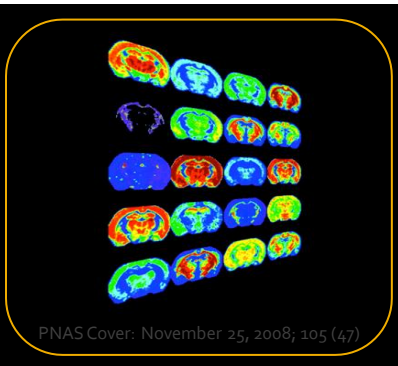


PNAS Cover: November 25, 2008; 105 (47)

BMG 744 - Spring 2011
David Stella
February 25th, 2011

Mass Spectrometry Imaging (MSI)

MSI: part I

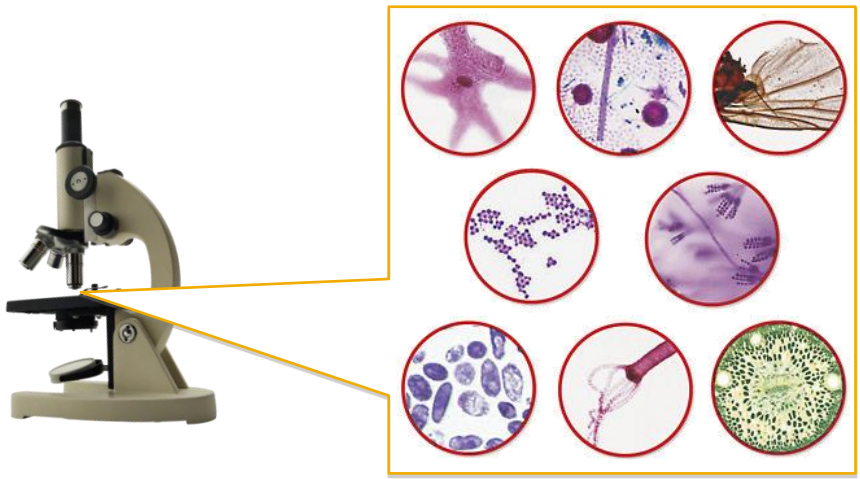
What usefulness is imaging in the pursuit of biological research?

Imaging Experiments

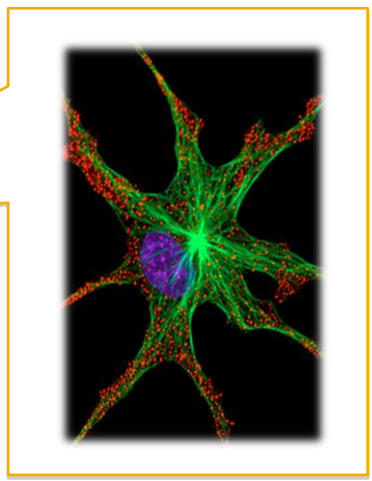
- What kind of questions can be answered?
  - What's there?
  - Who's there?
  - How much is there?
  - What changes occurred?
  - Others…???

Imaging and biological research: dissecting microscope

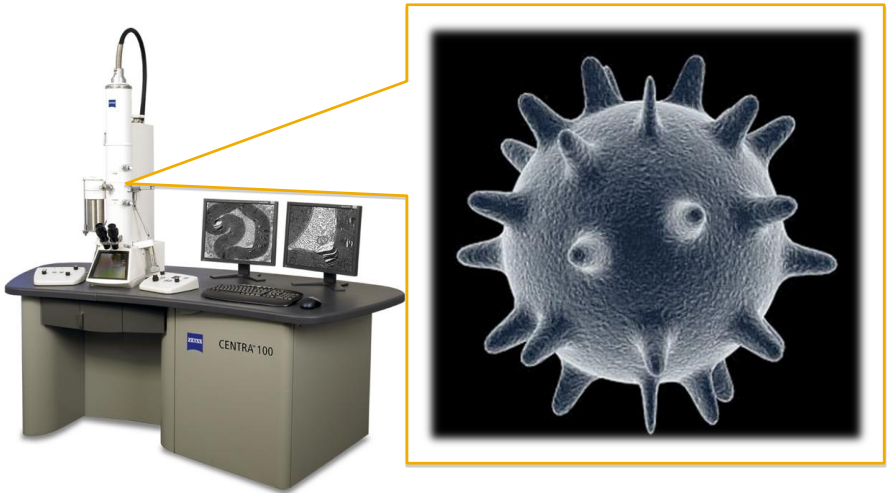
Imaging and biological research: light microscopy



