Biosciences at the Lawrence Livermore National Laboratory

Investigational Drug Steering Committee
Team Science Meeting
11 Jan, 2013

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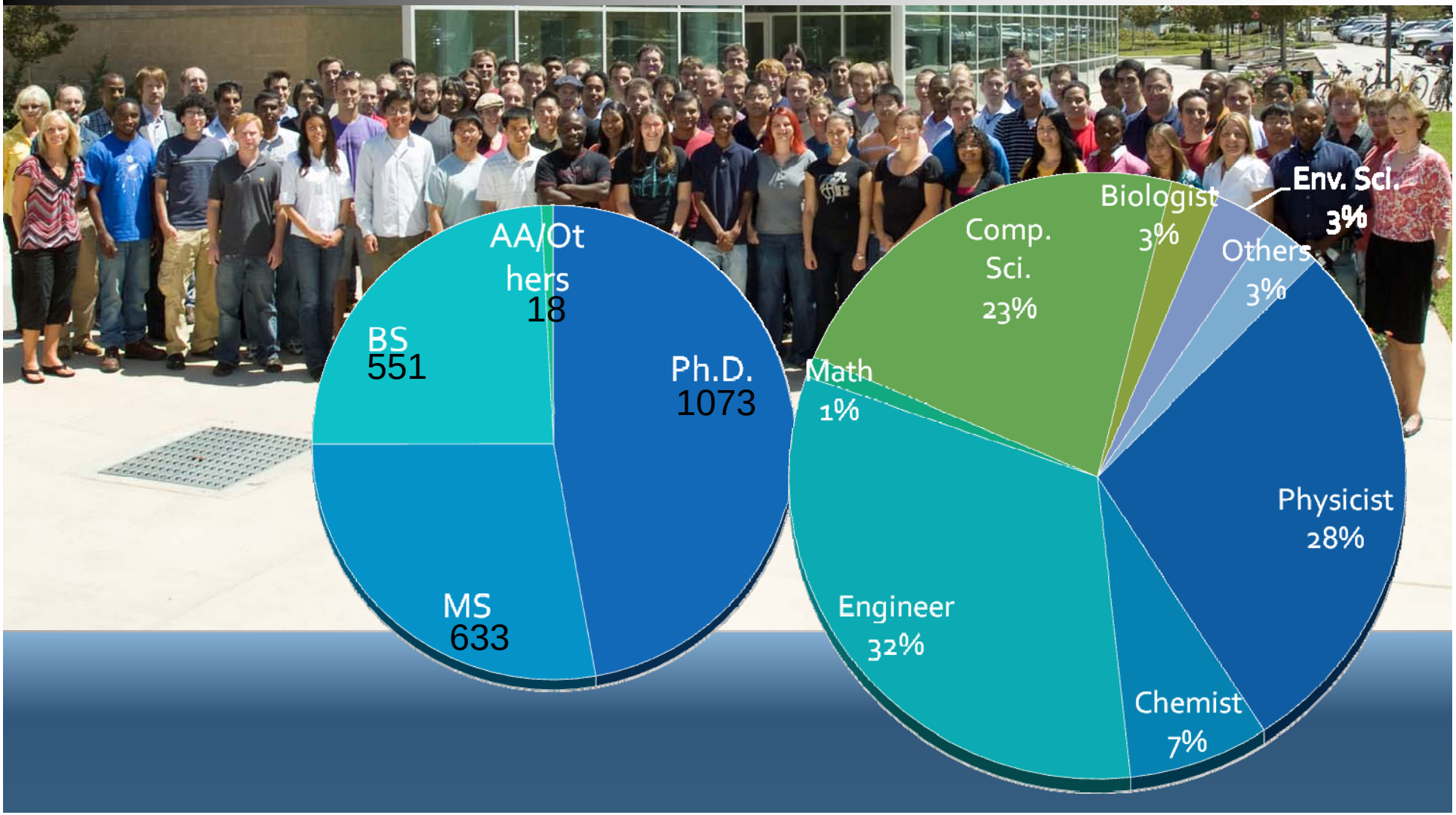
LLNL

- **Is a multi-sponsor FFRDC, managed by NNSA**

- **Our role…**
  - Provide enduring focus on issues in national security
  - Leverage multidisciplinary capabilities
    - Laser Sciences & Isotopic Sciences (National Ignition Facility)
    - High Performance Computing
    - Analytical and Measurement Sciences
    - Material Sciences
    - Advanced Engineering, Instrumentation & Diagnostics
    - Biosciences

LLNL has a broad, multidisciplinary workforce



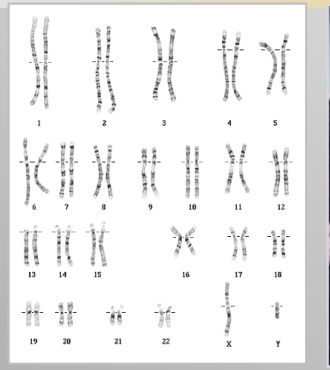
LLNL's Core Capabilities for the Biosciences

- **Multidisciplinary teams** including bioscientists, engineers, computational scientists, physicists, chemists . . .
- **Working at the interface** of engineering, materials, chemical and biological sciences
- **Detection & diagnostics** – detection and surveillance platforms, fit-for-purpose arrays…
- **High precision measurement/Instrumentation**— Accelerator Mass Spectrometry, single molecule spectroscopy, microfabrication & engineering
- **Computational biology**—Massively parallel computing resources and biocomputing expertise
- **Expertise in** medical devices, assay development, genomics, biological /chemical threats, and countermeasures

LLNL's Biological Research has a History of Coupling Technology Developed with Biological Research

- The original mission was to investigate the effects of ionizing radiation on humans
- Focus on goal driven research, developing technology that is coupled to solving problems of national significance in health and the environment

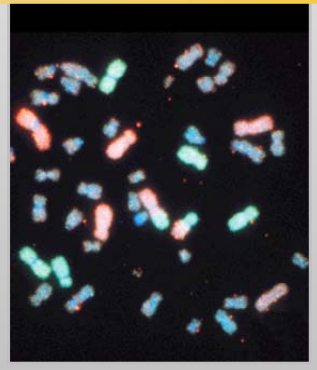
1970's



Radiation Effects

1980's

Automated Cell Sorting

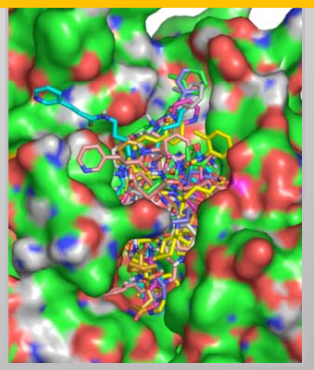


FISH Chromosome Painting

1990's

Human Genome Project

2000's



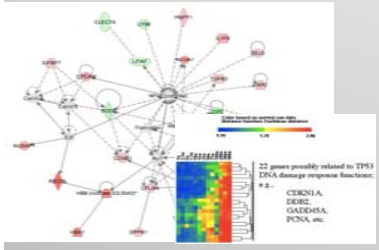
High Performance Simulations

Example: Accelerating Therapeutic Development

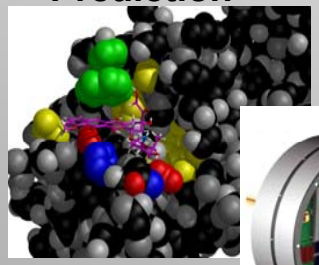
Now: 15 years *Our goal: 5 years*

Target discovery validation → Lead generation → Lead optimization → Preclinical development → Phase o

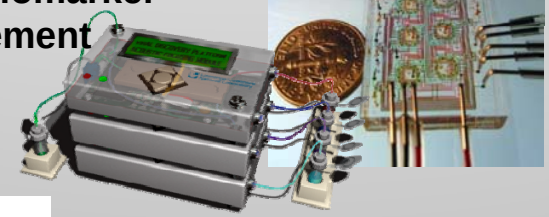
Pathway Characterization



Computational Prediction



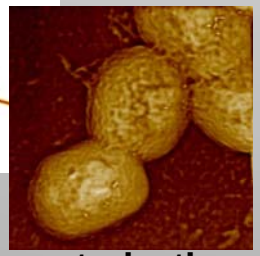
Rapid sample prep & biomarker measurement



