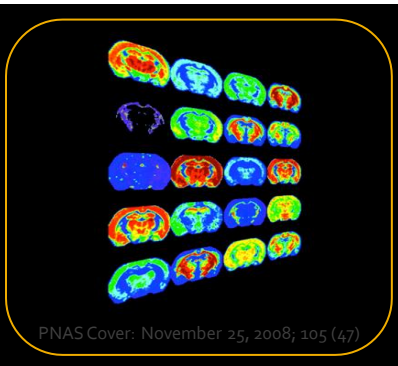


PNAS Cover: November 25, 2008; 105 (47)

BMG/PHR 744 - Spring 2012
David Stella
March 2nd, 2012

Mass Spectrometry Imaging (MSI)

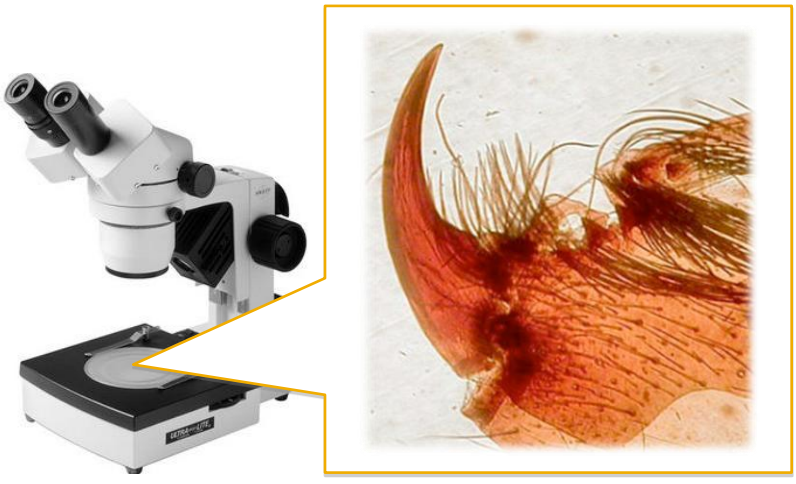
MSI: part I

What usefulness is imaging in the pursuit of biological research?

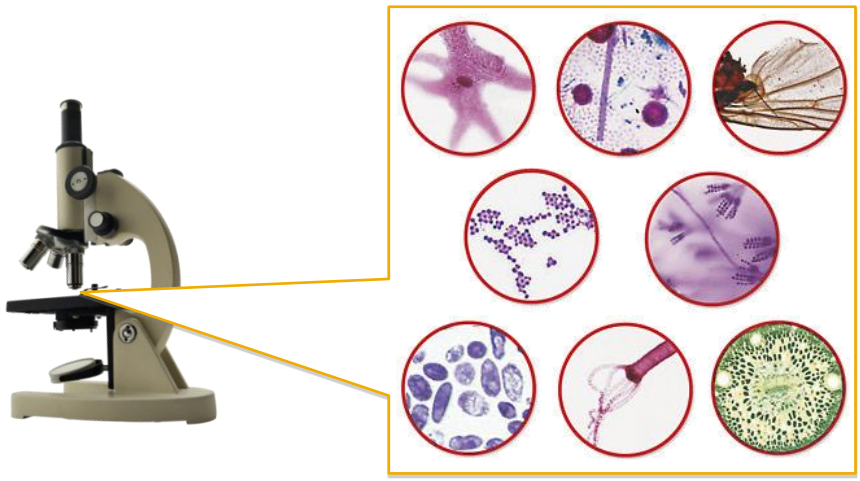
Imaging Experiments

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

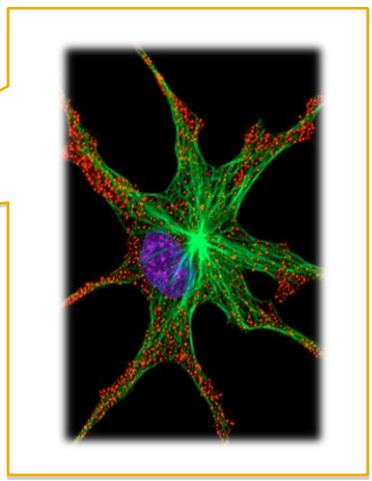
Imaging and biological research: dissecting microscope



