mode atomic force microscopy (TM-AFM) provide evidence for the viability of these methods. For both types of films, lines of polymer having lateral dimensions of ~20 μm and edge resolutions of < 1 μm are obtained. The polymer thicknesses are on the order of 20-50 nm depending on the number of iterative polymer grafting steps."

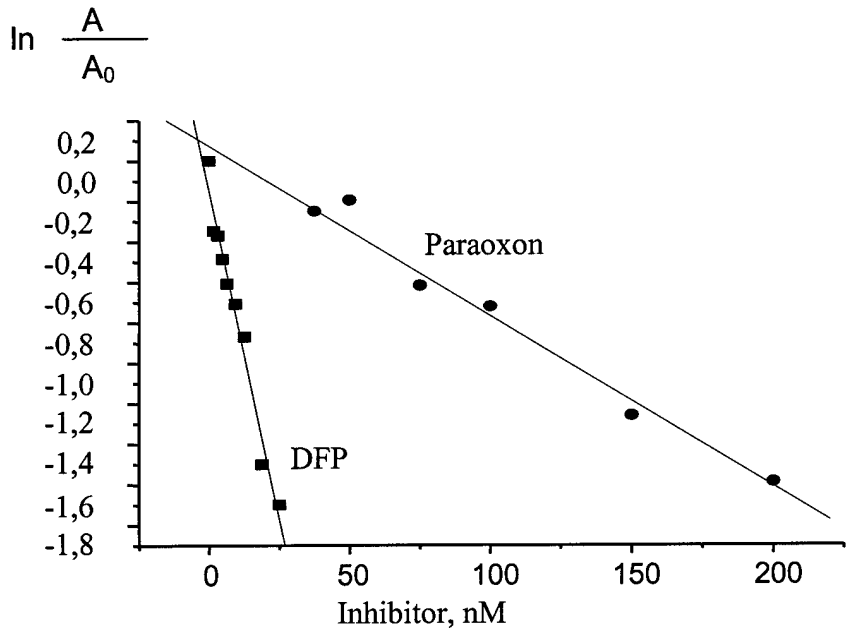
In addition to these studies, it has been observed that there is a critical balance between the expanded activities of mutant enzymes and the stability of the altered enzymes. In order to address these unwanted changes, a new gel based technology has been developed in the laboratory of Dr. Jim Wild, and it has passed its original screening as a means to identify the functional stability of enzymes in situ (within the bacterial cell). With this technology it is possible to

screen hundreds of second and third generation genetically engineered enzymes. Thus, for example, third generation mutations from the H254X – H257X libraries can be transferred to denaturant gels with varying concentrations of urea and their activity against paraoxon or coumaphos can be determined, thus linking functionality with stability.

Task 10: Develop Biosensing Systems for Discriminating Between Different Classes of Neurotoxins Based on Coupled AchE-OPH Enzyme Monitors

Activities relating to this task include collaborations with a team of Russian scientists at Moscow State University. This has led to an DOE-IPP (International Proliferation Protection) with Dr. Ilya N, Kurochkin and Dr. Sergei D. Varfolomeyev. It was possible to separate the effects of different inhibitors, using a combined recognition/discrimination strategy based on the joint action of acetyl-cholinesterase and organophosphate hydrolase enzymes. The detection ranges of these integrated biosensors were 1 – 200 nM for paraoxon, 1 – 25 nM for DFP, and 1 – 150 nM for carbofuran. In addition, it was possible to detect carbofuran concentration in mixed samples contained DFP.