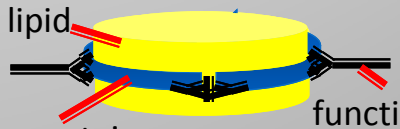
3D Organ Culture

BSL/3 Testing

AMS Microdosing

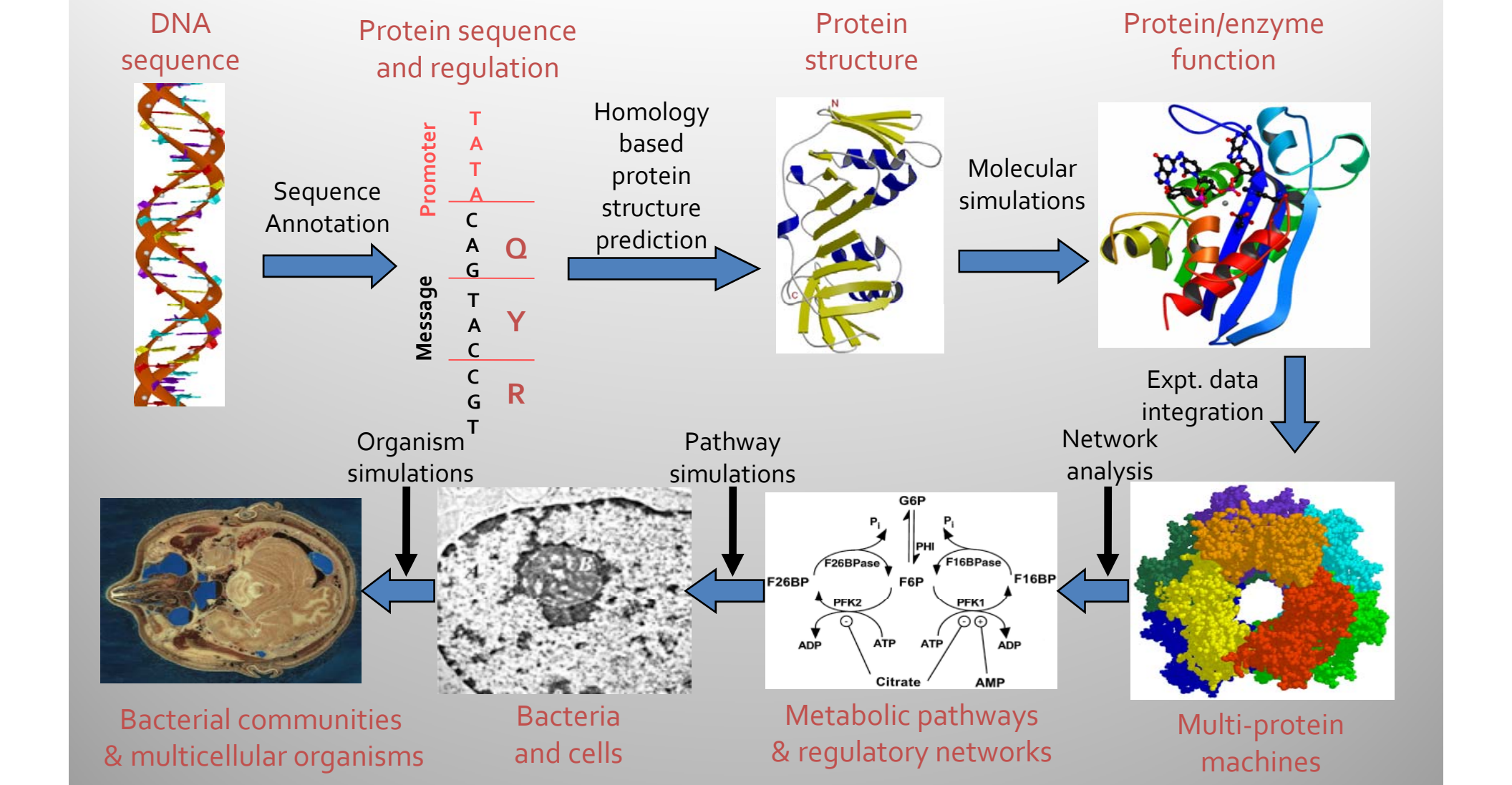


Structural Characterization



NLP's for Delivery, Imaging & Functional Studies

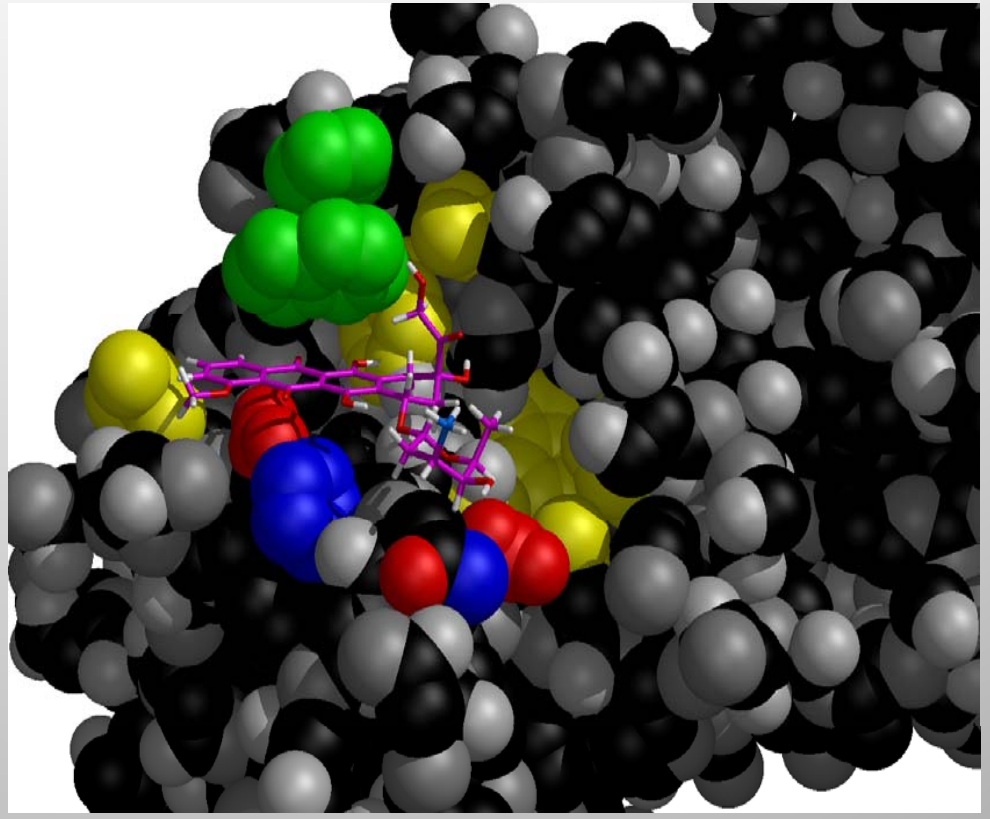
Computational Analysis and Simulation



HPC is Being Used to Design Affinity Reagents for Use in Detection Assays and Therapeutics

Utilizes DNA signature and protein structural data to identify target sites.

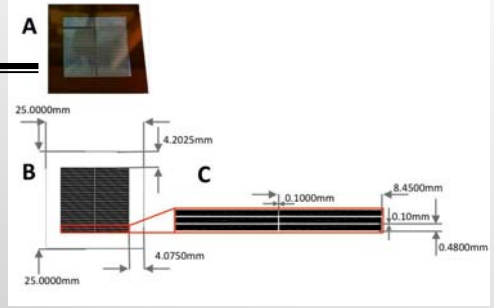
- More robust detection
- Longer shelf-life
- Better treatments



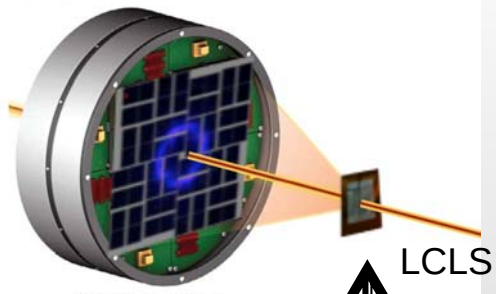
Protein structure information may allow developing synthetic ligands as interventions or detectors.

Developing Advanced Methods for Structural Characterization with XFELs to improve Models

Fixed-target support



LCLS detector



LCLS beam

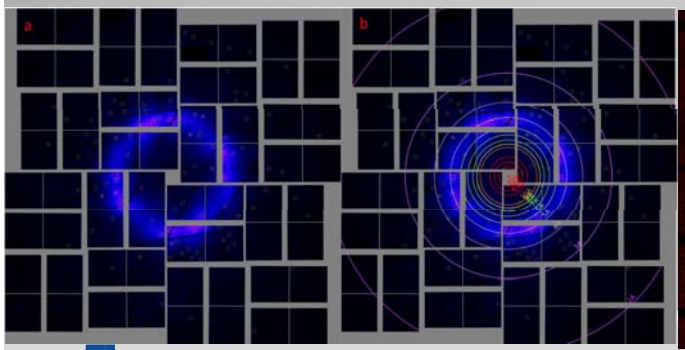
Apply sample

Why?

