SAND2006-0914P

"Technology-for-Biology @ Sandia"
(Bioscience & Biotechnology at Sandia)

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Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.

Sandia National Laboratories

Sandia Has Two Primary Sites

Kauai Test Facility,
Hawaii

Albuquerque,
New Mexico

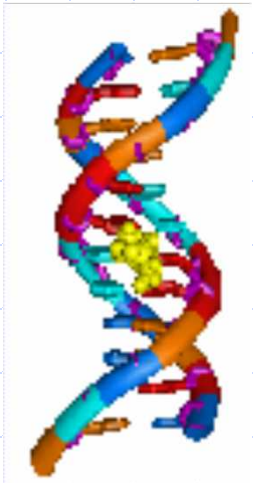
Livermore,
California

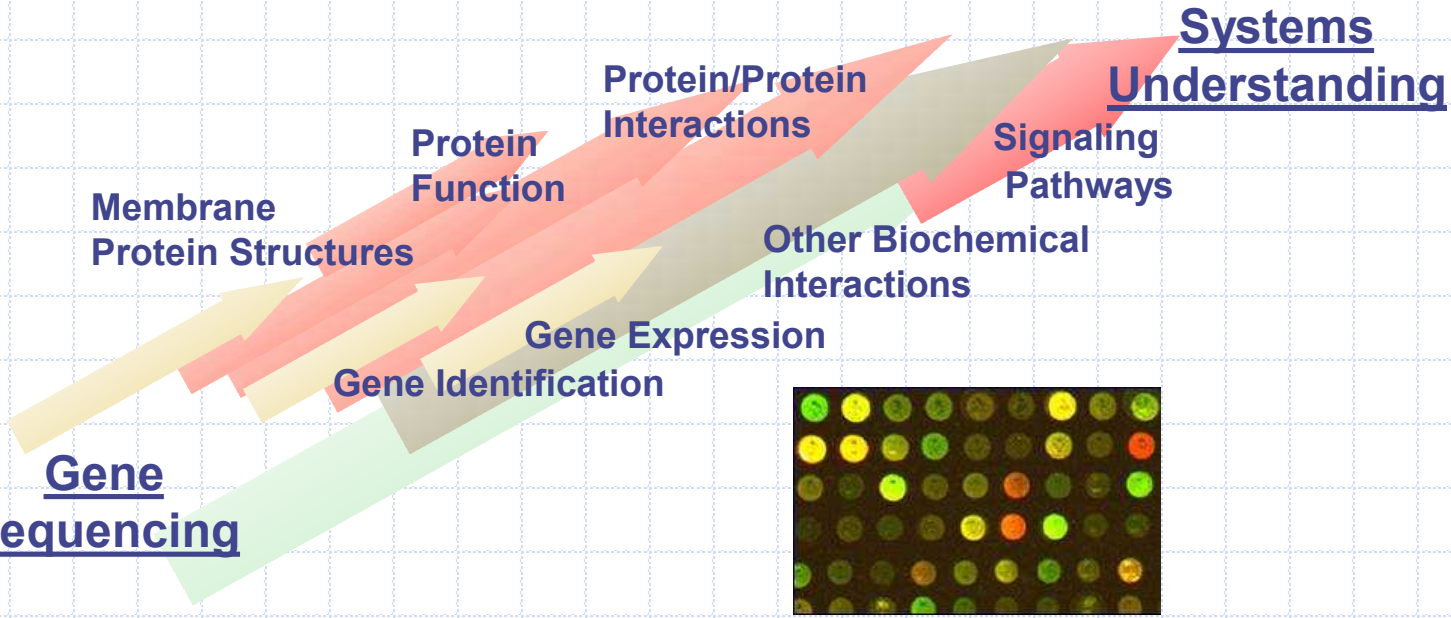
Tonopah Test Range,
Nevada

The Backdrop

From High-Throughput Experimental Methods to Systems-level Understanding

- DNA Sequencing
- Gene Expression Analysis With Microarrays
- Protein Profiling via High Throughput Mass Spectroscopy
- Protein-Protein Interactions
- Whole-Cell Response





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20-21 Sept, 2005

NanoTechnology

Today's Threats

- Bacterial
- Viral
- Chemical
- Radiation
- Bioengineered

Systems Approach

Solutions

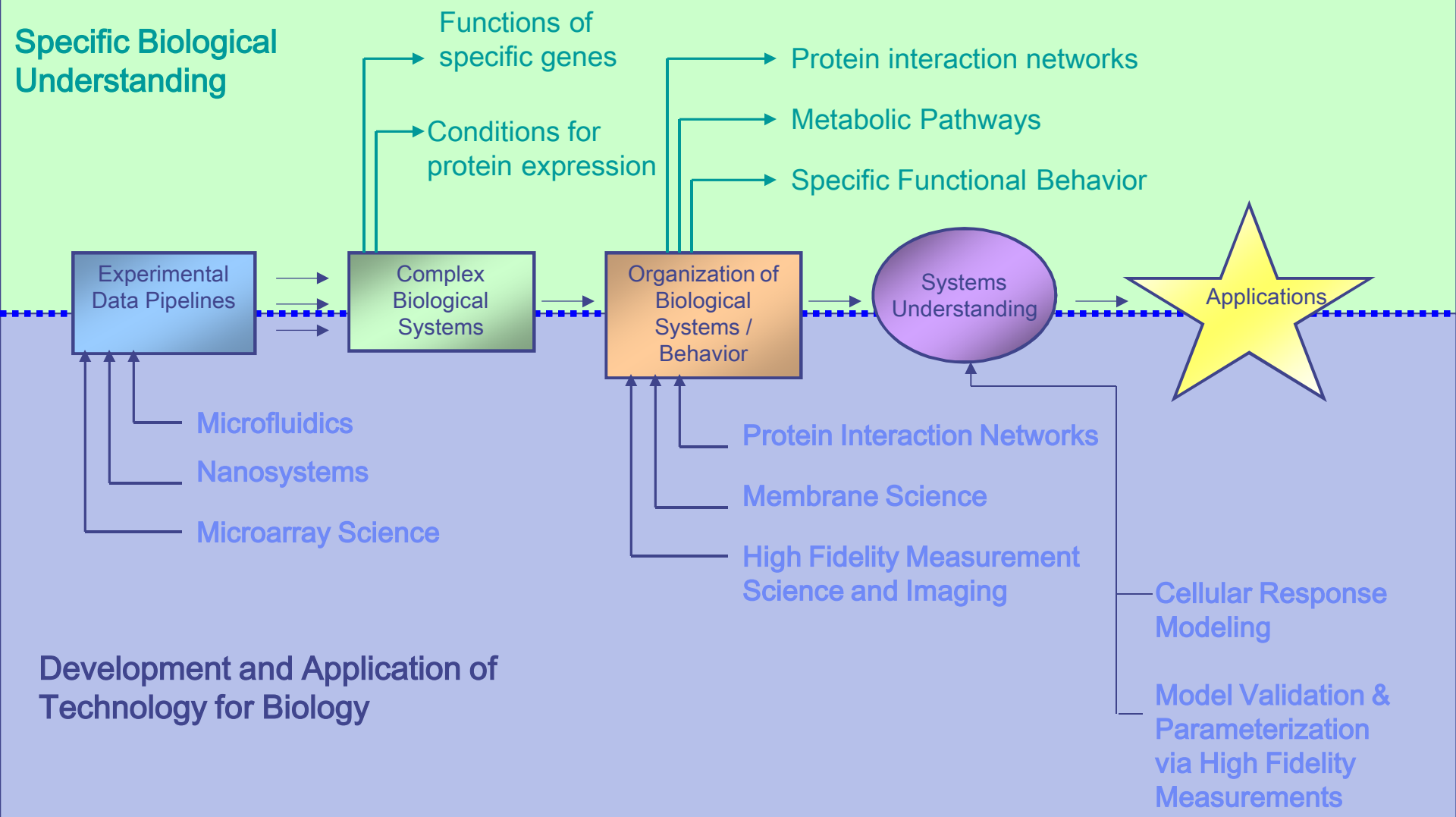
Physical Sciences
- •Decontamination
- •Protection
- •Sensors

Biological Sciences
- •Target Agent Characteristics
- •Biological Process Analysis
- •Bio-Technology Explosion
  - •Genomics
  - •Proteomics
  - •Systems Biology
  - •Physiologic Processes

Detection Technologies
Decontamination
Protection
Medical Countermeasures
Analysis
Drug Development
Drug Delivery

