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Workshop Agenda

Tuesday, September 26, 2006

Location: Ballroom South

7:30–8:00 am	Registration and Continental Breakfast	
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8:15–8:45 am	Challenges in Biodetection	José Olivares
Biodetection Sampling Systems		*Session Lead: Greg Kaduchak*
8:45–9:35 am	Overview of Biodetection Sampling Systems	Gary W. Long
9:35–10:00 am	Break & Poster Viewing	
Spectroscopy Systems		*Session Lead: José Olivares*
10:00–10:50 am	Overview of Spectroscopy Systems	Luis Garcia-Rubio
10:50–11:10 am	Break & Poster Viewing	
Systems Integration		*Session Lead: Kristin Omberg*
11:10–12:00pm	Overview on Systems Integration	David Cullin
12:00–1:00 pm	Lunch ***(location: La Terraza)***	
1:00–3:00 pm	Group Breakouts ***(location: see attached)***	
3:00–3:30 pm	Break	
3:30–5:00 pm	Groups Report to Assembly ***(location: Ballroom South)***	
5:00–6:00 pm	Poster Session and Reception ***(location: Ballroom North)***	
7:00–9:00 pm	Dinner (participants & guests) ***(location: New Mexico Room)***	

Workshop Agenda

Wednesday, September 27, 2006

Location: Ballroom South

8:00–8:30 am	Continental Breakfast	
8:30–8:35 am	Welcome and announcements	José Olivares
DNA Based Detection Technologies		*Session Lead: Hong Cai*
8:35–9:25 am	Overview of DNA Technologies for Biodetection	Stephen M. Apatow
9:25–9:45 am	Break & Poster Viewing	
Ligand Based Technologies		*Session Lead: Jennifer Martinez*
9:45–10:35 am	Overview on Ligand Based Technologies for Biodetection	Brian Kay
10:35–10:55 am	Break & Poster Viewing	
Transduction Systems		*Session Lead: Steve Graves*
10:55–11:45 am	Overview of Transducers for Biodetection	Larry Sklar
11:45–1:00 pm	Lunch ***(location: New Mexico Room)***	
1:00–3:00 pm	Group Breakouts ***(location: see attached)***	
3:00–3:30 pm	Break	
3:30–5:00 pm	Groups Report to Assembly ***(location: Ballroom South)***	
5:00 pm	Workshop adjourns	

Group Breakouts Locations

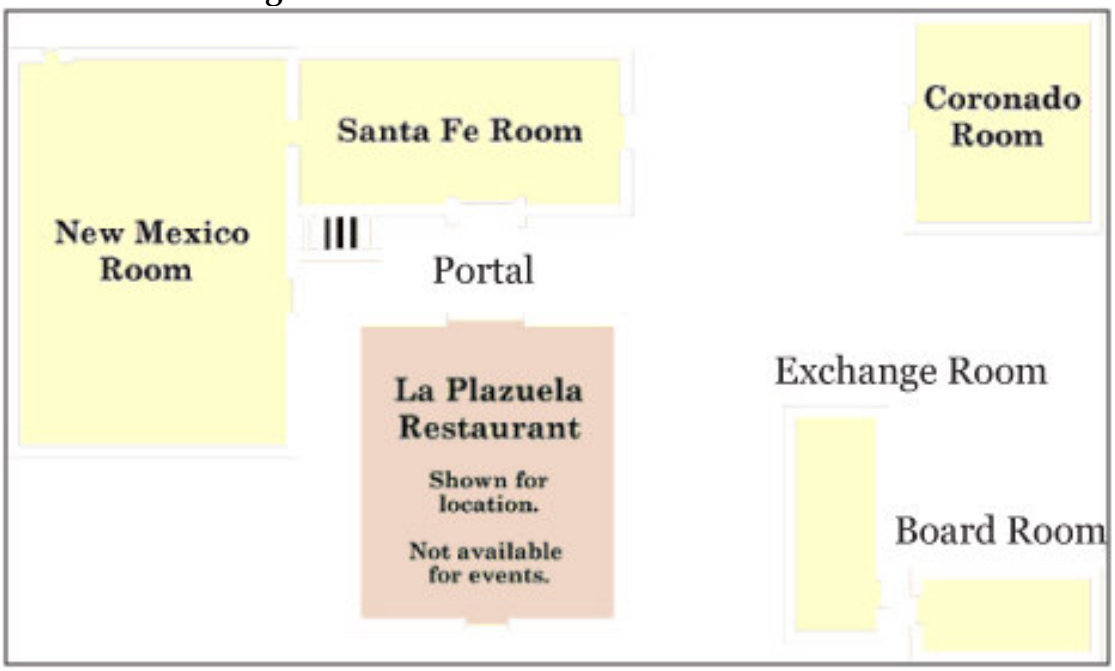
Tuesday, September 26, 2006

Biodetection Sampling Systems	
Discussion Lead: Greg Kaduchak	Ballroom South – West End
Discussion Lead: Gary W. Long	Ballroom North – East End
Spectroscopy Systems	
Discussion Lead: José Olivares	Stiha Room
Discussion Lead: Luis Garcia-Rubio	Ballroom South – East End
Systems Integration	
Discussion Lead: Kristin Omberg	Santa Fe Room
Discussion Lead: David Cullin	Ballroom North – West End

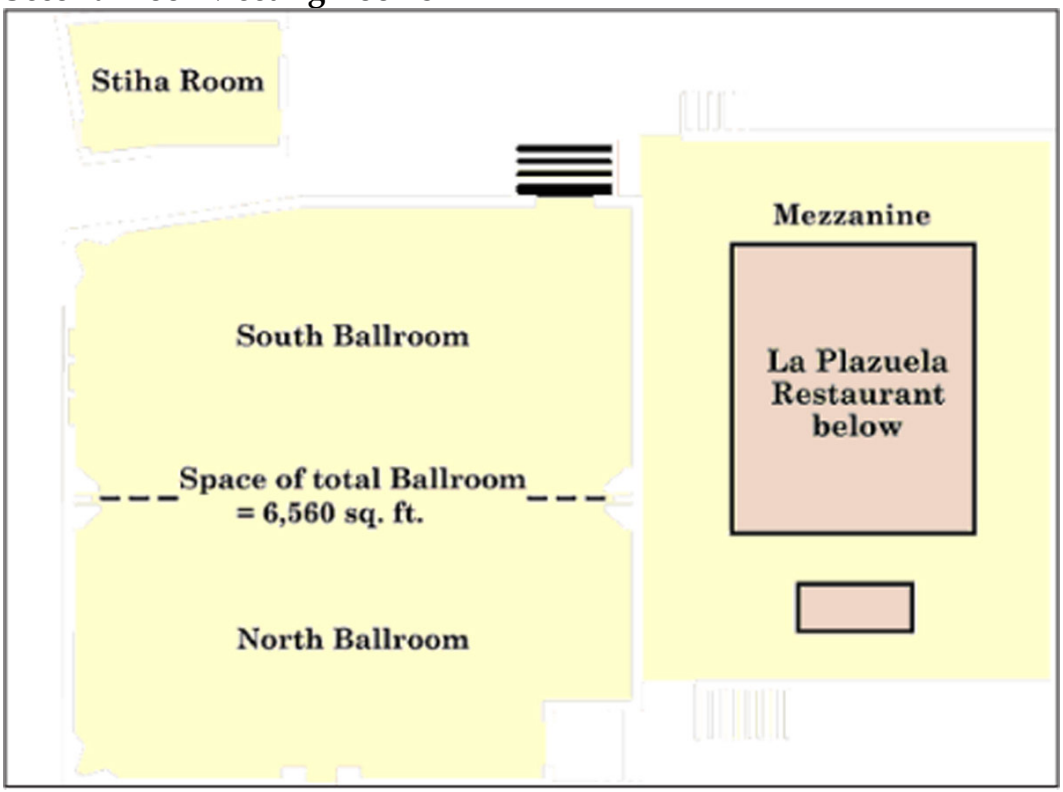
Wednesday, September 27, 2006

DNA Based Detection Technologies	
Discussion Lead: Hong Cai	Ballroom South – West End
Discussion Lead: Stephen M. Apatow	Ballroom North – East End
Ligand Based Technologies	
Discussion Lead: Jennifer Martinez	Stiha Room
Discussion Lead: Brian Kay	Ballroom South – East End
Transduction Systems	
Discussion Lead: Steve Graves	Santa Fe Room
Discussion Lead: Larry Sklar	Ballroom North – West End