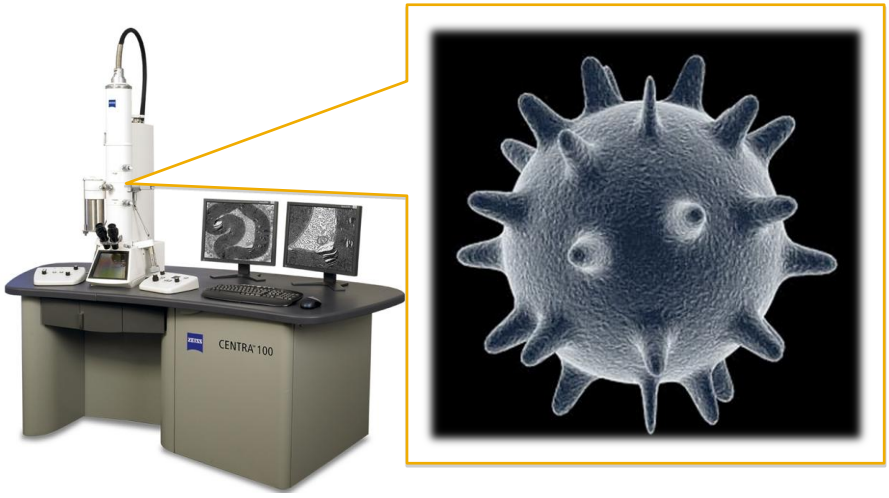
Imaging and biological research: light microscopy



Imaging and biological research: confocal microscopy



Imaging and biological research: electron microscopy



The technology

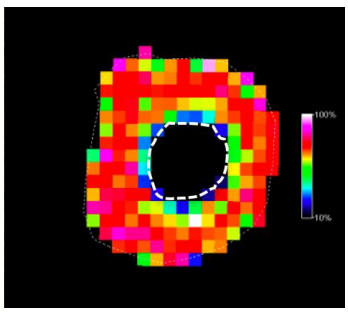
- Using MS to create pretty images.
- Comparable to traditional histochemistry techniques to identify macromolecular species present in tissue sections
- Diverse applications and approaches to answering biological research questions

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)

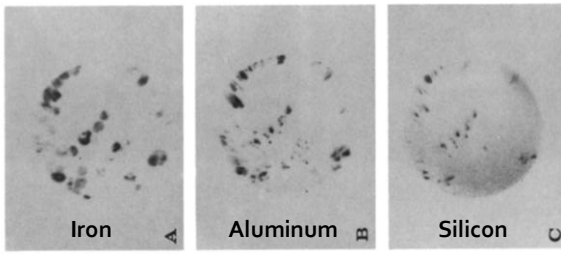
MALDI-TOF mass spectrometer



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

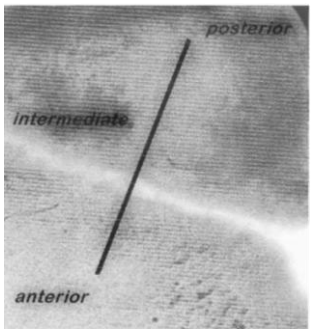
by Victor L. Roggli,* Peter Ingram,† Richard W. Linton,‡ William F. Gutknecht,† Pat Mastin,* and John D. Shelburne*

