How well do we REALLY understand MALDI?

Kevin G. Owens
Department of Chemistry
Drexel University
Philadelphia, PA

4th Annual NIST Polymer MS Workshop
National Institute of Standards & Technology
Gaithersburg, MD
November 9, 2005

Future Directions in the Soft Ionization of Polymers

In order to predict the future you really have to know the past.

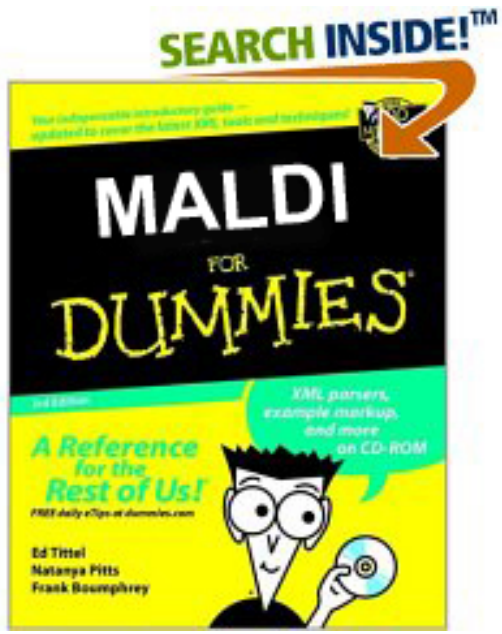
“Prediction is very difficult, especially if it's about the future”. Niels Bohr

“UnMALDIable” “It doesn’t fly”

The “black art” (or “magic”) of MALDI sample preparation

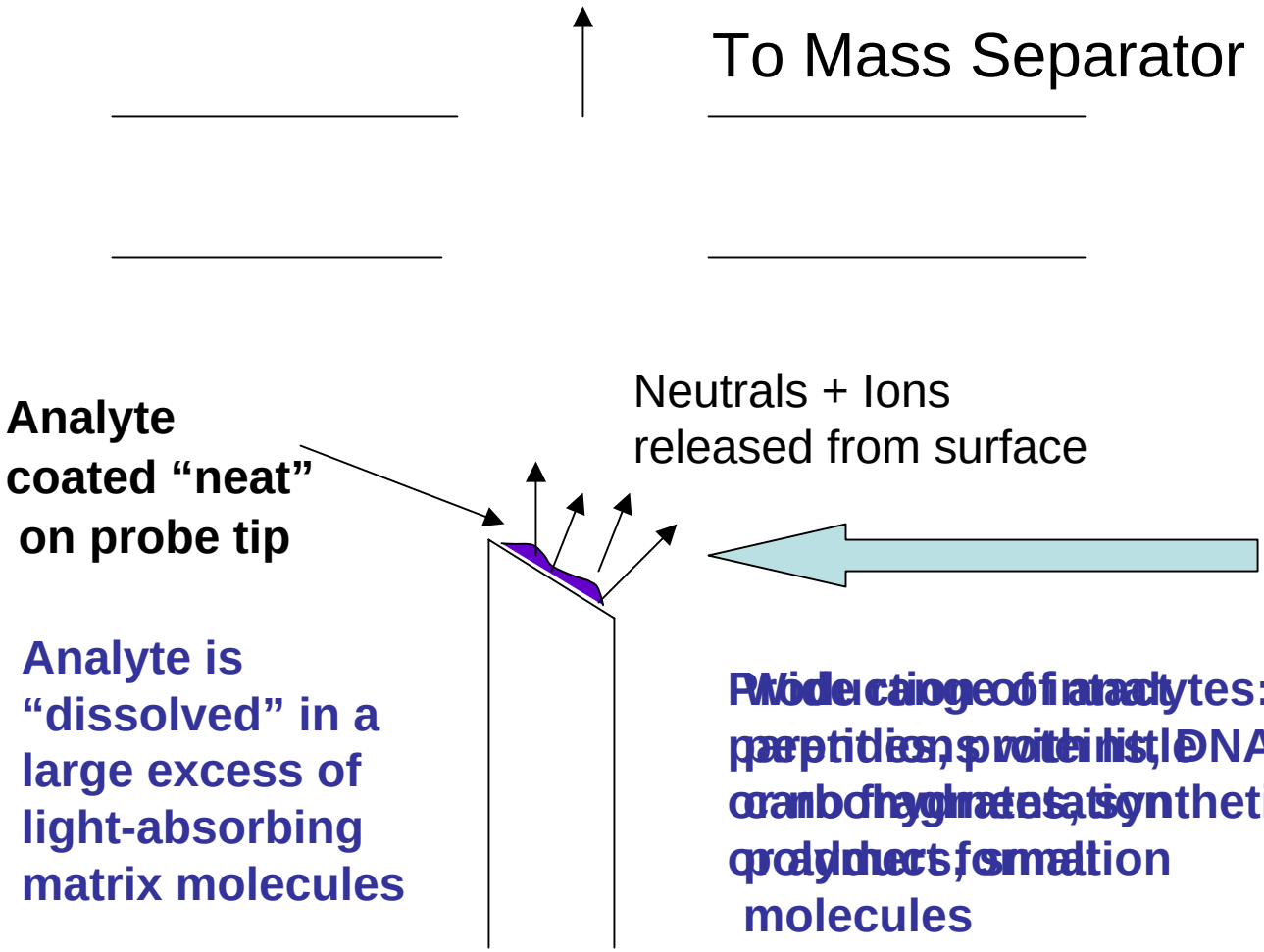
“Get the right seven experts in a room and the MALDI process will be understood”. Kevin Owens

A select (and small) group of people are working on fundamentals; some (many) users think everything is known about the process.



Laser Desorption Mass Spectrometry (LDMS)

Matrix Assisted Laser Desorption/Ionization (MALDI)



UV Lasers:

Nitrogen
Excimer
Nd:YAG (3rd+4th)
Doubled Dye

IR Lasers :

CO_2(10.6 μm)
Er:YAG (2.94 μm)

Laser Desorption Mass Spectrometry - Variations on a theme...