Imaging and biological research: confocal microscopy



Imaging and biological research: electron microscopy



The technology

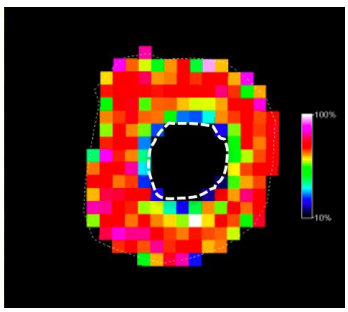
- Using MS to create pretty images.
- Comparable to traditional histochemistry techniques to identify macromolecular species present in tissue sections
- Diverse applications and approaches to answering biological research questions

Mass spectrometry

- What kind of questions can be answered?
  - What's there?
  - Who's there?
  - How much is there?
  - What changes occurred?
  - Others…???

Mass spectrometry imaging (MSI)

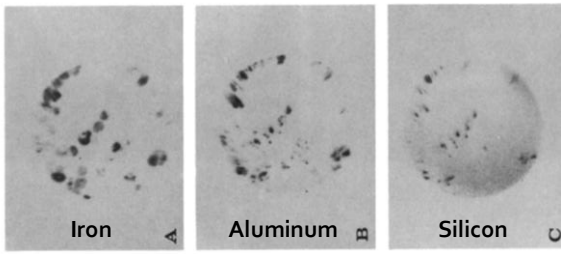
MALDI-TOF mass spectrometer



Chemical image

Early MSI: elemental analysis

Smoker's lung macrophages



Environmental Health Perspectives
Vol. 56, pp. 163-183, 1984

New Techniques for Imaging and Analyzing Lung Tissue

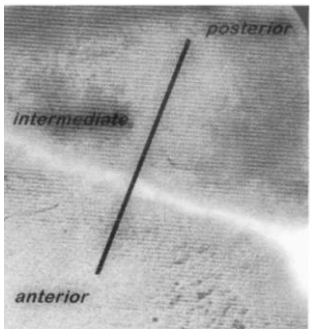
by Victor L. Roggli,* Peter Ingram,† Richard W. Linton,‡ William F. Gutknecht,† Pat Mastin,* and John D. Shelburne*

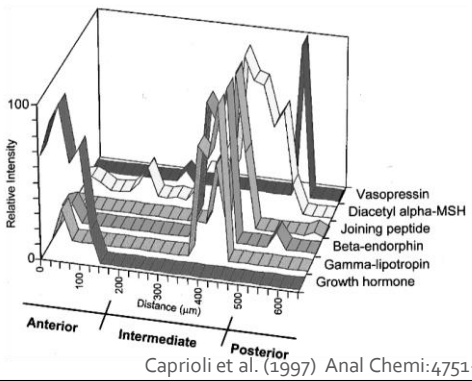
Early MSI: Bio-macromolecules

Molecular Imaging of Biological Samples: Localization of Peptides and Proteins Using MALDI-TOF MS

Richard M. Caprioli,* Terry B. Farmer, and Jocelyn Gile

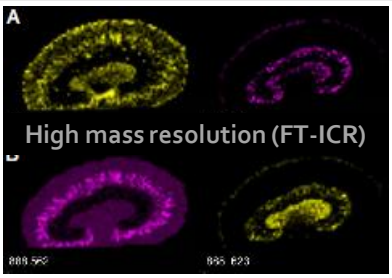
Anal. Chem. **1997,** *69,* 4751−4760

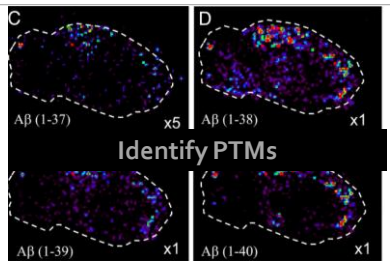


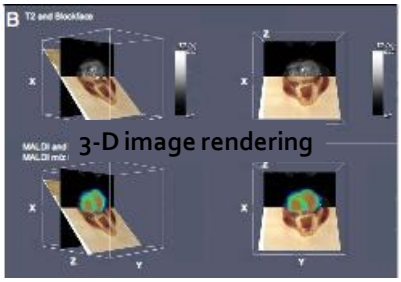


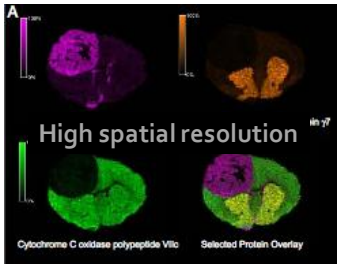
Caprioli et al. (1997) Anal Chemi:4751-60.