First Floor Meeting Rooms



Second Floor Meeting Rooms



The Future of Biodetection Systems
Workshop Overview

As the emergence of natural disease, the threat of bio-terror and the use of industrial facilities for non-peaceful purposes increases, our ability to monitor these threats is critical. Maintaining public health and national security requires the implementation of the best scientific and technological solutions possible. Key to this objective will be developing strategic investments in biodetection technology development.

The principle objective for this workshop is to develop a vision of the future of biodetection for the national security community by understanding the status of current science and technology in this area and developing an analysis of the gaps that need to be filled through strategic and targeted investments.

Through a workshop style program that brings together industry, academia, national labs, and federal agency personnel in an interactive process, participants will develop a roadmap for research and development investment in biodetection. These R&D initiatives will address areas of sampling technologies, DNA-based detection technologies, protein-based detection technologies, transducers, spectroscopy-based technologies, and systems integration.

The workshop's invited speakers will initiate the discussion by reviewing the science and technology in each one of the specified areas; providing an understanding of the challenges and gaps; and initiating a vision as to where the technology will be in 5-10 years with appropriate levels of R&D investments. Workshop participants will breakout into small groups each afternoon to further develop these areas and will report back to the main session on their outcomes.

Through this arrangement, as well as the poster session on Tuesday evening and the numerous opportunities for discussion added to the schedule, this workshop will allow participants to thoroughly explore opportunities for growth in the biodetection arena.

Speaker Biographies

Gary W. Long, Ph.D.

Senior Scientist/Vice President, Tetracore, Inc.

Dr. Long received a Ph.D. in microbiology in from Indiana State University and was a USPHS Research Fellow in Pathobiology at the Pritzker School of Medicine, The University of Chicago. While there, he worked on the immunology and immunodiagnosis of schistosomiasis. From 1982 to 1985 he was the head of the Department of Immunology at the U.S. Naval Medical Research Unit 2 in Manila, Republic of the Philippines. He performed research on immunology and pathology of schistosomiasis and epidemiology of drug resistant malaria. From 1985 to 1990 he studied cell mediated immunity to malaria at the Naval Medical Research Institute, Bethesda, MD. From 1990 to 1994 he was a Research Associate at the School of Hygiene and Public Health at The Johns Hopkins University where he developed techniques for the molecular detection and identification of infectious diseases. In 1994 he established the Molecular Diagnostics Group within the Biological Defense Research Program (BDRP) of the Naval Medical Research Institute. This program was one of the first to develop methods for rapid PCR identification of biological warfare agents. Dr. Long was named head of the Biological Defense Research Program in 1998. He left BDRP in 1999 and was one of the four founders of Tetracore, Inc. Dr. Long was a Lieutenant in the U.S. Navy from 1982-1990. He was an inspector for UNSCOM and performed five inspections of biological warfare facilities.

Luis Garcia-Rubio, Ph.D.

Professor, College of Marine Science, University of South Florida

Professor Luis Garcia Rubio is an internationally renowned researcher whose work has been featured in more than 300 papers and proceedings in the fields of polymer chemistry, sensor development, and modeling of complex biological systems. With a Bachelors degree in Chemical Engineering from the National University of Mexico (UNAM), and a Masters and Ph.D. from McMaster University (Hamilton, Canada), Dr. Garcia-Rubio held technical positions at Hoechst (MX), PEMEX (MX) and Xerox (CAN) before moving to the University of South Florida in 1984, where in addition to supervising more that 20 Ph.D. candidates and generating 18 patents & invention disclosures, he has served as a consultant to Smith & Nephew, Perkin-Elmer, Ortho Diagnostics, Kimberly Clark, Beckman-Coulter, Waters, and SC Johnson, amongst others.

In addition to his academic and research work, Prof. Garcia-Rubio was also a Principal and Co-Founder of Ocean Optics Inc, a manufacturer of high-resolution spectrometers. Founded in 1986, Ocean Optics grew into one of the premier suppliers of spectrometers for use in a wide variety of optical analyzers and systems. Upon completion of the sale of Ocean Optics to Halma PLC in June, 2004, Prof. Garcia-Rubio was able to focus his attention toward Claro and his lifelong goal of expanding the use of Biophotonics into disease detection and diagnosis.

Dr. Garcia-Rubio's team at the College of Marine Science, University of South Florida focuses on sensor development for real-time continuous monitoring of biological and environmental processes with particular emphasis on quantitative characterization of micron and submicron particles. This research couples state-of-the-art analytical techniques in spectroscopy and microbiology to provide a detailed characterization of microorganisms and cells. In addition to marine bio-particles, the technology developed through this research has important applications in veterinary applications and medical diagnosis.

Research Interests: Mathematical Modeling, Sensor Development and Instrumentation, Spectroscopy, Light Scattering, Remote Sensing, Microbial Detection, Micron and Sub-micron Particle Characterization

David W. Cullin, Ph.D.

Senior Vice President for Technology Transition, ICX Technologies, Inc.

Dr. Cullin was born in Allentown, Pennsylvania and graduated with a Bachelor of Science degree in chemistry at the University of Pittsburgh at Johnstown in 1984. He proceeded to The Ohio State University and earned Masters (1988) and PH.D. (1991) degrees in Physical Chemistry. His PH.D. dissertation focused on the High Resolution Spectroscopy of Substituted Cyclopentadienyl Radicals, gas phase spectroscopic studies of the electronic spectroscopy of jet-cooled radical species.

Upon receiving his Ph.D., Dr. Cullin took a position as a Research Chemist with the Naval Surface Warfare Center, Dahlgren Division (NSWCDD) in Dahlgren, VA. He spent the first five years at Dahlgren researching the use of photoactive bio-polymers for their use in optical storage and optical processing devices. In 1996, Dr. Cullin assumed duties as the NSWCDD Project Manager of the Air Base/Port Advanced Concept Technology Demonstration Program in support of the Joint Program Office for Biological Defense (JPO-BD). This program was later named the Portal Shield ACTD and is currently fielded to multiple sites around the world.

In October of 1998, Dr. Cullin accepted a position with the Joint Program Office for Biological Defense (JPO-BD) as the Program Director for the Critical Reagents Program (CRP). In that capacity he managed the procurement and fielding of all of the Department of Defense's reagent based consumables as well as a robust research and development program aimed at performance increases and cost savings in those reagent systems.

From June 2002 until December of 2005, Dr. Cullin assumed a position as the Joint Program Executive Office for Chemical and Biological Defense's (JPEOCBD) Director of Technology. His current responsibilities were to be the JPEOCBD lead for the Transition of New Technologies into JPEOCBD advanced development and procurement fielding, Horizontal Technology Integration, ACTD/ATD Generation and Coordination, Procurement of Non-Standard Equipment, JPEOCBD International Cooperation (Foreign Military Sales, Cooperative Development, Export License Requests), Intelligence and Threat Coordination and acquisition modeling and analysis.