Biosensor Sensitivity Toward Paraoxon and DFP

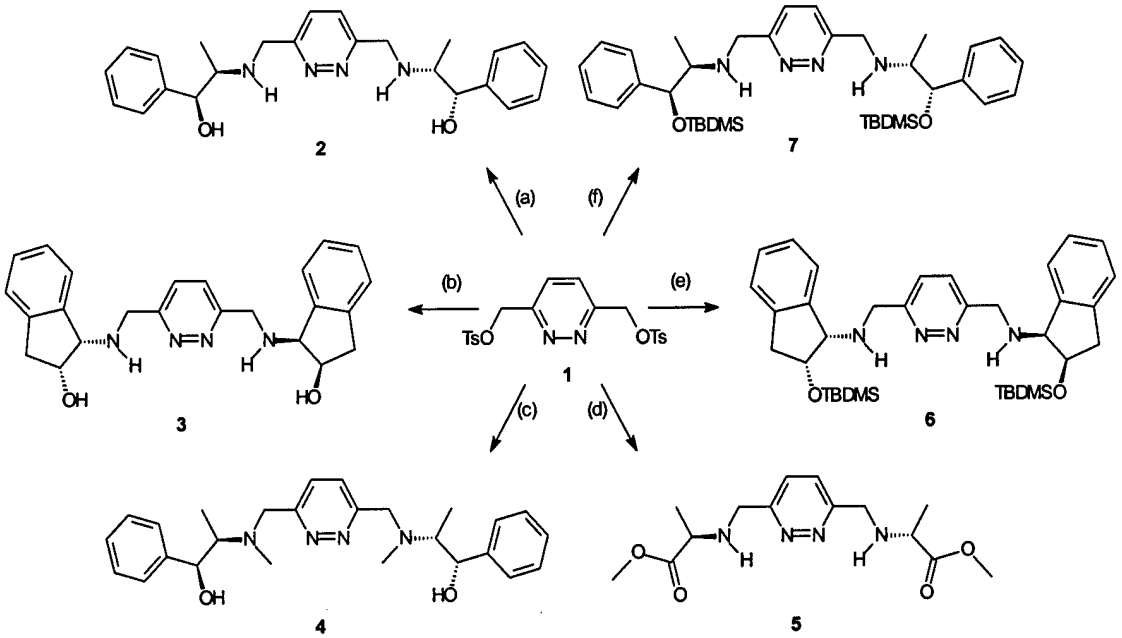


Task 11: Investigation of the application of Polynuclear Metal Complexes for the Detection and Destruction of Chemical Warfare Agents Biosensors for the Detection of Biotoxins in Environmental and Medical Application.

A growing number of investigations indicate that the stereochemistry of the phosphorus center of G-type nerve agents plays a key role in the toxicity of the compounds. For example, studies carried out on the phosphonylation of human acetylcholinesterase (HuAChE) using the four stereomers of GD (soman) shows that the reactivity of wild type HuAChE toward the S_P-soman diastereomers was 40000 to 75000 times higher than that toward the R_P-diastereomers. With this in mind, we have decided to investigate asymmetric catalysts that would selectively activate the hydrolysis of the toxic enantiomer of these agents. Seeking inspiration from nature, we have initiated an effort aimed at the preparation of dinuclear complexes that would mimic both the structure and activity the phosphotriesterase enzyme (PTE).

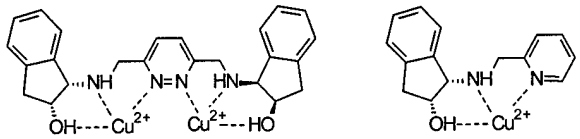
Through simple condensation reactions involving the bis(tosylate) **1**, we have been able to prepare a library of dinucleating ligands (*Scheme 1*). In addition, we have performed coordination studies which have allowed us to confirm the formation of chiral dinuclear Cu(II) complexes in the case of **2** and **3**.