Observations: low MW compounds work. As MW increases you need to increase the laser intensity, but at high intensity the compounds fragment

LD

Liquid matrix with solid particles (e.g. cobalt power or graphite)

MALDI

DIOS

SELDI

Ionic liquid matrixes

MELDI

Solid sample preparation

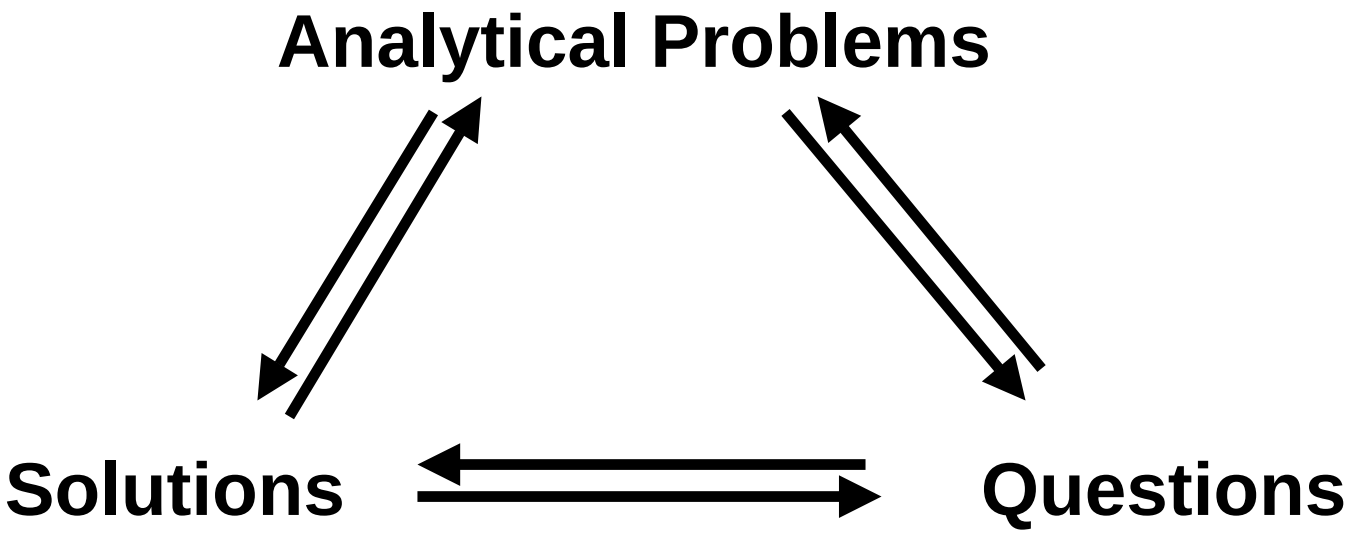
Hydrophobic surfaces

nanoparticle “Matrix”

Why are there variations on a theme?

- Because there are different types of analytes that pose different types of problems*/challenges/questions to be answered…

* "Problems are really just useful information at inconvenient times." Bill Erb, 26th Annual East Coast Ion Chemistry Conference, Department of Chemistry & Biochemistry, University of Delaware, Newark, DE, October 2005.


Analytical Problems
Solutions
Questions

Problem... We need to analyze higher MW (less volatile, more highly functionalized) analytes

Solution:

- Add a matrix (use MALDI)

Functions that the matrix must perform:

- Disperse the analyte
- Reduce/eliminate interaction with the sample surface
- Absorb the laser light
- Disintegrate/dissociate at low energy
- Desorb the analyte from the sample surface
- Ionize the analyte

"The matrix is what falls apart first." Rick King

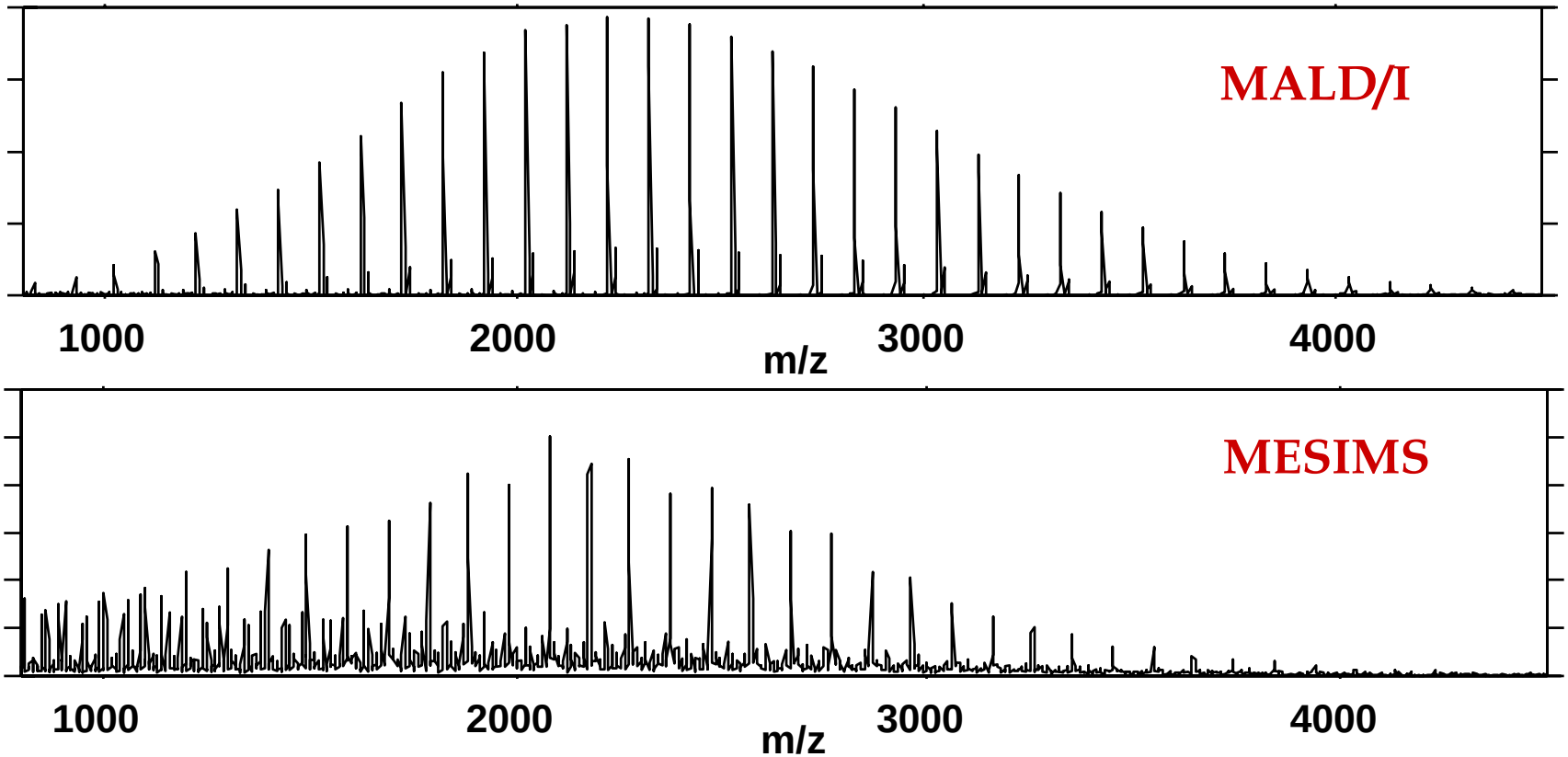
Problem... How do you select a “good” matrix for an analyte of interest?