In December of 2005, Dr. Cullin assumed his current position as the Senior Vice President for Technology Transition at ICx Technologies. In this capacity, Dr. Cullin is responsible for Detection Group Agency interactions, product development road-mapping and integration of detection products.

Stephen M. Apatow, Ph.D.

President and Director of Research and Development, Humanitarian Resource Institute

Stephen M. Apatow, President and Director of Research and Development, of the nonprofit organization Humanitarian Resource Institute, is a specialist in strategic planning and project development of initiatives associated with human medicine, veterinary medicine and U.S. and international law. Current programs include the internet based Biodefense Reference Library, Foreign Animal and Zoonotic Disease Center, Bioinformatics: Pathobiological Diagnostics Center and Biodefense Legal Reference Library. Educational resource development for the veterinary and medical community include the Foreign Animal Disease Online Course and the Zoonotic Disease Online Review. To enhance collaboration between Humanitarian Resource Institute and the international community of scholars, the Humanitarian University Consortium was formed to enhance the development of initiatives associated with economic, social, cultural and humanitarian issues worldwide. In 2004, Pathobiologics International was formed as the Consulting Arm of Humanitarian Resource Institute and the Humanitarian University Consortium.

Brian Kay, Ph.D.

Professor and Head of the Department of Biological Sciences, University of Illinois at Chicago

Dr. Brian Kay started his academic career in 1984 at the University of North Carolina-Chapel Hill as a cell and developmental biologist, using *Xenopus laevis* as a model system in which to study oogenesis and muscle differentiation. After 13 years, he moved to the Department of Pharmacology at University of Wisconsin-Madison where he research was applied to discovering inhibitors of signal transduction pathways. In October of 2001, he moved to the Biosciences Division at the Argonne National Laboratory, where he was a Senior Biochemist and Group Leader, and set up a high-throughput functional genomics effort using various display technologies. In 2005, he became Professor and Head of the Department of Biological Sciences at the University of Illinois at Chicago. He has authored 110 publications and reviews, co-edited three books, and been issued 15 patents.

Larry A. Sklar, Ph.D.

Regents Professor of Pathology and Distinguished Professor of Pharmacy
PI and Director New Mexico Molecular Libraries Screening Center
Associate Director of Basic Research, UNM Cancer Research and Treatment Center
PI Keck-UNM Small Animal Imaging Resource
Director of Biotechnology Integration, UNM Health Sciences Center

Dr. Larry A. Sklar is a graduate of the Stanford University Chemistry Department, was a Helen Hay Whitney Postdoctoral Fellow at the University of California and the Baylor College of Medicine, and was a tenured member of the Immunology Department at The Scripps Research Institute before being jointly recruited to UNM by the Department of Pathology, the UNM Cancer Research and Treatment Center, and LANL in 1990. Dr. Sklar received an AHA Established Investigator Award for his pioneering real-time analysis of ligand-receptor interactions; he directed the National Flow Cytometry Resource (NFCR) at LANL. He has more than 250 publications and patents in the areas of leukocyte biology, molecular assembly in signal transduction and cell adhesion, and new technology for molecular assembly and drug discovery. He is currently UNM Regents Professor of Pathology and Distinguished Professor in the UNM College of Pharmacy. As CRTC Associate Director of Basic Research, he is responsible for coordinating four research programs and nine shared resources in the CRTC. As Senior Advisor/Director of Biotechnology at UNM SOM he has been responsible for integrating and coordinating shared resource activities. Teams he leads engaged in small molecule discovery have recently been designated as an NIH Roadmap Molecular Libraries Screening Center and a Keck-UNM Small Animal Imaging Resource. Trained as a physical chemist, but working with industrial partners, physicians, biomedical researchers, and National Lab engineers and physicists, he has a long history translating between medical and non-medical researchers.

Speaker Presentations

Biodetection Sampling Systems

Gary W. Long, Ph.D.
Tetracore, Inc.

Detection and identification of infectious agents is performed on a diverse range of complex materials. The methods used for sample collection and preparation for analyses are varied depending on source, analytic technique and performance goals. Commonly used methods for collection and preparation of forensic, environmental and clinical specimens will be discussed. Some unique difficulties posed by clinical and veterinary specimens will also be described.

Spectroscopy Systems for Biodetection

Luis H. Garcia-Rubio, Ph.D.
College of Marine Science, University of South Florida

Spectrophotometric methods and devices provide both, an alternative and a complement to molecular techniques for the identification and classification of microorganisms and cells. As such, spectrophotometric methods have been used in a variety of ways and configurations to detect specific molecules and to correlate the measured spectral patterns to phenotypical characteristics of microorganisms. This presentation reviews the principles and the state of the art of spectroscopy technologies such as UV/VIS, Light Scattering, Fluorescence, Raman, and MS, for biodetection applications together with novel spectrometer designs aimed at increasing the information content of spectral data. The advantages and limitation of the different techniques are discussed in context of the sensitivity and specificity requirements for the early detection of pathogens and infectious diseases. Successful applications of spectrophometric methods for bioagent detection and for the diagnosis of infectious diseases such as Malaria (parasite infection), Dengue Fever (viral infection), sickle cell anemia (genetic disease) and others are presented and discussed. The considerable implications of spectroscopy technologies, their evolution, and their potential for real-time in-situ monitoring of physiological parameters, telemedicine, epidemics, and other applications are also presented and discussed.

Systems Integration

David W. Cullin, Ph.D.
ICX Technologies, Inc.

Domestic and world events over the past ten years have drastically changed the operational requirements necessary for the nation's biological defense systems. Previously, biological defense was the domain of the Department of Defense (DoD) as they protected troops against largely state threat scenarios. With the increase of terrorist and insurgent threats, we have seen a convergence of needs, from bio-defense systems, for the DoD and in protection of the homeland.

This discussion will focus on the evolution of bio-defense systems over the past fifteen years. The talk will begin with an examination of how the threat has changed and how existing systems have evolved in response to the new threat. This will lead to a discussion of the various component systems which make up the overall bio-defense architecture. Each component system will be described along with a discussion of how those technologies have matured over the past several years. Finally, a series of ideas about future technology and system requirements will be discussed. These concepts will be geared toward providing effective and sustainable systems in the future.

DNA Technologies for Biodetection

Stephen M. Apatow, Ph.D.
Humanitarian Resource Institute

An accurate comprehension of the microbial threats presenting a challenge to the U.S. and international community is fundamental to diagnostic competency and the integrated role of the medical and veterinary professions in biodefense. The lack of validated, field tested molecular detection technologies is now the reference point for risk management discussions associated with emerging infectious diseases, bioterrorism, national and international security. The potential for severe outbreaks of high consequence pathogens, the impact on public health and disruptions to international commerce, underscore the importance of these technologies for surveillance, containment and control. The current threat demands a thorough review of available technologies, targeted research and development to address the immediate needs as well as future optimization of the global public health infrastructure.