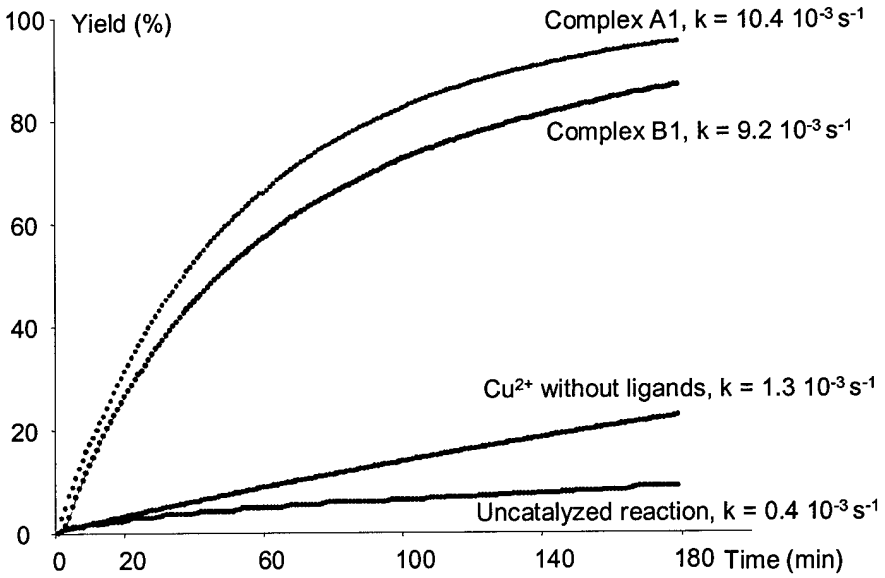
Scheme 1



(a): (1*R*-2*S*)-(-)-norephedrine, Na_2CO_3, MeCN; (b): (1*S*-2*R*)-(-)-cis-1-amino-2-indanol, Na_2CO_3, MeCN;
(c): (1*R*-2*S*)-(-)-ephedrine, Na_2CO_3, MeCN; (d): L-alanine methyl ester Na_2CO_3, MeCN;
(e): ((1*S*-2*R*)-(-)-cis-1-amino-2-indanoxy)-dimethyl-*t*-butylsilane, Na_2CO_3, MeCN;
(f): (1*R*-2*S*)-(-)-norephedrinyldimethyl-*t*-butylsilane, Na_2CO_3, MeCN.

Following the preparation of these dinuclear complexes, we have started to evaluate their ability to promote the hydrolysis of organophosphate nerve agent surrogates. In order to simplify these experiments and evaluate the activity of the catalysts only, we have centered our efforts on the decomposition of achiral substrates such as paraoxon (diethyl-*p*-nitrophenylphosphate). We have been able to confirm that complex **A1** acts as a catalyst and promotes hydrolysis of the substrate. Its activity is modest as it provides only a 4-fold acceleration over the uncatalyzed reaction with a catalyst loading of 50%. These reactions have been monitored by pH-Stat method through the amount of base added to the reaction in order to maintain constant pH.





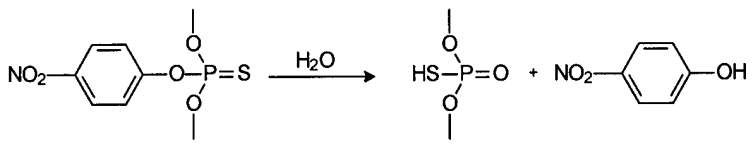


Figure 1: *Advancement of the hydrolysis reaction of dimethyl parathion as a function of time*

More recently, we have turned our attention to the case of parathion (dimethyl-*p*-nitrophenylthiophosphate) and discovered that its activation by **A1** can be readily observed. Experiments carried out at pH 8 and 60°C were followed by pH-stat methods. With a catalyst loading of 50%, hydrolysis of the substrate was complete within three hours. Pseudo-first order kinetic rate constant have been calculated and indicate that catalyst **A1** provides a 25-fold acceleration over the uncatalyzed reaction (Figure 1). Interestingly, while complex **A1** by far outperforms a simple solution of Cu^{2+} ion without ligand, we found that the mononuclear derivative **B1** displays an activity comparable to that of **A1**.

We have evaluated several cations including Cu(II), Cd(II), Pd(II), Pt(II) and have found that Pd(II) provides the greatest catalytic enhancement in the hydrolysis of dimethyl parathion while Cd(II) show no activity at all. Comparison of the first order kinetic rate constant shows that, with Pd(II), a 500-fold enhancement is obtained over the uncatalyzed reaction. Preliminary studies suggest that such an activity is maintained when the Pd(II) ions are complexed by ligand **3**.

In an effort to identify potential molecular recognition units for chemical warfare agents, we have investigated the use of trimeric perfluoro-*ortho*-phenylene mercury (**C**) as a receptor. So far, we have discovered that **C** complexes bis(2-hydroxyethyl)sulfide, a surrogate of HD. We have also found that **C** complexes dimethyl parathion. This has been confirmed by X-ray analysis of the adducts which shows, in both cases, coordination of the sulfur atom to the mercury centers of **C** (Figure 2).