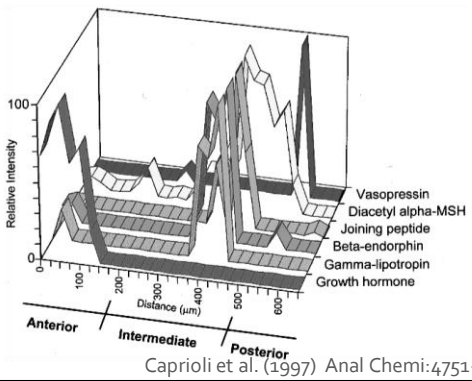
Early MSI: Bio-macromolecules

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

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

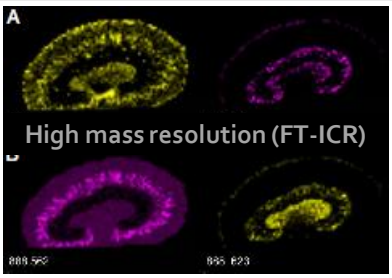
Anal. Chem. **1997,** *69,* 4751−4760

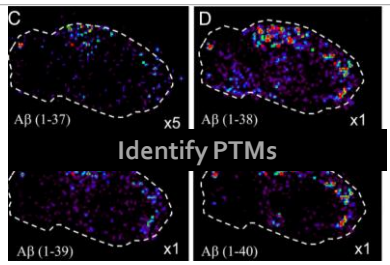


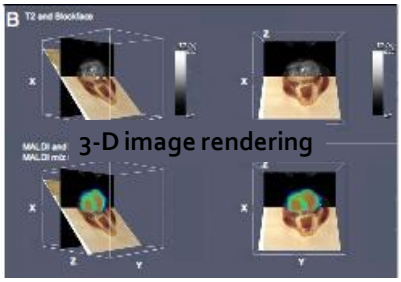


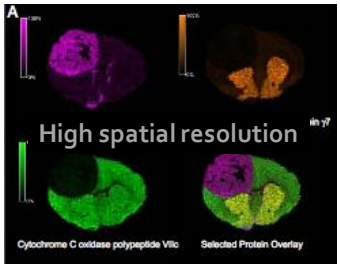
Caprioli et al. (1997) Anal Chemi:4751-60.

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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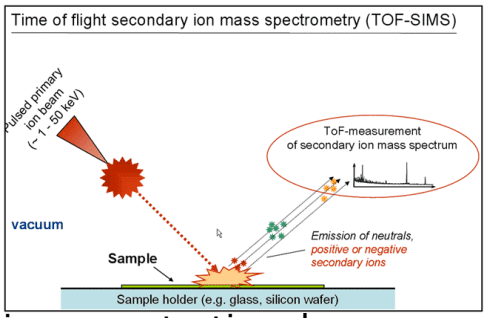
SIMS

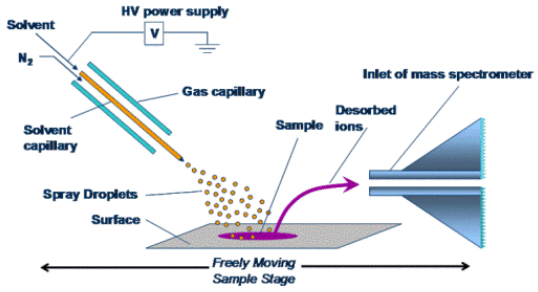
- Used in early studies (elemental analyses)
- Very high resolution (>50 nm)
- Principle of ionization: collated ion beam



- 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

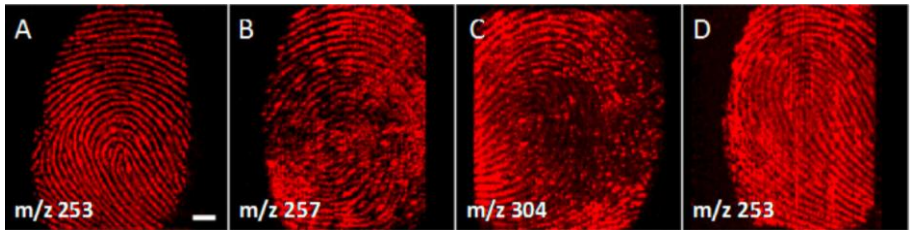
Ifa et al. (2008) PNAS Brevia:805.