Combinatorial Libraries as a Source of Diagnostic Probes and Inhibitors

Brian Kay, Ph.D.
University of Illinois at Chicago

From combinatorial libraries of peptides, proteins, and small molecules, it is possible to identify ligands that bind target proteins. Through various display technologies, such as phage-display, it is possible to screen libraries of combinatorial peptides and engineered proteins (proteins that contain combinatorial peptide sequences within a given stable structure) by affinity selection and isolate molecules that bind selectively and tightly to target proteins in a few weeks' time. Interestingly, the selected interacting molecules ligands often bind the target at sites of protein-protein interaction or within catalytic pockets; consequently, such molecular entities can be formatted in assays for detecting the target, as well as used *in vitro* or *in vivo* as inhibitors. Several examples of this technology will be described for the detection and treatment of pathogens, including the use of peptide ligands to block anthrax toxin and its host receptor, *Plasmodium* invasion, and for the species-specific detection of *Bacillus* spores. It is also possible to screen libraries of combinatorially synthesized small molecules (i.e., heterocycles, carbohydrates, etc.) for binding in a variety of formats (i.e., microtiter plate wells, arrays, intact cells, mass spectrometry). Several examples of successful screens will be presented.

Cellular Principles of Signaling: Living Cells as Biosensors

Larry A. Sklar, Ph.D.
University of New Mexico

Cells and cell networks function as exquisitely sensitive detectors, responding to single photons, piconewton forces, and single digit numbers of molecular recognition events. These processes are made possible through a number of principles that include: amplification and differential amplification and differential stoichiometry of signaling components; redundant extracellular recognition pathways that may include arrays of molecular recognition, antennae, and sequential signal processing steps which amplify or enhance the display or presentation of input signal from one cell type to another; redundant intracellular pathways or networks that compensate for the failures of individual pathways; compartmentalization of intracellular processes which may accumulate or localize signaling elements; heterogeneity of signaling media which include two dimensional arrays, diffusion in two and three dimensions, and compartments which are specialized to communicate between the media; desensitization processes which may adjust gain and terminate or recycle signaling elements for reuse in another location or another time; and dampening processes by which interacting pathways may cancel one another out. Biological systems may function in autocrine, endocrine, and juxtacrine modes, i.e. through self regulation, through production of mediators that act on distance cells, and through nearest neighbor regulation. Cells, as sensors, do not exist in a static format. They may multiply (proliferate), change their form (differentiate), die as a response to environmental toxicity, or program their own death (apoptosis), presumably to prevent the proliferation of an injurious phenotype. As appropriate, specific examples will be drawn from paradigms of acute host defense involving the interactions between neutrophils and bacteria.

Poster Presentation Abstracts

Magnetic Bead Based High Throughput Viral RNA Isolation Enables Superior Animal Pathogen Molecular Detection

Xingwang Fang
Ambion, The RNA Company (Applied Biosystems), Austin, Texas

Traditional RNA isolation methods by phenol/chloroform extraction are not amenable to high throughput due to the difficulty of phase extraction procedures and high risk of cross contamination. Solid phase binding methods using glass fiber filters and paramagnetic beads have thus been developed to replace phenol/chloroform extraction for high throughput RNA isolation. The use of magnetic beads eliminates common problems associated with filter-based methods such as filter clogging, large elution volume, and inconsistent RNA yield. Magnetic beads provide better RNA binding and higher and more consistent RNA yields, thus providing a superior method for high throughput RNA isolation. Elution in only 20-50 ìl concentrates the purified RNA for convenient streamlining of downstream applications. Ambion's MagMAX™ magnetic bead-based high throughput nucleic acid isolation technology provides a highly efficient and effective method for viral RNA isolation from low titer samples of various biological sources such as blood, serum, plasma, and nasal and cloacal swabs and is easily adaptable for manual and robotic high throughput processing. The ability to perform high throughput viral RNA isolation from diverse sample matrices enables rapid animal pathogen detection. Here we present data demonstrating the simplicity, sensitivity, efficiency, and efficacy of this technology for animal pathogen molecular detection using diverse biological sample types.

Delivering Antibodies of Defined Specificities

J.Ayriss, T.Woods , A.Bradbury and P.Pavlik
Bioscience Division, Los Alamos National Laboratory, Los Alamos, New Mexico

Selection of specific antibodies from phage display libraries can be used for generating vast numbers of binding ligands against an unlimited number of targets. At our current setting, automated procedures enable selection against 24 targets at a time; producing an infinite number of potential binders that require further screening. Current standard screening procedures (e.g. ELISA) are low throughput and are not capable of accommodating high-throughput or multiplexing needs, even when automated. In order to overcome the bottlenecks associated with high-throughput screening, a ELMS technology was developed that includes autoinduced expression of binders in 96 well format, one-step fluorescent labeling, uniform coating of microbeads with antigens and multiplexed HT flow cytometry screening. The ELMS technology, on the other hand, completely lacks washing and purification steps. We see the ELMS to be the most advantageous for screening of antibodies with defined specificities, where binding against numerous targets is characterized simultaneously. In order to demonstrate the application of ELMS technology; 300 single chain antibodies (ScFv[1]s), generated against an antigen, have been screened against a panel of 4 highly homologous and 4 unrelated antigens.

Nanotube Based Optical Sensing via Reversible Doping

Satish Chikkannanavar, Leif Brown, Yuan Gao, Hsing-Lin Wang and Stephen K. Doorn
Chemistry Division, Los Alamos National Laboratory, Los Alamos, New Mexico

A new approach to nanotube-based optical sensing is demonstrated, which depends on reversible redox quenching of nanotube fluorescence coupled to analyte binding chemistry. Carbon nanotubes exhibit reversible fluorescence quenching upon interaction with dye-ligand complexes consisting of a redox active dye molecule covalently bound to a biological receptor ligand. The interaction between the dye-ligand complexes and target protein analytes introduced into nanotube solution induce recovery of the quenched fluorescence. We demonstrate this approach to nanotube-based optical sensing using the biotin-avidin test system. Nanomolar sensitivity is attained with high specificity for the targeted analyte. We discuss the results of our finding which make this a highly versatile approach for nanoscale biosensing applications.

Force and Fluorescence Spectroscopy at the Single Protein Level

Mircea Cotlet[1], Christopher Jarzynski[2], Geoff Waldo[3], Jim Werner[4] and Peter Goodwin[4]
[1]Chemistry Division; [2]Theoretical Division; [3]Bioscience Division; [4]Materials Physics Applications Division, Center for Integrated Nanotechnologies; Los Alamos National Laboratory, Los Alamos, New Mexico

Atomic force microscopy (AFM) can be used to measure the forces required to mechanically unfold single protein and polymer molecules[1]. Typically this is done using an AFM probe to pull on a single polymer molecule tethered between the probe tip and a surface and measuring force versus molecular extension. Single molecule fluorescence measurements (SMF) can be used to investigate enzymatic turnovers, protein folding or the photophysics and photochemistry at the most detailed level where the ensemble averaging is completely removed[2]. Single molecule methods (AFM and SMF) are each independently emerging as powerful tools to analyze intricate details of biophysical processes but, as powerful as the two techniques are individually, they each suffer from specific limitations that can be overcome by their application in concert. Here we combine single molecule force microscopy and single molecule fluorescence detection in order to understand the relationship between the fluorescence and folding stability of green fluorescent proteins (GFPs). GFP's chromophore is formed autocatalitically only after the protein has folded into a correct can-like structure[3]. Therefore the fluorescence of GFP is related to an intact protein structure. The combination of these two methods is expected to lead to new physical understandings of both the free energy landscape and the interplay between mechanical stress, protein conformation. Our understanding about the folding stability of these experimental systems may generate information with direct help for the creation of GFP-based molecular force sensors, i.e. fluorescence proteins which can report on the intensity of a force at the single molecule level by means of their intrinsic fluorescence. This poster will summarize our progress to date and future directions.