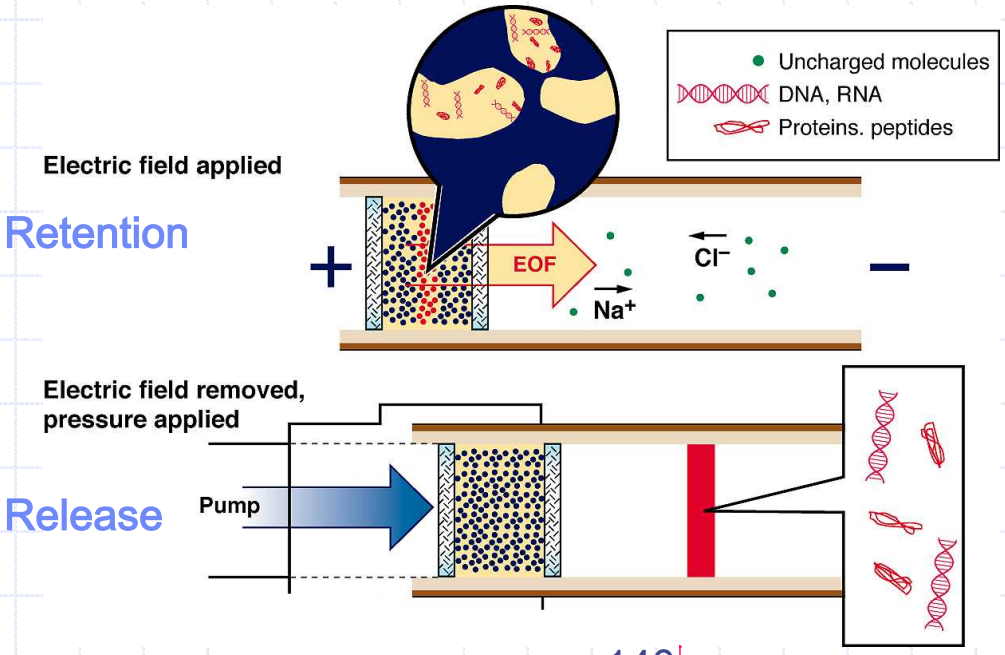
One *PIECE* at a time → Broad Application

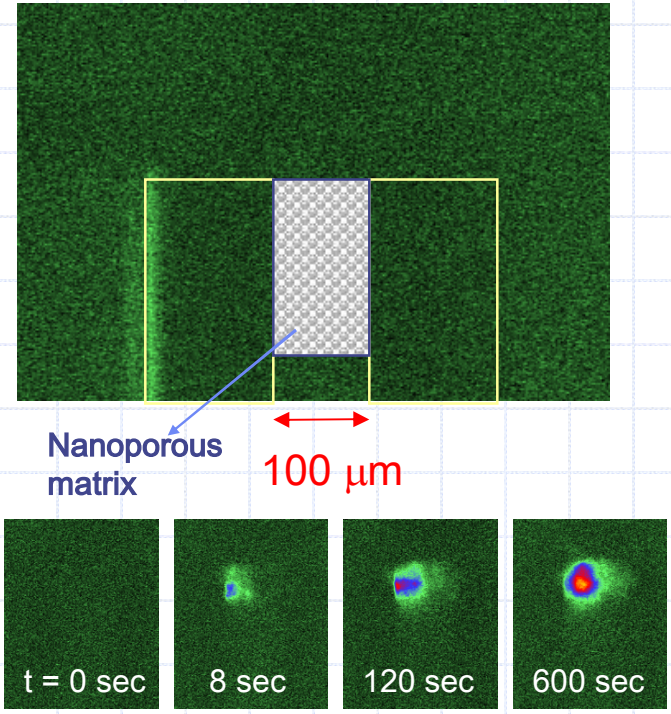
The Technology-Enabled Life Science Revolution

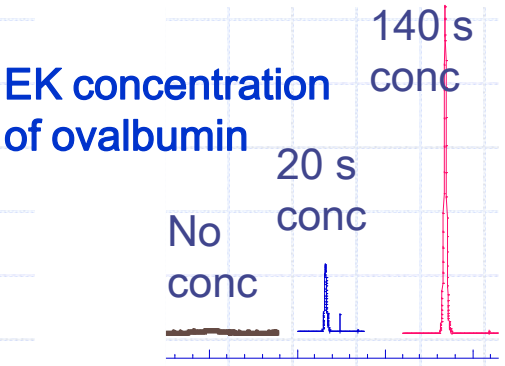


Concentrating Biomolecules

We discovered a new phenomenon enabled at microscale- An electric field addressable retention and release that can be used for concentrating proteins



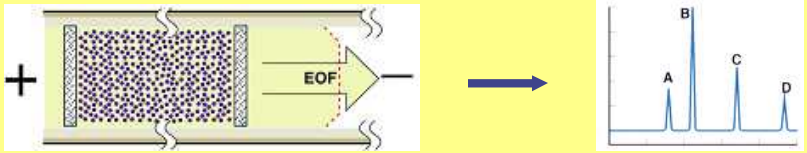




Up to 100-fold concentration in minutes

Microchip Chromatography

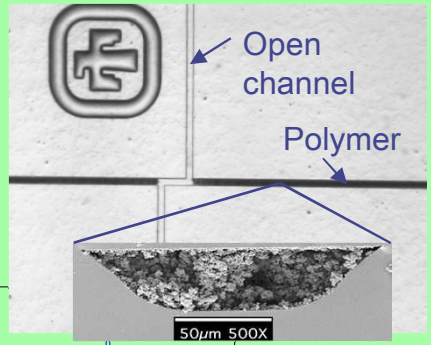
Chromatography: sorting molecules in a packed column



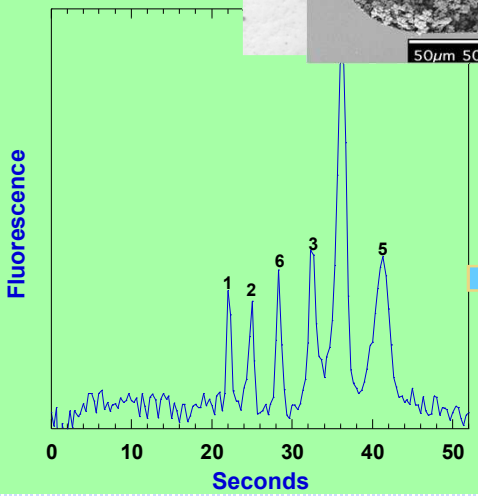
Commercial Chromatography System

- 100-fold more reagents
- Same analysis takes ~9 min.

Miniaturized Chromatography System



Throckmorton, Shepodd, Singh
Anal. Chem. 2002, 74, 784-789

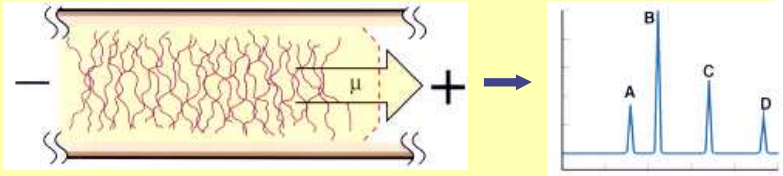


6 bioactive peptides separated in 45 sec

Microchip analysis: faster, better, potentially cheaper and uses tiny amounts of reagents

Gel Electrophoresis in a Chip

Proteins are sorted by size as they migrate through a porous sieving gel

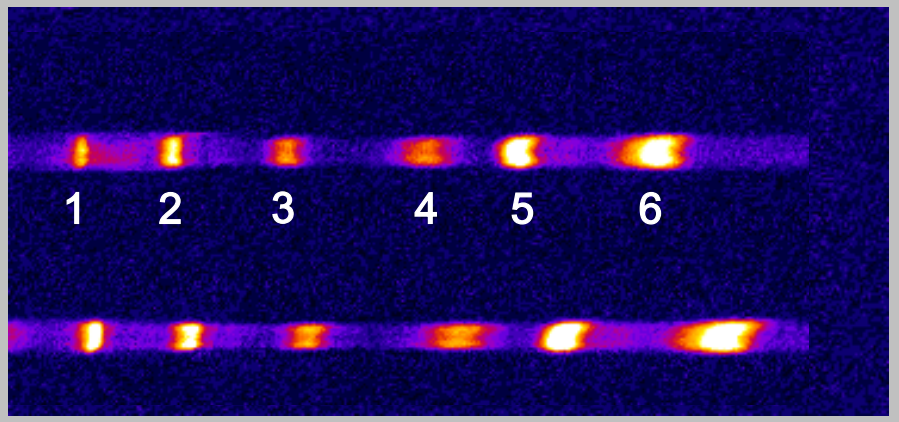


Conventional Method

- Large sample amounts
- Takes hours
- Proteins can't be recovered easily

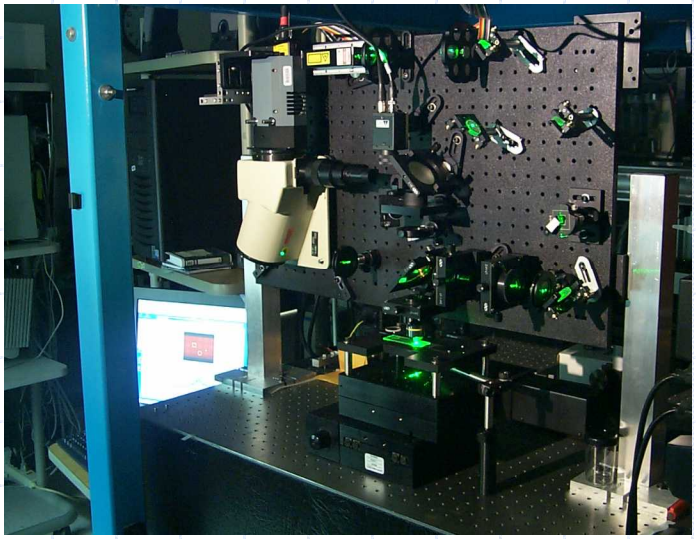
Microchip Method

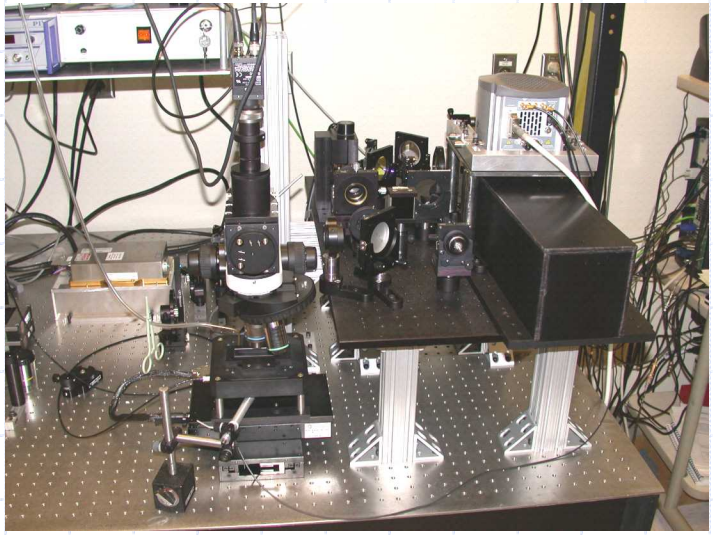
30 sec separation of 6 proteins (molecular mass 20,000-200,000) in ~1 mm long channel