MSI today









Seeley et al. (2008) PNAS:18126-31.

What ionization source is commonly used?

- SIMS - Secondary Ion MS
- DESI - Desorption ElectroSpray Ionization
- MALDI - Matrix-Assisted Laser Desorption Ionization
- MALDESI
- LAESI -Laser Ablation ElectroSpray Ionization

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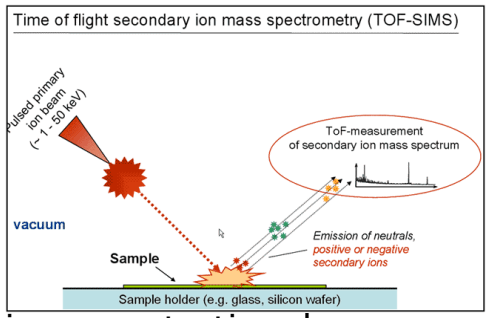
SIMS

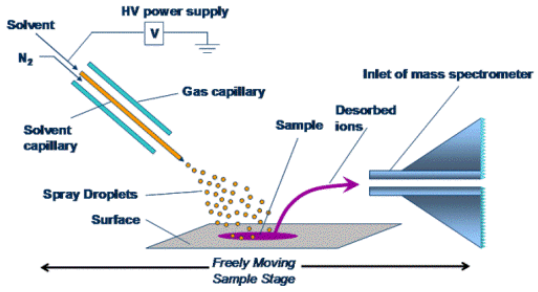
- Used in early studies (elemental analyses)
- Very high resolution (>50 nm)
- Principle of ionization: collated ion beam



- Destructive, penetrating, low mass range

DESI

- Moderate resolution (20 - 300 µm)
- Principle of ionization: Charged solvent spray



- Surface molecules, multiply charged, low - mid mass range

A pretty darn cool MSI experiment…

DESI image

Ink image

Computer-generated from preceding image

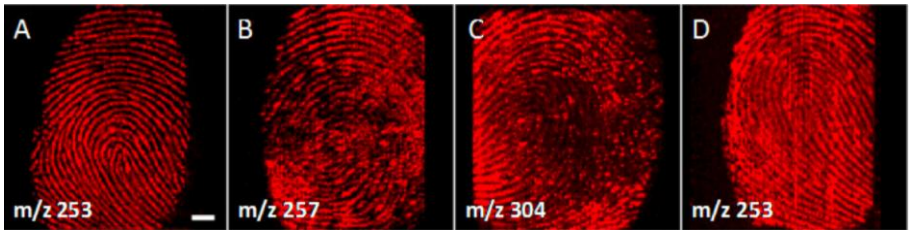
Ifa et al. (2008) PNAS Brevia:805.

A pretty darn cool MSI experiment…

Latent Fingerprint Chemical Imaging by Mass Spectrometry

Demian R. Ifa, Nicholas E. Manicke, Allison L. Dill, R. Graham Cooks*

Science AAAS

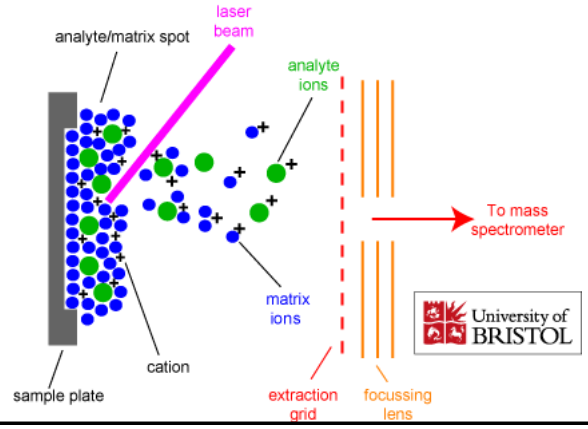


Fatty acid on glass	Explosive on plastic	Cocaine on adhesive tape	Fatty acid on adhesive tape

Ifa et al. (2008) PNAS Brevia:805.