1. Rief, M.; Gautel, M.; Oesterhelt, F.; Fernandez, J.M. and Gaub, H.E., *Reversible unfolding of individual titin immunoglobulin domains by AFM.* Science, 1997. **276**(5315): p. 1109-12.
2. Ambrose, W.P.; Goodwin, P.M.; Jett, J.H.; Van Orden, A.; Werner, J.H. and Keller R.A.., *Single molecule fluorescence spectroscopy at ambient temperature.* Chemical Reviews, 1999. **99**(10): p. 2929-2956.
3. Tsien, R.Y., *The green fluorescent protein*, Annu. Rev. Biochem.; 1998; **67:** p.509-544

Single Molecule Studies of Antigen-Antibody Binding: The Potential for Highly Quantitative Multiplexed Biosensors

Jamshid P. Temirov[1], Andrew Bradbury[2], James Werner[1]

[1]Materials Physics Applications Division, Center for Integrated Nanotechnologies; [2]Bioscience Division; Los Alamos National Laboratory, Los Alamos, New Mexico

We have been studying the binding of individual fluorescently labeled antigens to surface immobilized antibodies using wide-field fluorescence imaging. The fluorescence time history at an individual binding site can be used to calculate a binding affinity. While these measurements are of biophysical interest, they also demonstrate the feasibility of single molecule biosensors. This single molecule approach to biosensing could have distinct advantages, which include:

- Since individual antigens are literally counted, these measurements are highly quantitative. Unlike Enzyme-Linked ImmunoSorbent Assays (ELISAs), there is no need for signal amplification (which introduces both noise and measurement uncertainty).
- As a single fluorescent molecule can be spatially located to within ~1 nm of accuracy (See Yildiz et al, *Science* 2003), this could provide a means of reducing the contribution of non-specific binding to a bioassay, as only antigens that are spatially co-localized with known antibody locations are counted.
- As these single molecule affinity measurements are based upon kinetic properties (on/off times) and not total fluorescence intensities, they are not subject to limitations in dynamic range customary of most fluorescent methods.
- A different antibody (that recognizes a distinct target) could be located in every 1 by 1 micron square of a surface, meaning then in 1x3 slide could have 10^9 different recognition elements. For a standard 100 by 100 micron field-of-view of a high NA microscope objective this still leads to potentially 10^4 different recognition elements imaged simultaneously.

Facilities Monitoring for Bio-aerosol Detection

Perry C. Gray[1]
[1]*Decision Applications Division, Los Alamos National Laboratory, Los Alamos, New Mexico*

A Microfluidic Device for Continuous Capture and Concentration of Microorganisms from Water

Ashwin K. Balasubramanian, Kamlesh A. Soni, Ali Beskok, Suresh D. Pillai
BioMicroFluidics Laboratory, Texas A&M University, College Station, Texas

Water quality is critical for human health and habitation in numerous applications including the municipal water distribution systems, beverage and food industries, and space exploration missions. Technologies for monitoring the physical, chemical and microbial environments of such systems are of critical importance. One of the major challenges is to have reliable, efficient and cost effective procedures for water quality monitoring that can detect pathogens in water distribution and/or storage systems in a continuous manner. Significant research has been carried out on the development of specific, sensitive and high throughput technologies to detect pathogens in water. These include biosensors, microarray technologies, and PCR-based assays. One of the key limitations associated with currently available pathogen detection technologies is the small volumes sampled by these methods (10 – 50 μL volume). Efficient detection requires pathogen levels in the analyzed volume to be within the sensitivity threshold of the detection assay. Hence, large volumes with dilute pathogen levels need to be sufficiently concentrated down to smaller volumes, for current detection methods to have any practical value. This requires development of pathogen non-specific (generic) concentration methods for microorganisms.

In this study, a microfluidic device based on electrophoretic transport and electrostatic trapping of charged particles has been developed for continuous capture and concentration of pathogens from water. Reclaimed and bottled water samples at pH values ranging from 5.2–6.5 were seeded with bacteria (*E. coli, Salmonella, Pseudomonas*) and viruses (*MS-2* and *Echovirus*). Negative control and capture experiments were performed simultaneously using two identical devices. Both culture based methods and real-time PCR analysis were utilized to characterize the capture efficiency as a function of time, flowrate, and applied electric field. Based on differences between the capture and negative control data, capture efficiencies of 90% to 99% are reported for *E. coli, Salmonella, Pseudomonas*, and *MS-2*, while the capture efficiency for *Echovirus* was around 75%. Overall, the device exhibits 16.67 fold sample volume reduction within an hour at 6 mL/hr. This results in a concentration factor of 15 at 90% capture efficiency. Scaling of the device to sample 5 L/hr can be achieved by stacking 835 identical microchannels. Power and wetted volume for the prototype and scaled devices are also presented. The device can thus function either as a filtration unit or as a sample concentrator to enable the application of real-time detection sensor technologies.

Rapid Prototype: Virulence, Antibiotic Resistance and Genetic Engineering Chip

Crystal Jaing, Shea Gardner, Chitra Manohar, Peter Williams, Nisha Mulakken, Kevin Mcloughlin, Kat Swan, Mark Wagner, Pauline Gu, Phillip Banda* and Tom Slezak

Pathogen Bioinformatics, CB Division, NHI and Livermore Microarray Center, CMLS, Lawrence Livermore National Lab, Livermore, California*

The purpose of this study is to prototype a high-density oligo array to detect the presence of known virulence and antibiotic resistance genes, and vectors for bacterial genetic engineering. More than 760 Hidden Markov Models were built from known pathogen virulence and antibiotic resistance gene families and used to search for gene homologues in various organisms. Initial prototyping was performed using a NimbleGen high-density array (390,000 spots) and 4 BSL-2 organisms. Probe set for the first chip included 10 virulence genes from the 4 BSL-2 bacteria, negative controls from *Thermotoga maritima* and random oligos, positive control oligos from the 4 bacterial genomes, and carefully designed mismatch probes. Probe success rates exceeded 99% across the 4 target organisms. Longer probes (50-60mer) tend to work better than shorter probes (30-40mer). In addition, longer probes tolerated 1-3 base mismatches. Mutations near the 5′ end of the probe appeared to have much stronger effects than near the 3′ end in binding specificity. Chip design for antibiotic resistance genes and bacterial vectors is ongoing. Limits of detection and environmental sample amplification will be studied in the second generation chip.

Functional Reconstitution of Transmembrane Proteins into Bilayers on Nanoporous Silica Microbead Supports

Ryan W. Davis, Adrean Flores, Todd A. Barrick, Jason M. Cox, Susan M. Brozik, Gabriel P. Lopez, and James A. Brozik*
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The introduction of functional transmembrane proteins into supported bilayer based biomimetic systems presents a significant challenge for biophysics. Among the various methods for producing supported bilayers, liposomal fusion offers a versatile method for the introduction of membrane proteins into supported bilayers on a variety of substrates. In this study, the properties of protein containing unilamellar phosphocholine lipid bilayers on nanoporous silica microspheres are investigated to determine the effects of the silica substrate pore structure and the curvature on the stability of the membrane and the functionality of the membrane protein. Supported bilayers on porous silica microspheres show a significant increase in surface area on surfaces with structures in excess of 10 nm as well as an overall decrease in stability resulting from increasing pore size and curvature. Comparison of liposomal and detergent mediated introduction of purified bacteriorhodopsin (bR) and the human type 3 serotonin receptor (5HT3R) are investigated focusing on the resulting protein function, diffusion, orientation, and incorporation efficiency. In both cases, functional protein with near native diffusion constants are observed, however, the reconstitution efficiency and orientation selectivity are significantly enhanced through detergent mediated protein reconstitution. The results of these experiments provide a basis for bulk ionic and fluorescent dye based compartmentalization assays as well as single molecule optical and single channel electrochemical interrogation of transmembrane proteins in a biomemetic platform.