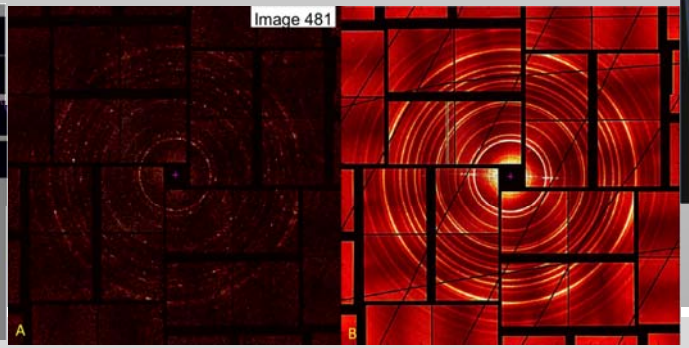
- Allows 2D crystallography
  - Membrane proteins in a native environment!
- Reduced sample consumption
  - 100s µg vs 10s to 100s mg
- Room temperature protein structures!
  - Possibly more biologically relevant

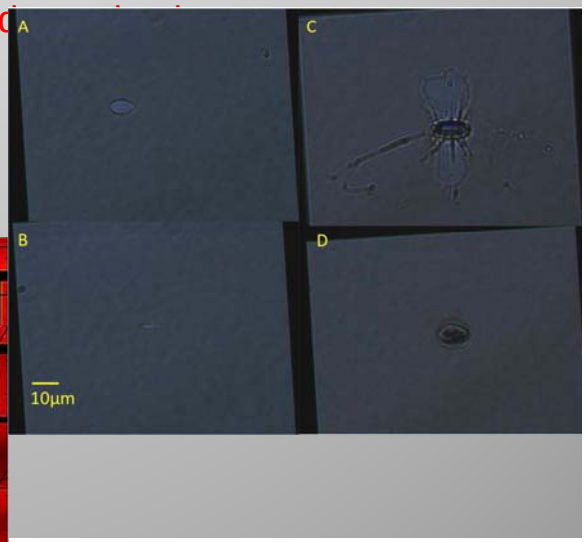
Thorough characterization of mechanical damage pathways and implications for structure

3D crystallography results from proteins involved in putative virulence pathways to atomic resolution



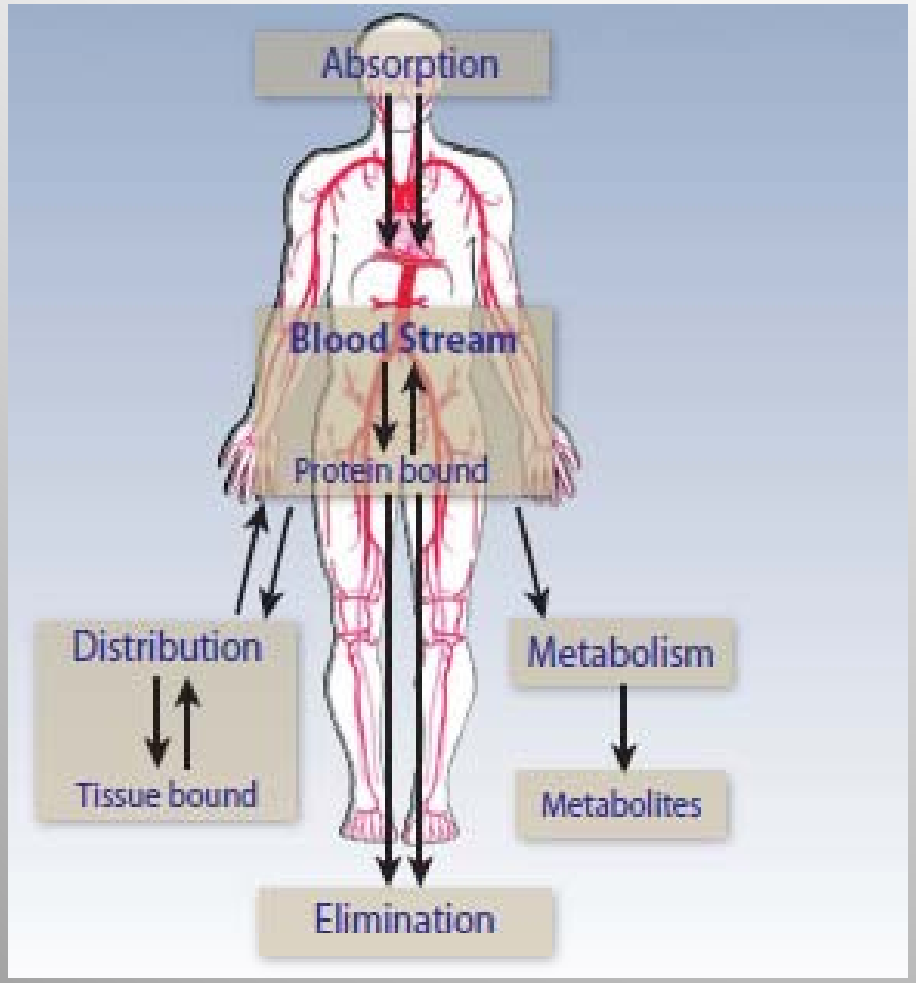
2D crystallography results from a membrane protein (bacteriorhodopson) to molecular resolution (6Å)





NNSA

Microdosing Allows Early Human Testing and Reduces Extrapolation from Animals

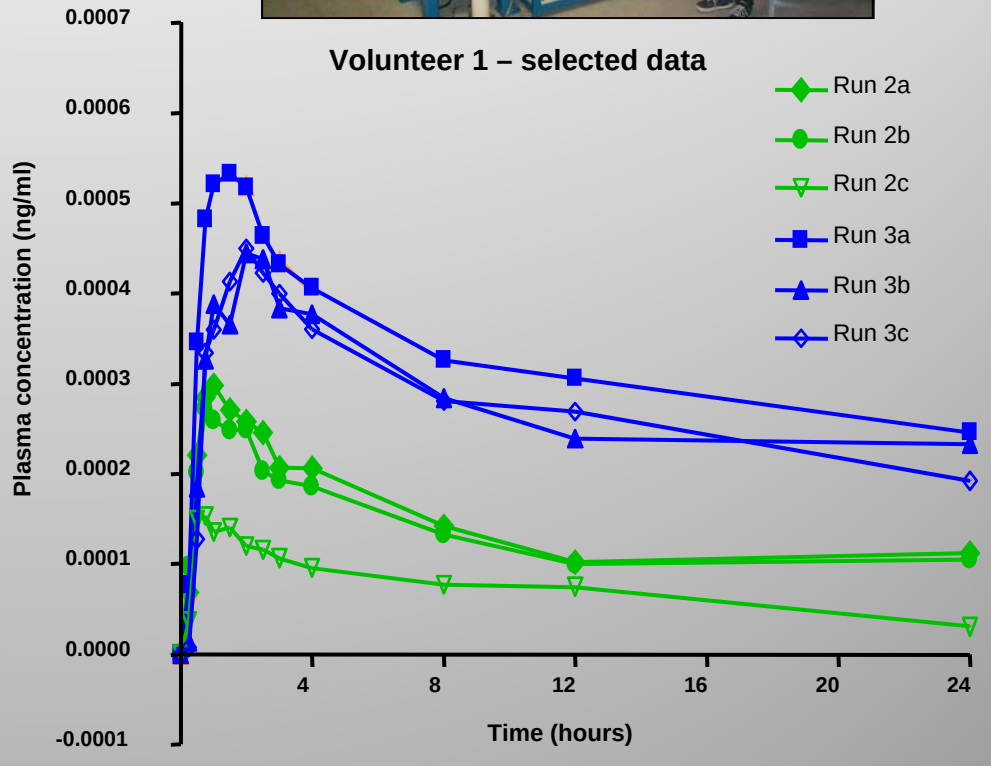


- Administration of low doses (/100th the anticipated therapeutic dose) to healthy volunteers
- Many benefits including :
  - ➢Reduced animal testing
  - ➢Reduced drug synthesis
  - ➢Early assessment of efficacy/safety
- Difficult without extremely sensitive methods of analysis

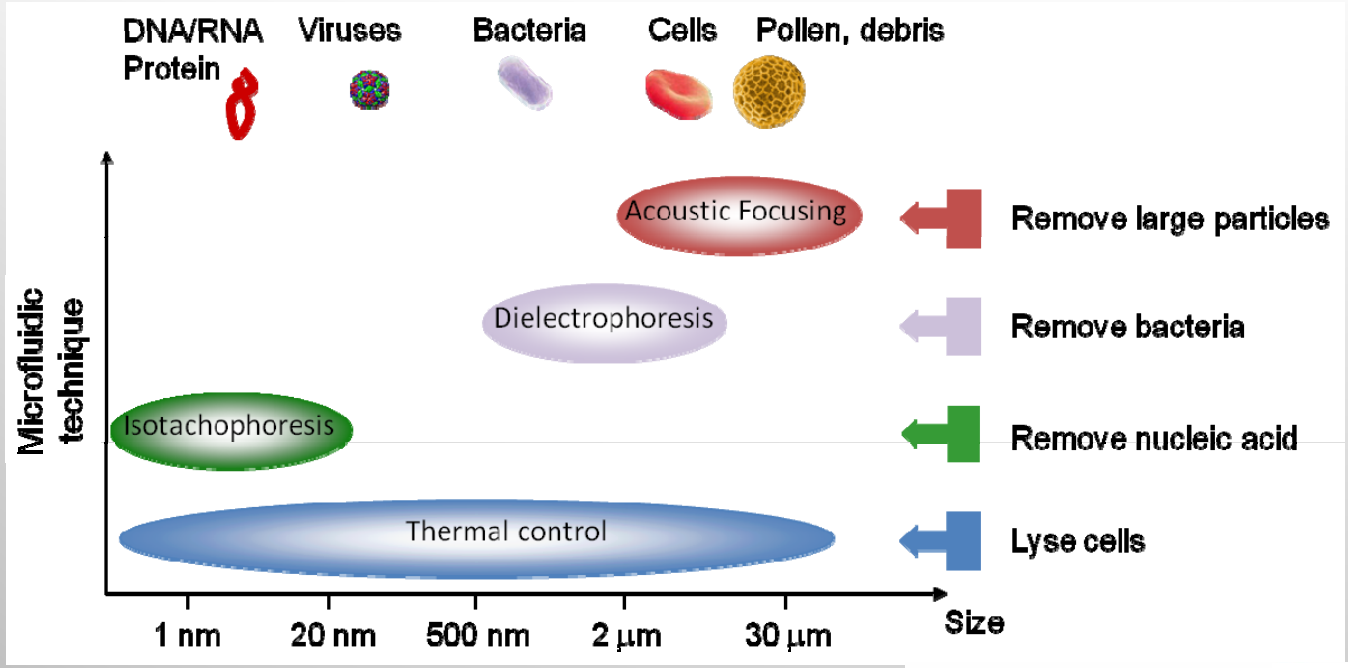
AMS Enables Microdosing

Quantifies extremely low concentrations of labeled molecules

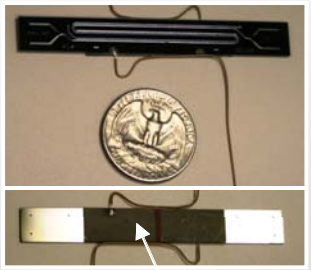
- Type of mass spectrometer that analyzes ions at very high energies through use of a linear acceleration stage
- Measures isotope ratios
- Analysis of attomole quantities of chemical or biological entities in µg - mg sized samples with high precision
- Directly determines drug safety & relevance in humans

