

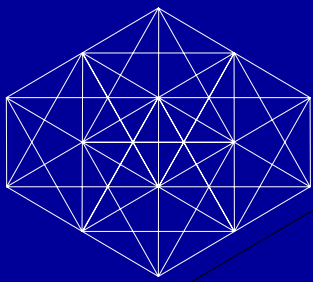
# Integrated Biosystems Research

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Proteomics

Integrative Proteomics

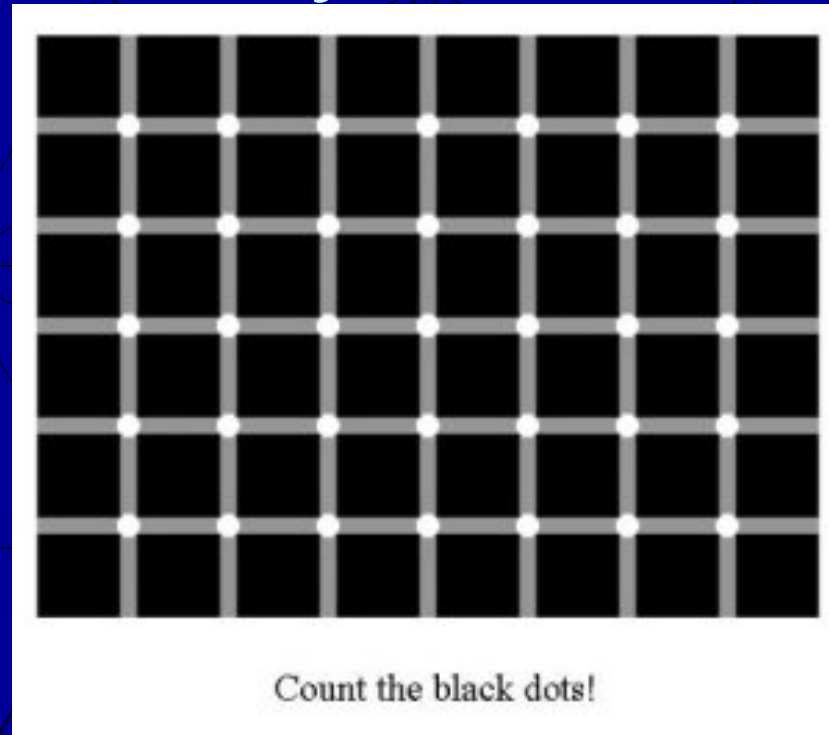
Team Report



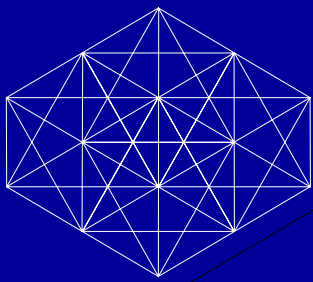
# Integrated Biosystems Research

## Proteomics Definition

What exactly is Proteomics?



As many explanations as there are black dots.



# Integrated Biosystems Research

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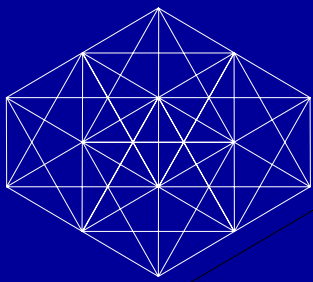
## Proteomics Team Members

Presenters: Mark A. Hayes – ASU  
Serrine Lau – U of A

### Contributors:

Mike Berens, TGen  
Peter Pingerelli, BNI  
George Tsaprailis, U of A  
David Craig, TGen  
Mike Mobley, Biodesign  
Ron Lukas, BNI

David F. Smith, Mayo  
Randy Nelson, IBI  
Dan Brune, ASU  
Stan Burns, BNI  
Yhengping Yi, ASU  
Ritu Pandey, U of A



# Integrated Biosystems Research

## Proteomics Research Interests

### Barrow Neurological Institute

- \*Clinical, translational, and basic neuroscience
- \*Whole system neuroscience (neuronal circuits of higher order functions, such as vision, motor control, addictive behavior)
- \*Molecular and cellular biology, electrophysiology, neuro-oncology and pharmacology

### Mayo Clinic Scottsdale

- \*Cancer, cancer biology
- \*Neurology
- \*Immunology
- \*Molecular Endocrinology

### Intrinsic Bioprobes, Inc.

- \*Biomarker Discovery
- \*Population Proteomics
- \*Pharmacokinetics
- \*Broad range of diseases

### The Translational Genomics Research Institute

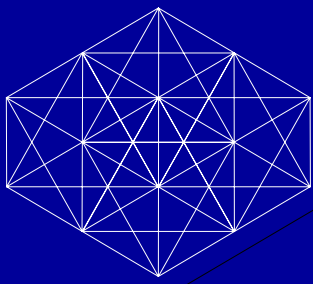
- \*Broad range of topics
- \*Cancer, neurology, aging, metabolic, autism
- \*Highly collaborative

### University of Arizona & Related Institutions

- \*Medical School
- \*Diverse breadth & depth of interests

### Arizona State University & The Biodesign Institute

- \*Fundamental Research Oriented
- \*Metabolic diseases
- \*Cancer
- \*Broad range of diseases



# Integrated Biosystems Research

## Proteomics Resources

### Barrow Neurological Institute

- \*Biochemical protein analysis

- \*'Established' new MS proteomics capabilities

Focused on neurodegenerative & neuromuscular diseases, neuro-oncology

- \*Pingerelli & Burns for details

- \*Collaborative

### Mayo Clinic Scottsdale

- \*General utilizes facilities In-house (Minnesota)