Overcoming Challenges for Rapid Detection of Pathogens in Food and Environmental Samples

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Detection of pathogens in food and environmental samples needs to be fast, sensitive, accurate, user-friendly and cost-effective. The major challenges to consider during development of novel detection methods include: a) low prevalence of pathogens in theses samples; b) low numbers of pathogens; c) non-homogeneous distribution of pathogens in the sample; d) possibility to apply the method for the analysis of samples of different nature (negligible or low sample matrix effects); e) low regulatory detection limits set at 1 cell per 25 g. The obvious way to overcome these challenges is to develop method that can effectively separate and concentrate pathogens from big volume/surface samples followed by amplification and sensitive detection and confirmation.

Several new strategies tested in our laboratory are presented for separation/concentration such as: immuno- and bacteriophage-based biosorbents; density gradient centrifugation (flotation), filtration and centrifugation. Both bioluminescent and molecular methods are reviewed as possible rapid detection techniques. Pros and cons of different techniques are discussed.

Enabling Informed Selection of Equipment for the National Guard Civil Support Teams

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The Civil Support Team (CST) is a federally funded State Army and Air National Guard unit established to support civil authorities in the event of a weapon of mass destruction (WMD) attack. The mission of the CST is to supplement the local and federal response capabilities and is routinely required to rapidly investigate and identify unknown objects/agents in the exclusion zone. The CSTs are deployed with 60 pounds of gear and personal protection equipment. In addition, the CSTs are required to transport equipment capable of detecting specific contaminants. The detection equipment is vital to the mission, but must meet a set of standards including ruggedness and portability. Currently the CSTs possess rapid identification capabilities for chemical, biological, radiological, and explosive agents/objects technologies which were deployed from 1999-2001. With a request for $5 million to congress the CST would like to improve their current equipment with technologies that better fit their needs.

In cooperation with Defense Threat Reduction Agency (DTRA) and the National Guard Bureau (NGB), Los Alamos National Laboratories (LANL) initiated a market survey of non destructive evaluation (NDE) and non destructive testing (NDT) technologies to be utilized by the CST. The final product for DTRA is a thorough evaluation of the NDE/NDT technologies market to give an overall proposal regarding best-in-class technologies for prospective CST deployment, well developed recommendations for the adaptation of commercial equipment for military use**,** and information to allow for knowledgeable purchasing.

We will present the metrics by which technologies are "scored" regarding their usefulness in the exclusion zone for detection of various threats. One of the most important outcomes from this project will be the identification of technology gaps for use by our CSTs.

Detector Selection Using Logical Decisions® Software

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Effective detection of chemical, biological, and radiological warfare agents is becoming increasingly important in current times. The Civil Support Team (CST) is a federally funded State National Guard established to assist local and state authorities in the case of a terrorist event or threat. Identification of unknown substances is one of the CST's primary missions, and it relies upon effective detection equipment. The task of selecting the best equipment from the large number of detectors available on the market is cumbersome, but necessary. Towards that end Los Alamos National Laboratory (LANL) conducted a non-destructive evaluation of chemical, biological, and radiological detectors for the CST. LANL used Logical Decisions® by Decision Support Software because it offered a formal and defensible approach for selecting the best detectors. Derived from the Multi-Attribute Utility Theory (MAUT), Logical Decisions® uses a systematic method of evaluating each detector based on a series of attributes. Through consultation with subject matter experts, attributes for the detectors were determined and prioritized, along with metrics to evaluate the detectors by. Logical Decisions® generated a ranking of the detectors using the weighted attributes. Thus, the detectors to be validated in the field were identified. As proof of principle, three field usable biological detectors were ranked through this process and analysis of the results demonstrates the utility of this tool.

Trace Explosive Detection Using Surface Plasmon Resonance (SPR)

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Sensitive detection of trace High Explosives (HEs) requires high affinity and specificity for individual HEs. Our approach is to use biomimetic surfaces containing proteins and/or DNA, combined with SPR readout. Some of the useful features of the biopolymers include their ease of preparation, stability, target affinity and selectivity, and low cost of production.

As the first step towards a protein-based explosives detection platform, we are using antibodies against TNT and RDX. The antibodies are tethered to a dextran layer on a gold chip. The surface refractive index is monitored using a Biacore instrument. Upon introduction of an HE, the refractive index changes are recorded and plotted in real time. Association and dissociation curves are different for each HE, and provide unique "signatures" which can be described mathematically and matched to each individual HE with high accuracy.

A Next Generation Bioforensic Sample Collection and Tracking Device

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In case of a bioterrorism-related event, first responder units such as the FBI's Hazardous Materials Response Team are called in to collect samples/evidence at the scene. Unfortunately, current sample collection technologies and methods tend to be quite time consuming, error-prone, and costly. To address these problems and to allow for rapid, cost-efficient, and reliable high volume field sample collection, Los Alamos National Laboratory recently developed a handheld, high-tech loaded, multi-purpose sample collection and tracking device. This poster will provide an overview of this patented technology, addressing its features, benefits, and potential application areas.

Synthetic Approach Towards a Protocell: Gene Modulated Amphiphile Production

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The goal of the project is to transform inanimate molecules into an organized system resembling living units through a bottom-up approach. In particular, the coupling of two man-made chemical systems, one mimicking metabolism and the other heredity, inside of a dividing chemical compartment is needed. Our initial strategy attempts to genetically regulate the conversion of synthetic precursors into fatty acid based lipids inducing the formation, growth and division of vesicle systems. Precursor molecules are designed to undergo photolytic conversion into protocellular building blocks via a metabolic mediated process using a ruthenium organo-metal complex as a cofactor. Specifically, we are attempting to reduce picolinium ester based lipid like precursors into fatty acids with $[Ru(bpy)_3]^{2+}$ sensitized electron transfer from a sequence dependent nucleic acid. Photolysis kinetics may ultimately show a rate dependency on nucleic acid sequence, providing the system a mechanism for evolutionary control. Herein we describe synthetic efforts towards this fatty acid based protocell along with preliminary experimental results that include the first example of a nucleic acid catalyzed photo-cleavage of picolinium ester generating carboxylic acid.

Quantitative Assessment of SNP Discrimination for Computational Molecular Beacons

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The ability to discriminate nucleic acid sequences is necessary for a wide variety of applications: high throughput screening, distinguishing genetically modified organisms (GMOs), molecular computing, differentiating biological markers, fingerprinting a specific sensor response for complex systems, etc. Hybridization-based target recognition and discrimination is central to the operation of nucleic acid microsensor systems. Therefore developing a quantitative correlation between mis-hybridization events and sensor output is critical to the accurate interpretation of results. Additionally, knowledge of such correlation can be used to design intelligent sensor systems that incorporate mis-hybridization noise into system design.

In this work, using experimental data produced by introducing single mutations (single nucleotide polymorphisms, SNPs) in the probe sequence of computational catalytic molecular beacons (deoxyribozyme gates) [Stojanovic & Stefanovic, 2003], we investigate correlations between free energy of the target-probe complex and the measured fluorescence of the deoxyribozyme gate. We also explore statistical and coding theory based algorithms for determining the SNP location based on the measured fluorescence value for a given SNP.

*Authors contributed equally to this work.