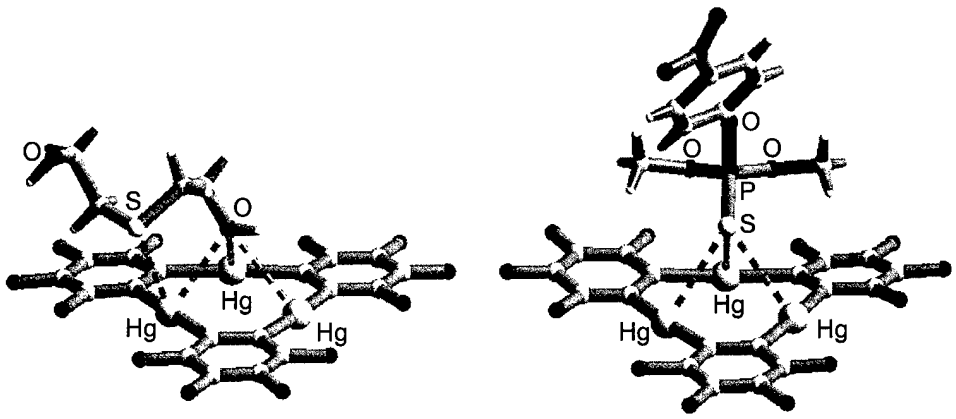
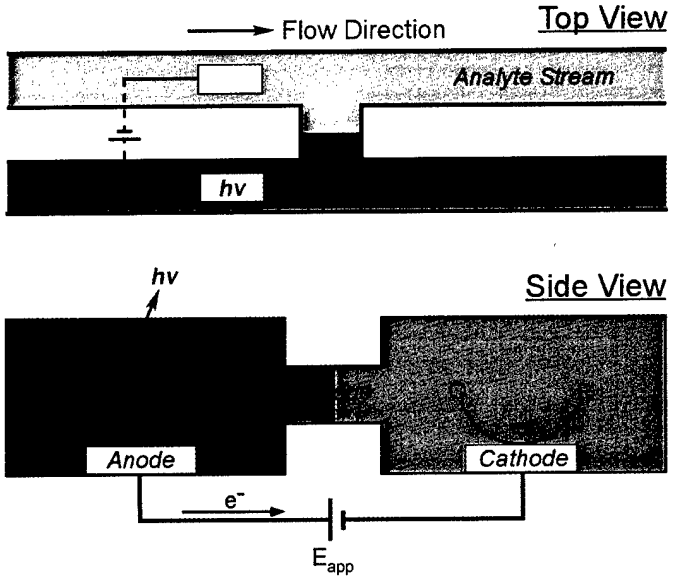


Figure 2: Host-guest complex formed between C and: bis(2-hydroxyethyl)sulfide, a HD surrogate (left); dimethylparthion (right).

Task 12: Develop Peptide and Antibody-Based Biosensors for the Detection of Biotoxins in Environmental and Medical Applications

We have discovered a new method for detecting redox-active targets electrochemically and report their presence photonically via electrogenerated chemiluminescence (ECL). This approach does not require the target to directly interact with the reactants required for ECL, which greatly expands the number of analytes that can be reported by ECL. The detection limits are presently in the nanomolar range, but with a more sensitive detector this can likely be lowered to the picomolar level. In this report we have physically separated the ECL reporting reaction from electrochemical detection events. This is accomplished by placing electrodes in each of two separate channels, which are connected by a stagnant plug of solution in a crossover channel (*Scheme 2*).

Scheme 2



A two-channel, two-electrode microfluidic device that detects analytes electrochemically and reports their presence photonically.

This approach expands the number of analytes that can be detected, because even those that might interfere with the ECL cocktail are accessible. Moreover, this two-channel approach opens up the possibility of using

completely different solution conditions (for example, different solvents or electrolytes) in the detection and reporting channels. Two key findings have come out of this project thus far. First, it is possible to take advantage of the useful laminar flow characteristics of microfluidics to detect at least three different analytes, each confined to their own separate laminae. This might be important if different solvents were required for each target or if precipitation would occur if two of the targets were in the same bulk-phase solution. Second, the measurements reported here were all obtained under continuous flow conditions, which opens up the possibility of real-time detection. Coupling this general strategy with microfluidics also has the general advantage of low power requirements and portability. The results reported here clearly indicate that this new ECL-based analysis approach can be used for quantitative analysis of redox-active targets with high reliability and stability. At present, target selectivity is accessible only via the different redox potentials of the target analytes. However, there are a number of means for improving selectivity. For example, there are many examples of using chemically modified electrodes for this purpose. Moreover, the fraction of the detection column leading up to the electrode could be modified in such a way that specific analytes could be preconcentrated or separated prior to detection.