### Intrinsic Bioprobes, Inc.

- \*New molecular recognition/mass spectrometry technology
- \*Individual & population mapping
- \*High through-put capacity

### The Translational Genomics Research Institute

- \*Nothing specific to proteomics

- \*Extensive tissue and sample bank available for collaborative work

### University of Arizona & Related Institutions

- \*Large coordinated effort across several internal organizations

- \*Full, state-of-the-art facilities being developed

- \*High-throughput capacity

- \*Separate slides on resources

### Arizona State University & The Biodesign Institute

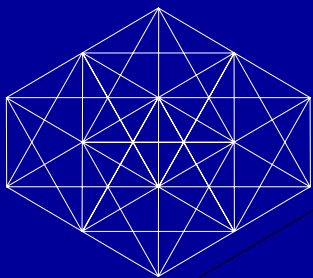
- \*Primarily fundamental research-oriented facilities

- \*Standard MS (high & low res.) & separations

- \*No significant high-throughput capacity

- \*Strong interest in building capacity and capabilities

Also: Sun Health, VA Hospital, Banner Health



# Integrated Biosystems Research

## Proteomics

**BIO5**

**CHEMISTRY**  
The University of Arizona



**UA Mass Spectrometry Consortium**  
 Co-Directors: **S. Lau Ph.D** and **V. Wysocki Ph.D**  
 Sponsors: **ARL, AZCC, BIO5, Chemistry, SWEHSC**