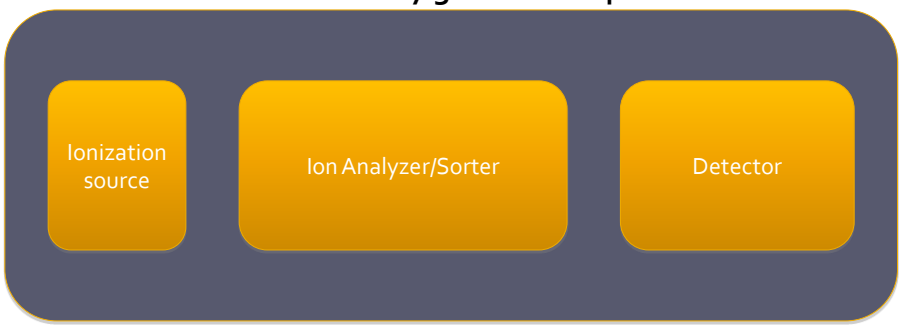
MALDI

- Currently the more commonly used ionization source in MSI



Analyzers/detectors

Basic schematic of any given mass spectrometer

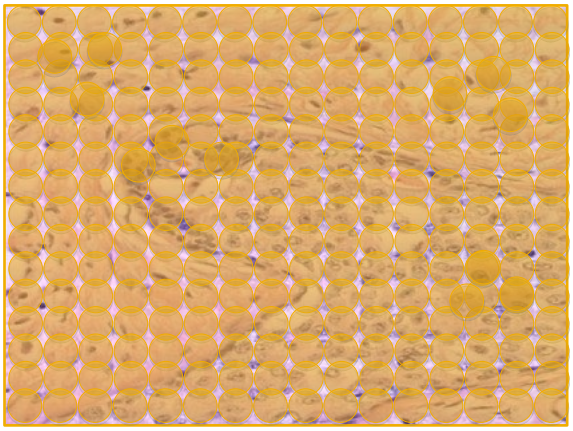


What MS platforms can you think of and how they can be used for MSI?

"Profiling" versus "imaging"

= MALDI matrix

BREAST CANCER TISSUE

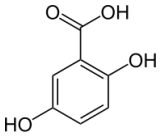


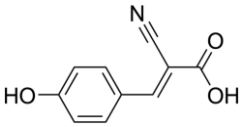
"Profiling" versus "imaging"

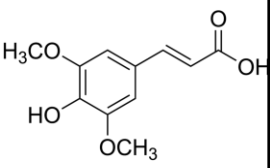
- Profiling:
  - Limited, directed information
  - Rapid analysis → high throughput
  - Useful for clinical applications and biomarker discovery.
- Imaging:
  - Extensive, high-resolution
  - Time consuming, laborious
  - Useful for investigative research (and fingerprints…)

Matrix choices - depends on desired analysis

- DHB- 2,5-dihydroxybenzoic acid
  - Commonly used for small molecules
- CHCA- α-Cyano-4-hydroxycinnamic acid
  - Commonly used for peptides and small proteins
- SA- Sinapinic acid
  - Commonly used for peptides and whole proteins (<100 kDa)







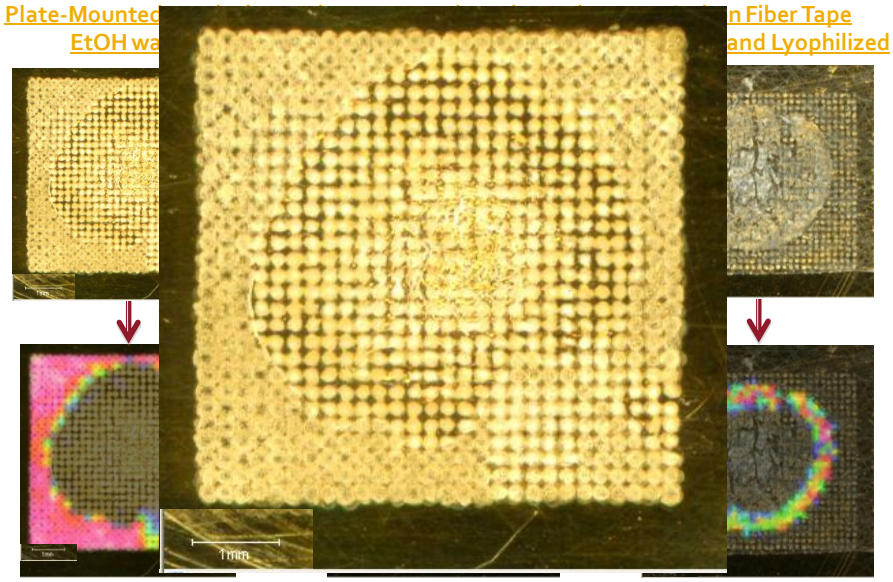
Matrix solvent/acid

- Acetonitrile is the solvent of choice
  - Range of percentage of solvent
    - Depends on application (tissue types)
  - Sometimes the solution is augmented with different additives including detergents
- Acid is also present in the matrix solution
  - Commonly formic acid
  - Promotes ionization
  - Ranges of percentage is also possible (upwards of 10%)