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

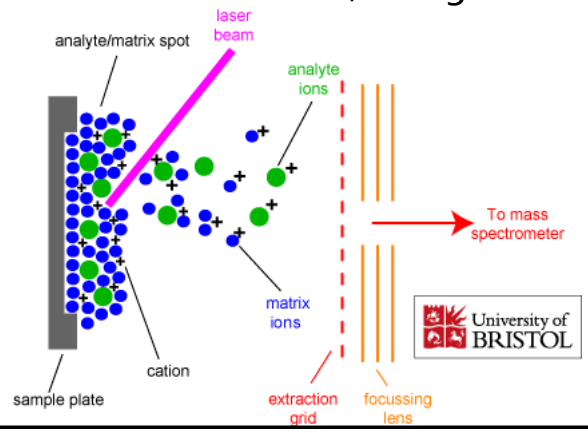


Fatty acid on glass	Explosive on plastic	Cocaine on adhesive tape	Fatty acid on adhesive tape

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

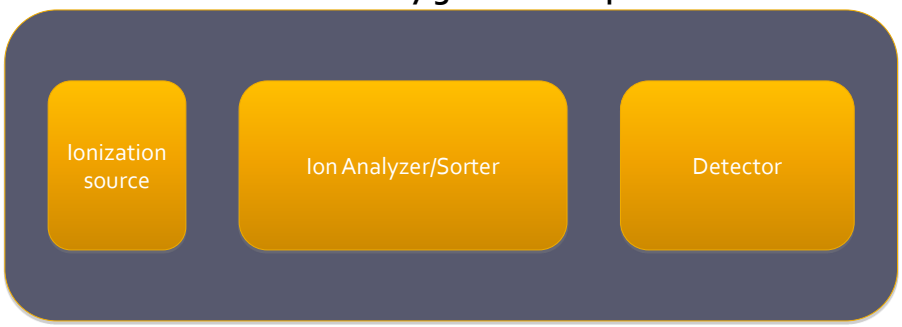
MALDI

- Currently the more commonly used ionization source in MSI (biological research)



Analyzers/detectors

Basic schematic of any given mass spectrometer

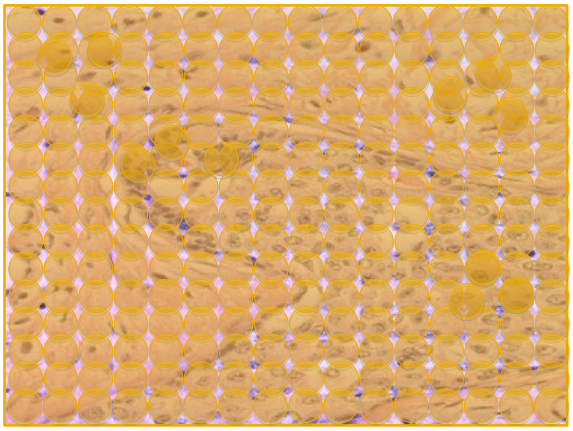


What MS platforms can you think of and how they can be used for MSI?

"Profiling" versus "imaging"

= MALDI matrix

BREAST CANCER TISSUE

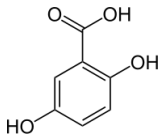


"Profiling" versus "imaging"

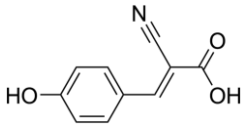
- Profiling:
  - Limited, directed information
  - Rapid analysis → high throughput
  - Useful for clinical applications and biomarker discovery.
- Imaging:
  - Extensive, high-resolution
  - Time consuming, laborious
  - Useful for investigative research (and fingerprints…)