We foresee that if the detection stream is allowed to flow much faster than the reporting stream, there will be a higher steady-state current at the detector electrode and thus a higher ECL intensity. To achieve this scenario, however, a true salt-bridge that allows electrochemical contact, but hinders the hydrostatic connection, will be required. Work towards the improvement and extension of the analytical capability of this microfluidic sensor is currently underway in our laboratory.

Task 13: Detection of Bacterial Pathogens Using Bacteriophage Arrays and Biofluorescence

Burkholderia cepacia (synonym *Pseudomonas* cepacia) was first identified as the causal agent of bulb rot of onions. This same bacterium is now recognized as an important pathogen in nosocomial infections and in patients with cystic fibrosis. *Burkholderia cepacia* has also become an important bioremediation agent because of its catabolic ability and a potential biocontrol agent for plant pathogens because of its ability to promote plant growth and inhibit the growth several plant pathogens. Therefore, *B. cepacia* is among the rare group of plant pathogens that are also human pathogens and soil saprophytes.

Using a polyphasic taxonomic approach, *B. cepacia* isolates have been grouped into seven genomovars; distinct species that are collectively regarded as the *B. cepacia* complex (BCC). Genomovar classification has assisted in

the elevation of members of the BCC to four newly named species (e. g. *B. multivorans B. stabilis*, *B. vietnamiensis,* and *B. ambifaria* previously genomovars II, IV, V, and VII, respectively). Genomovars III, VI, and I are pending a binomial species name designation. Approximately 85% of strains isolated from colonized cystic fibrosis patients belong to genomovar III and *B. mulitvorans* with genomovars IV, VI, and VII not commonly found in sputum samples of colonized patients. *Burkholderia cepacia* and *B. gladioli* are commonly found in highly organic soils. The objective of this study was to determine the range of *B. cepacia* complex species that could be isolated from organic soils planted to onions. Additionally, soil samples were screened for the presence of *B. cepacia* complex species-associated bacteriophages. Soil samples were collected from six different fields with a long history of planting to onions. Four of the fields were sampled several times during the growing season over a two-year period, whereas the remanding two were sampled several times during the growing season for one year. Sixteen soil samples were taken at each time period at a depth of 7.5 cm along an X transect and mixed to form a composite sample for each of the fields. The samples were processed and plated to selective medium. Individual colonies were isolated and presumptively identified as *B. cepacia* complex or *B. gladioli* based on morphology and fatty acid methyl ester profiles. Isolates (133) were further analyzed to determine species within the *B. cepacia* complex by using rRNA and *recA* gene-targeted PCR. Isolates were also genotyped by using PFGE and RAPD typing. Representative genomovar III isolates were tested for the presence or absence of the Burkholderia cepacia epidemic strain marker (BCESM) marker by PCR. Six of the 133 presumptive isolates could not be identified as *B. cepacia* complex or *B. gladioli.* Among the remaining 127 isolates 31 genomovar I, 29 genomovar III, 54 genomovar VII, and 13 probable *B. pyrrocinia* were identified. Ten of 11 genomovar III tested for the presence of the BCSEM marker were positive.