Spotting/spraying matrix

- Multiple technologies available:
  - Hand-spotting
  - TLC spraying
  - Sublimation
  - Precision mechanical spotting:
    - Acoustic devices.
    - Chemical printers
    - Inkjet printers

Many options = OPTIMIZATION!



Slide adapted and modified from Kyle Floyd

MSI: part II

Application of the technology

A true application of the technology

- The greatest project in the history of science
  - Led to and continues to lead to scientific journal articles
  - Facilitated the funding of multiple research projects
  - Advanced the field of vision science
  - AND…….

A true application of the technology

The University of Alabama
at Birmingham

By the authority of the Board of Trustees
on the recommendation of the faculty of the
University of Alabama at Birmingham Graduate School
the degree

Doctor of Philosophy

with all the accompanying rights and privileges
has been conferred upon

David Ray Stella

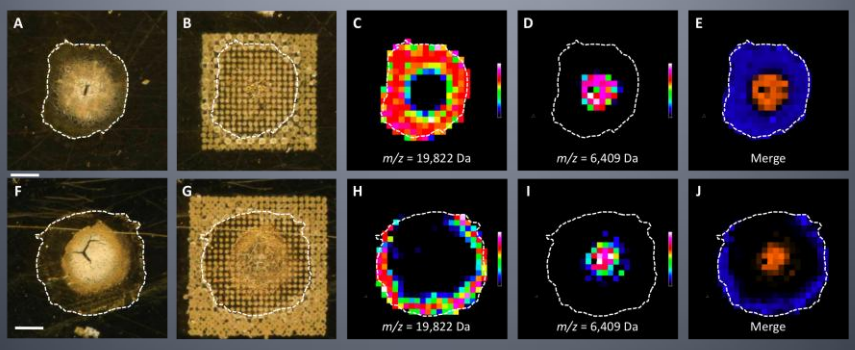
In Testimony Whereof, this diploma is issued with the seal of the University
and the signatures authorized by the Trustees hereunto affixed.

Given at the University at Birmingham on the
eighteenth day of December, 2010.

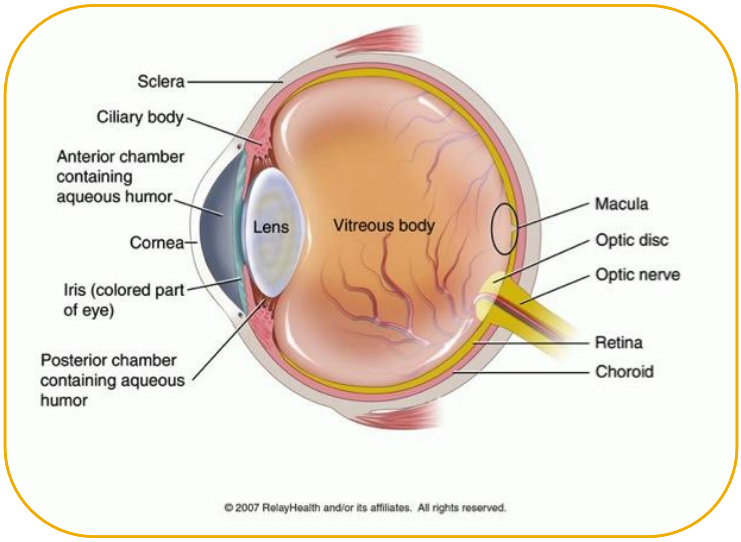
Carol Z. Garrison
President

Bryan D. Noe
Dean, Graduate School

LENS CATARACT: BIOCHEMICAL ANALYSIS OF THE ALPHA CRYSTALLINS



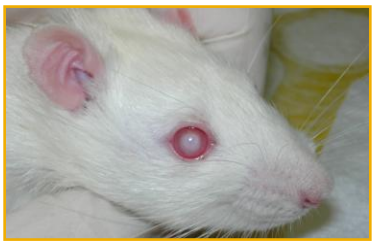
Anatomy of the eye

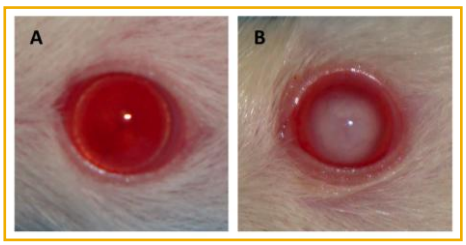


What was the project?