- Tiny sample (nL)
- Takes less than a minute
- Easily integrated and automated

Hyperspectral Imaging





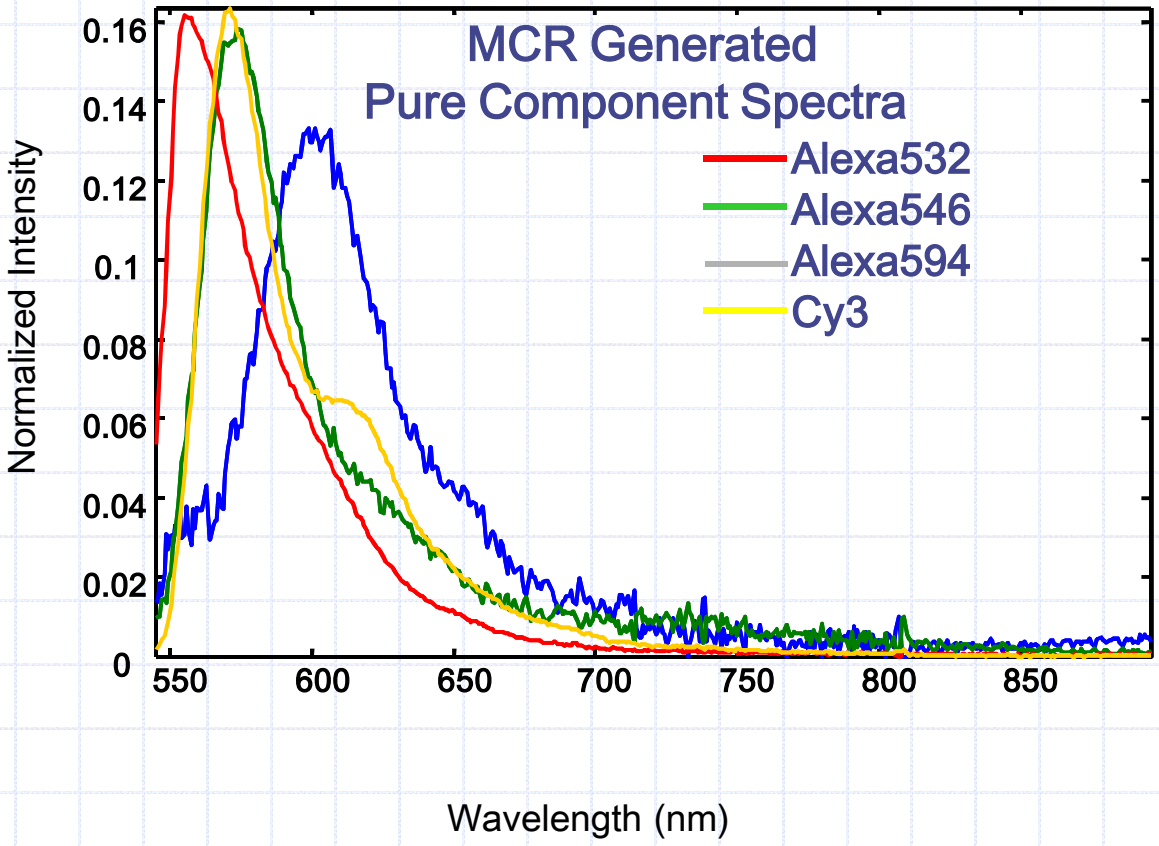
Collect an entire spectrum at each pixel, use multivariate data analysis to separate overlapped spectra into pure spectra of each emitting species and generate corresponding concentration maps

- fully confocal design
- high throughput prism spectrometer
- electron-multiplying CCD imager
- custom high-speed readout mode

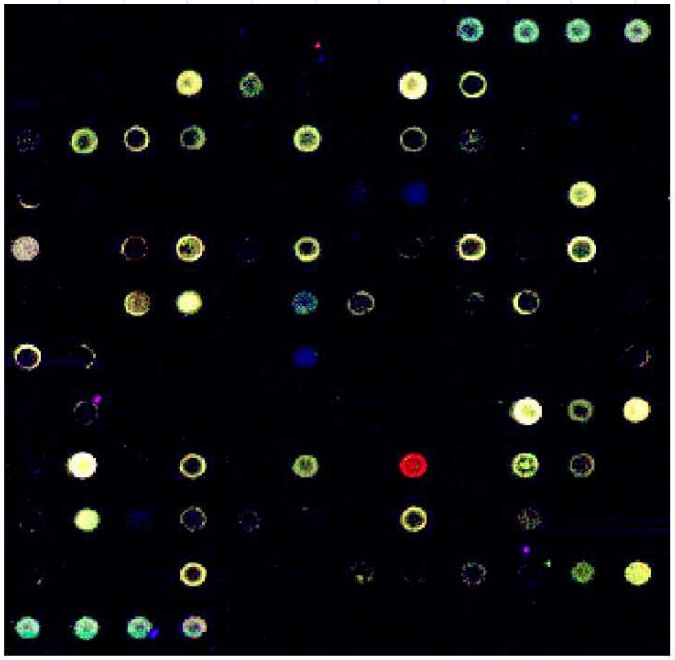
- Allows visualization of spatial patterns of overlapped pigment component spectra in live cells at 250 nm resolution
- Can follow many tags simultaneously
- Discrimination of autofluorescence or other impurity emissions
- Compatible w/ multivariate data analysis

Hyperspectral Scanning

Multiple Green Dye Hybridization

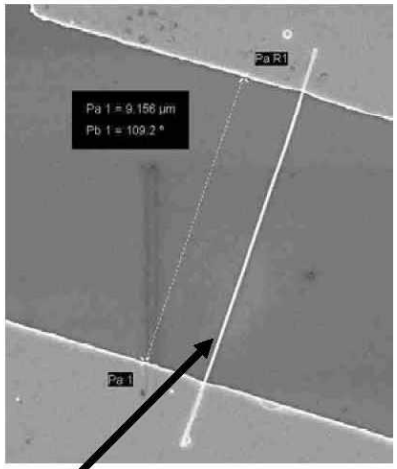


RGB Image of Alexa Dye Concentration Maps

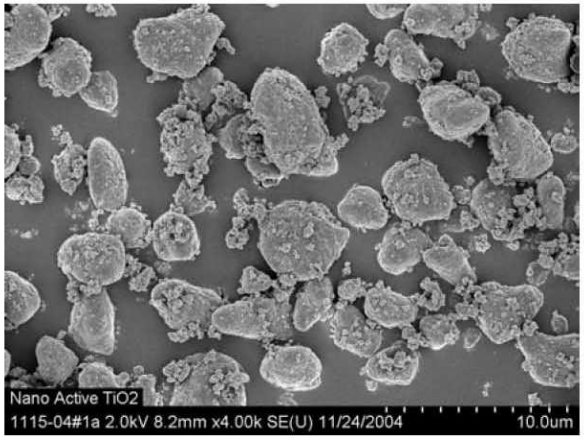


Nanotechnology Initiatives

- Nanofilament Based CB Detector
- Evaluation of Permeation Behavior of Polymeric Nanocomposites for Protective Applications
- Nanoactive particles for chemical decontamination
- Electronically Controlled Microarrays for High Throughput Detection of Biological Warfare Agents