Matrix choices - depends on desired analysis

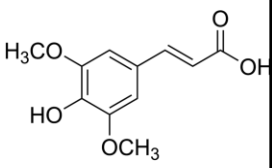
- DHB- 2,5-dihydroxybenzoic acid
  - Commonly used for small molecules



- 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)



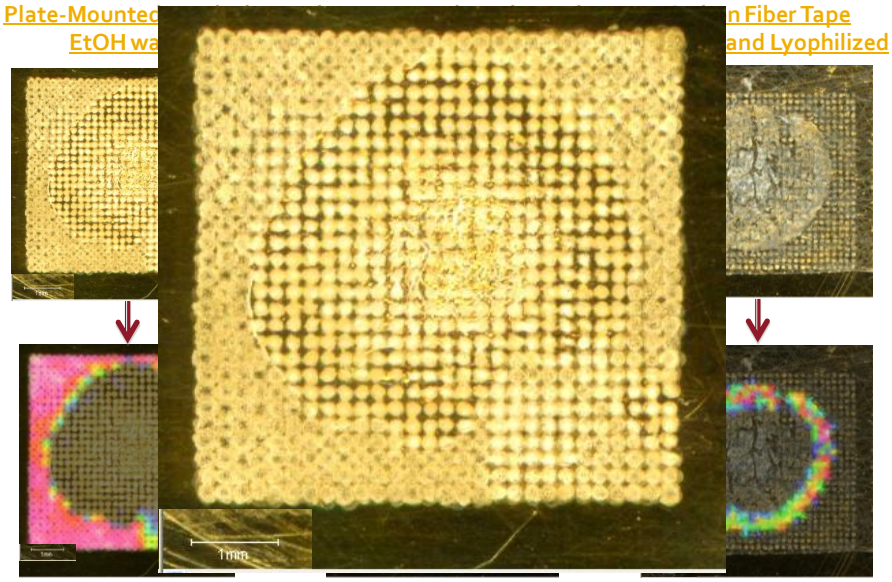
Matrix solvent/acid

- 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…….