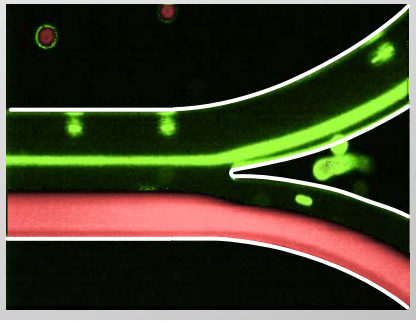


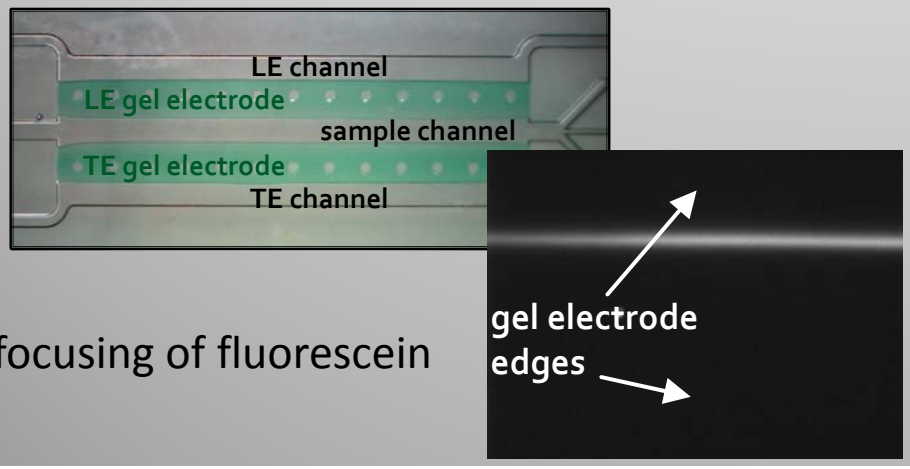
Biological Sample Preparation for Diagnostics



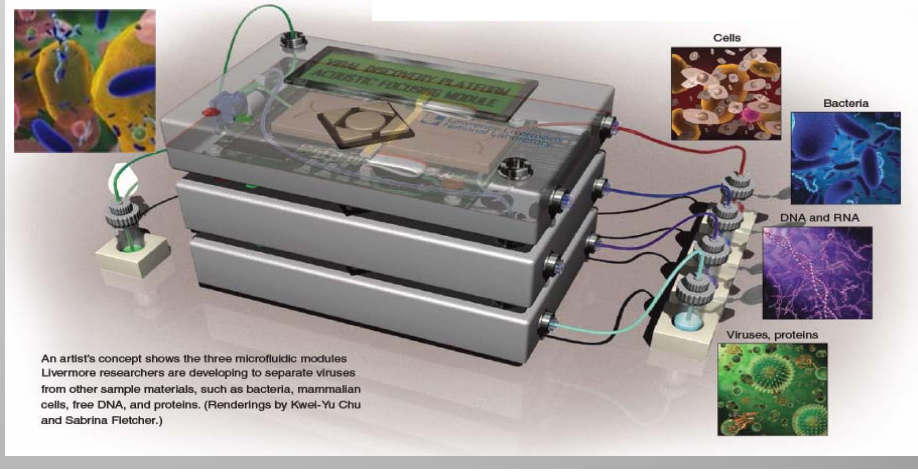
Microfluidic chip



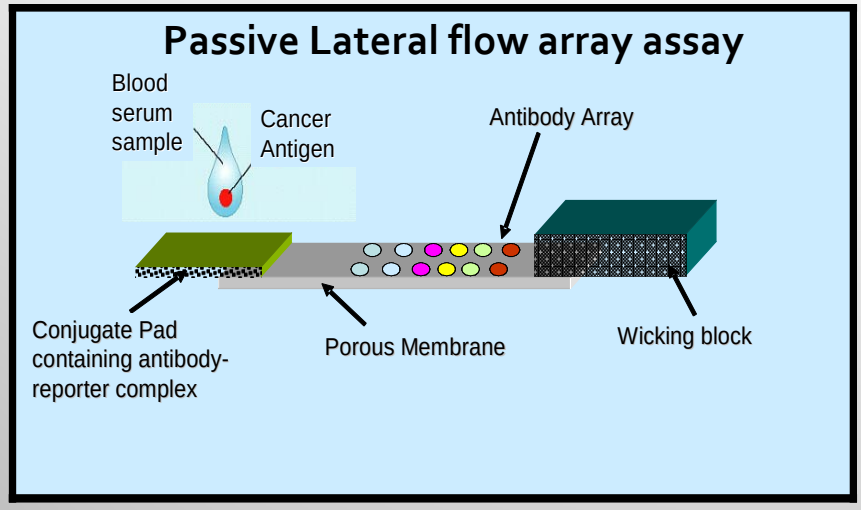


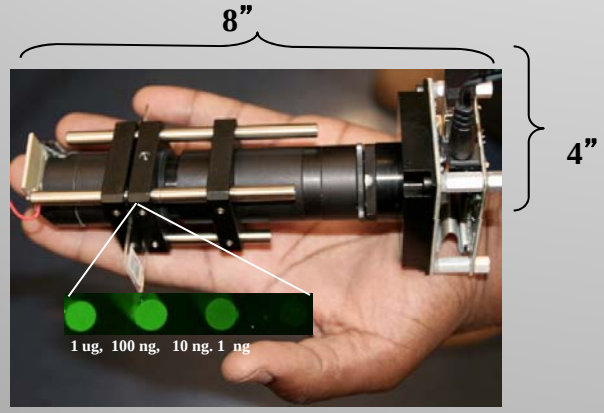


focusing of fluorescein



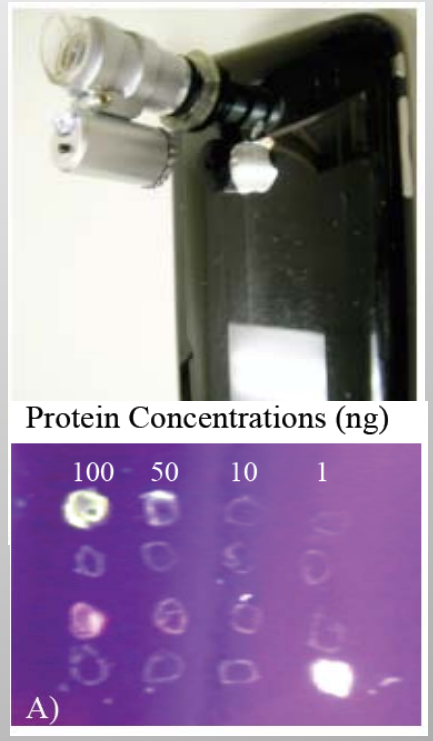
Flow –Channel Protein Assay: Developing Disposable Detection Assays for Biodosimetry and Cancer Diagnostics