SEM of 100 nm diameter GaN Nanowire



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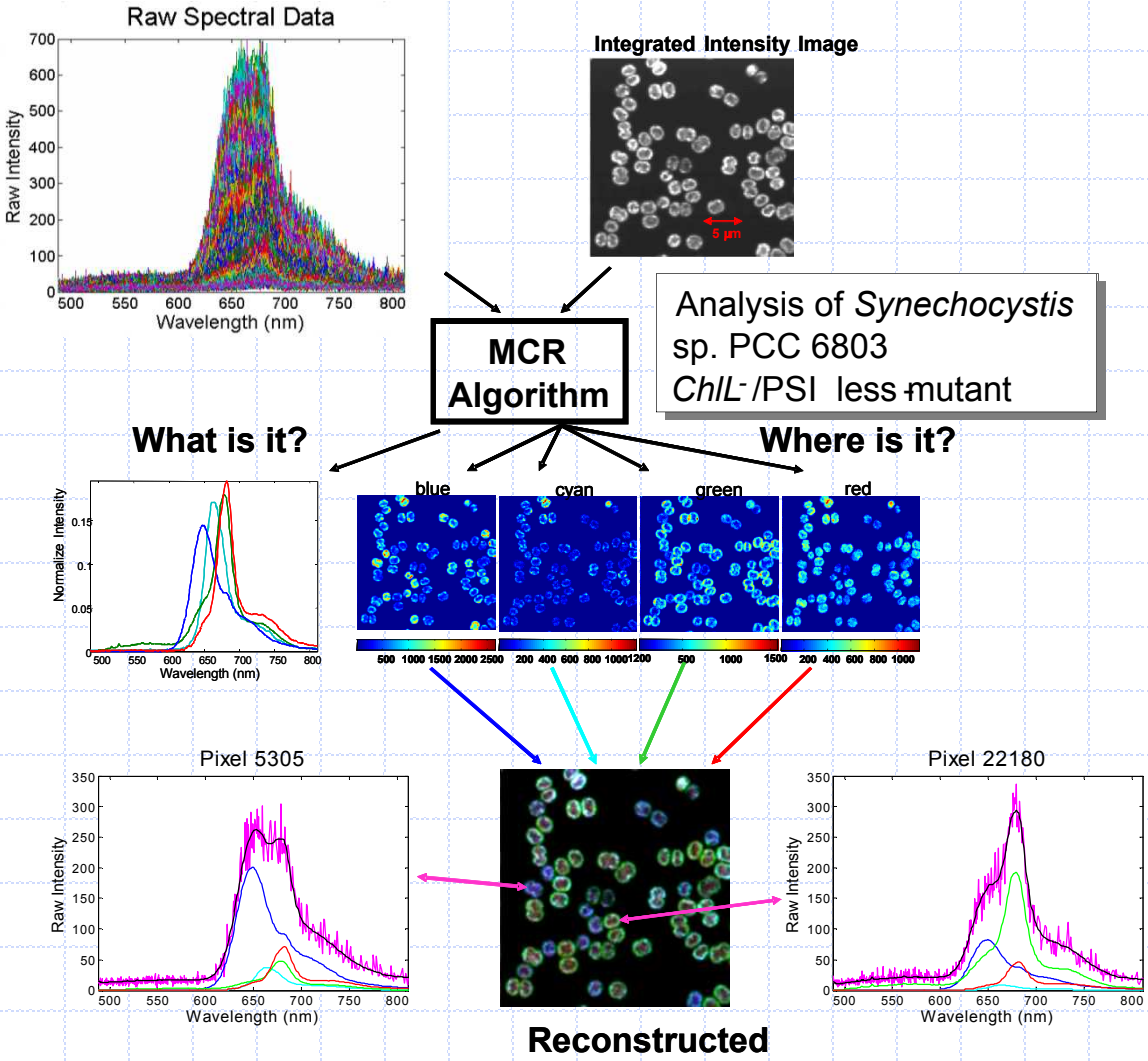
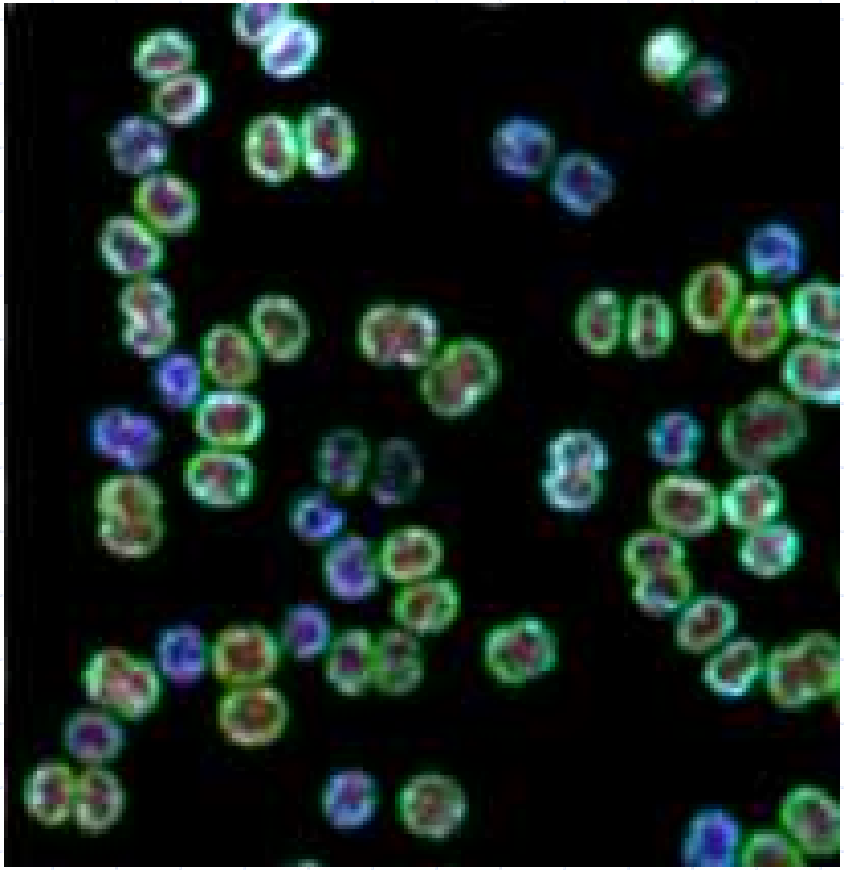
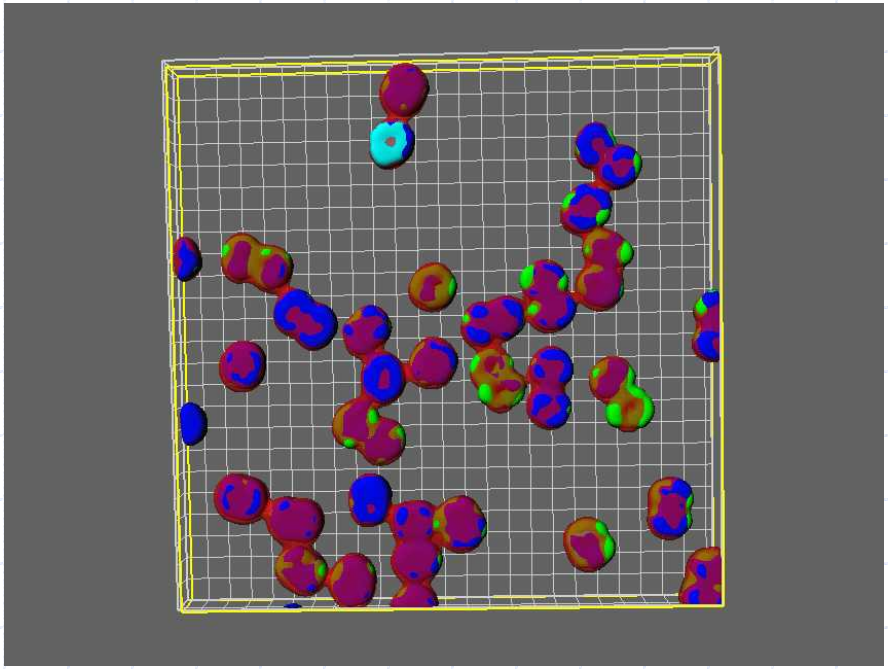
Hyperspectral Confocal Imaging of Live Cells



- 3D hyperspectral *in vivo* imaging of bacteria demonstrated
- Resolution = 0.25 µ xy, 0.6 µ z, 40 ms for entire cell
- Multivariate curve resolution (MCR) derives pure emission spectra and concentrations without *a priori* information
- Extreme spectral and spatial overlaps are resolved
- Quantitative images achieved without crosstalk
- Preliminary kinetic imaging of *Synechocystis*

Detailed 3d Information for *Individual Cells*

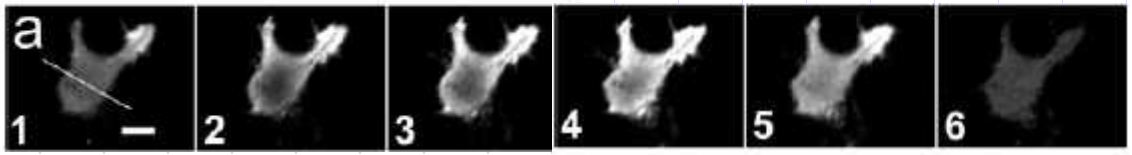




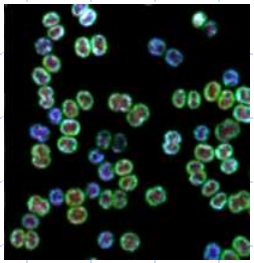
Rate Constant Determination

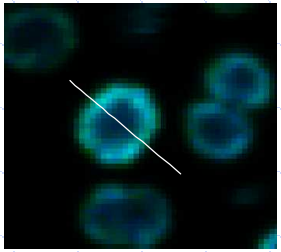
- Rate constants & diffusion coefficients can be obtained from single color imaging over time.
- Hyperspectral imaging allows simultaneous tracking of multiple components and thus inference of protein interaction correlations.
- Will use method of Gemperline to estimate multiple rate constants and diffusion coefficients of key pathway species.
- High-throughput, high-resolution.