To date, soil enrichment experiments have yielded bacteriophages for genomovar III, VII, and I isolates. Phage susceptibility testing indicates that the genomovar III bacteriophages exhibit a narrow host range. However, several genomovar I showed broad-host range and were able to form a lysogenic state in a clinical genomovar III isolate. This study demonstrates that *B. cepacia* complex species co-exist in the natural environment and that bacteriophages with inter-genomovar host-range also exist in the environment. The potential for horizontal gene transfer by *B. cepacia* phages with inter-genomovar host ranges could potentially provide a mechanism for gene transfer in natural environment

During the Spring 2002 semester, five students have worked in our laboratories and learned how to isolate bacteriophage from soil extracts, purify the bacteriophages and extract DNA for sequencing. The first group of students

has completed the sequence of phage 781 and is nearly finished with the sequence of phage 43. Students have continued to isolate and purify phages, and genomic libraries have been made for five newly isolated phages. Plans are to have 17 new students in the fall of 2002 working on the project to learn not only molecular biology techniques but also the thought process of scientific discovery. As part of the project students are acquainted with the scientific literature to address topics, and students are taught to critically evaluate methods used to determine if the results obtained justify the conclusions. Our goal to is to develop not only their technical skills but also their conceptual and critical thinking as scientist.

Task 14: Develop Discriminative Organophosphate Neurotoxin Biosensing Systems Based on Multiple OPH Enzyme Monitors

The investigation of enzyme immobilization on different surfaces used in biosensor development provides the critical basis for forming biorecognition elements. This work involves the integration of biochemical recognition by layer-by-layer assembly of enzymes and redox polymers. This collaborative project resulted in the following publication.

Characterization of Assembly of Redox Polymer/Oxidoreductase Nanocomposite Thin Films Used for Biosensor Applications. A. L. Simonian*, A. Revzin, J. R. Wild, J. Elkind, and M. Pishko. 2002.

Abstract: Layer-by-layer electrostatic assembly of the nanocomposite thin films consisting of bilayers of redox polymer (RP) and oxidoreductase enzymes, glucose oxidase (GOX), lactate oxidase (LOX) and pyruvate oxidase (POD), was investigated. Multi-layer nanostructures were fabricated on gold surfaces by deposition of an anionic self-assembled monolayer (SAM) of 11-mercaptoundecanoic acid (MUA), followed by electrostatic attachment of cationic redox polymer (RP), poly[vinylpyridine Os(*bis*-bipyridine)$_2$Cl-co-allylamine] (PVP-Os-AA), and anionic oxidoreductase enzymes. Surface plasmon resonance (SPR) spectroscopy, Fourier transform infrared external reflection spectroscopy (FTIR-ERS) and electrochemistry were employed to characterize assembly of nanocomposite films. The minimum concentration of sensor components necessary for complete layer formation were found to be 0.9 mg/ml and 0.05 mg/ml for RP and GOX respectively. Surface concentration of GOX was found to be 2.4 ng/mm^2 for the first enzyme layer and 1.96 ng/mm^2 for the second enzyme layer, while values of 10.7 ng/mm^2 and 1.3 ng/mm^2 were obtained for POD and LOX respectively. The apparent affinity constant for adsorption of GOX was found to be $8x10^7$ M^{-1}. FTIR-ERS was used to verify the incorporation of GOX and its conformational stability inside of the nanocomposite thin films. A flow-through cell of the

SPR instrument was modified by additions of Ag/AgCl reference and Pt counter electrodes, with the gold-coated SPR chip serving as a working electrode. This enabled real time observation of assembly of sensing components and immediate electrochemical verification of sensor activity. Amperometric response was obtained with a sensitivity of 0.197μA cm^{-2} mM^{-1} for a linear range of 1-10 mM of glucose. The SPR studies also showed that no deterioration or degradation of the nanocomposite RP/GOX structure when stored in aqueous environment occurred over the period of three weeks.

Key Research Accomplishments

The Texas A&M University System Digital EMS

- Concept Definition, Design, and Development of Deployable Telemedicine System
- Reengineering of DEMS systems for Liberty Co. information processing requirements. A separate software engineering effort is in progress to provide this capability.
- Ability to manage multiple simultaneous remote connections from civilian, military or DTS clients.
- Implementation of new communications protocol and graphical support for Telestration and remote drawing capability on video overlays.
- Implementation of Version 1.0 of ICM with communications transparency and intelligent multiplexing.

The Texas A&M University System Detection and Remediation of Chemical and Biological Threat Agents Program

- Developed two-channel microfluidic system.
- Developed new, highly sensitive method for detecting electroactive compounds.
- Fabricate critical detection component for integrated biosensor.
- Fabricate bead-based DNA-capture module for microfluidic system.
- Demonstrate selective DNA recognition.
- Demonstrate generality of approach for other types of biomolecular recognition.
- Synthesized a collection of chiral dinucleating ligands and confirmed that they form dinuclear metal complexes when mixed with transition metal cations.