- You must understand the chemistry of the MALDI process
  - The MALDI plume is a “reaction vessel”
- The sample preparation must provide favorable conditions for desorption
  - Owens/Hoteling et al. Solubility is important
- Chemistry of ionization - favorable reactions for ion formation
  - Knochenmuss model
  - Relative physical-chemical properties of the matrix and analyte (e.g., proton affinity, cation affinity, etc.)

Questions (sample preparation) to consider:

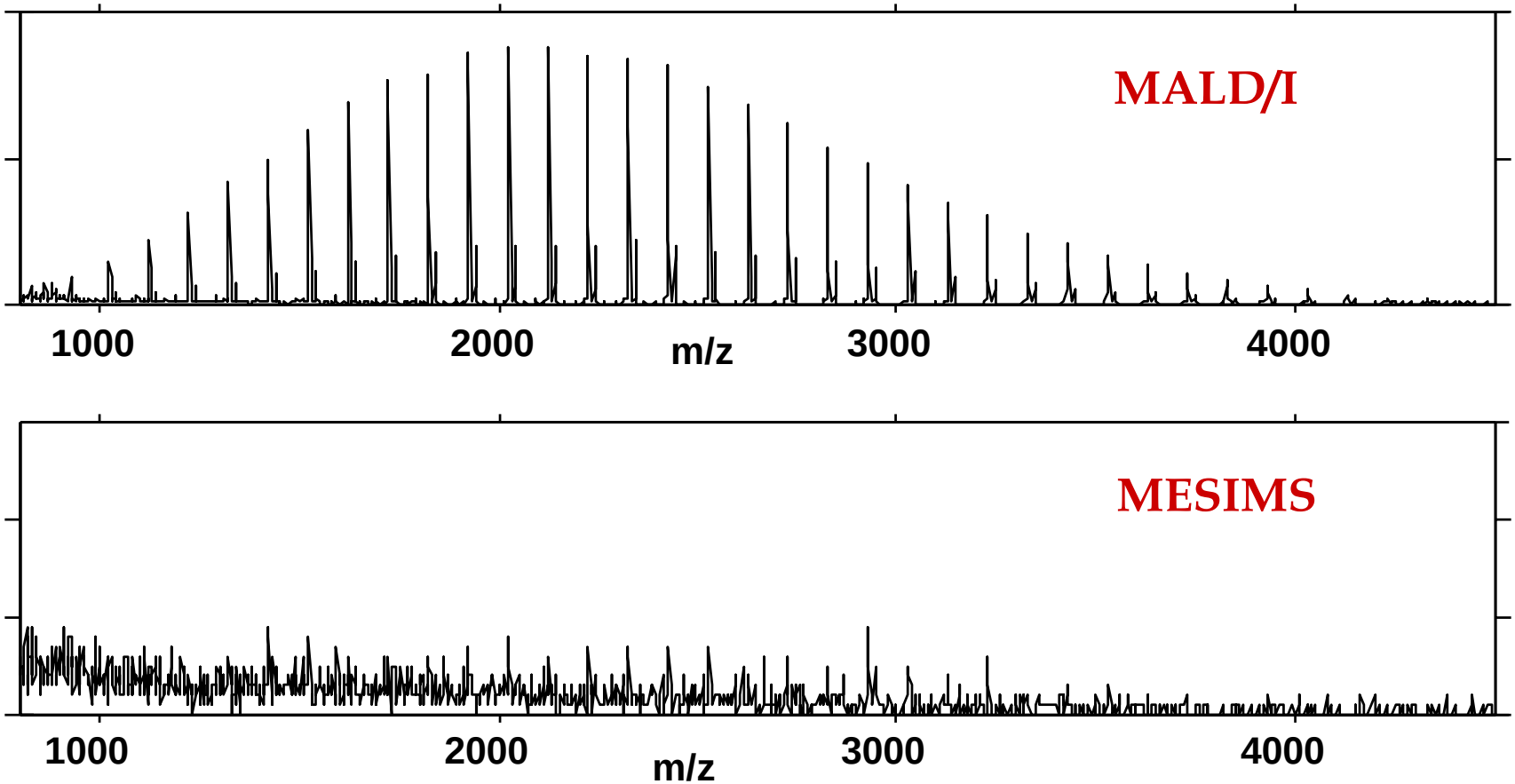
- Do the analyte molecules have to be molecularly isolated within the matrix?
- Are the analyte molecules solubilized by the matrix?
- How big of an analyte cluster (aggregate) is too big?
- Do the analyte molecules have to be soluble to be analyzed by MALDI?
- Is the analyte inside the matrix crystal, or simply on the surface?
- Does it matter?
  - Fluorescence microscopy
  - **MESIMS**
  - **Layered sample preparation studies (using ES deposition)**