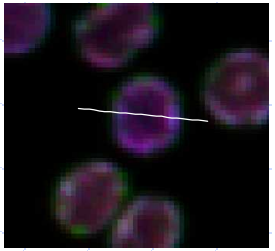
Time Series: one GFP-labeled protein (Schneider & Haugh)



Time Series (dark/light): hyperspectral *Synechocystis*

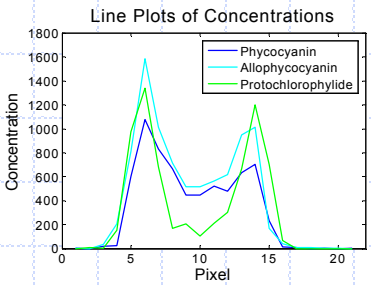




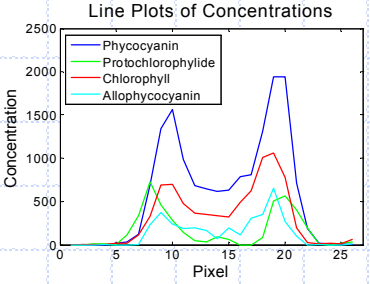


Spatial distribution of multiple correlated pigments

t = 0



t = 8 hr



Modeling Complex Biological Systems
Challenges

- Formulate cellular response model via interacting particles
  - one particle per biomolecule, protein complex, or protein machine
  - track diffusion & reactions
  - realistic cellular geometries for prokaryotic cells (microbes)
- Model carbon fixation process in *Synechococcus*
- Develop a general cell modeling tool
  - applicable to cellular systems where localization is important
  - enable full cell model (up to many millions of particles)
  - computationally efficient
  - designed for parallel platforms
  - open source

Stochastic Particle Dynamics

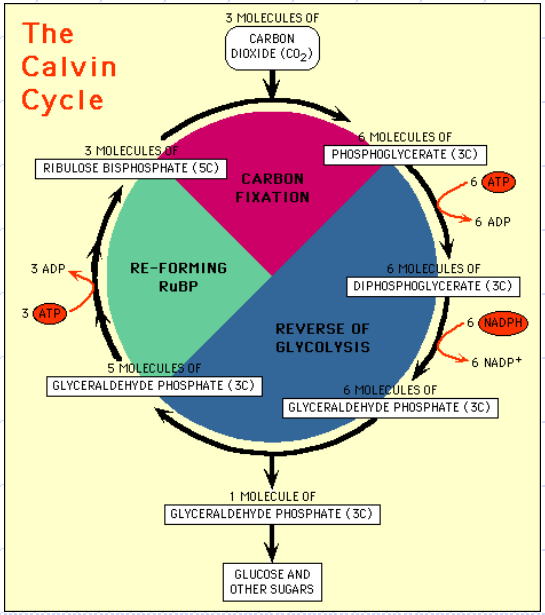
Carbon Fixation: 3 Components of Model

Calvin cycle reactions

$6CO_2 + 6RuBP \rightarrow 12PGA$

$2PGA \rightarrow$ Glucose

$10PGA \rightarrow 6RuBP$

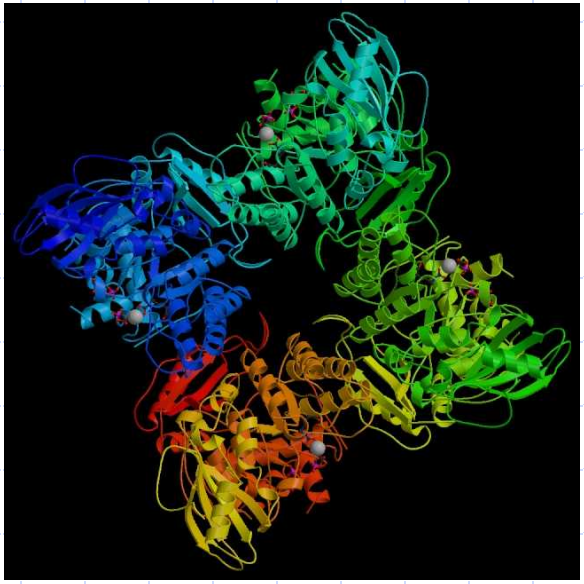


Rubisco

dual-purpose enzyme

carboxylase & oxygenase

function of μ, T (e.g. carboxylase rxn rate increases with decreasing T)



Carbon concentration

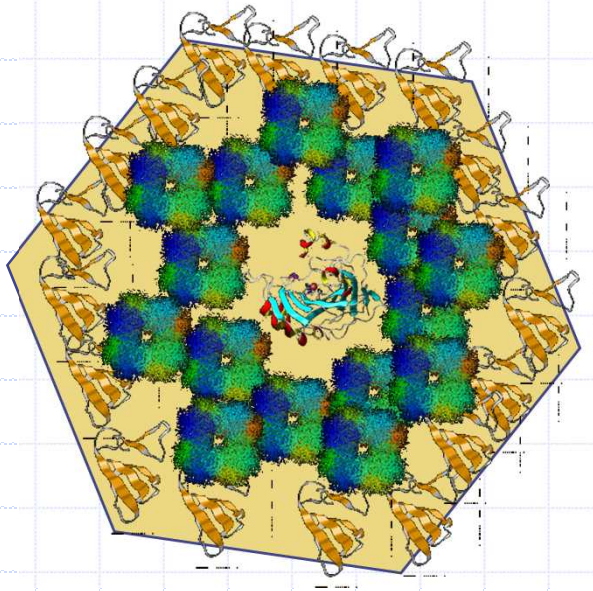
HCO_3 in cytosol

CO_2 in carboxysome

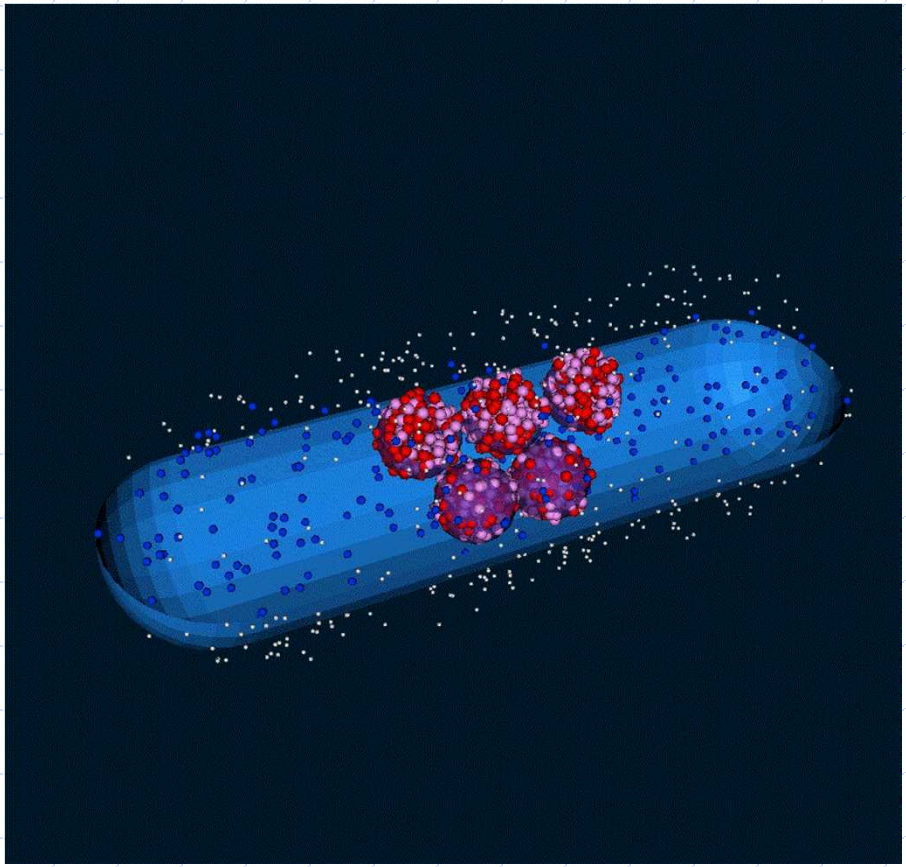
requires spatial effects

requires stochastic

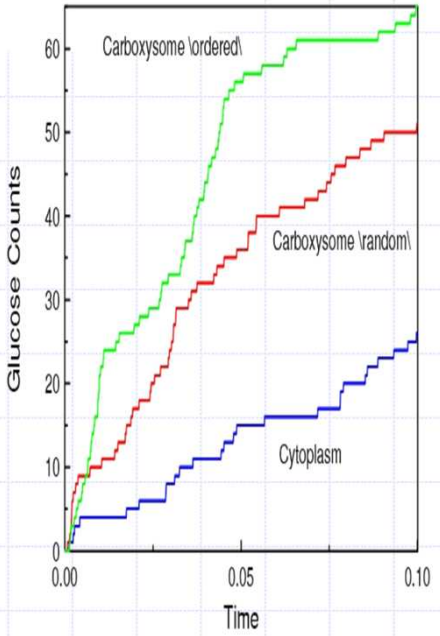
~50 CO_2 per carboxysome



Synechococcus Carboxysome Model



- Carbonic anhydrase
- RuBisCO
- Activated RuBisCO
- Ribulose
- HCO_3, CO_2
- Glucose



Molecular Design

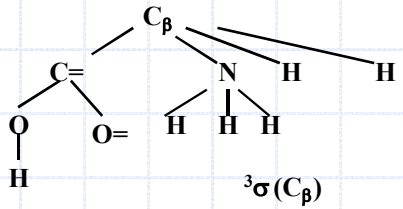
- Design materials with better aging and reliability profiles (DOE/DP/ACS)
- Design sensor for the detection of aging chemical biproducts (DOE/DP/ACS)
- Biological network & protein-protein interaction prediction (DOE/GTL)
- Bio- and chem-informatics algorithms (DOE/MICS)
- Screening and testing for endocrine disrupting compounds and design replacement (EPA)
- *Biologically active compound design (NIH)*
- *Sensor design for chemical/biological warfare agents (DHS, NIH-U19)*
- *Protein design for virus detection and phenolic compounds nitration (DARPA)*