Reportable Outcomes

The Texas A&M University System Digital EMS

1. ICM patent application.
2. Provisional patent for Deployable Telemedicine System

The Texas A&M University System Detection and Remediation of Chemical and Biological Threat Agents Program

1. M. L. Amirpour; P. Ghosh; W. M. Lackowski; R. M. Crooks; M. V. Pishko "Mammalian Cell Cultures on Micropatterned Surfaces of Weak-Acid, Polyelectrolyte Hyperbranched Thin Films on Gold" *Anal. Chem.* 2001, *73*, 1560-1566.
2. P. Ghosh; W. M. Lackowski; R. M. Crooks "Two New Approaches for Patterning Polymer Films using Templates Prepared by Micro-Contact Printing" *Macromolecules* 2001, *34*, 1230-1236.
3. "Characterization of Assembly of Redox Polymer/Oxidoreductase Nanocomposite Thin Films Used for Biosensor Applications," *Analytica Chimica Acta,* January 8, 2002.
4. Publication: "C_2-chiral dinucleating ligands with a 3,6-disubstituted pyridazine core" Alexandre Picot and François P. Gabbaï, *Tetrahedron Letters,* **2002**, *43(1)*, 11-13.
5. G. H. Seong; W. Zhan; R. M. Crooks "Fabrication of Microchambers within Microfluidic Systems using Photopolymerized Hydrogels: Application to DNA Hybridization" *Anal. Chem.* **2002**, *74*, 3372-3377.
6. W. Zhan; G. H. Seong; R. M. Crooks "Hydrogel-Based Microreactors as a Functional Component of Microfluidic Systems" *Anal. Chem.*, May, **2002.**
7. Characterization of Assembly of Redox Polymer/Oxidoreductase Nanocomposite Thin Films Used for Biosensor Applications. A. L. Simonian*, A. Revzin, J. R. Wild, J. Elkind, M. Pishko. 2002.
8. W. Li, Y. Li, C. M. Hill, K. T. Lum, and F. M. Raushel, "Enzymatic Synthesis of Chiral Organophosphothioates from Prochiral Precursors" *Journal of American Chemical Society*, ***124***, 3498-3499 (2002).
9. F. M. Raushel, "Bacterial Detoxification of Organophosphate Nerve Agents" *Current Opinion in Microbiology*, 5, 288-295 (2002).
10. G. H. Seong; W. Zhan; R. M. Crooks "Fabrication of Microchambers within Microfluidic Systems using Photopolymerized Hydrogels: Application to DNA Hybridization" Anal. *Chem.* **2002**, *74*, 3372-3377
11. T. Sacquety; R. M. Crooks "A Brief History of Chemical Weapons" *J. Chem. Ed.*, October, **2001**.

12. W. Zhan; G. H. Seong; R. M. Crooks "Hydrogel-Based Microreactors as a Functional Component of Microfluidic Systems" Anal. *Chem.* **2002**, *74*, 4647-4652.
13. W. Zhan; J. Alvarez; R. M. Crooks "Electrochemical Sensing in Microfluidic Systems Using Electrogenerated Chemiluminescence as a Photonic Reporter of Redox Reactions" *J. Am. Chem. Soc.*, June **2002**.
14. G. H. Seong; R. M. Crooks "Efficient Mixing and Reactions within Microfluidic Channels Using Microbead-Supported Catalysts" *J. Am. Chem. Soc.*, July, **2002**
15. A.L. Simonian, A. Revzin, J. R. Wild, J. Elkind, and M. V. Pishko. "Characterization of Oxidoreductase/ Redox Polymer Electrostatic Film Assembly by Surface Plasmon Resonance. Spectroscopy, FTIR, and Ellipsometry on Gold." Anal.Chim.Acta, 2002, 466:201-212.
16. Won-Gun Koh, Alexander Revzin, Aleksandr Simonian, and Michael Pishko, "Control of Mammalian Cell and Bacteria Adhesion on Substrates Micropatterned with Poly(ethylene glycol) Hydrogels", *Biomedical Microdevices,* 2002.
17. A.L. Simonian, E.N. Efremenko, and J.R. Wild. "Separation of Neurotoxin and Heavy Metal Ion Effects in Enzyme based Biosensors". 2001. Analytica Chimica Acta.
18. Alexandre Picot and François P. Gabbaï, "C_2-chiral dinucleating ligands with a 3,6-disubstituted pyridazine core" *Tetrahedron Letters,* 2002, *43(1)*, 11-13.
19. Alexandre Picot and François P. Gabbaï, "C_2-chiral dinucleating ligands with a 3,6-disubstituted pyridazine core" 223rd American Chemical Society Abstract ORGN 278.
20. B. Rowan; M. A. Awheeler; R. M. Crooks "Patterning Bacteria within Hyperbranched Polymer Film Templates" *Langmuir*, July, 2002.
21. J. Heo; K. J. Thomas; G. H. Seong; R. M. Crooks "A Microfluidic Bioreactor Based on Hydrogel-Entrapped E. coli: Cell Viability, Lysis, and Intracellular Enzyme Reactions: *Anal. Chem.*, July, **2002**.