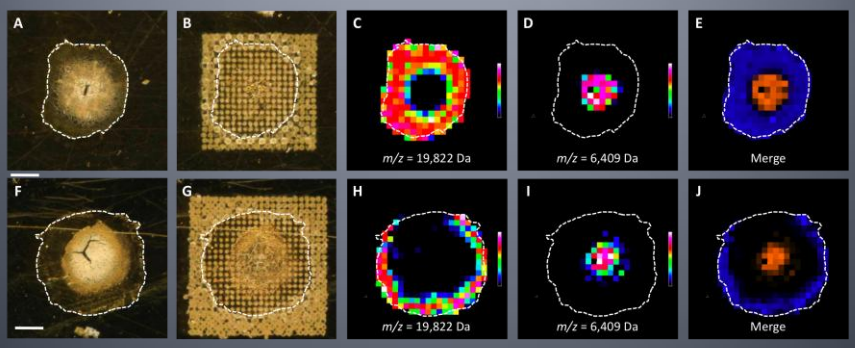
LENS CATARACT:
BIOCHEMICAL ANALYSIS OF THE
ALPHA CRYSTALLINS

DAVID RAY STELLA

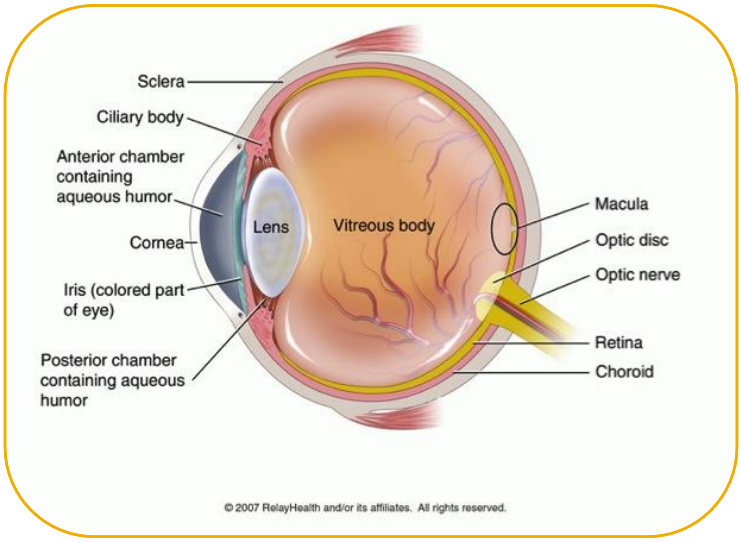
UNIVERSITY OF ALABAMA
AT BIRMINGHAM

2010

LENS CATARACT: BIOCHEMICAL ANALYSIS OF THE ALPHA CRYSTALLINS



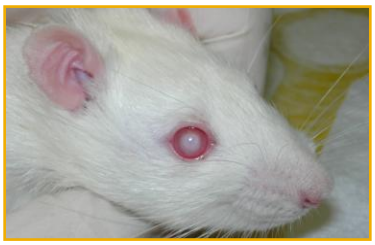
Anatomy of the eye

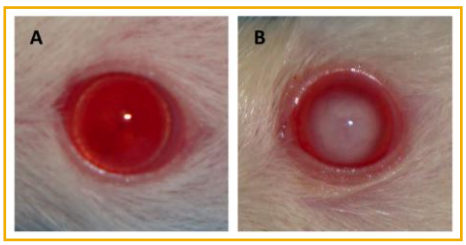


What was the project?

- 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

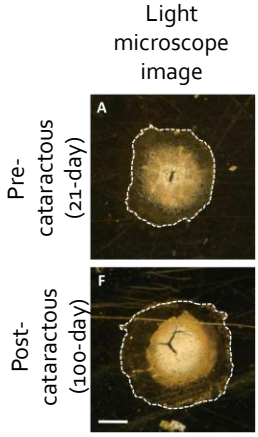
Rat model of cataract disease.





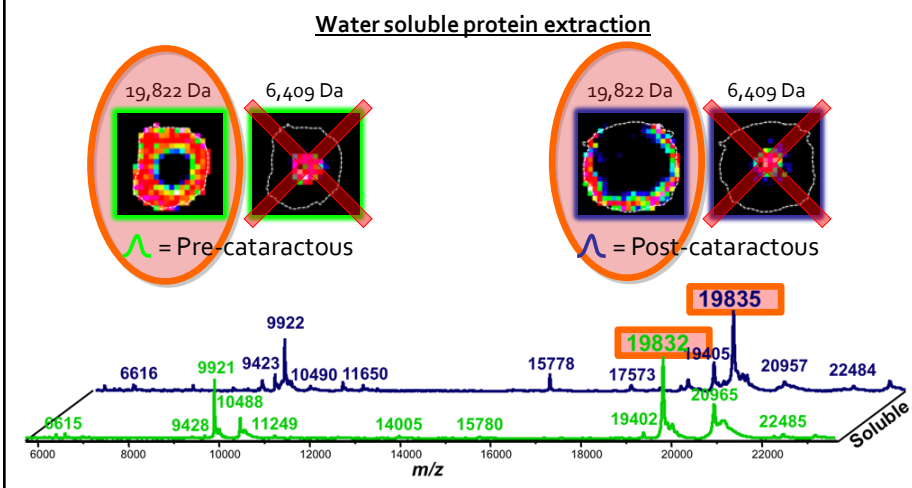
- ICR/f rat (Ihara/Inherited Cataract Rat, strain-f)
  - Model of age-related disease.
  - Spontaneously develops cataracts by 10 weeks of age.
    - Possible result of early oxidative insult.
    - Compare 21-day vs. 100-day