- Understand more about the protein localization in the ocular lens.
  - Interesting lens facts:
    - From "womb to tomb"
    - No protein turnover
    - Limited translated proteome
      - Expanded PTM proteome though!
    - Predominantly alpha crystallin proteins
      - small heat-shock proteins

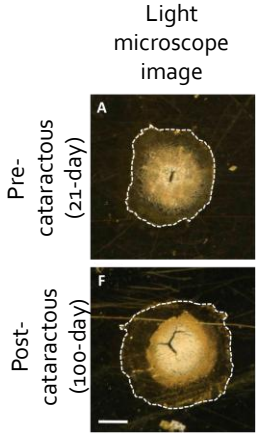
Rat model of cataract disease.





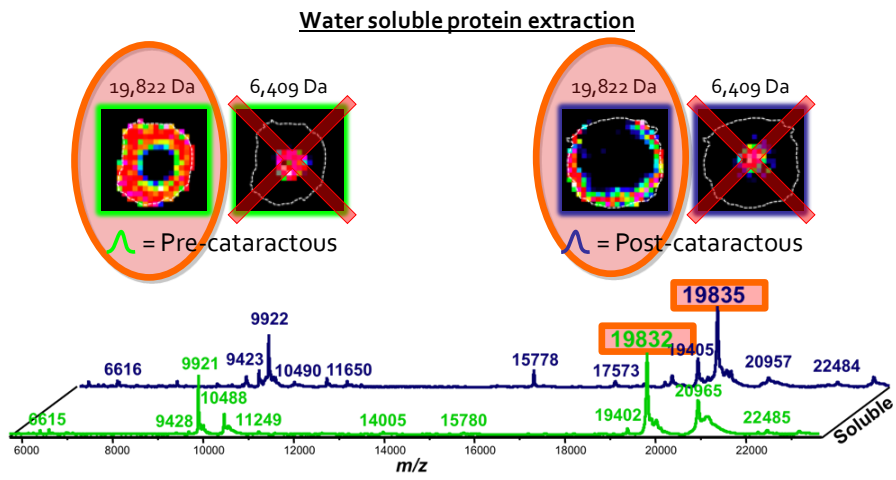
- ICR/f rat (Ihara/Inherited Cataract Rat, strain-f)
  - Model of age-related disease.
  - Spontaneously develops cataracts by 10 weeks of age.
    - Possible result of early oxidative insult.
    - Compare 21-day vs. 100-day

MALDI-MSI of the ICR/f rat lens



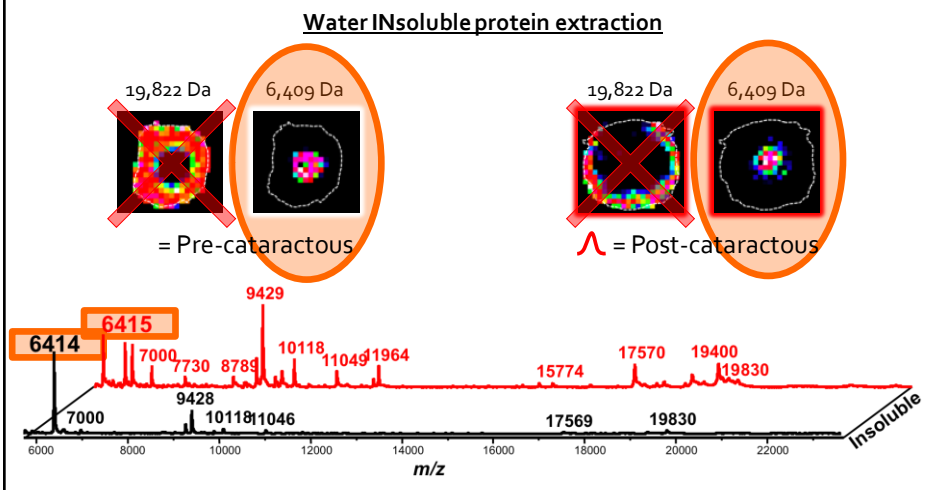
Stella et al. (2010) Invest Opth Vis Sci:5153 - 61.