Waveguide Based Optical Biosensor for Detection of Breast Cancer Markers: System Optimization

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Early detection of breast cancer is limited by the lack of sensitive and rapid techniques. Approaches like mammography do not account for intrinsic differences associated with disease manifestation between patients. Use of tumor markers (proteins/carbohydrate upregulated during cancer) for diagnosis has been suggested. However, inadequacies in experimentation and lack of sensitive technology limit such an application. A comprehensive study involving appropriate controls and robust technology is required for the application of these markers to cancer detection. The sensor group at Los Alamos has developed a waveguide-based optical biosensor for the sensitive and rapid detection of markers such as cholera toxin. The antigen is sandwiched between a capture antibody (immobilized on a waveguide surface) and a detection fluorescent antibody. The assay is developed using fluid lipid bilayer membranes for the sensing film. The purpose of the current study is to adapt the sensor to the detection of breast cancer markers in nipple aspirate fluid (NAF) from patients allowing for a sensitive and non-invasive method of early detection. For this, we obtained recombinant and native carcinoembryonic antigen (CEA, cancer marker) and optimized DOT-blot and ELISA techniques for its detection in a sandwich immunoassay format. Subsequently, the antigen was tested on the optical biosensor and sensitivity and specificity compared to those obtained by biochemistry. The superior sensitivity (5 pM) and short time (5 min) required for the biosensor has allowed for the application of an Institutional Review Board application to test detection limits in NAF from patients and normals. Also, we propose to screen for other specific markers in NAF by biochemical and mass spectrometry methods. This study aims to provide utility, reliability and concentration data for such markers between normals and patients thereby allowing for their use in disease detection.

High Throughput Affinity Reagent Selection for the Proteome: Binders Against Specific Post-translational Modifications: The Sulfotyrosine Example

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Many cellular activities are controlled by post-translational modifications, the study of which is hampered by the lack of specific reagents. Sulfotyrosine is one such post-translational modification, important in many extracellular protein-protein interaction, including HIV infection. We have examined the possibility of selecting post-translation modification specific antibodies from large phage antibody libraries, using sulfotyrosine as a test case. After screening over 8000 selected clones, using a number of different selection strategies, we were able to isolate a single specific scFv, from two different selections. This is able to recognize sulfotyrosine independently of its sequence context in test peptides and four different tested proteins using both ELISA and western blotting. Antibody reactivity is lost by treatment of target proteins with sulfatase, or by carrying out binding in the presence of tyrosine sulfate, whereas, tyrosine or tyrosine phosphate do not inhibit the activity of the antibody.
The isolation of this antibody signals the vast potential of phage antibody libraries in the isolation of such specific reagents, although the extensive screening required indicates that such antibodies are extremely rare.

Developing M13 Phage Display Platform for GFP Based Affinity Reagents

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The ability of different M13 phage proteins to display GFP derivatives were tested by displaying GFP, and GFP variants containing a peptide epitope (myc) recognized by a monoclonal antibody (9e10), in seven different display vectors. These comprised the N or C terminus of p3, the C terminus of p6, the C terminus of p9, and the N terminus of p3 using a hybrid display system using either a TAT transporter leader or an SRP leader. In these latter display systems, the leader is responsible for exporting the displayed GFP to the periplasm, and p3 is responsible for display. We have devised protocols to analyze display levels using ELISA against anti-GFP antibody, anti-SV5 tag antibody and the anti-myc antibody. We have also developed selection protocols to determine the efficiency of display on the different platforms. We find that GFP and myc-GFP are displayed on phage, and recognizable by their antibodies, when displayed on the N terminus of p3 using, pelB leader, SRP leader and in the TAT hybrid system using E & K coils. The SRP leader appears to be most effective when the folding robustness of the displayed protein is decreased, and this is the system most likely to be used for future selection experiments.

Antibody Binding Loop Insertions as Diversity Elements

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In the use of non-antibody proteins as affinity reagent scaffolds, the diversity has generally been derived from random amino acids encoded by oligonucleotides. Such diversity is used either to replace surface amino acids present within the scaffold, or as insertion elements at specific sites within the protein. Although specific binders of high affinity have been selected from such libraries, random oligonucleotides often encode stop codons and amino acid combinations which affect protein folding. Recently it has been shown that the third complementarity determining region (HCDR3) from specific antibody heavy chains can be transplanted into heterologous proteins and so confer the specific antibody binding activity to the created chimeric protein. We have explored the use of such HCDR3s as diversity elements. We first show that we are able to similarly insert a specific HCDR3 from a lysozyme binding antibody into GFP, and create a fluorescent protein able to bind lysozyme. Subsequently, we have developed a PCR method able to harvest random HCDR3s and insert them at predefined sites in any protein, using GFP as an example. The majority of such GFP chimeras are fluorescent, indicating that the HCDR3 does not disrupt correct folding. The availability of this method sets the stage for the use of libraries containing HCDR3s as diversity elements for selection experiments.

The Creation of a Novel Fluorescent Protein by Guided Consensus Engineering

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Consensus engineering has been used to increase the stability of a number of different proteins, either by creating consensus proteins from scratch, or modifying existing proteins so that their sequences more closely match a consensus sequence. In this work we have created a novel fluorescent protein based on the consensus derived from the alignment of 32 fluorescent proteins with homology greater than 62% to a monomeric Azami Green (mAG) fluorescent protein, using the sequence of mAG to guide the selection of amino acids at positions of ambiguity and also to retain a monomeric product. This consensus green protein is extremely well expressed, monomeric and fluorescent with red shifted absorption and emission characteristics compared to mAG. Although it is slightly less stable than mAG, it is also more readily expressed. It is remarkable that a functional consensus protein sequence can be created from scratch for a protein belonging to a family with such a highly cooperative structure, and in which single mutations can have dramatic effects on function. This study further illustrates the power of consensus engineering to create stable proteins using the subtle information embedded in the alignment of similar proteins.

Reengineering Phage Display by Replacing Helper Phage with Bacterial Packaging Cell Lines

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The use of helper phage results in phagemid particles which are both genetically and phenotypically heterogenous. We have been able to eliminate helper phage by using "bacterial packaging cell lines" which contain M13 based helper plasmids. These can package phagemid particles as efficiently as helper phage, but without helper phage contamination, resulting in genetically pure phagemid particle preparations. Furthermore, by using constructs differing in the form of g3 they contain, the display from a single library can be modulated between monovalent, phagemid-like, display to multivalent, phage-like, display levels without any further engineering. These plasmids will allow the elimination of helper phage from selection protocols, facilitating automation, the optimization of selection by matching display levels to diversity, and the effective use of packaged phagemid particles as means to transfer genetic information at an efficiency approaching 100%.

The Kalypsys High Throughput Protein Production Robot

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LANL purchased a high throughput protein production robot last year using funds from DOE. This has the capacity to express and purify the equivalent of 96 x 1 liter cultures. Bacteria are fermented to OD550 20-40 by sparging air/oxygen mixtures. Subsequently tubes are loaded into the robot, and all subsequent steps from centrifugation, lysis, column loading washing and elution are automated.

Selecting GFP Based Binders Using T7 Phage Display

Minghua Dai, Csaba Kiss and Andrew R.M. Bradbury
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Filamentous phage display is not very effective at the display of proteins usually expressed in the cytoplasm. As T7 is a cytoplasmic phage, it has been prospected as being more efficient for the display of such proteins. We have used T7 to display a number of proteins including, GFP and GFP modified to contain either the myc epitope, recognized by the mAb, 9E10 and an anti-lysozyme antibody binding loop (CDR3). These positive controls can be displayed in T7 phage and were used to optimize selection conditions. After dilution of 1000 positive phages in 10e11 negative phages, 10-50% of the selected phages are positive after 4 rounds selection. This demonstrates that binders can be selected using T7 phage display system.