MALDI versus MESIMS: PMMA/DHB



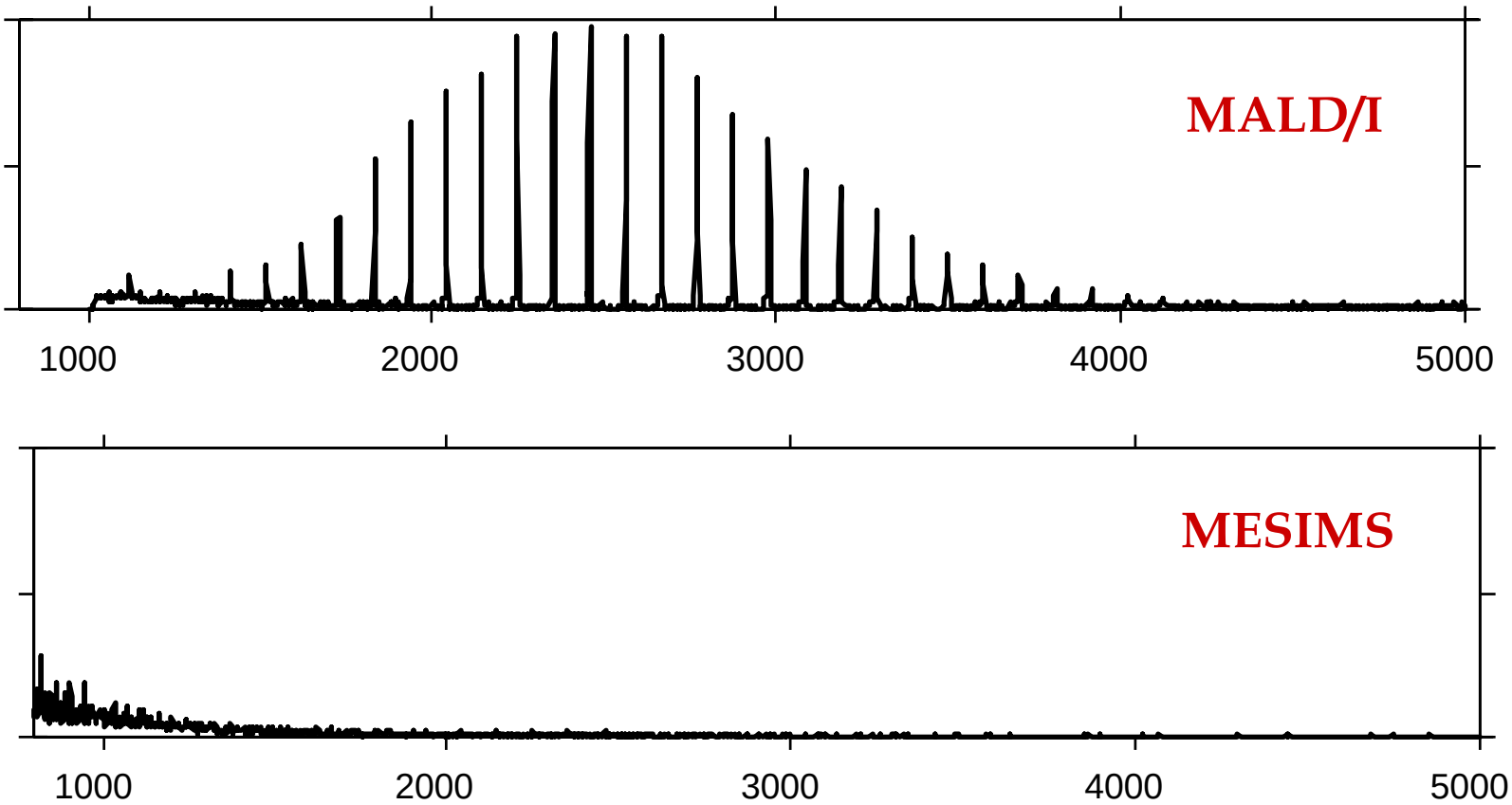
- MESIMS can be effective for PMMA with DHB