MALDI-MSI of the ICR/f rat lens



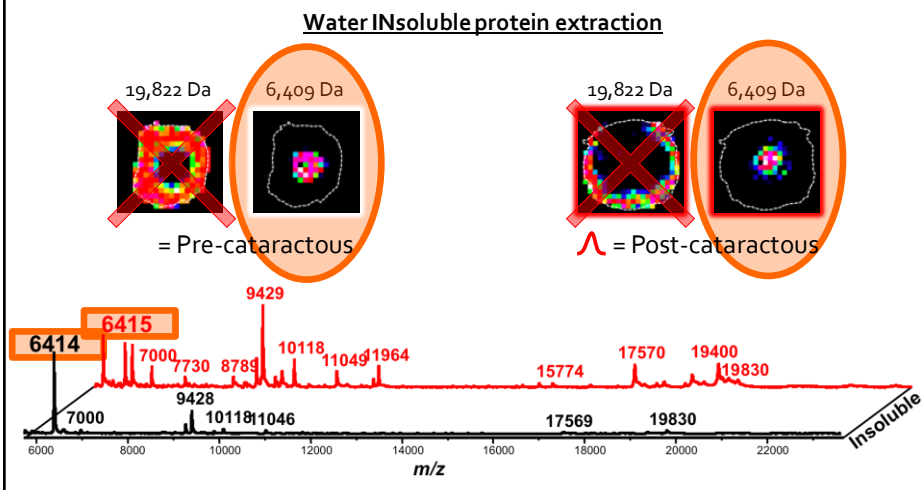
Stella et al. (2010) Invest Opth Vis Sci:5153 - 61.

MALDI-TOF solubility profiles: water soluble



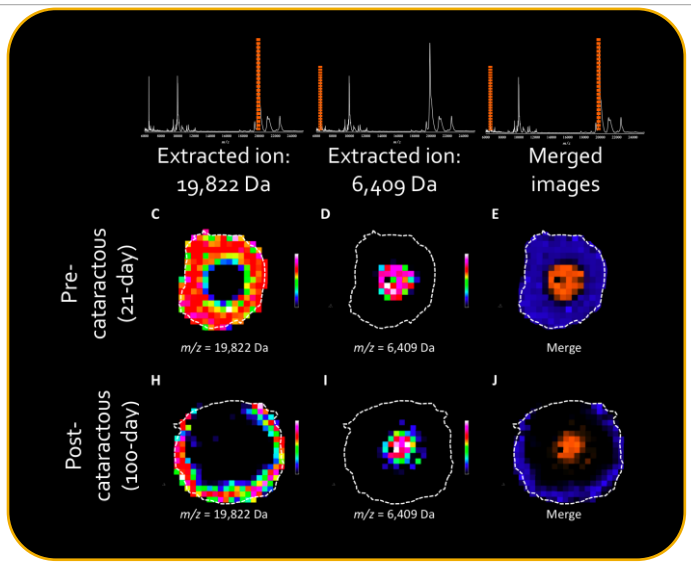
Stella et al. (2010) Invest Opth Vis Sci:5153 - 61.

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"

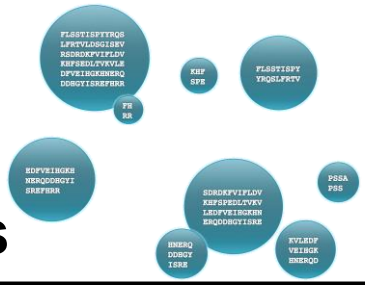
Using a high-resolution FT-ICR mass spectrometer:

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

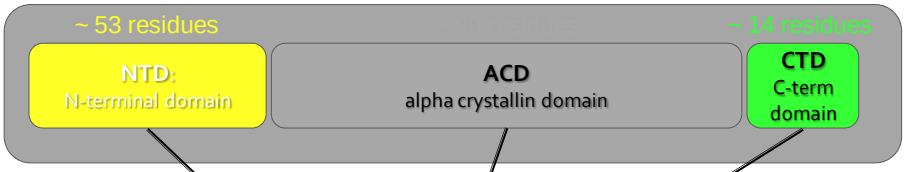
Protein ID	Predicted Mass (Da)*	Residues
Crystallin, alpha A	5,053.50	1-42
Crystallin, alpha A	6,409.19	1-53
Crystallin, alpha A	6,565.29	1-54
Crystallin, alpha A	9,284.76	1-78
Crystallin, alpha A	9,421.80	1-79
Crystallin, alpha A	10,110.11	1-85
Crystallin, alpha A	11,041.61	1-93
Crystallin, alpha A	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

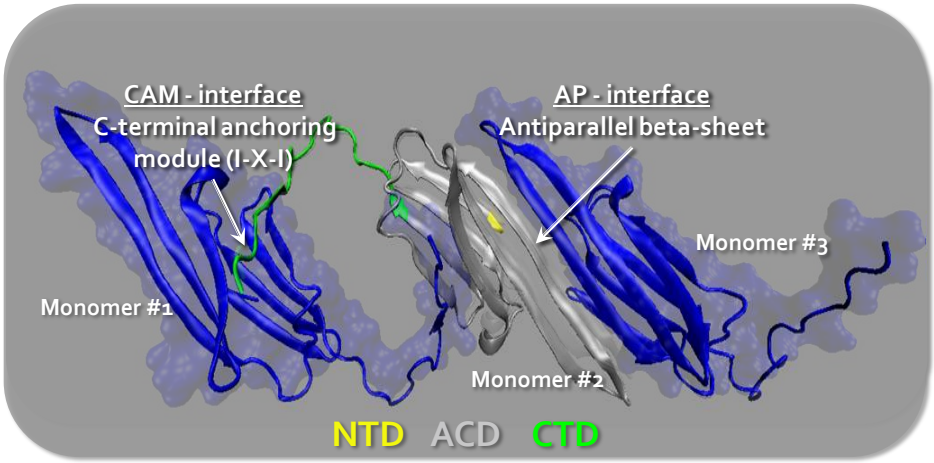


Substrate recognition and oligomerization

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

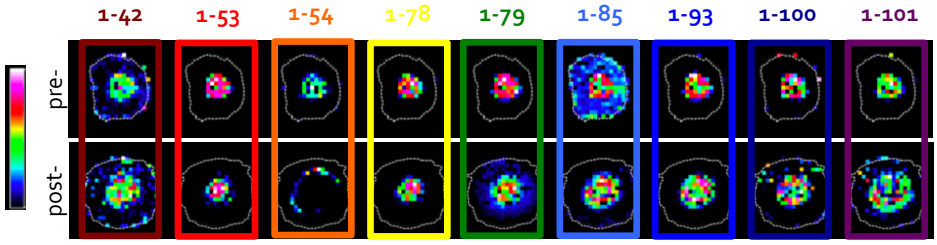
Partial bovine αA-crystallin Structure: Dimerization Interfaces



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



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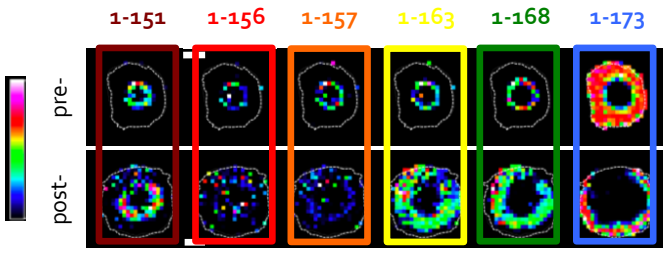
SPYYRQSLFRTVLDSGISEVRSDRDKFVIFLDVKHFSPEDLTVK
VLEDFVEIHGKHNERQDDHGYISREFHRRYRLPSNVDQSALSC
SLSADGMLTFSGPKVQSGLDAGHSERAIPVSREEKPSSAPSS

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!