Libraries of GFP containing antibody binding loops were constructed and used to carry out selections. ELISA results showed that positive binders against different antigens. Binding was confirmed in preliminary surface plasmon resonance experiments.

Progress on Fluorobodies: Intrinsically Fluorescent Binders Based on GFP

Csaba Kiss, Minghua Dai, Hugh Fisher, Emanuele Pesavento, Jen Martinez, Rosa Valero, Lisa Phipps, Nileena Velappan, Leslie Chasteen, Peter Pavlik and Andrew R.M. Bradbury
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Antibodies are the most widely used binding ligands in research. However, they suffer from a number of problems, especially when used in molecular diversity techniques. These include low expression levels, instability and poor cytoplasmic expression, as well the inability to detect binding without the use of secondary reagents. The use of GFP as a scaffold would resolve many of these problems. However, due to the destabilization of GFP folding upon the insertion of extraneous sequences, it has not been possible to use standard GFP as an effective scaffold. Initial attempts to insert diversity into an extremely stable form of GFP (Superfolder GFP) and use phage display were unsuccessful. We have now overcome these problems and have succeeded in selecting GFP based binders in proof of principle experiments which preserve both fluorescence and binding activity. These bind their targets specifically as shown by ELISA, FLISA and flow cytometry, with affinities (measured using surface plasmon resonance) in the nanomolar range.

Fluorescent proteins only become fluorescent when correctly folded. This property becomes extremely useful in the design, selection, screening and use of fluorescent binders, in particular:

- Making libraries; diversity compatible with folding can be selected, screened or monitored
- Monitoring the selection process
- Analyzing expression and affinity of selected fluorescent binders
- Assessing functionality: if it is fluorescent, it is functional
- As a downstream detection signal in e.g. immunofluorescence, FLISA, flow cytometry, biosensors

These binders hold tremendous potential in many different fields, including proteomics and high throughput selection projects, such as the GTL protein production and affinity reagents facility.

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History of Santa Fe

(Courtesy of Santafe.com)

Kiowa poet N. Scott Momaday remarked that the American West "is a place that has to be seen to be believed, and it may have to be believed in order to be seen."

The City of Santa Fe was originally occupied by a number of Pueblo Indian villages with founding dates between 1050 to 1150.

The "Kingdom of New Mexico" was first claimed for the Spanish Crown by the conquistador don Francisco Vasques de Coronado in 1540, 70 years before the founding of Santa Fe. Coronado and his men also traveled to the Grand Canyon and through the Great Plains on their New Mexico expedition.

Spanish colonists first settled in northern New Mexico in 1598. Don Juan de Oñate became the first Governor and Captain-General of New Mexico and established his capital in 1598 at San Juan Pueblo, 25 miles north of Santa Fe. When Oñate retired, Don Pedro de Peralta was appointed Governor and Captain-General in 1609. One year later, he moved the capital to present-day Santa Fe. New Mexico was part of the empire of New Spain and Santa Fe was the commercial hub at the end of which linked Mexico City with its northern province.

During the next 70 years, Spanish soldiers and officials, as well as Franciscan missionaries , sought to subjugate and convert the Pueblo Indians of the region. The indigenous population at the time was close to 100,000 people, who spoke nine languages and lived in an estimated 70 pueblos, many of which exist today.

In 1680, Pueblo Indians revolted against some 2,500 Spanish colonists, killing 400 of them and driving the rest back into Mexico. The conquering Pueblos sacked Santa Fe and burned most of the buildings, except the Palace of the Governors. Pueblo Indians occupied Santa Fe until 1692-93, when don Diego de Vargas reestablished Spanish control.

When Mexico gained its independence from Spain, Santa Fe became the capital of the province of New Mexico. Trade was no longer restricted as it was under Spanish rule and trappers and traders moved into the region. In 1821 William Becknell opened the 1,000 mile-long Santa Fe Trail.

On August 18, 1846, in the early period of the Mexican American War, an American army general, Stephen Watts Kearny, took Santa Fe and raised the American flag over the Plaza. Two years later, 1848, Mexico signed the Treaty of Guadalupe Hidalgo ceding New Mexico and California to the United States.

In 1851, Vicar Apostolic, and later Archbishop of Santa Fe, Jean B. Lamy, arrived in Santa Fe. Eighteen years later, he began construction on the Saint Francis Cathedral, one of 45 churches he built in New Mexico. Built in the French Romanesque style, the building is alien to the Spanish heritage of Santa Fe, but is still one of its greatest landmarks. Constructed on the site of an adobe church destroyed in the Pueblo Revolt, the Cathedral was built of locally quarried stone. Portions of the old adobe parish church (La Parroquia), remain in the form of the Chapel of Our Lady of the Rosary, which houses a wooden stature of the Virgin know as La Conquistadora, Our Lady of the Conquest. La Conquistadora was first brought to Santa Fe in 1625 and was returned to the city by the armies of don Diego de Vargas during the reconquest of 1692-93.

For 27 days in March and April of 1862, the Confederate flag of Brigadier General Henry H. Sibley flew over Santa Fe until he was defeated by Union troops. With the arrival of the telegraph in 1868 and the coming of the Atchison, Topeka and the Santa Fe Railroad in 1880, Santa Fe and New Mexico underwent an economic revolution. Corruption in government, however, accompanied the growth, and President Rutherford B. Hayes appointed Lew Wallace as a territorial governor to "clean up New Mexico." Wallace did such a good job that Billy the Kid threatened to come up to Santa Fe and kill him.

New Mexico gained statehood in 1912 and Santa Fe has been the capital city since statehood.

Ten years before Plymouth Colony was founded by the Mayflower Pilgrims, Santa Fe, New Mexico was established as the seat of power of the Spanish Empire north of the Rio Grande. Santa Fe is the oldest capital city in the United States and the oldest European community in the U.S. west of the Mississippi. The Palace of the Governors, on the north side of the Plaza, is the oldest public building in the United States.

Santa Fe has been a seat of government under the flags of Spain, Mexico, the Confederacy, and the United States of America. Courtesy of City of Santa Fe

History of La Fonda Hotel

La Fonda Circa 1929 Courtesy Museum of New Mexico. Neg. # 46955

When Santa Fe was founded in 1607, official records show that an inn, or la fonda, was among the first businesses established.

More than two centuries later, in 1821, when Captain William Becknell and his retinue forged a commercial route across the plains from Missouri to Santa Fe, they were pleased to find comfortable lodging and hospitality at la fonda on the Plaza. Literally the inn at the end of the Santa Fe Trail, La Fonda still occupies the southeast corner of the Plaza where travelers of all descriptions have been welcomed for almost 400 years.

"La Fonda' circa 1905 Courtesy Museum of New Mexico. Neg. # 13040

The current La Fonda was built in 1922 on the site of the previous inns. In 1925 it was acquired by the Atchison, Topeka & Santa Fe Railroad, which leased it to Fred Harvey.

From 1926 to 1968, La Fonda was one of the Harvey Houses, a renowned chain of fine hotels. Since 1968, La Fonda has been locally owned and operated and has continued a tradition of warm hospitality, excellent service and modern amenities while maintaining its historic integrity and architectural authenticity.

A travel writer once said, "Like vintage wine, La Fonda only improves with age...it is definitely an authentic Santa Fe heirloom."

For more information, pick up one of our "History of La Fonda" brochures.

Art of La Fonda

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Map of Santa Fe