**Advisory Committee**  
 TB, Ph.D, **GTB**, Ph.D, **KD**, Ph.D, **DG**, Ph.D, **DV**, Ph.D



**TM, Ph.D – Staff Engineer Senior**

**Proteomics Facility**  
 Director: **GT**, Ph.D,  
 Assoc. Director: **LB**, Ph.D  
 TBN: Ph.D.

**Inorganic Mass Spectrometry**  
 Geo Science

**Molecular Mass Spectrometry**  
 Old Chemistry  
**AS**, Ph.D, **MM**, M.S, 2 B.S

**BIO5 GATC**

**ARL Biocomputing**

**BIO5 Service Core**  
**FH**, B.S, **AH**, B.S,  
**KK**, Ph.D

**Pharmacy/Chemistry Technology Development**  
**YF**, M.S, **BL**, Ph.D,  
**AH**, Ph.D, TBN: M.S

**Chemistry User-based Facility**  
 TBN: B.S

**Water Contamination Center**  
 Gould Simpson

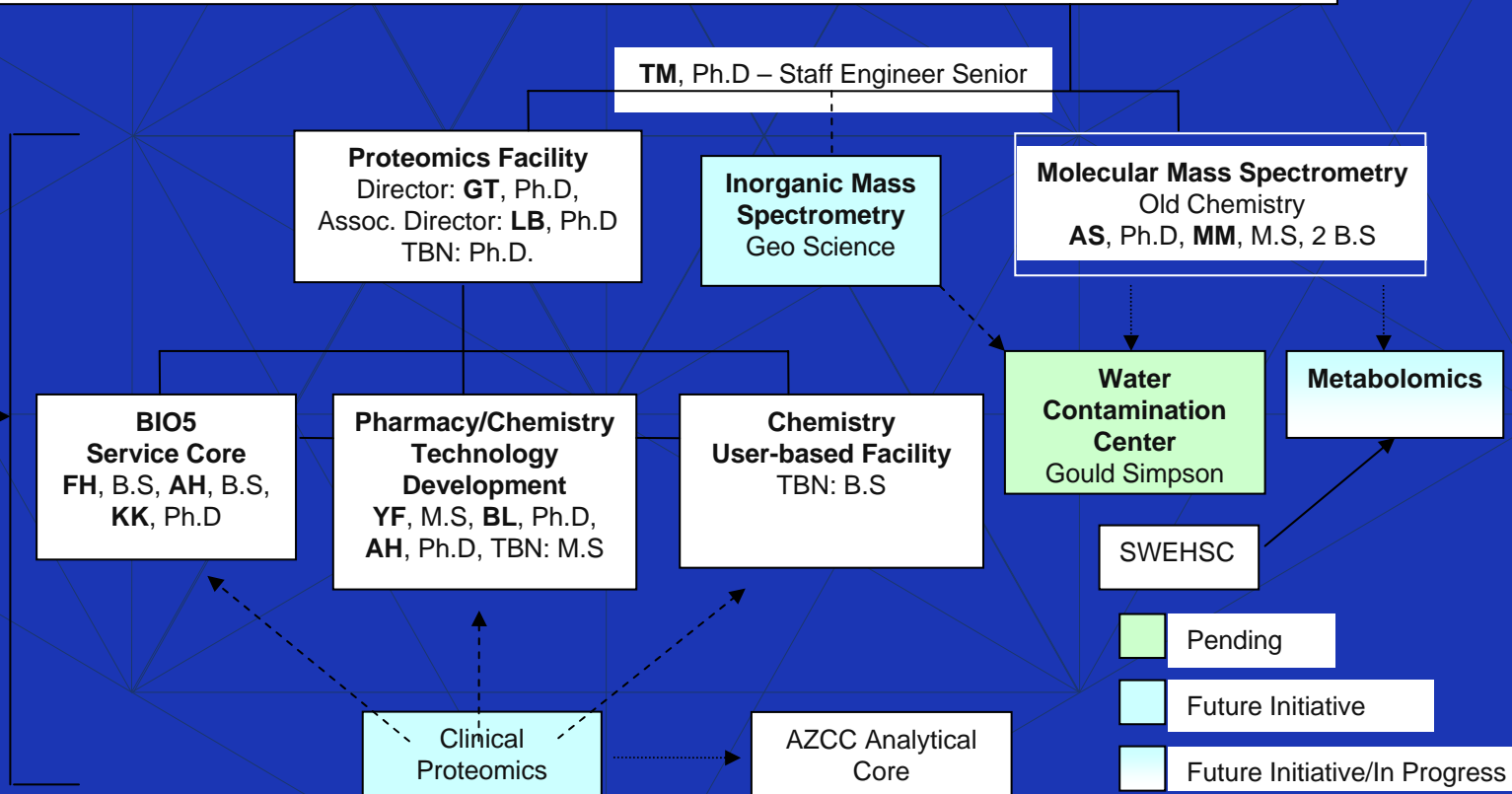
**Metabolomics**

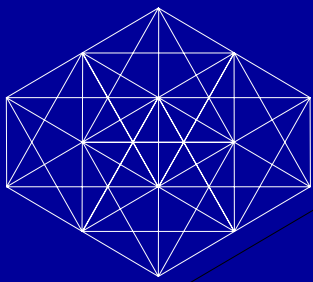
**SWEHSC**

**Clinical Proteomics**

**AZCC Analytical Core**

- Pending
- Future Initiative
- Future Initiative/In Progress



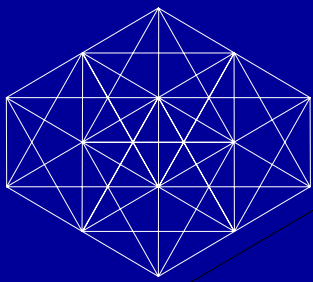


# Integrated Biosystems Research

## Proteomics U of A Resources and Services

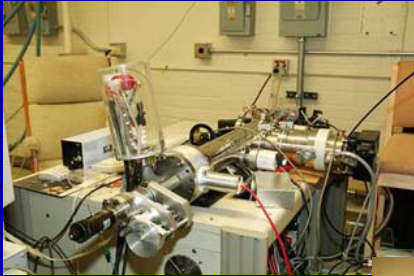
- LC-MS/MS and Mupdit (LC-LC-MS/MS) proteome profiling
- PTMs (SALSA, P-Mod, Xtandem)
- MALDI-TOF MS mapping
- 1D, 2D-SDS PAGE and DIGE Analysis
- LIMS & bioinformatics
- Technology Development & Instrument building





# Integrated Biosystems Research

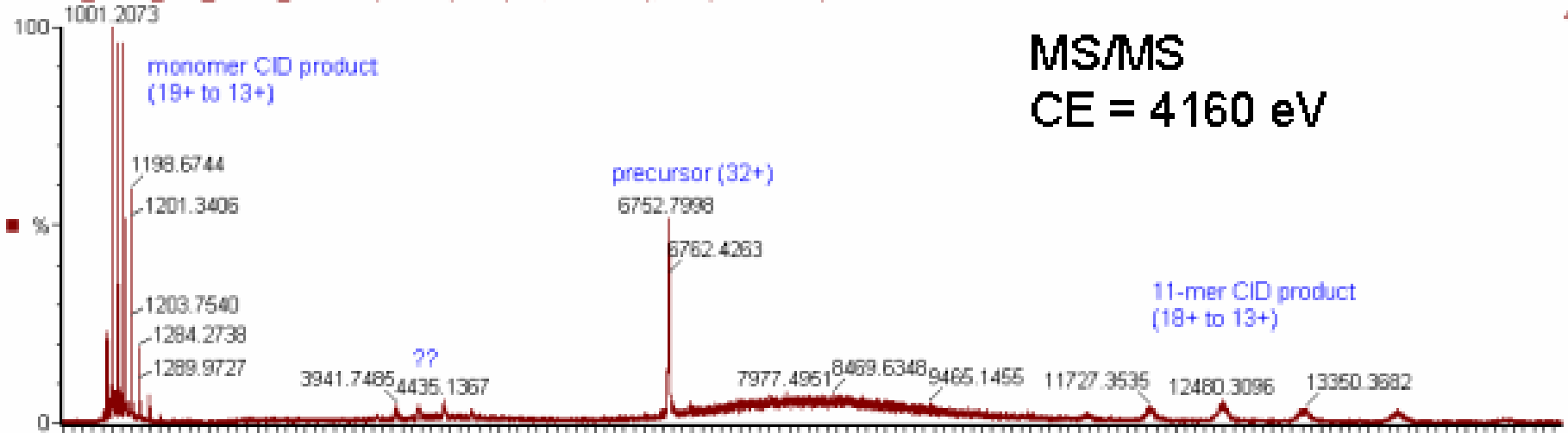
## Proteomics U of A Technology Development

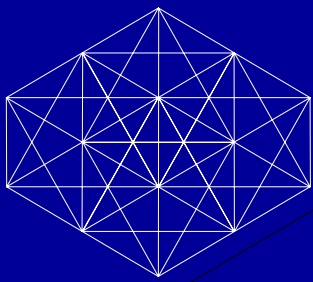


- Gas phase fragmentation; improve MS/MS sequencing algorithms
- Surface Induced Dissociation for whole protein complex characterization
- H/D exchange to probe peptide structure and Protein-protein interactions
- Protein array development

051109\_SHSP\_181\_MSMS\_2 1184 (52.284) Sm (SG, 15x15.00); Cm (1044:1223)