QSAR: Choice of the molecular descriptor

- 2D and 3D topological descriptors
  - 100+ of such descriptors (Codessa, Molconn-Z)
  - Daylight fingerprints

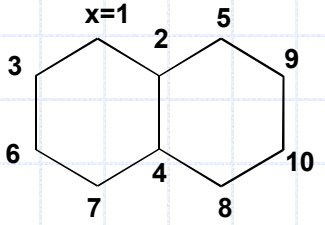
- Fragmental descriptors
  - Group contribution
  - Tripos Hologram and Zefirov's subgraphs
  - Glen & Bender Featured trees
  - NCI open browser PASS descriptors
  - Signature (JCICS 1994 and JMGM 2002)
    - rooted subgraphs of predefined diameters (height)
    - cycle closure is coded
    - computationally efficient (JCICS 2003-a)
    - structures are uniquely characterized (canonized) with signatures of height greater than graph diameter (JCICS 2004)
    - signature is the only reversible fragmental descriptor (JCICS 2003-b)

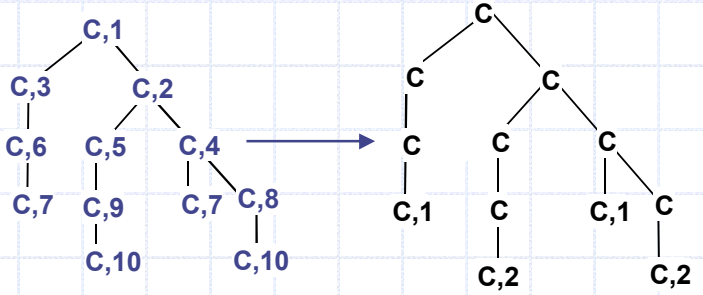
Glycine (H_3N-$C_\beta H_2$-COOHH)



Atomic signature

$^0\sigma(C_\beta)$ = C
$^1\sigma(C_\beta)$ = C(C=NHH)
$^2\sigma(C_\beta)$ = C(C=(O=O) N(HHH) H() H())
$^3\sigma(C_\beta)$ = C(C=(O=()O(H)) N(H()H()H()) H() H())

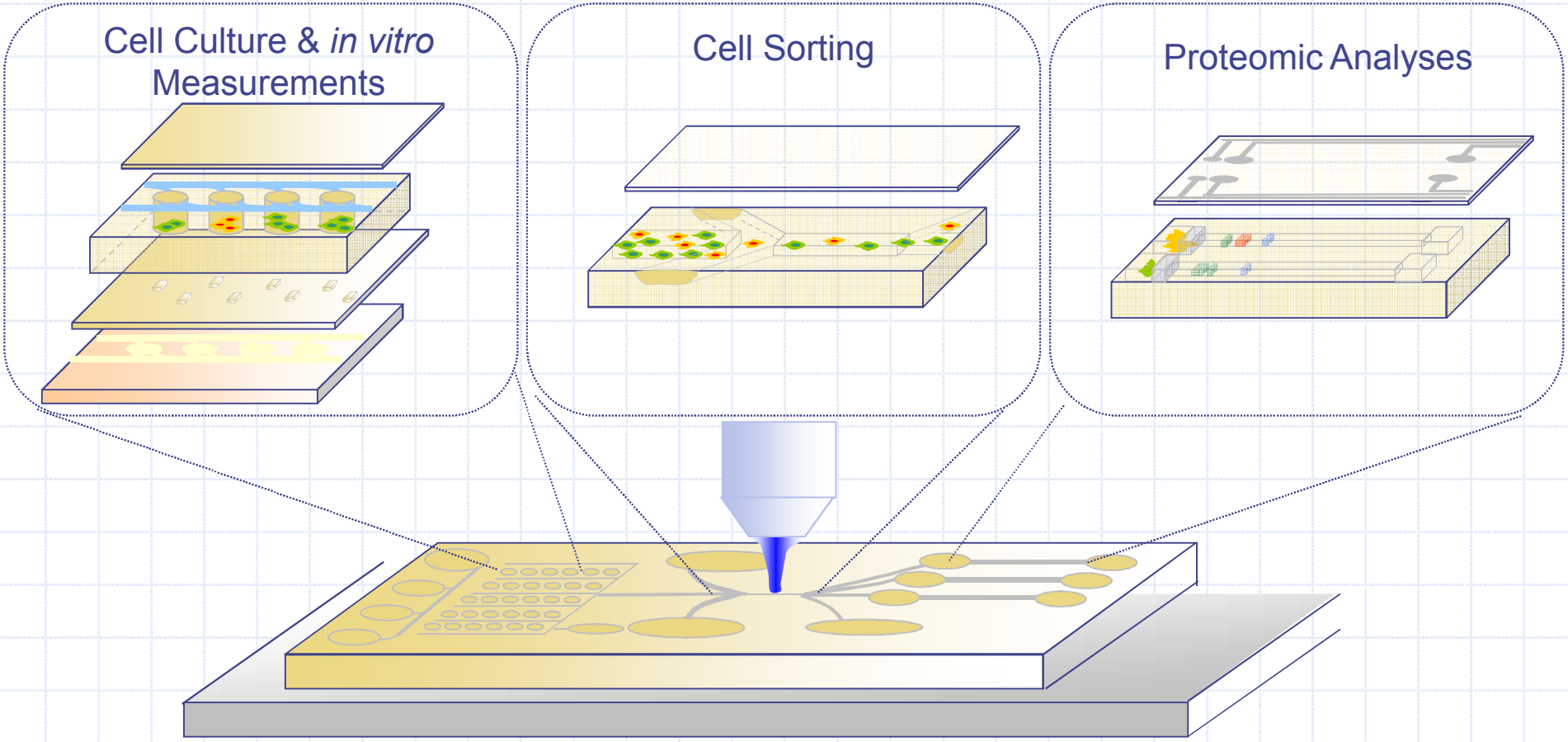




The PubChem Challenge

- Using pharmaceutical molecular descriptors (signature atom neighborhoods, and topological indices) SIGNATURE was used to reverse engineer the molecular structures of 800 drug compounds.
- This proved that molecular descriptors are not a “secure” representation of patentable chemicals for the proposed NIH PubChem database (aimed at creating an on-line library of chemicals with potential pharmaceutical implications).
- This challenge was published in Nature Review Drug Discovery (March 2005) and the focus of an ACS symposium organized by C. Lipinsky and T. Oprea convened to discuss the issue.
- NIH dropped the idea of employing molecular descriptors for PubChem and is proceeding using full molecular structures.

Putting It All Together - The Microscale Immune System Laboratory



Integrating Sandia-developed Lab-on-a-Chip modules to enable quantitative biology measurements.

Sandia Technology Components

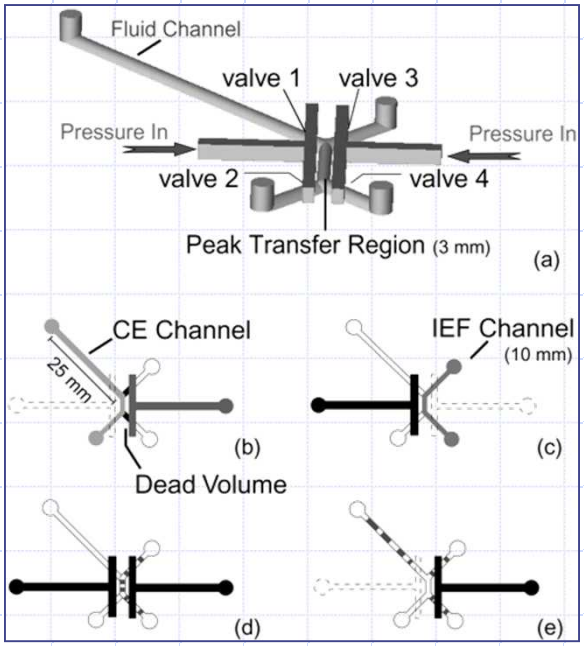
Microengineered Platforms:

integrated biologically compatible microsystems
quantitative on-chip protein analysis
cellular platforms

Systems Engineering:

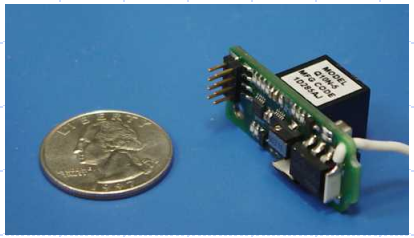
microChemLab
BioBriefcase (w/ LLNL)

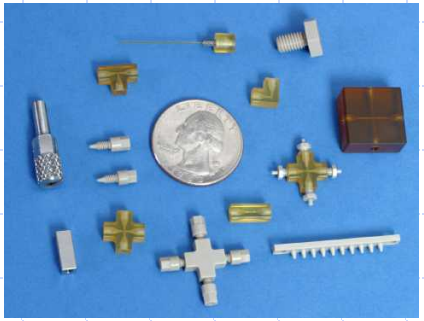
MIMS Grand Challenge[11]



μChemLab Grand Challenge[12]

Chip interfaces





Compact hardware

[11]Wang, Choi, Han. 2004. *Anal. Chem.*, 76(15): 4426-4431.
[12]Renzi *et al.*, 2005. Anal. Chem. 77(2): 435-441.