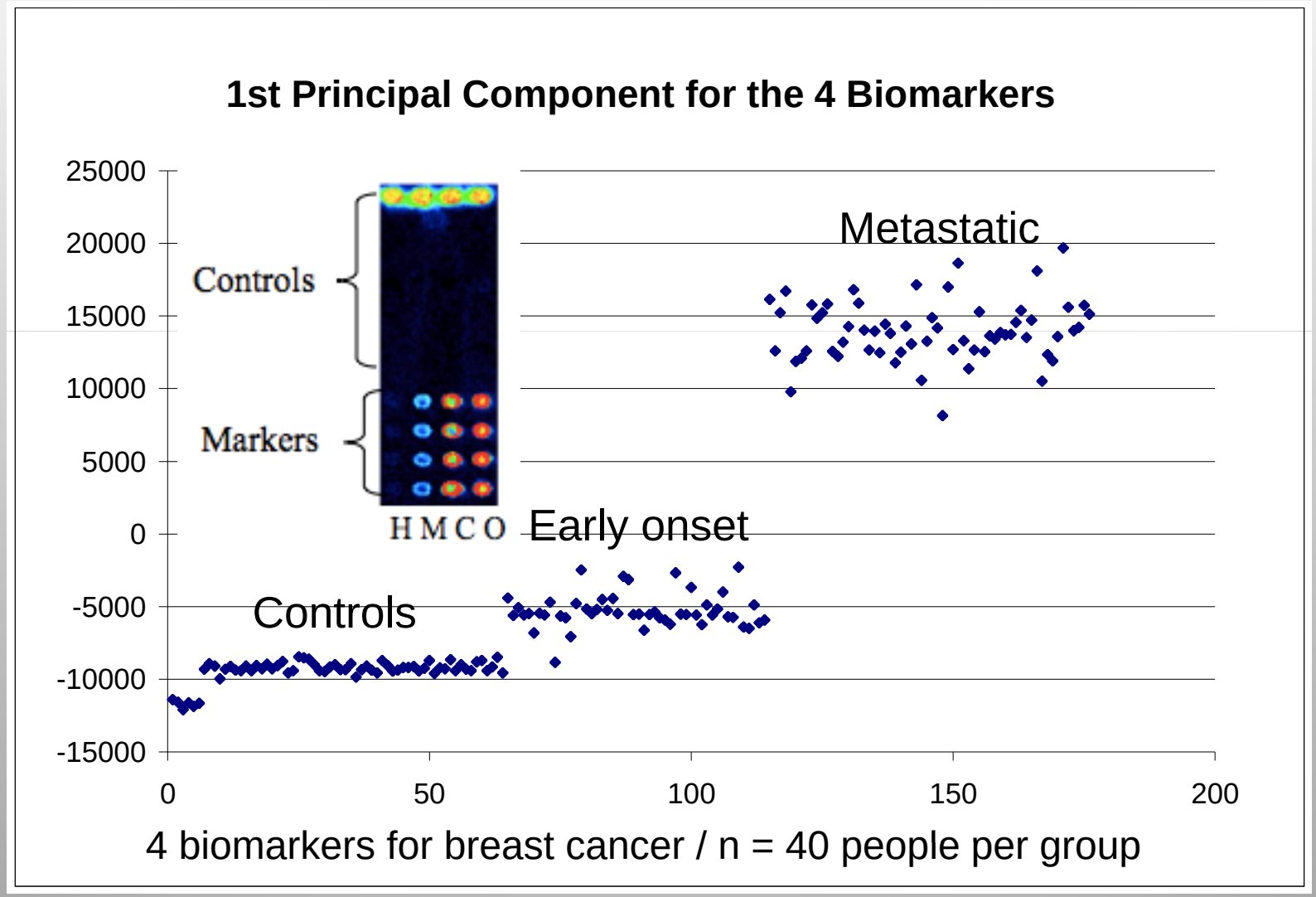


Hand-held detector for array-based assays

Next generation device uses simple microfluidics for smart phone-based diagnostics



Assays can be Applied to Serum-based Breast Cancer Diagnostics



Rao, R., et al., (In preparation) Passive-flow breast cancer screens capable of discriminating between normal from metastatic breast cancer in serum.

Lawrence Livermore Microbial Detection Array (LLMDA) has Probes to Detect all Known Viral and Bacterial Organisms

5,964 microbial species

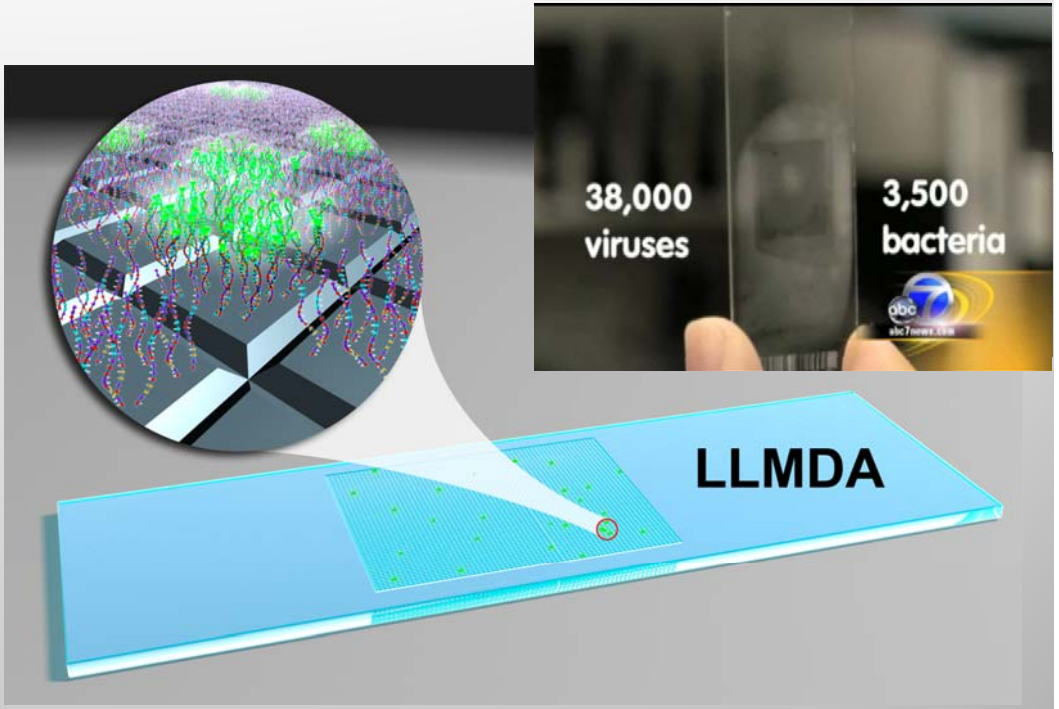
- 3,368 viral species
- 2,223 bacterial species
- 136 fungi

387,156 total probes

- Probes are 50-65 bases long
- Unique regions from viral and bacterial sequences used
- >15-50 probes per each sequence

Enabled by Bioinformatics

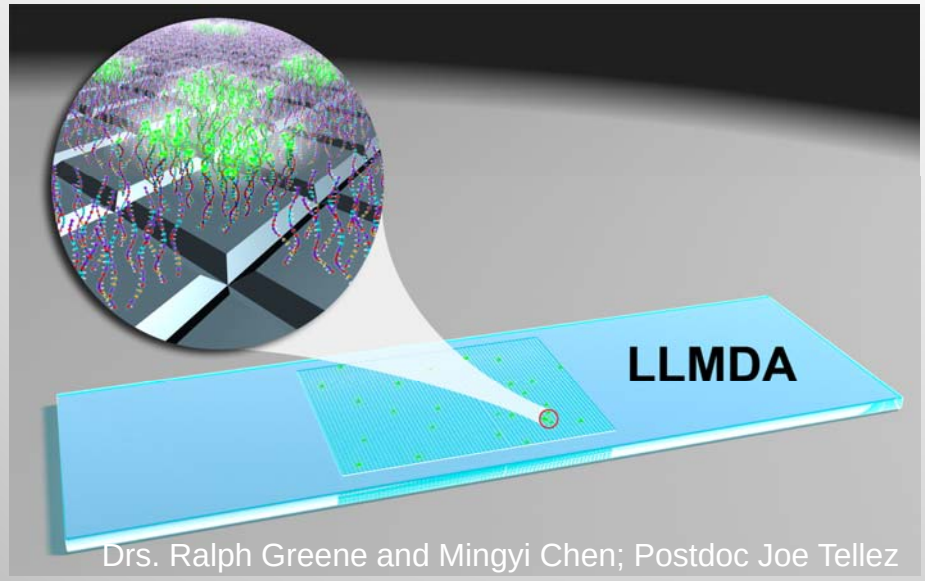
- Expertise in DNA signatures and biostatistics
- Large Cluster computing used



A Microbial Detection Array (MDA) for Viral and Bacterial Detection. Gardner SN, Jaing CJ, McLoughlin KS, Slezak TR. *BMC Genomics* 2010, **11**:668doi:10.1186/1471-2164-11-668, published Nov 25, 2010. http://www.biomedcentral.com/1471-2164/11/668.

- More than 20 collaborations with academia, government agency and pharmaceutical companies
- Multiple licensing discussions underway.

LLMDA used in Collaboration with UC Davis to Analyze Infectious Agents in Lymphoma



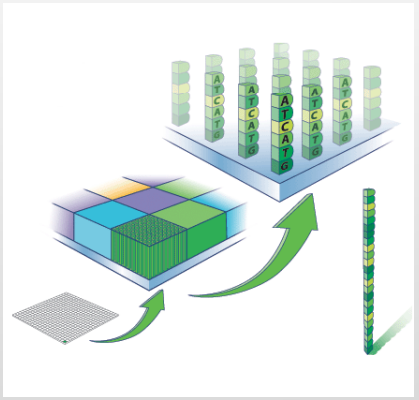
Drs. Ralph Greene and Mingyi Chen; Postdoc Joe Tellez

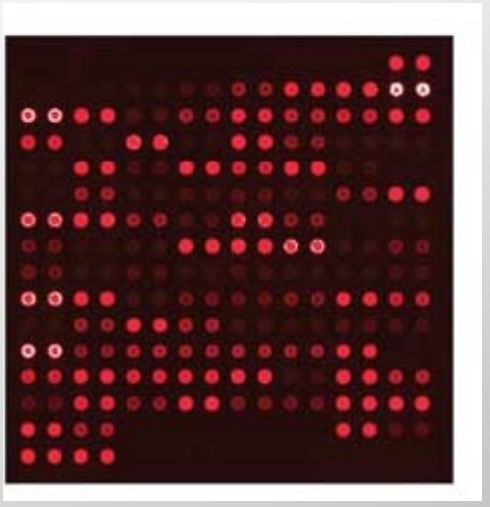
Non-Hodgkin's lymphoma, malignancy of bone marrow-derived cells which results in unregulated replication and expansion of these cells in lymphoid tissue and beyond.