TOF MSMS 6752.00ES+  
44.1

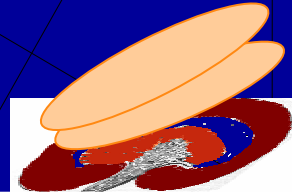




# Integrated Biosystems Research

## Proteomics

1. A Leica CM 1480 cryostat was used to cut 14 $\mu$ m sections from snap frozen tissue.



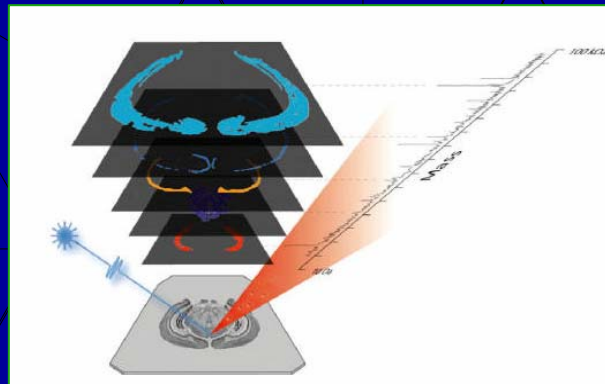
Additional Layers of matrix

Kidney section

in EtOH & Triton X-100

MALDI plate

2. 14 $\mu$ m tissue sections are positioned on top of sinapinic acid in EtOH. Additional layers of sinapinic acid in 50% acetonitrile are placed on top.



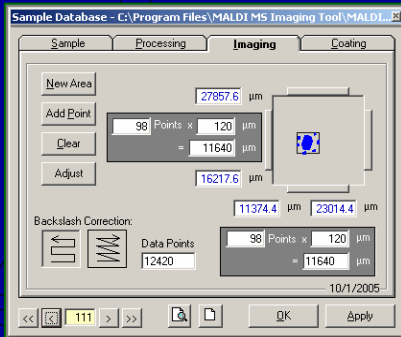
5. The MALDI laser fired 25 shots at each pixel in a grid as determined in the setup protocol. Each pixel contains a spectrum. This collection of spectra is exported as an image file that is imported into BioMAP.

Tissue preparation & image acquisition

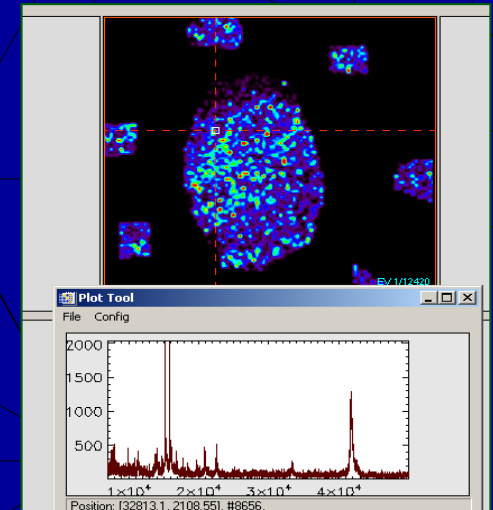
ABI Voyager-DE™ STR



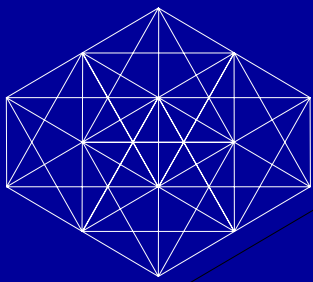
3. An Applied Biosystems Voyager-DE was used to acquire data



4. MMSIT was used to acquire spectra. In this example, spectra were acquired every 120 $\mu$ m along both the X and Y, shaded blue, calibration spots.



6. Image analysis by BioMAP

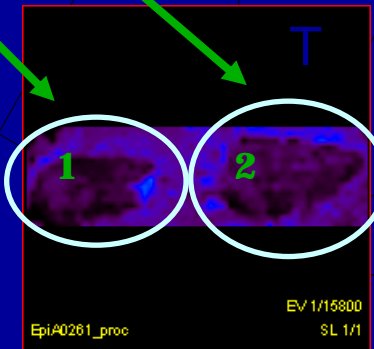


# Integrated Biosystems Research

## Proteomics

## MALDI-TOF-MS-Based Tissue Imaging and Protein Profiling

Eker rat  
OSOM  
kidney areas

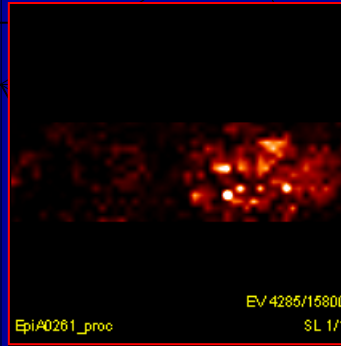


Tissue Image  
ion density map  
2-53 kDa

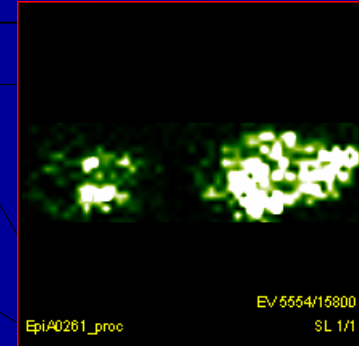
validation

discovery

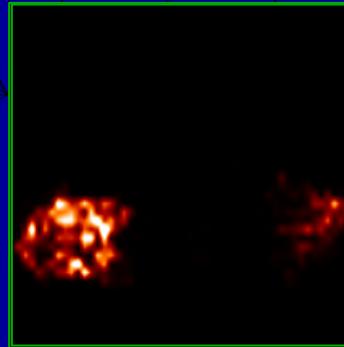
Differentially expressed  
proteins localized



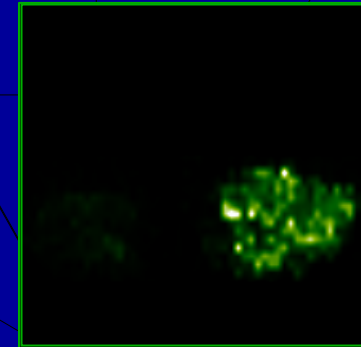
Ion density map IP-10  
8673 Da  
Increases in tumors, 12.3x