Sandia Technology Components

Advanced Measurements:

highest spectral resolution (3nm) 3D confocal microscopy
world leader in multivariate analysis
simultaneous wide-field imaging
in silico electrical and electrochemical measurements on living cells

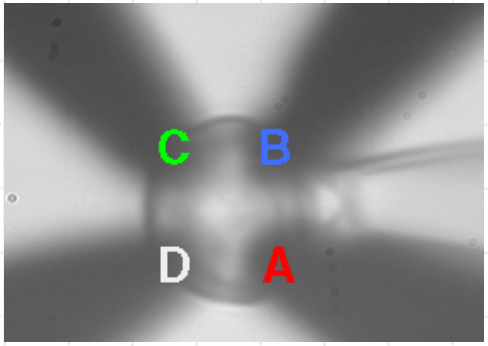
Computational Biology:

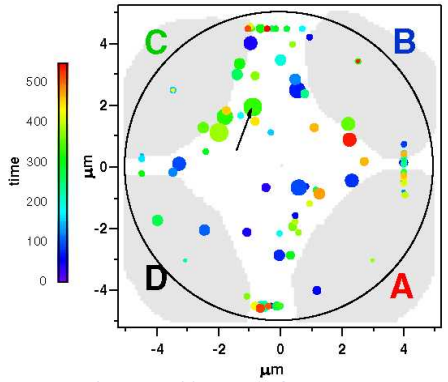
protein-protein interactions
spatial reaction-diffusion simulations
gene regulation network inference

In Silico Electrical & Electrochemical Measurements of Living Cells

Electrochemical detection scales well with miniaturization (L^2 vs L^3 for optical)

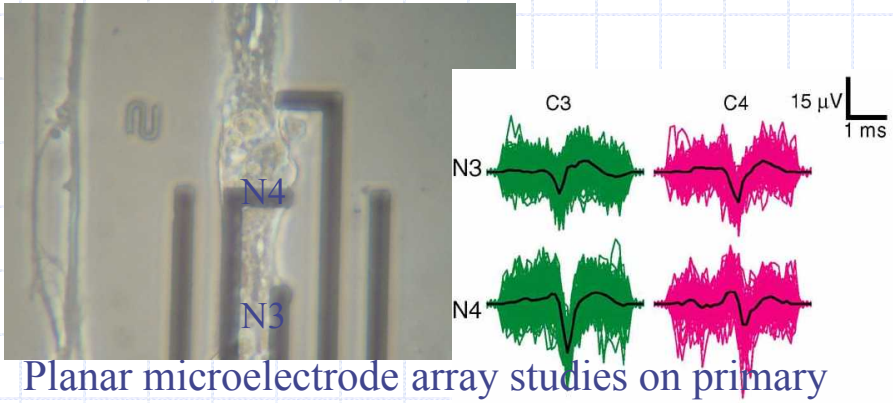
Detection methods can be non-invasive (extracellular) or invasive (intracellular)





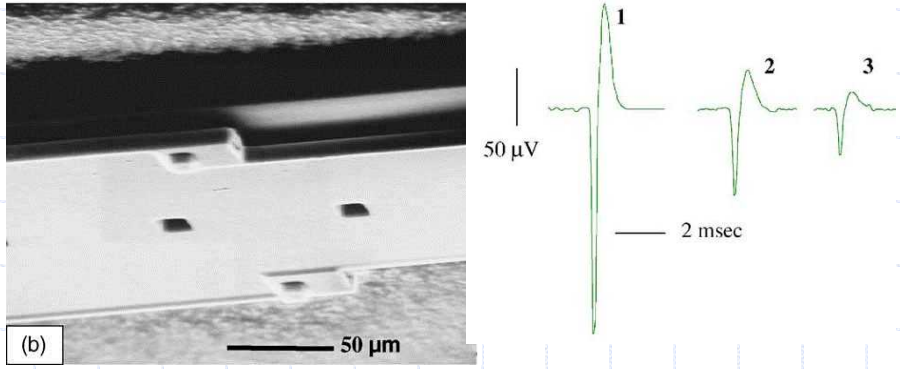
Oxidation of exocytosed noradrenaline from chromaffin cells

*AF Dias, CD James, Nanotech., **13**, pg. 285, 2002.*



Planar microelectrode array studies on primary hippocampal neurons

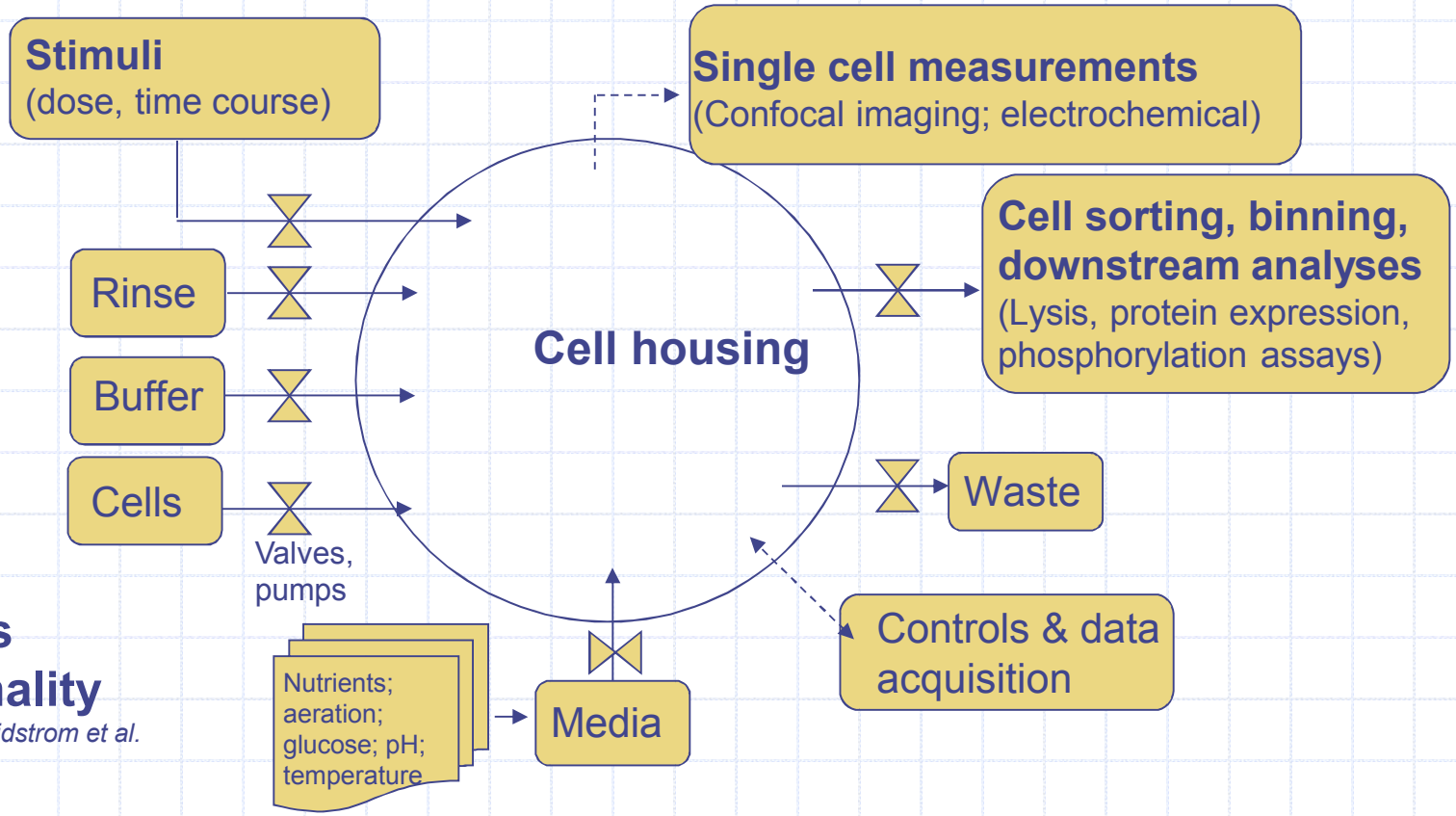
CD James et al., IEEE Trans Biomed Eng, 51, 2004



PolySi neural probes for *in vivo* measurements

Okandan et al., J NeuroSi Meth, 142, 2005.