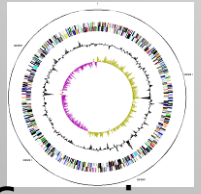
- Analyze viruses and/or bacteria in control lymphoid tissues, indolent lymphomas and aggressive lymphomas
- Identify unique groups of commensal and pathogenic microbial species that might be associated with each sample group
- Compare the relative abundances of microbial families between normal vs indolent vs progressive lymphomas to determine whether higher abundance of certain microbes in tissues correlates with lymphomas and the progression of lymphomas.

LLNL Bioinformatics is Playing a Key Role in Detection & Diagnostics Architecture

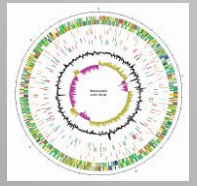
- Facilitate strain-collection access via LLNL collaborations with sequencing centers
- Conduct shift/drift and genetic engineering detection studies
- Protein structure function pipeline







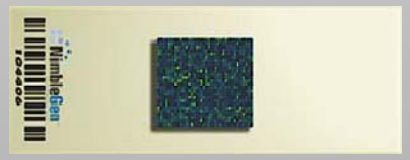
Genomics



Bioinformatics

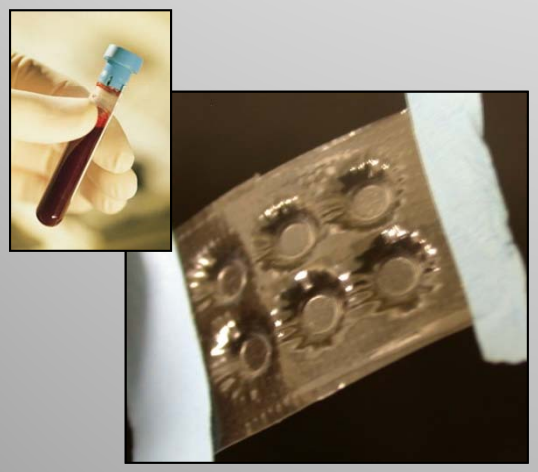
Validated assays

```
18021  GACTTTTTAA GGACTGTAGT AAGATCATTA CTGGTCTTCA TCCTACACAG GCACCTACAC
18081  ACCTCAGCGT TGATATAAA. TTCAAGACTG AAGGATTATG TGTTGACATA CCAGGCATAC
                                                                  sc_6900
18141  CAAAGGACAT GACCTACCGT AGACTCATCT CTATGATGGG TTTCAAAATG AATTACCAAG
                                                                  sc_6900
18201  TCAATGGTTA CCCTAATATG TTTATCACCC GCGAAGAAGC TATTCGTCAC GTTCGTGCGT
                                                                  sc_6900
18261  GGATTGGCTT TGATGTAGAG GGCTGTCATG CAACTAGAGA TGCTGTGGGT ACTAAC..TA
                                                                  sc_6900
```

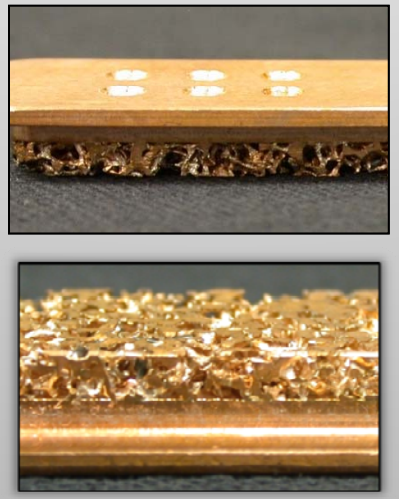


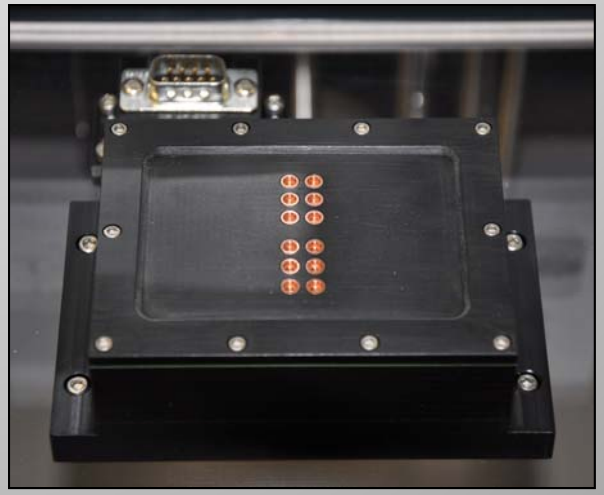
Fast PCR - Sample to Answer in <10 minutes

- Eliminate long ramp times by porous media flow
  - Allows PCR to be governed by diffusion and enzyme processivity
- Optical (real-time) detection
- Architecture can be ruggedized and miniaturized
- Target <10 minutes STA - including sample prep & detection



Disposable plastic insert for PCR (5µL wells)



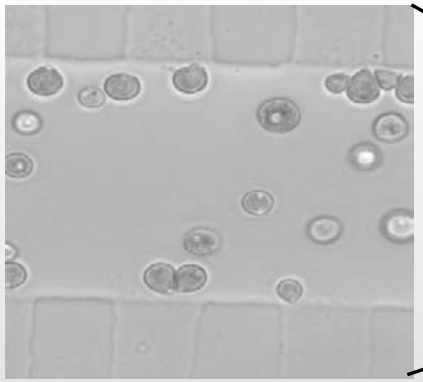


Fluidics system for convective heat transfer

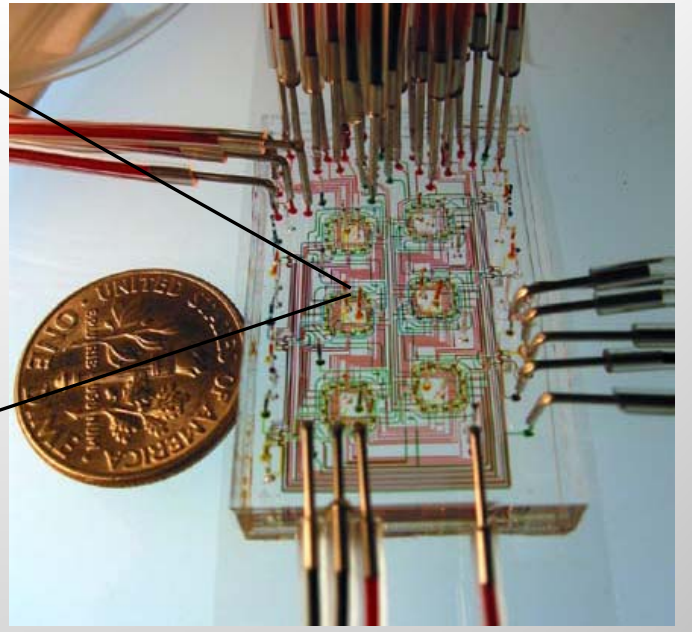
Microfluidic Cell Culture Platform

Enables Studies of:

- Host Cell-Pathogen interactions
- Viral Evolution, anti-infective resistance…
- Biochemistry and Physiology
- Drug safety