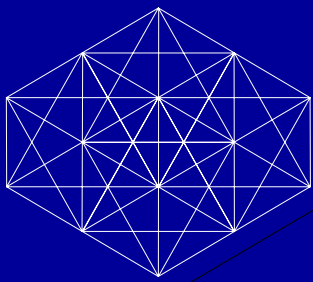
Ion density map thioredoxin  
11542 Da  
No reported change



Ion density map  
9580 Da



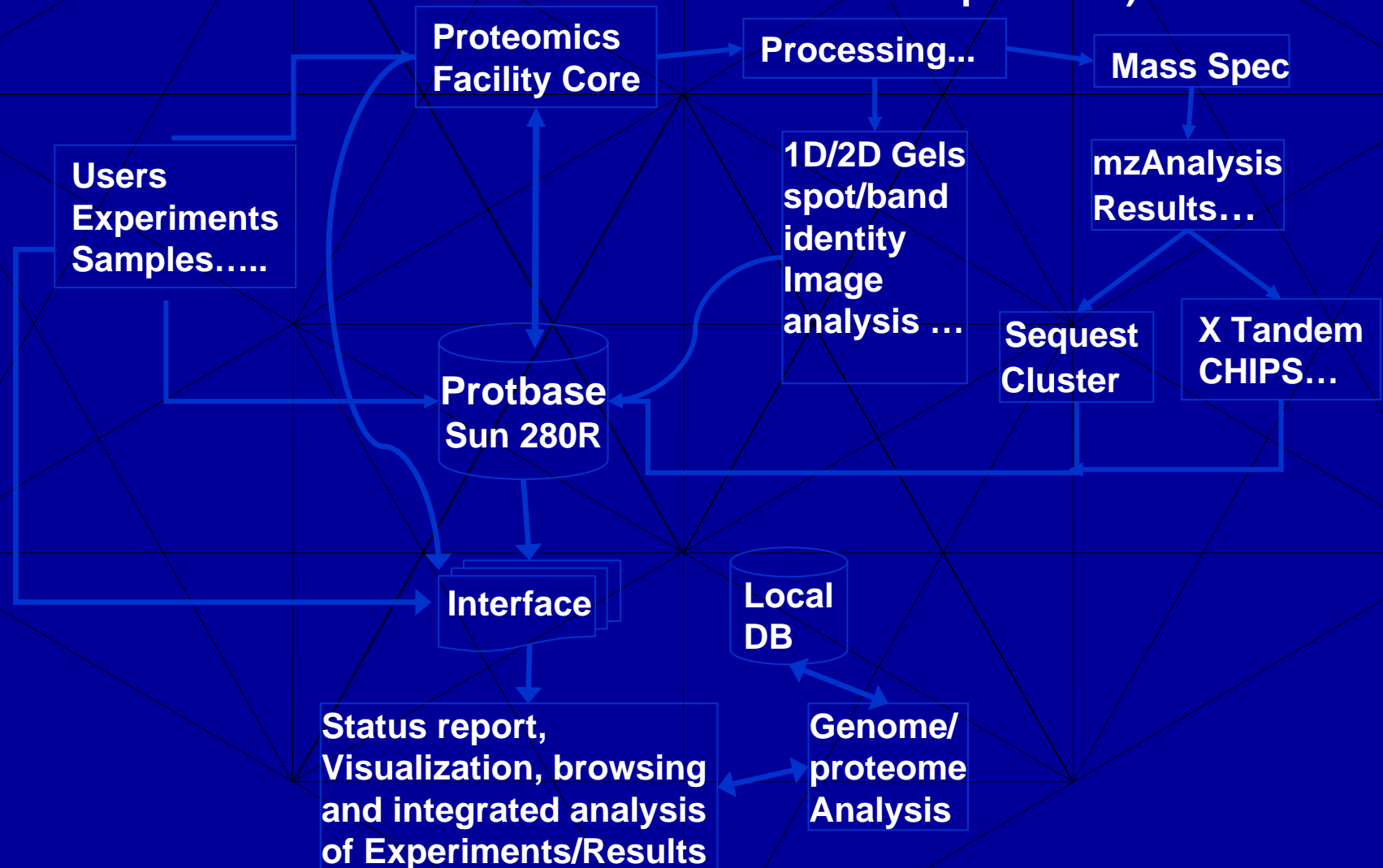
Ion density map  
8720 Da

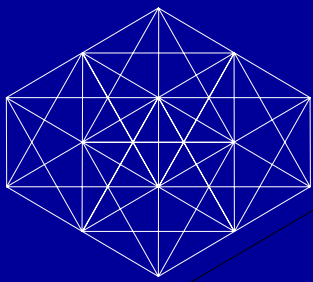


# Integrated Biosystems Research

## Proteomics

Data Flow and Data Storage based on MIAPE (Minimum Information About Proteomics Experiment) Protocol





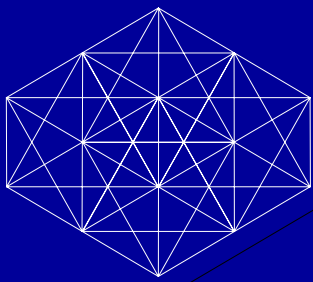
# Integrated Biosystems Research

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## Proteomics Regional Needs