MALDI vs. MESIMS: PMMA/Ferulic Acid



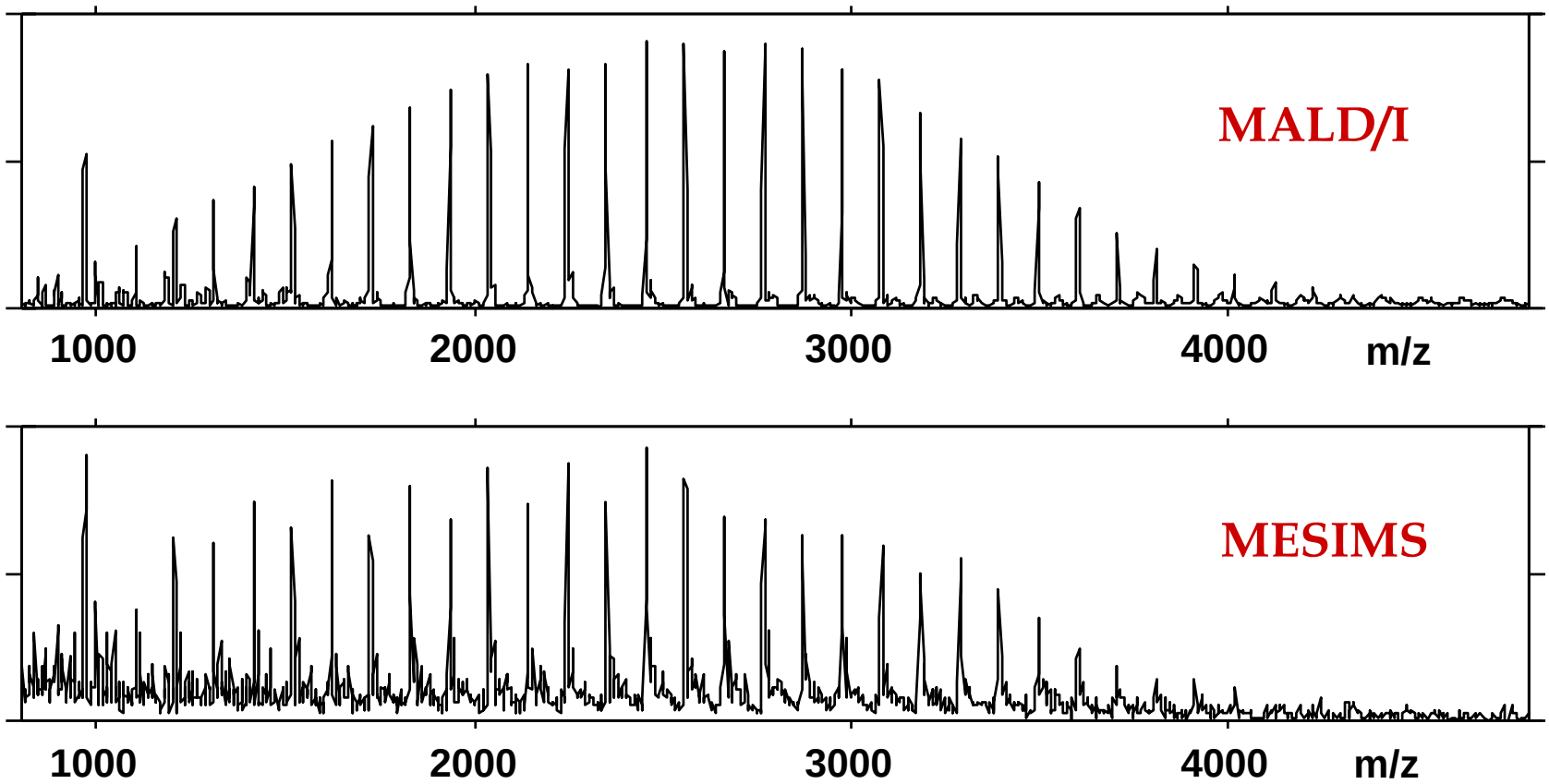
- No PMMA at the Ferulic acid matrix surface

MALDI vs. MESIMS: PS/Dithranol



- No PS2450 at the Dithranol matrix surface.

MALDI vs. MESIMS: PS/DHB



- MESIMS can be effective for PS2450 with DHB.

MESIMS Results Summary

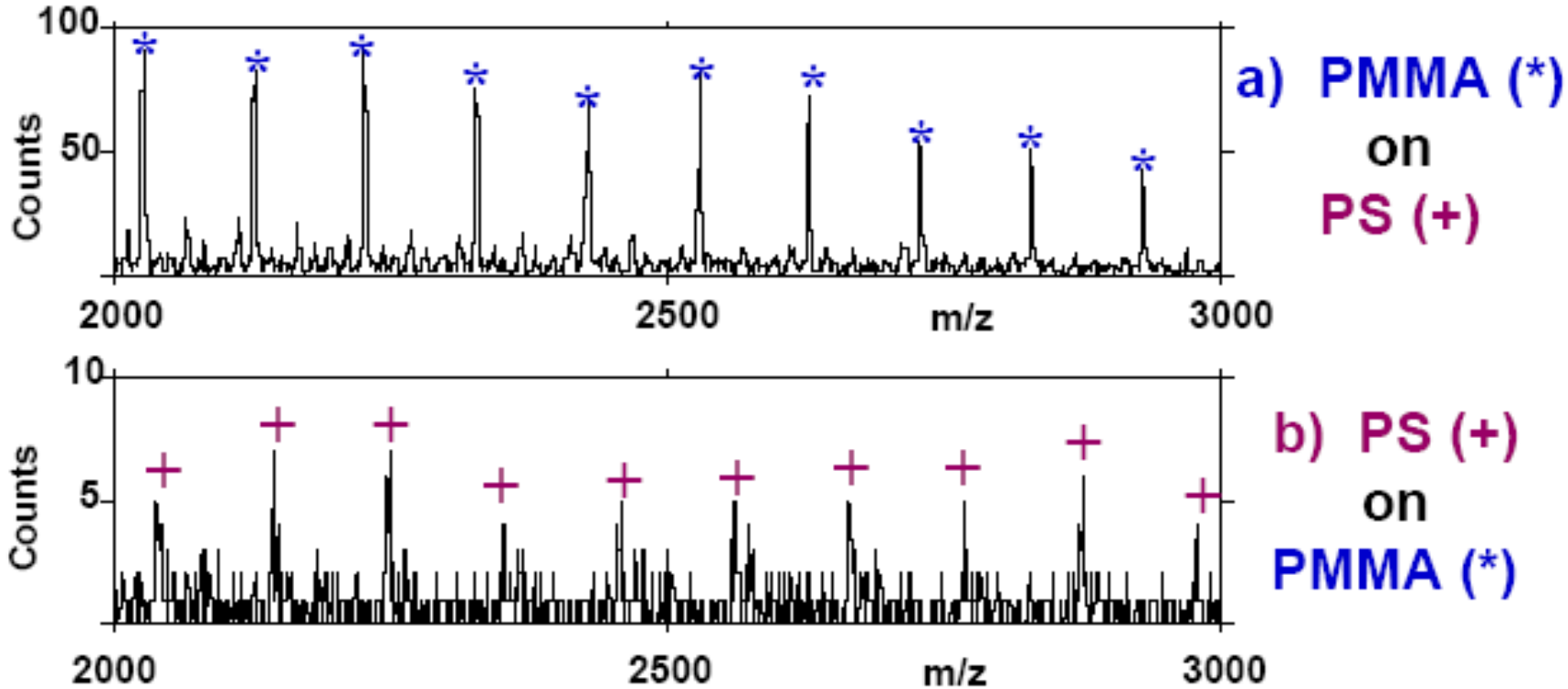
Are Polymer Ions Observed By MESIMS?