A. L. Simonian, A. Revzin, J. R. Wild, J. Elkind, M. Pishko. Characterization of Assembly of Redox Polymer/Oxidoreductase Nanocomposite Thin Films Used for Biosensor Applications. *Analytica Chimica Acta,* January 8, 2002.

CONCLUSIONS

The Texas A&M University System Digital EMS

Efforts throughout the year on the civilian DREAMS vehicle have been focused on transitioning the current system from prototype development into a fielding unit for deployment in Liberty Co., TX. A set of communication tests were performed throughout the county to characterize and profile the communications coverage provided by the cell modem infrastructure that will be used for the initial deployment. Additionally, a new software development effort was begun to transition the current DREAMS patient information subsystem to accommodate new Liberty Co. data requirements.

Work was also focused on initial development, design, and implementation of the Deployable Telemedicine System (DTS). This system was built and demonstrated at the 2002 American Telemedicine Association meeting. The concept of the DTS is to move the functionality of the DREAMS vehicle and/or the DREAMS physician station into a rugged and transportable set of containers that can be shipped and deployed in remote areas in minimal time and with minimal effort.

Work was begun to design and build a military DREAMS vehicle based on the HMMWV platform. This system will accommodate some of the same functionality used within the civilian prototype with the additional functionality to support DoD use requirements for fielded ambulances.

The Texas A&M University System Detection and Remediation of Chemical and Biological Threat Agents Program

There are a series of interplinary teams of research scientists, engineers, and educators in the Texas A&M University System that are involved in rapid detection of chemical and biological threat agents, first-responder emergency medical training, environmental detoxification, equipment/personnel decontamination, and protecting America's food supply. This consortium is concerned about multiple threat agents which pose world-wide environmental, agricultural, and human health threats as the result of military or terrorist action, as well as natural biological blooms or accidental releases. Existing research teams have established a wide spectrum of working collaborations with corresponding colleagues at the federal level, in NATO, and around the world. In addition to possessing sophisticated scientific and engineering capabilities, these teams are highly funded in the interconnected aspects of the

management and destruction of a wide variety of threat agents. The breadth of these various working teams ranges from environmental risk assessment and hazardous materials training to deciphering the fundamental mechanisms of action for important decontaminating enzymes and genes.

Under the direction of the DREAMS project, the TAMU System program has made significant strides in the development of discriminating detection systems and decontamination processes for chemical warfare agents. New enzymes are being constructed to neutralize specific neurotoxins and develop enzyme-based biosensor systems. As each component of fundamental science matures, DREAMS researchers are partnering with local private companies, federal agencies, and academic colleagues through a series of SBIR and STTR programs to develop such deployable systems as enzyme-based decontaminating fogs, foams, and decontamination towelletes. These systems are expected to be useful for both military and civilian protection. These products and those developed from currently emerging discoveries will be integrated into the DREAMS Interact ambulance, an advanced emergency medical response vehicle that supports coordinated emergency communications, remote patient monitoring, and provides a platform for distributing and utilizing advanced chemical and biological systems for the detection, destruction, and decontamination of weapons of mass destruction.