### Consensus:

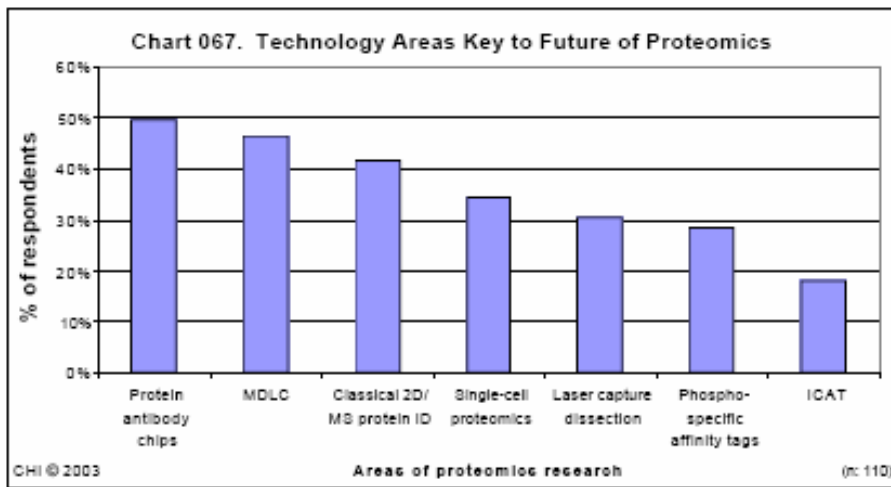
- Build capacity & accessibility for accepted state-of-the-art capabilities
  - Separations (classic, arrays), MS, bioinformatics, structural biology, molecular modeling
- Instigate new capabilities that are unique & provide a competitive advantage
  - Drive projects with clinical/biological research
  - Individual faculty for technology/capability development
- Integrate & collaborate efforts
  - bioinformatics, equipment, facilities, personnel



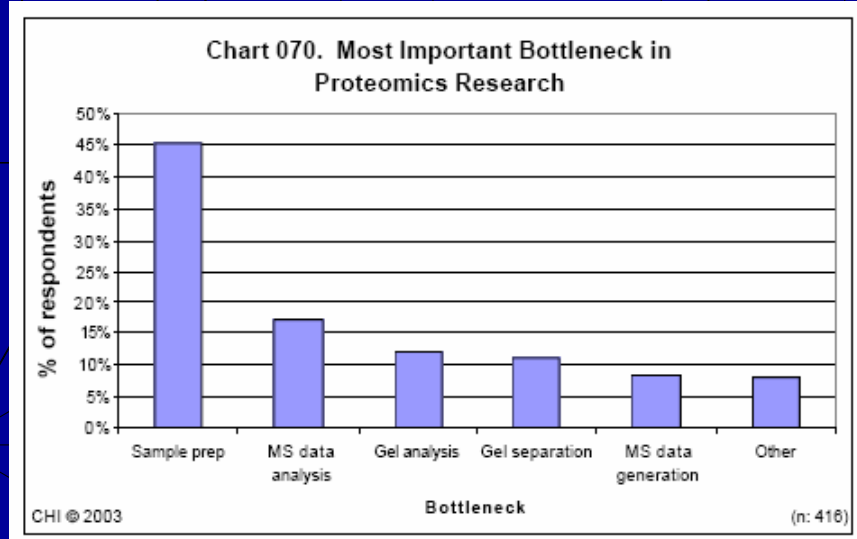
# Integrated Biosystems Research

## Proteomics Regional Needs

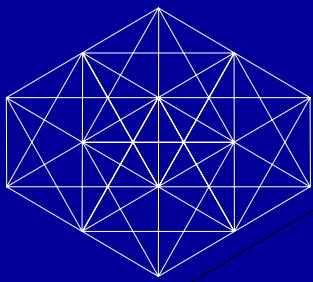
### Current Market Thoughts/Information:



Broad spectrum of technologies considered



Sample prep is key: one goal here today



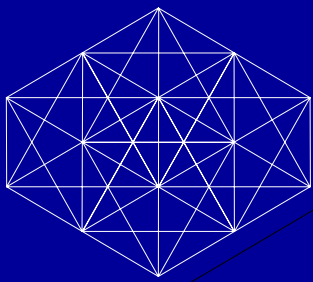
# Integrated Biosystems Research

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## Proteomics Regional Synergy

### Comments/ Suggestions:

- Truly unique opportunity with cooperation of major metropolitan ‘players’
- Cooperation/ communication key: leverage without redundancy/ negative competition
- Utilize resources at each institution to maximum benefit: bioinformatics as potential example
- Local ‘example projects’ to kick-start the HUPOs
- ‘Brain storming’ consortia:



# Integrated Biosystems Research

## Proteomics Proposed Consortia

### Collection & Preparation of Human Peripheral Blood for Proteomics

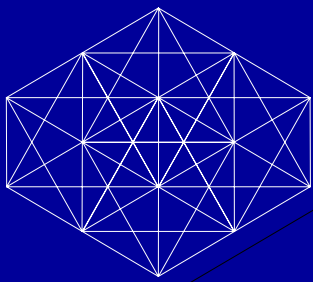
Charter: Align up to 3 clinical sites and 2 proteomics laboratories for demonstration project on variance in MS-based and antibody-based proteomics analysis for up to 5 different analytes in peripheral blood.

Needs: Principal Investigators; Clinical Research Coordinator; laboratory technicians (2); up to \$500,000.

Duration: 16 months

#### Milestones & Deliverables:

3 months- Up to 5 different analytes identified in up to 5 different clinical projects; experimental design finalized for up to 5 different projects; clinical sites identified; IRB protocols submitted;  
6-12 months- peripheral blood specimens collected; 12-16 months- proteomics analyses of analytes in peripheral blood collected according to different standardized protocols. Recommendations for "Best Practices" in collection and processing of Peripheral Blood for proteomics



# Integrated Biosystems Research

## Proteomics Proposed Consortia

### Tissue Collection & Preparation for Signal Transduction Proteomics

Charter: Align up to 3 surgical sites and 2 proteomics laboratories for demonstration project on variance in MS-based and antibody-based proteomics analysis for up to 5 different analytes in freshly-collected human tissues from surgical procedures.