Polymer	PEG	PPO	PEF	PVAc	PTMEG	PMMA	PS	PBD	PDMS
MW:	1000	2025	1500	1500	1800	2900	2450	1300	1500
DPBD				yes	no	**no**	yes	no	yes
Ret A		no *				no *	**no**	**no**	yes
Dith			yes	**no**	**no**	**no**	**no**	yes	no
IAA	yes	yes	**no**	**no**	**no**	**no**	**no**	yes	yes
FA	yes	yes	yes	yes	**no**	**no**	**no**	yes	yes
CHCA	yes	yes	yes	yes	yes	yes	yes	yes	yes
DHB	yes	yes	yes	yes	yes	yes	yes	no	yes
TU	yes		**no**	**no**	yes				

no * indicates no polymer observed due to segregation of cation

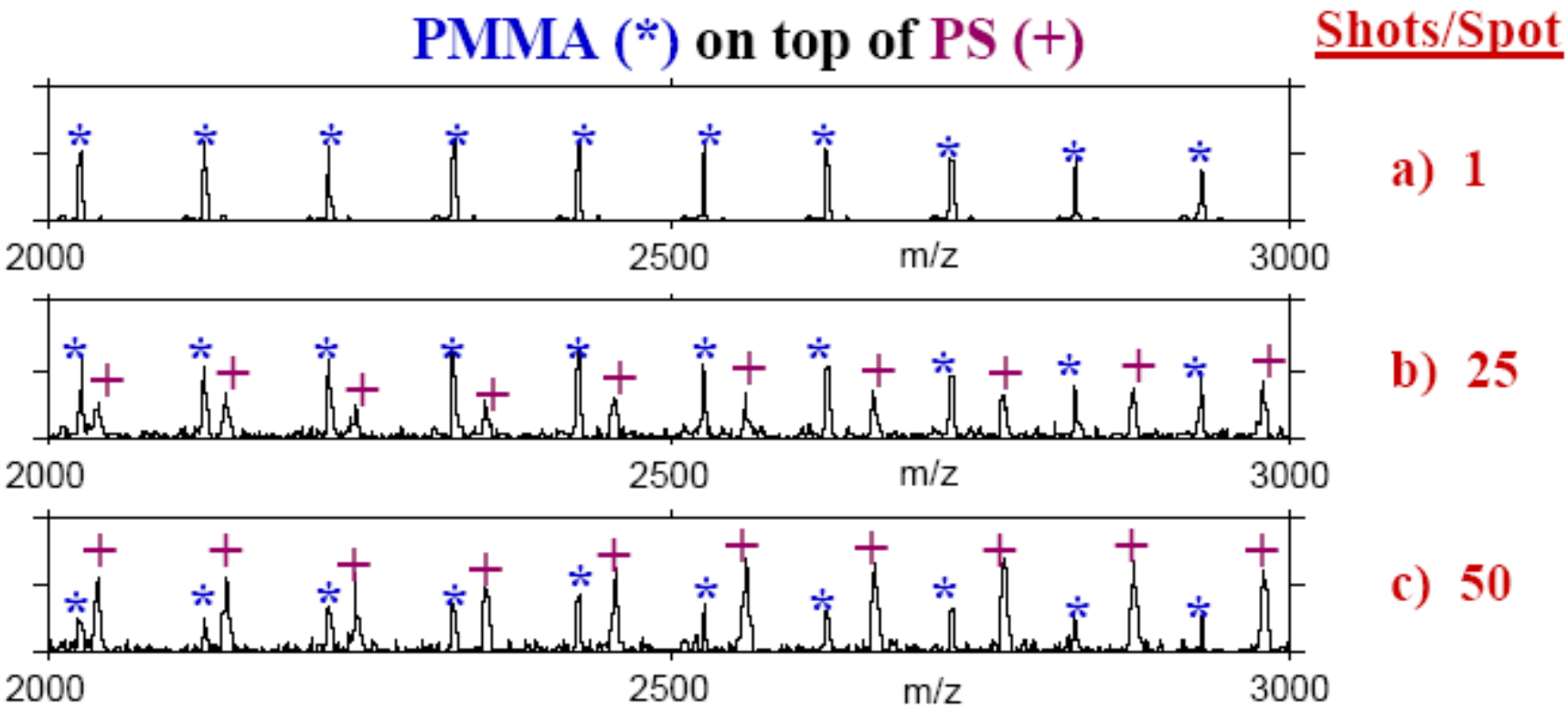
- The ‘no’ values in the center of the table may indicate similar Hildebrand Solubility

Layered Samples- MESIMS Analysis



Electrospray deposition of two layer samples

Layered Samples- MALDI Analysis



Electrospray deposition of two layer samples

Questions (sample preparation) to consider:

- Do you need to match the matrix to the analyte?
- Is electrospray deposition required to use MALDI quantitatively?

It depends on the sample

Air dried (dried drop) sample preparation is fine if you have a good match between the matrix, analyte (cationization reagent) and solvent.

"I never said most of the things I said". Yogi Berra

Questions (desorption) to consider:

- Are the molecules desorbing from the matrix?
- Does the matrix play a role as a “dispersant” to decrease the analyte/analyte interactions and increase the ease of desorption from the sample surface?
- Does it matter that the desorption process is a “supersonic molecular beam”?

- The supersonic molecular beam properties of the MALDI plume enables it to act as a “reaction vessel”.
- In a PSD experiment- what matrix is chosen determines the degree of fragmentation- differences in velocity leads to the effectiveness of collisions

Questions (ionization) to consider:

- Does MALDI simply release “pre-formed” ions from the sample?
- Is protonation/cationization a solution-phase or gas-phase process?
- Are ion-molecule or molecule-molecule reactions involved in the gas phase?
- What is the importance of the identity of the cationization agent?
- When are other ionization processes, such as protonation, anionization, electron transfer, important for a particular analyte?