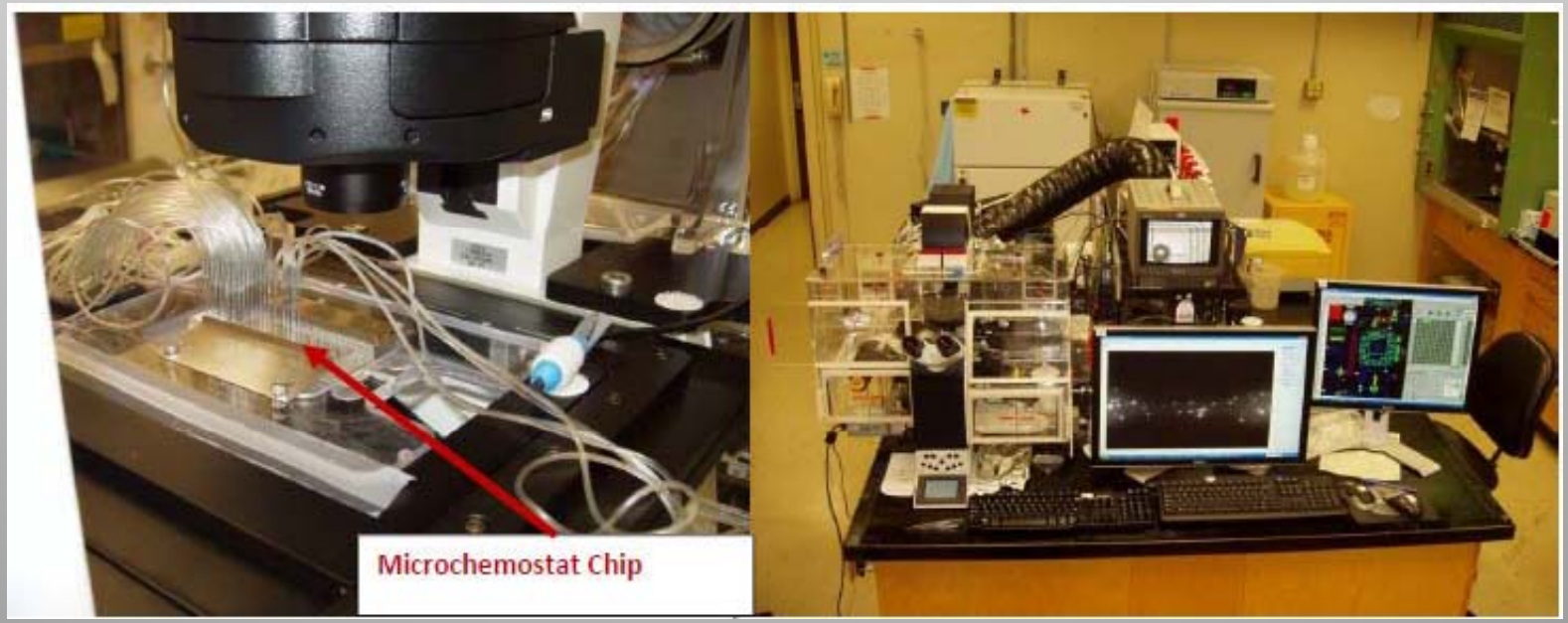


Cells in Perfusion Chamber

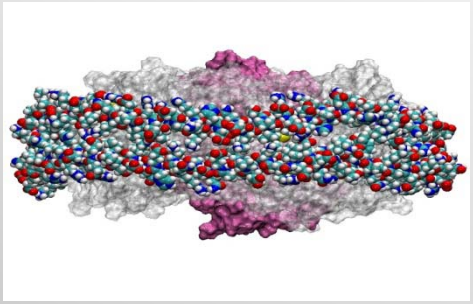


Micro-Fluidic Cell Culture Chip

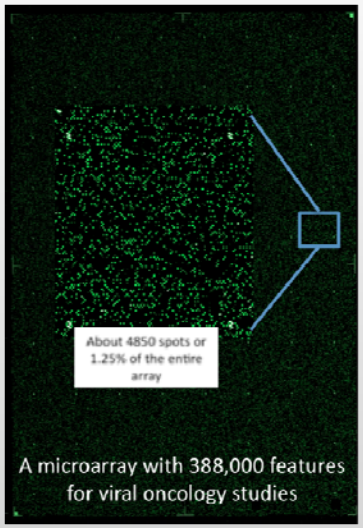
- 16 devices per chip
- Each device consists of 16 addressable chambers
- Each chamber has ~150 pL, total Device ~5 nL



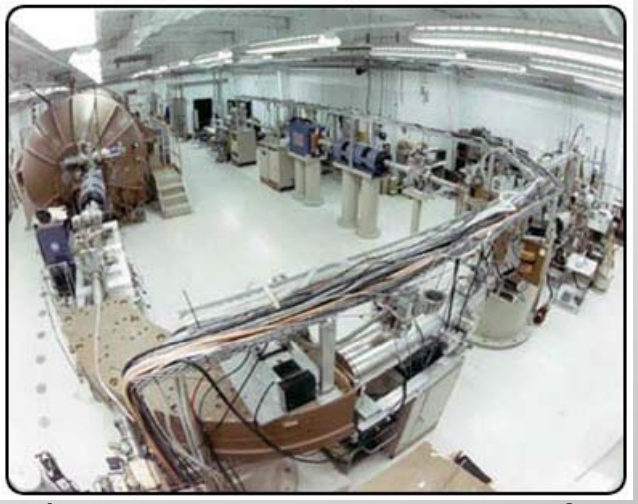
Collaborations with the University of California, Davis Comprehensive Cancer Center



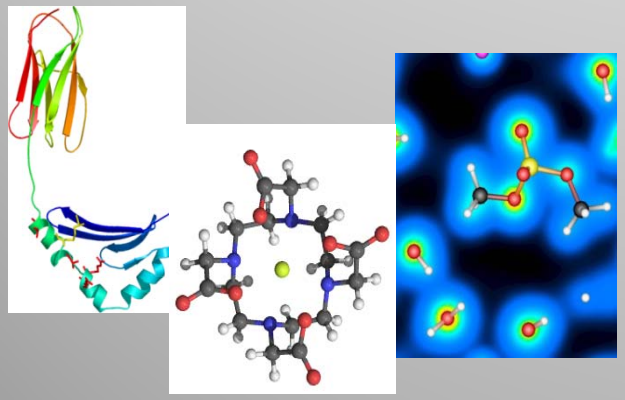
NLPs for imaging, immune modulation and drug delivery



High density microarrays for viral oncology



Accelerator Mass Spectrometer for Chemotherapy/diagnostic development



Computational Biology for Rational Drug Design

Proton radiotherapy

Optical Biopsy Endoscopy

We believe Team Science requires an ingrained institutional culture

- We hire people with the personality that fits the team environment
  - Training begins with Postdoctoral scientists
- Team work is a key part of performance management
  - We reward teams as much as we do individuals
- We administratively organize to allow fluid movement of SME's among programs/projects
  - We use a matrix environment where every person fits in a discipline organization which is responsible for career management and is also assigned to a program/project where that subject matter expertise is needed
- Facilities are organized to ensure interaction among disciplines
  - Multi disciplinary teams are either housed together
- Career management considers the team environment

What helps drive success

- Enthusiastic Leadership
  - Needs to be well understood responsibilities
  - Passion for the project
- Communication, communication and more communication..
- Working towards a common vision
  - Group developed understanding of the problem, critical path, challenges
- Setting goals and understanding requirements
  - Everyone needs to have the same understanding
- Understanding strengths and weaknesses of the team
  - Leadership needs to understand this
  - Collaboration
- Mentoring
- Rewarding success for the team
- Strong Management support

Challenges

- Most of us were not trained to work in teams
- Keeping communication and interaction among all team members going
- Walking the line between individual needs and project needs
  - Reward team success as often as we do individual
  - We emphasize long-term, high risk projects that are large but support individual

Questions

Our goals for the Biomedical Sciences:

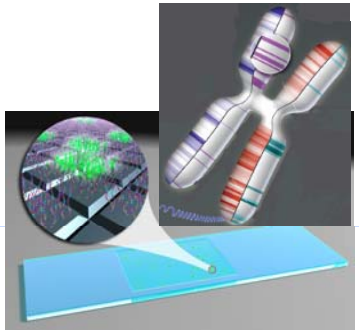
Work at the interface of the physical, chemical, biological and engineering sciences to solve important problems in public health

- Characterize key biochemical and molecular networks and enable simulation of biological networks, definition of new biomarkers, and understanding of disease processes
- Develop advanced deployable instrumentation for measurement of indicators of pathogens, early disease detection, and toxic chemical exposure
- Develop and translate technologies that will accelerate the preclinical development phases of drug development and more accurately predict safety and efficacy in humans
- Collaborate to quickly discover and deploy new countermeasures (assays, devices and therapeutics)

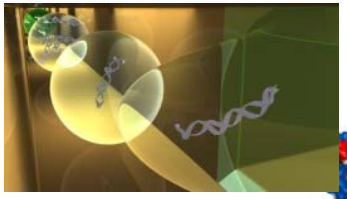
LLNL has a Tradition of Transitioning Cutting Edge R&D to Operational Capabilities for Public Health