MALDI-TOF solubility profiles: water soluble



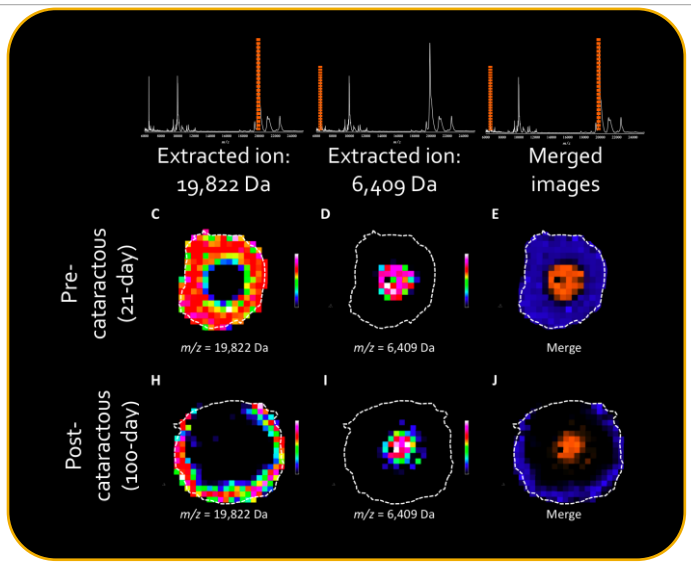
Stella et al. (2010) Invest Opth Vis Sci:5153 - 61.

MALDI-TOF solubility profiles: water INsoluble



Stella et al. (2010) Invest Opth Vis Sci:5153 - 61.

OK, so who are these proteins?



technologies/techniques are available for identifying these peaks

"Top-Down" Proteomics

Obtain protein identity ***without*** proteolytic digestion.

"chemistry in the gas phase"

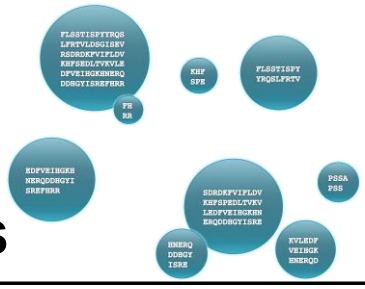
Using a high-resolution FT-ICR mass spectrometer:

1. Measure mass of the "whole" protein - "Precursor ion"
2. Measure masses of multiple "fragment products" = "Fragment ions"

Precursor Ion

Crash ion into inert gas

Fragment Ions



Top-Down Protein Assignment

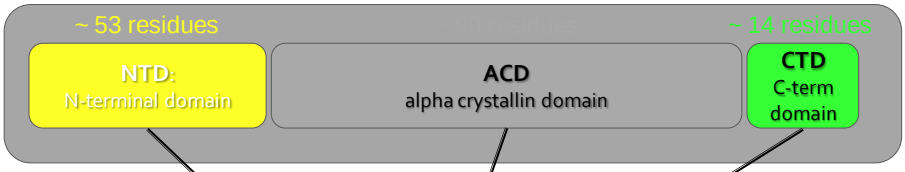
Protein ID	Predicted Mass (Da)*	Residues
Crystallin, alpha A	5,053.50	1-42
Crystallin, alpha A	6,409.19	1-53
Crystallin, alpha A	6,565.29	1-54
Crystallin, alpha A	9,284.76	1-78
Crystallin, alpha A	9,421.80	1-79
Crystallin, alpha A	10,110.11	1-85
Crystallin, alpha A	11,041.61	1-93
Crystallin, alpha A	11,842.04	1-100
Crystallin, alpha A	11,956.08	1-101
Crystallin, alpha A	17,562.77	1-151§
Crystallin, alpha A	18,043.96	1-156§
Crystallin, alpha A	18,200.06	1-157§
Crystallin, alpha A	18,823.44	1-163§
Crystallin, alpha A	19,393.70	1-168§
Crystallin, alpha A	19,822.89	1-173§

Full length →

* = N-terminal acetylation included (+42.01 Da)

Stella et al. (2010) Invest Opth Vis Sci:5153 - 61.