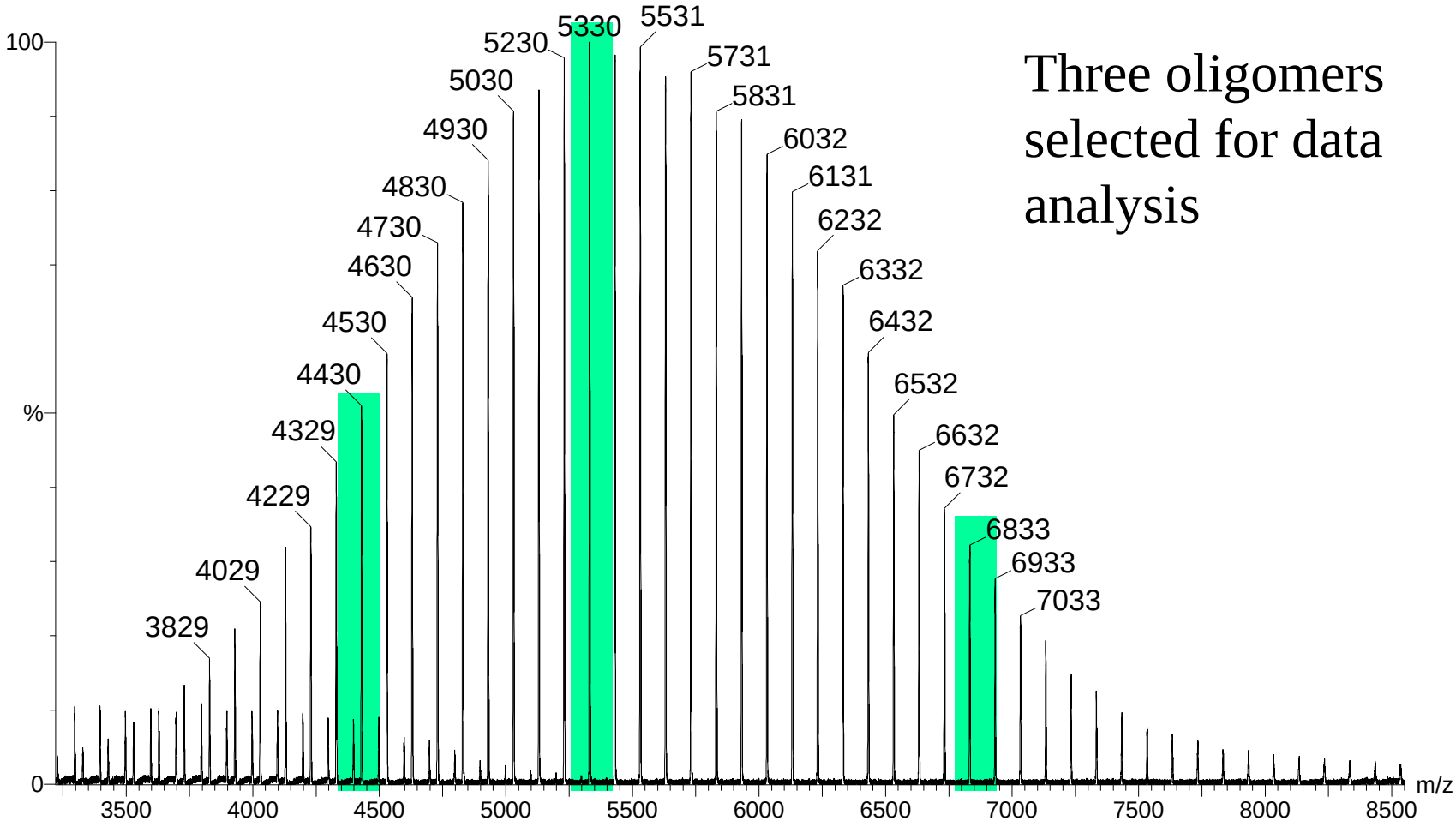
Questions (ionization) to consider:

- What is the ionization efficiency of the MALDI experiment?

“1 molecule in 10,000 is ionized”

  - Post-ionization experiments on peptides
  - **PMMA Salt-to-Analyte (S/A) ratio studies**
  - **Analysis of polystyrene w/o added Ag**