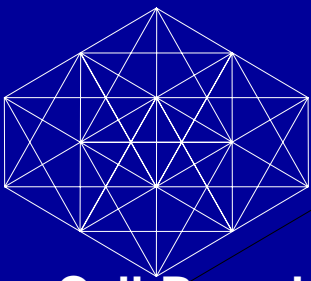
Needs: Principal Investigators; Clinical Research Coordinator; laboratory technicians (2); up to \$500,000.

Duration: 16 months

#### Milestones & Deliverables:

3 months- Up to 5 different analytes identified in up to 5 different clinical projects; experimental design finalized for up to 5 different projects; clinical sites identified; IRB protocols submitted;

6-12 months- tissue specimens collected; 12-16 months- proteomics analyses of analytes in tissue collected and processed according to different standardized protocols. Recommendations for "Best Practices" in collection and processing of tissue for proteomics.



# Integrated Biosystems Research

## Proteomics Proposed Consortia

### Cell-Based Proteomics and Development of Model Systems

**Charter:** Align up to 3 cell culture laboratories and 2 proteomics laboratories for demonstration project on variance in MS-based and antibody-based proteomics analysis for up to 5 different analytes in cultured human cells. Demonstrate utility of culture model systems for hypothesis-testing of proteomics outcomes (this is a very wide-open topic of use for cancer biologists, neuroscientists, endocrinologists, experimental cardiologists, etc).

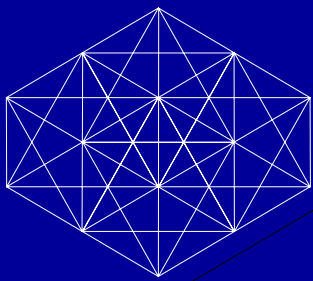
**Needs:** Principal Investigators; Clinical Research Coordinator; laboratory technicians (2); up to \$500,000.

**Duration:** 16 months

#### **Milestones & Deliverables:**

**3 months-** Up to 5 different analytes identified in up to 5 different projects amenable to cell culture testing; experimental design finalized for up to 5 different projects; laboratory sites identified; IRB protocols submitted;

**6-12 months-** cell lysates collected; **12-16 months-** proteomics analyses of analytes in cell lysates collected according to different standardized protocols. Recommendations for "Best Practices" in collection and processing of cell lysates for proteomics. Recommendations for cell-based models applicable to specific clinical research disciplines.



# Integrated Biosystems Research

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## Proteomics Proposed Consortia

### CSF Biomarkers in the Neurosciences

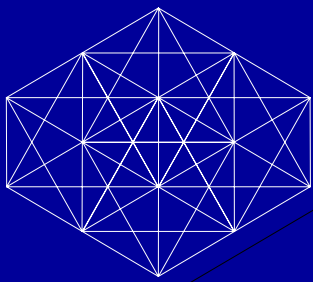
Charter: Align up to 3 clinical sites and 2 proteomics laboratories for demonstration project on variance in MS-based and antibody-based proteomics analysis for up to 5 different analytes in cerebral spinal fluid (CSF).

Needs: Principal Investigators; Clinical Research Coordinator; laboratory technicians (2); up to \$500,000.

Duration: 16 months

#### Milestones & Deliverables:

3 months- Up to 5 different analytes identified in up to 5 different clinical projects; experimental design finalized for up to 5 different projects; clinical sites identified; IRB protocols submitted;  
6-12 months- CSF specimens collected; 12-16 months- proteomics analyses of analytes in CSF collected according to different standardized protocols. Recommendations for "Best Practices" in collection and processing of CSF for proteomics.



# Integrated Biosystems Research

## Proteomics Proposed Consortia

### Emerging and Developing Technologies in Proteomics

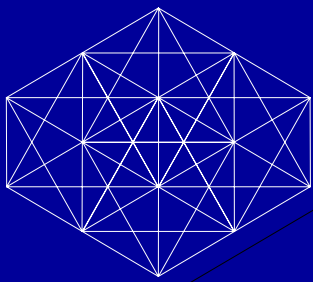
Charter: Recruit scientific team (clinical, laboratory, technical) to be available to team-evaluate new and emerging technologies for applicability in Arizona Proteomics Consortium.

Needs: Consortium Director; Administrative Assistant (25%); \$40,000 .

Duration: standing

#### Milestones & Deliverables:

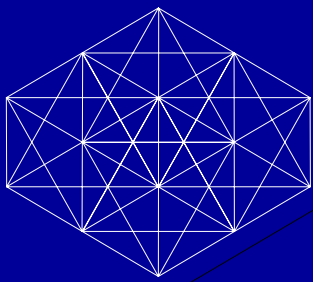
The Consortium for Emerging and Development Technologies in Proteomics serves the research community through its prompt and thorough evaluation of new proteomics approaches to biosciences. The Consortium recommends beta-testing sites, bio-specimens for evaluation, and potential key strategic users of the new technology. This resource would be a central conduit for commercial contacts to engage the broader bioscience research community in Arizona related to proteomics.