The Goal –High Fidelity & High Throughput, Customizable for Different Applications



Systems functionality

(adapted from Lidstrom et al. Nature 2002)

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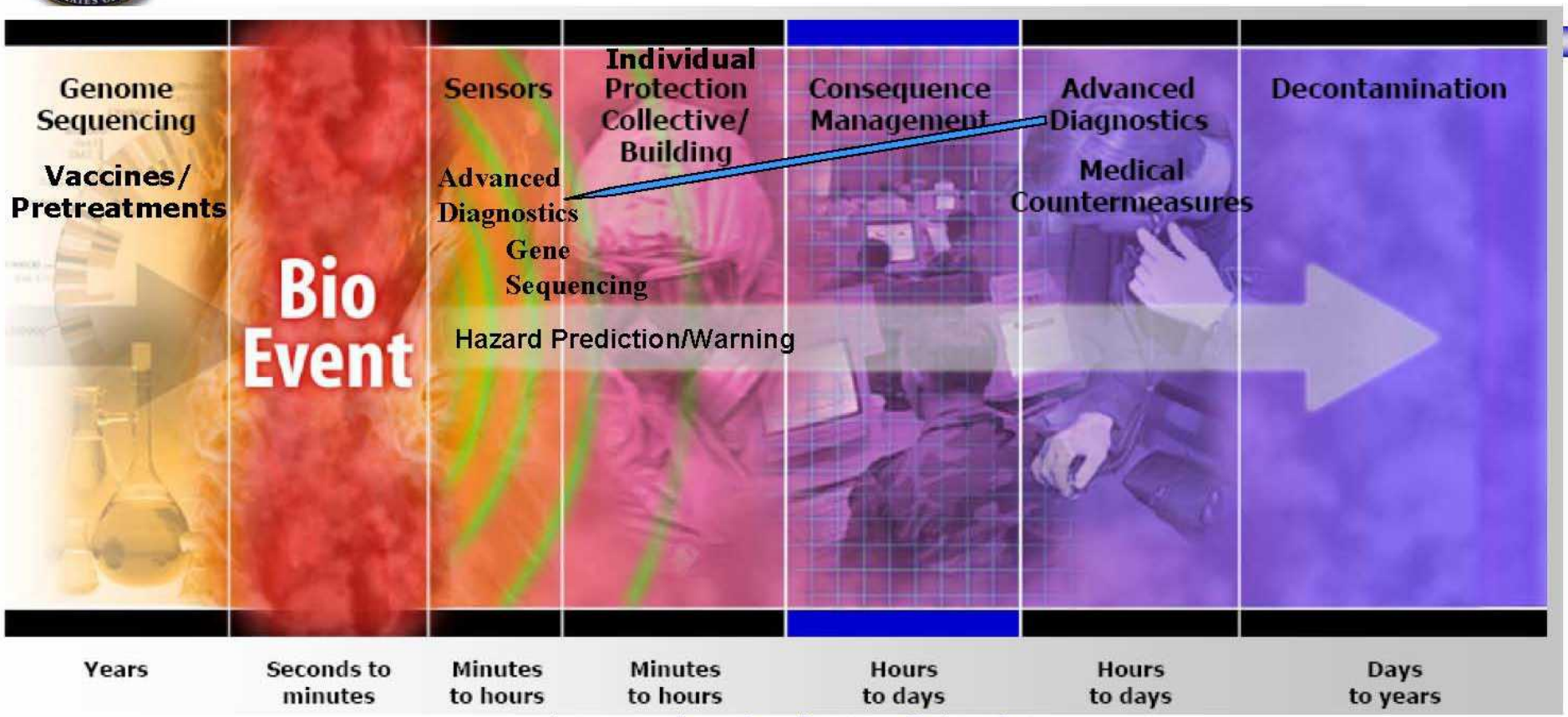
Bio-Technology Integration



Leveraging the State-of-the-Art

- Proteomics
- Genomics
- Metabolomics
- Toxicogenomics
- Multi-spectral analysis
- Bioinformatics
- PCR/Immuno-assays
- Bioscavengers
- Microarrays
- Nanomaterials
- Broad spectrum disinfectants
- Anti-viral/Antisense therapeutics
- Multi-agent vaccines
- Epidemiology
- Self-decontaminating coatings
- Host-response pathways

Partnerships & Cooperation to Leverage the State-of-the-Art

- Service Labs
- International
- Industry
- Academia
- Other Federal Agencies

Broad Spectrum Therapies for Biodefense *(Cont'd)*

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- **Basic Research/Science**
  - Directed at pathogen host response
  - Find novel intervention points
- **Applied Research/Science**
  - Expanding technologies
  - Speed the cycle from discovery to license application
- **Advanced Science/Tech Development**
  - Quick wins
  - Strategy to deliver products with IND approval
  - Advanced Component Development and System Demonstration

Strategic Partnerships

- UTMB/Galveston
  - NIH/NIAID RCE: “vaccines, therapeutics, & diagnostics”
  - BSL4, Infectious Disease Expertise
- University of Pittsburgh
  - One of 9 NIH/RM Molecular Libraries Screening Network Centers
  - Focused around rapid and high-throughput testing of compounds for therapeutics
  - Sandia is doing informatics and molecular modeling
- University of New Mexico
  - One of 9 NIH/RM Molecular Libraries Screening Network Centers
  - High throughput flow cytometric screening & automation
  - Virtual screening and cheminformatics for small molecule drug discovery
  - Probe Chemistry and Synthetic Optimization (Arterburn, ChemDiv)

Rational Drug Cocktail Design For Treatment Of Viral Hemorrhagic Fevers

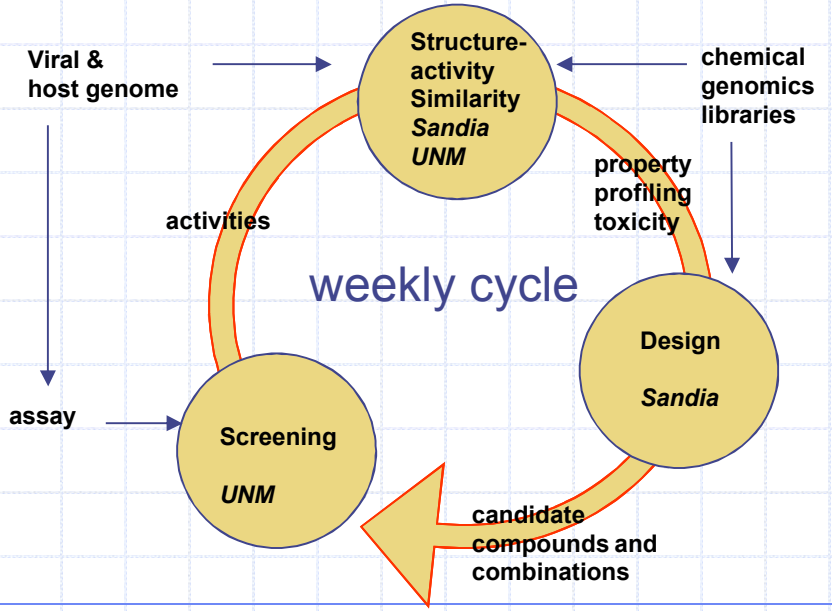
Sandia National Laboratories

The University of New Mexico

DoD CBDP and the Army Research Office (ARO)

Objective:
Fast bug-to-drug in silico drug design and experimental screening protocol for treatment of viral hemorrhagic fevers (VHFs).

Description of effort:
- Using novel inverse structure-activity approach and high throughput focus reduction screening assay identify optimum lead molecules and combination of leads that reduces VHFs infectivity.
- Approach driven by virus/host interactions (prevent virus entering host, prevent viral RNA to be released, alter viral RNA replication)



Benefit to warfighter:
- Protect warfighter against VHFs (currently no FDA-approved treatments)
- Propose a drug design protocol that can be brought into acquisition and fielded worldwide

Challenges:
- Experimental screening hits prioritization
- Structure-activity relationship accuracy

Maturity of Technology: TRL3. Conduct in vivo screening of lead compounds on Vero E6 cells.

Major goals/milestones and deliverable:
- A computational/experimental protocol that reduces the drug development timeline. (Year 1, month 4)
- Deliverable: An optimum list of lead compounds and combination of leads for VHFs treatment. (Year 1, month 12)

Proposed Funding ($K): 2700
Sandia: 1500
UNM: 1200 (750 Virology/screening, 450 Biocomputing)

PI contact Info: Dr. Jean-Loup Faulon, (925) 294 1279
jfaulon@sandia.gov

For More Information …

gsheffe@sandia.gov

2006 Medical Science and Technology (S&T) Chemical and Biological Defense Transformational Medical Technologies Initiative Fund (TMTIF)
Solicitation #: **W911NF05R0011**

The purpose of this Broad Agency Announcement (BAA) W911NF-05-R-0011 is to solicit proposals for the Department of Defense (DoD) Fiscal Year 2006 Medical Science and Technology (S&T) Chemical and Biological Defense Transformational Medical Technologies Initiative Fund (TMTIF). The DoD conducts a vigorous medical research program in chemical and biological defense with the goal of **protecting the warfighter from disease and biological and chemical warfare agents**. The Chemical Biological Defense Program seeks to **develop counter-measures** that can be brought into acquisition an d fielded worldwide. These products must be regulatory compliant, robust, and highly effective at a reasonable cost. Successful candidates must have a clear path to regulatory approval, production and end user utility. They must all be amenable to use in a military environment. This BAA is focused on developing **medical counter-measures to genetically engineered or non-traditional toxins, virulence factors and microorganisms as biological warfare (BW) threat agents**. This includes a faster bug-to-drug approach. It is anticipated that these counter-measures would include pre-treatments (including **vaccines**), **therapeutics** and **basic science** to characterize the nature of the threat and identify key targets for intervention or disruption of these agents. The agent classes that are to be focused on are: intra-cellular bacterial pathogens, hemorrhagic fever viruses and bioregulators. Approaches to the objectives for this area include studying the genetic diversity and pathogenicity of natural isolates, identifying common structural elements of specific agents or classes of agents (preferred), elucidating common virulence mechanisms (such as type III secretory proteins), identifying functional domains in toxins and virulence factors, and using this information to develop rapid and effective medical countermeasures protecting against genetically engineered or emerging BW threats. Through this BAA the **DoD CBDP and the Army Research Office (ARO)** expect to make several awards for one- to two-year performance periods, subject to the availability of appropriations. Awards may be made as contracts or grants. Single-year, stand-alone proposals are encouraged; multi-year proposals will be considered. A total of up to approximately $63 million is anticipated to be available under this solicitation. It is anticipated that funding will be between $1M to $4M per award.