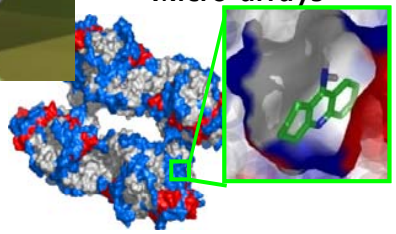
Foundational S&T

Threat Agent Science and Novel Assays

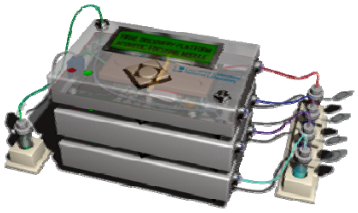


Enabling Bioinformatics & Micro-arrays





Computational Biology & Molecular Modeling



Bio-Instrumentation

Programmatic and National Impact

BioWatch

NARAC/IMAAC

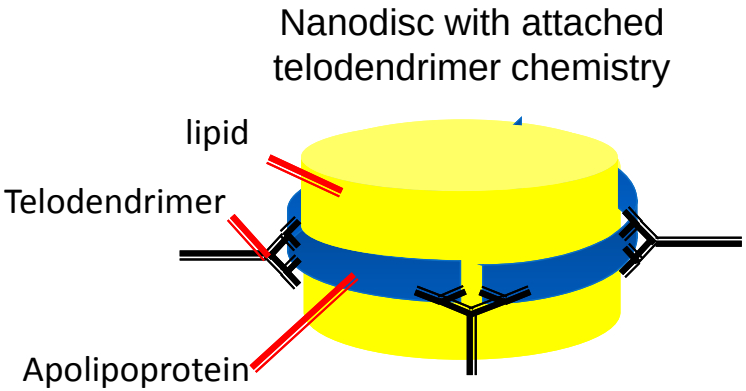
Handheld PCR

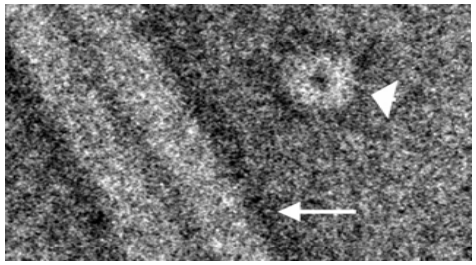
Joint Genome Institute

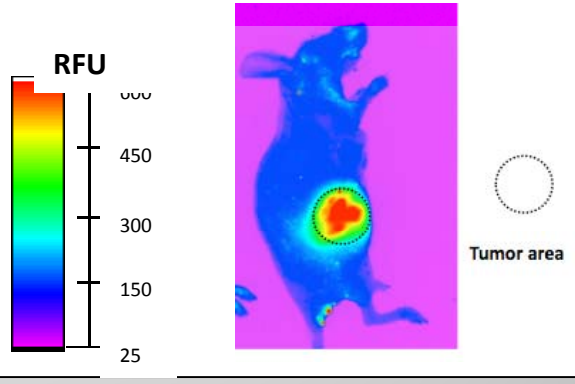
Forensic Science Center

Biodefense Knowledge Center

Development of Novel Nano-platforms for Biotechnology







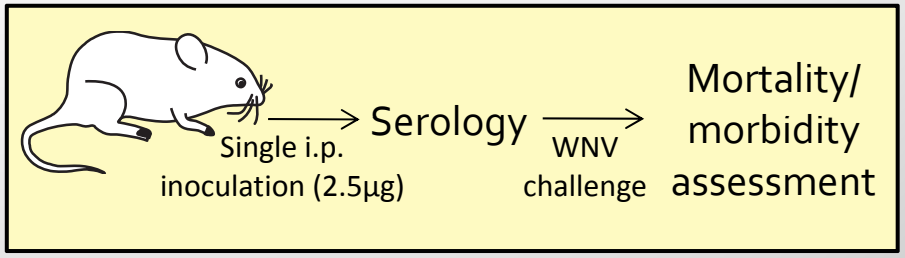
- Novel cell-free methods for the rapid assembly of apolipoproteins into <25 nm for in vitro and in vivo biochemistry.
- Telodendrimer chemistry to specifically tune the size and monodispersity of the nanodisc. The telodendrimer also allows a platform for the attachment of targeting peptides, labels and immunomodulatory drugs.
- These disc have shown to be a valuable resource for the production and solubilization of membrane bound proteins. They have also proven useful for in vivo delivery of drugs and imaging reagents.

Nanolipoprotein Particles (NLP) as Novel Vaccine Countermeasures

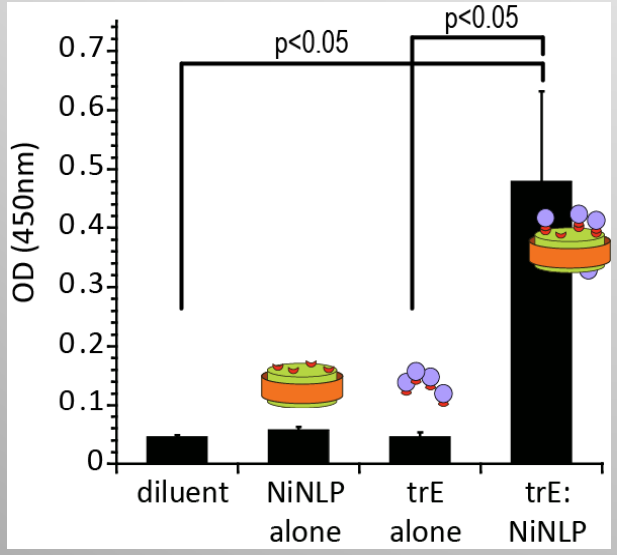
Model antigen - West Nile virus envelop protein (E):

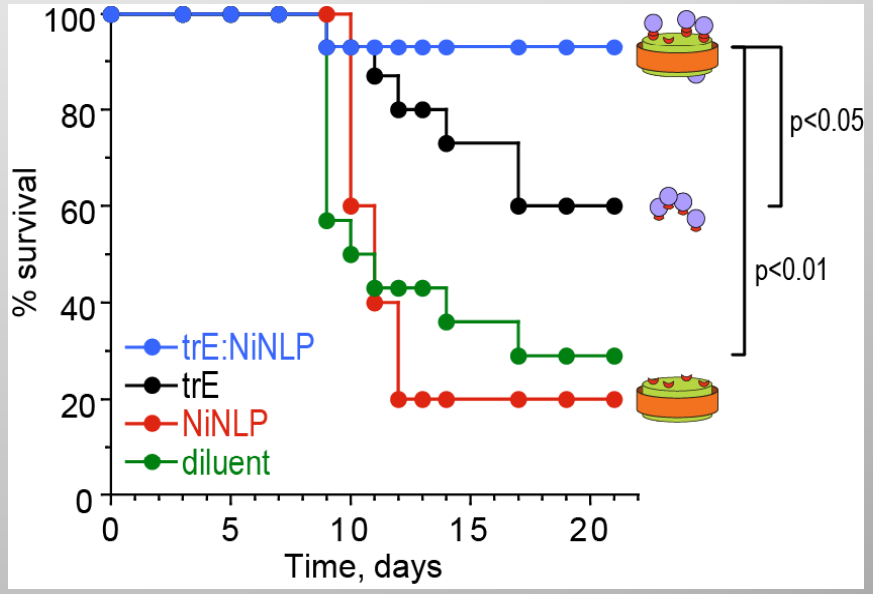
- Surface of virus exclusively displays E protein
- His-tagged, truncated E (trE) conjugated to NiNLPs

In vivo studies (Swiss Webster mice)



α-trE Ab Production



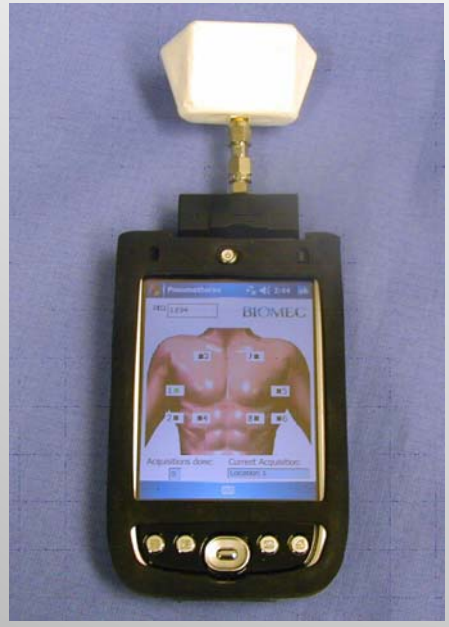


Diluent	n=15
NiNLP	n=5
trE	n=15
trE:NiNLP	n=15

Fischer NO et al (2010) Bioconjugate Chem. 21, 1018-22

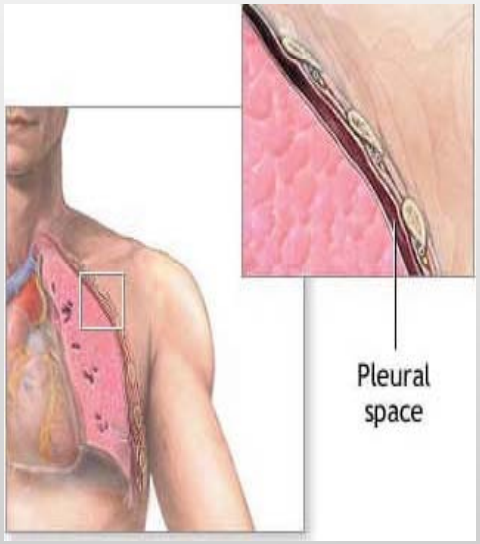
MUIR Pneumothorax Detector is Successfully Transitioning to a Clinical Product

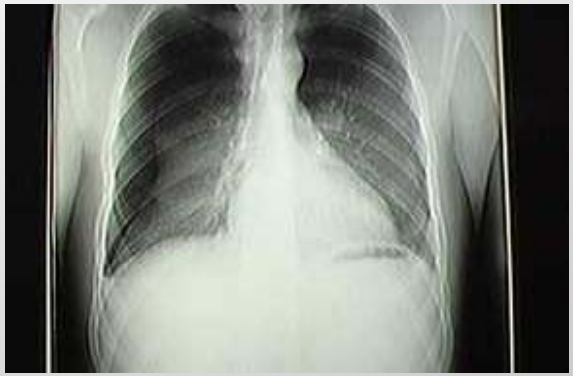
Out of hospital diagnosis for traumatic chest injuries



Early device Concept

Currently CRADA





Chest x ray of a pneumothorax. [Ref. Educational Computing for Health Technologies teaching web site, Michigan State University] Note the pleural line visible on the patient's right side (image left side).

LLNL in collaboration with Electrosonics Medical, Inc, Detroit Sinai-Grace Hospital, and Detroit Receiving Hospital

2007 R&D 100 Award,
2008 Federal Laboratory Consortium Award