# Integrated Biosystems Research

## Proteomics

# Questions and Answers?



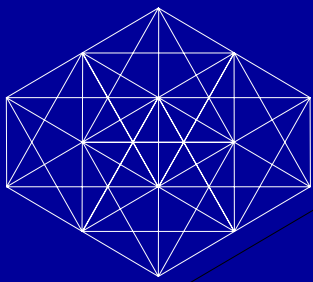
# Integrated Biosystems Research

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## Proteomics

### Auxiliary Slides

- Detailed capabilities
  - IBI
  - BNI
  - ASU

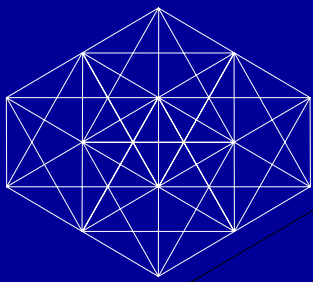


# Integrated Biosystems Research

## Proteomics Detailed Resources

### IBI Hardware Resources

- Lin MALDI-TOFMS (2)
- Ref MALDI-TOFMS
- MALDI-TOF/TOFMS
- MALDI-TOFMS (Custom; HV)
- ESI-QIT (Nanospray; HPLC)
- SPR-Biomolecular Interaction Analysis (BIA)
- BIA/MS
- 96-well parallel robotics (5)
- Protein extraction/processing (Device/Manufacture)



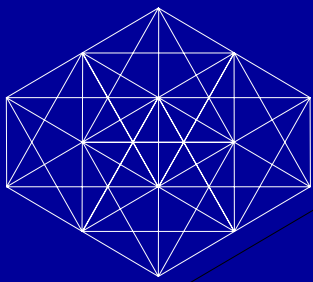
# Integrated Biosystems Research

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## Proteomics Detailed Resources

### IBI Staffing

- Ph.D. Senior Scientist/PI (6)
- MBA Administration/Manufacturing (2)
- BS/BA Interns/Researchers (1-4)



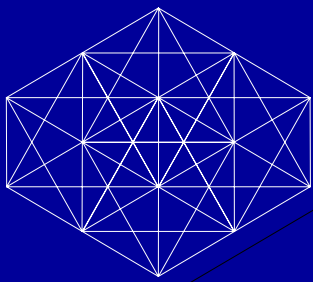
# Integrated Biosystems Research

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## Proteomics Detailed Resources

### IBI Core Competencies

- Proteomics/Clinical Proteomics
- Technology Development & Manufacture
- Mass Spectrometry
- Biomolecular Recognition (Interaction Analysis)
- Protein Characterization
  - Structure
  - Quantitation
- HT Targeted Proteomics
  - Diversity in Human Proteins
  - Biomarker Discovery and Validation



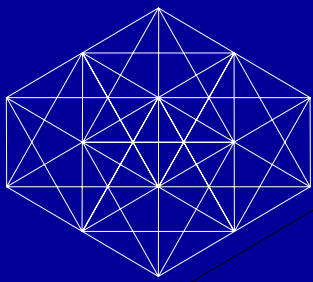
# Integrated Biosystems Research

## Proteomics Detailed Resources

### IBI Research Programs

- Advanced Technologies
  - Broadband & Multiplexed Assays: IBI
- Advanced Technologies
  - SPR-MS Arrays: NCRR
- Biodefense Agent Assays
  - Staphylococcal enterotoxins: NIAID
- Cardiovascular Disease
  - Apolipoproteins/MI Markers: NHLBI
- Cancer
  - Ovarian/Breast Biomarkers: NCI
- Diabetes
  - Type II Biomarker: NIDDK
- Population Proteomics
  - Diversity in Healthy: IBI



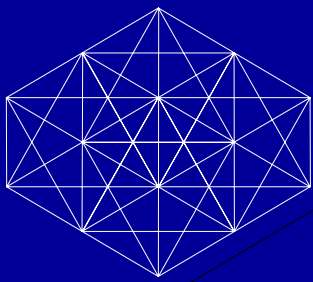


# Integrated Biosystems Research

## Proteomics Detailed Resources

### IBI Commercial & Collaborations (2005)

- Biosite
  - MI Biomarkers
- Ortho Clinical Diagnostics (J&J)
  - Cardiovascular Markers
- Amgen
  - Platform Technologies/Proprietary Assays
- Beavis Informatics LTD
  - Bioinformatics
- Yale
  - Bioinformatics
- Vanderbilt
  - Cancer Biomarker Validation
- U Wisconsin
  - Alcohol Markers
- Burlo Garofolo Pediatric Institute (Trieste Italy)
  - Technologies
- ASU
  - Protein Characterization



# Integrated Biosystems Research

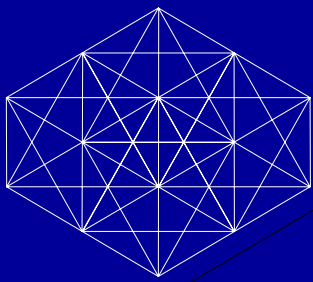
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## Proteomics Detailed Resources

### Barrow Neurological Institute

- Instrument and software listing:

- Bruker Ultraflex II MALDI TOF/TOF MS with tissue imaging capabilities for cells and tissue extracts
- Bruker HCTplus ESI-Ion Trap MSn
- The PROTEINEER™ suite; automated spotter 1 L
- Cryostat Leica CM3050
- Laser Capture Dissection Microscope (**LCM**) Zeiss
  
- Accelrys- Protein Modeler
- Matlab – Data Analysis
- PathwayAssist™ pathway analysis software
- Mascot - Matrix Science



# Integrated Biosystems Research

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## Proteomics Detailed Resources

### Arizona State University

- MALDI-TOF (Voyager DE STR from Applied Biosystems)
- triple quadrupole electrospray mass spectrometer
- 2D-HPLC-NSI-LTQ-FTICR (Fourier Transform Ion Cyclotron Resonance):
  - LTQ fast scanning, high sensitivity, capable of MS<sub>n</sub> (n=1-9)
  - FTICR: Accurate mass and high resolution
- MALDI-LTQ: fast screening, high sensitivity, capable of MS<sub>n</sub> (n=1-9)