sHSP Structure: Domains

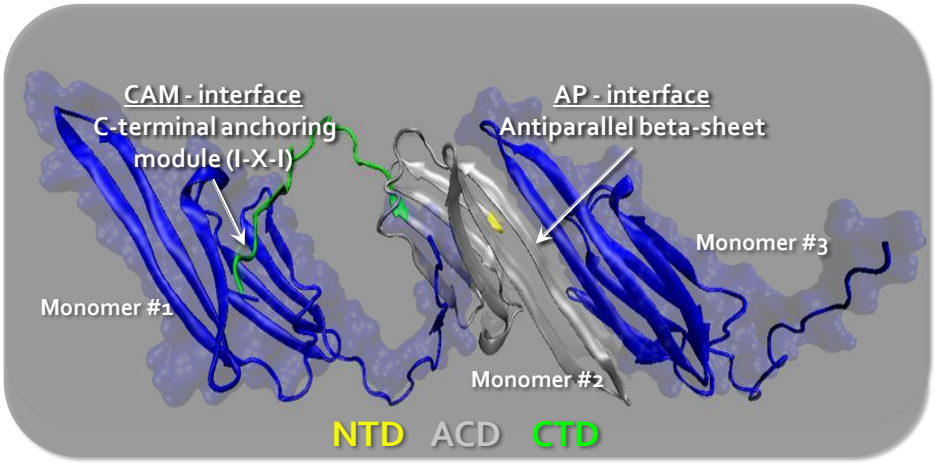


Substrate recognition and oligomerization

Core structural domain

- NTD - mostly substrate recognition
  - Only 25 -30% sequence identity
  - 1.5 Å RMSD with 10 sHSP structures
- CTD - mostly oligomerization
  - I/V/L-X-I/V/L ("I-X-I") motif - Found in 96% of >4400 sHSP sequences

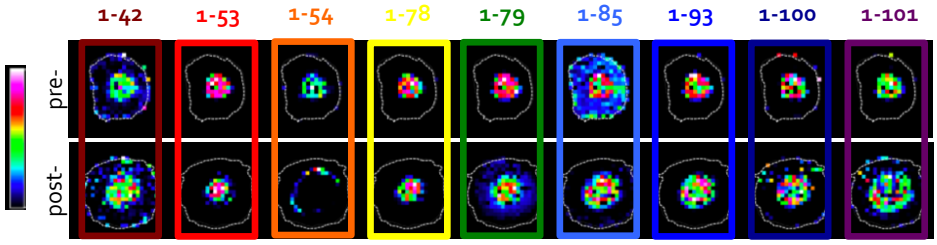
Partial bovine αA-crystallin Structure: Dimerization Interfaces



Laganowsky et al. (2010) Prot. Sci. :1031-43
Image generated using PDB: 3L1E with VMD 1.8.6 software

Localization of Validated αA-crystallin Protein Species

1. Nuclear localization



MDVTIQHPWFKRALGPFYPSRLFDQFFGEGLFEYDLLPFLSSTI
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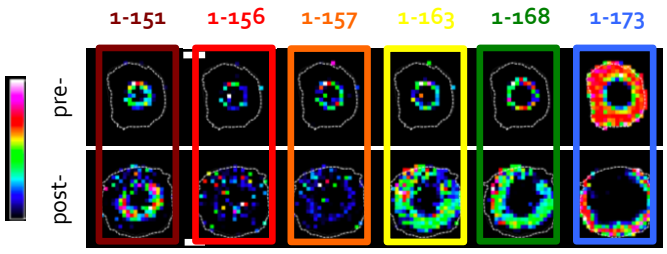
VLEDFVEIHGKHNERQDDHGYISREFHRRYRLPSNVDQSALSC
SLSADGMLTFSGPKVQSGLDAGHSERAIPVSREEKPSSAPSS

NTD ACD CTD

Stella et al. (2010) Invest Opth Vis Sci:5153 - 61.

Localization of Validated αA-crystallin Protein Species

2. Cortical/Nuclear-ring localization



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VLEDFVEIHGKHNERQDDHGYISREFHRRYRLPSNVDQSALSC
SLSADGMLTFSGPKVQSGLDAGHSERAIPVSREEKPSSAPSS

NTD ACD CTD

MS Imaging Study Summary: Biologically funcational PTMs

- 14 αA-crystallin truncation products were observed in the ICR/f rat lens.
  - Independent of cataract state.
- αA-crystallin with greater than 22 C-terminally truncated residues were:
  1. localized within lens nucleus.
  2. enriched in the water-insoluble fraction

Overall

- MSI is providing researchers new tools
- The field has rapidly expanded over the last 5-10 years.
  - An increasing interest is leading to technological breakthroughs
    - Matrix spotting technologies, novel ionization techniques, novel MALDI matrices
- Has across-the-board capabilities - clinical and basic research!