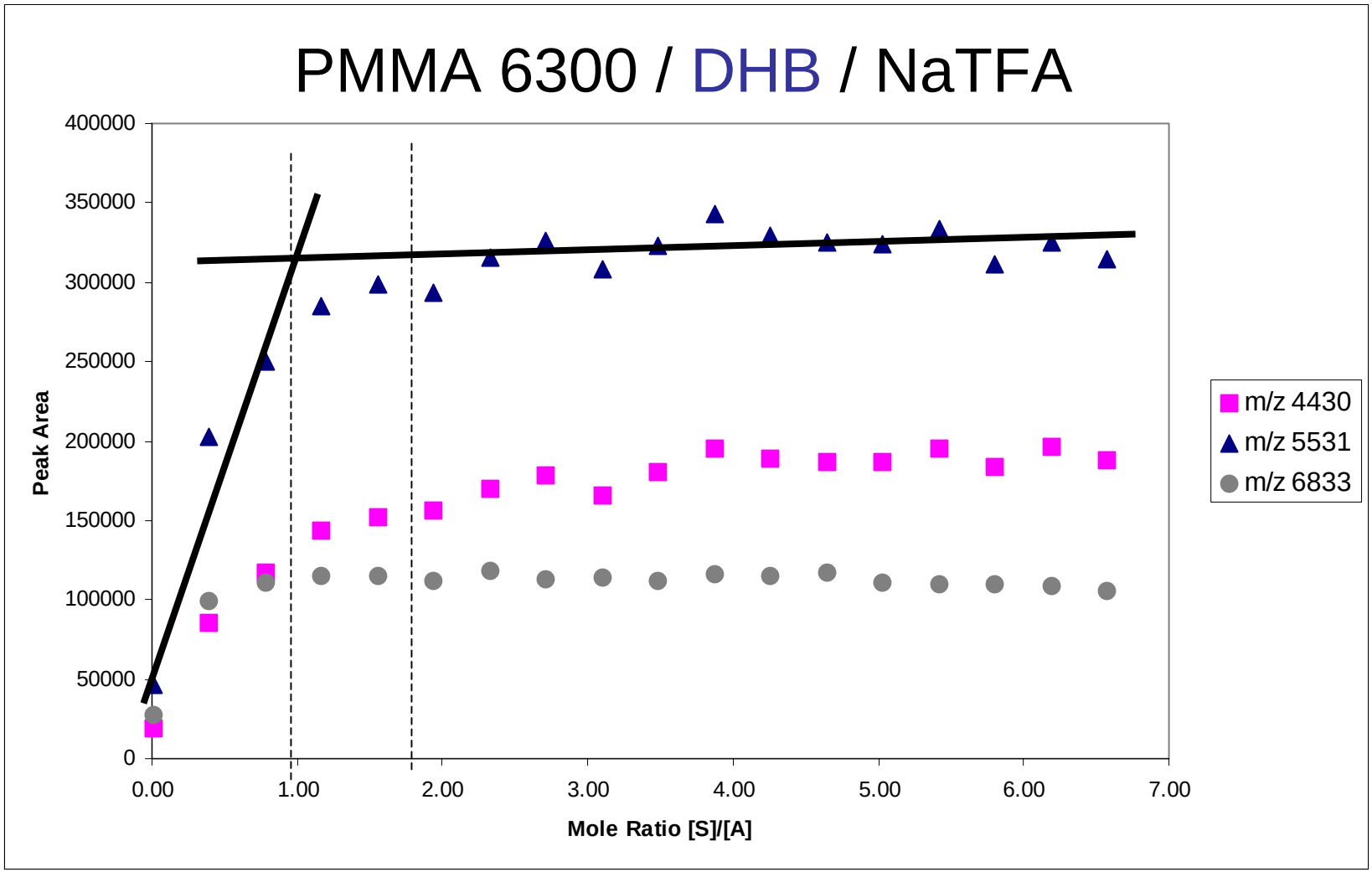
PMMA 6300



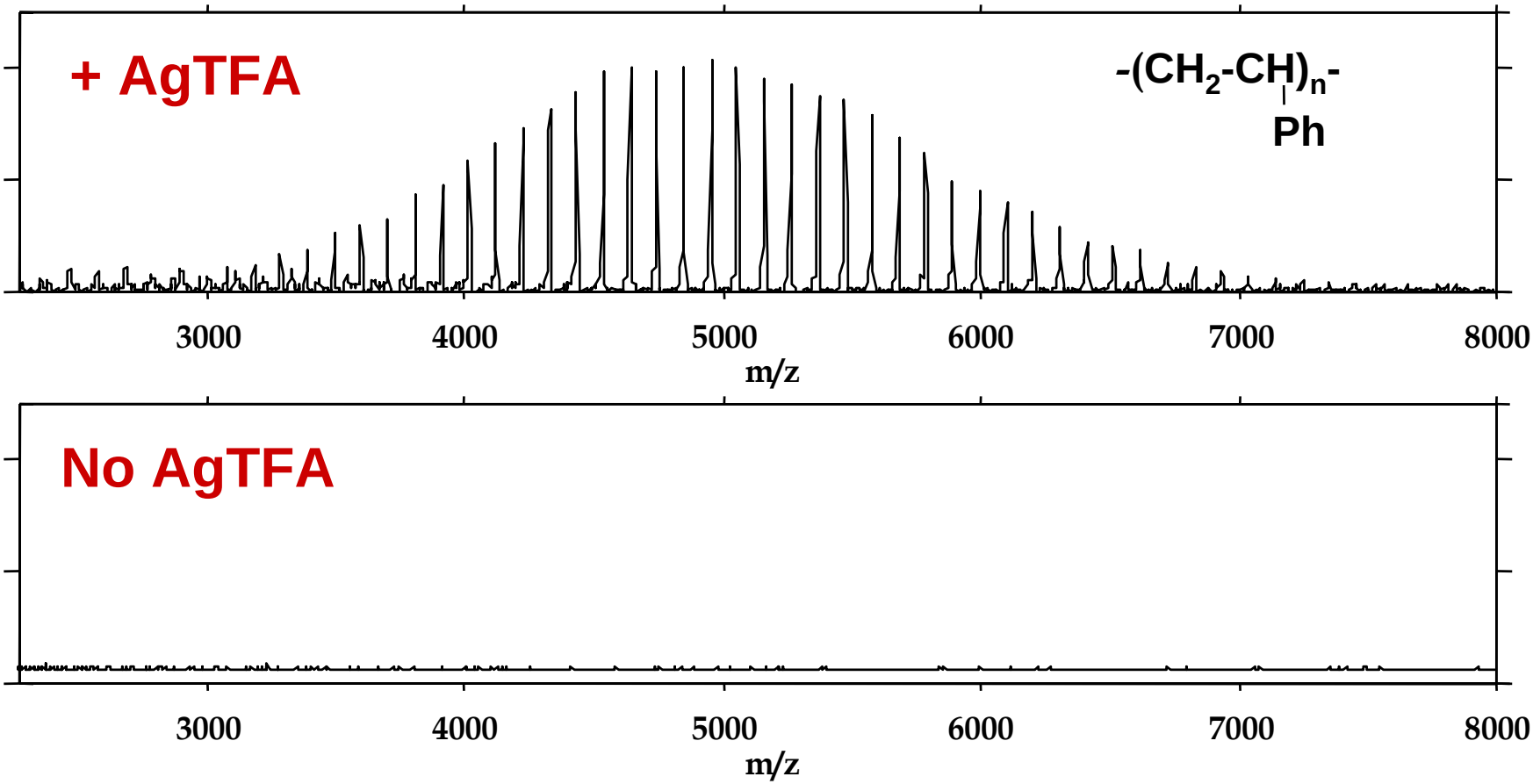
Three oligomers selected for data analysis

Salt Titration: The “Ideal Case”

- [M]/[A] = 1227
- NaTFA as the cationization reagent
- Peak-area plots of three different oligomers



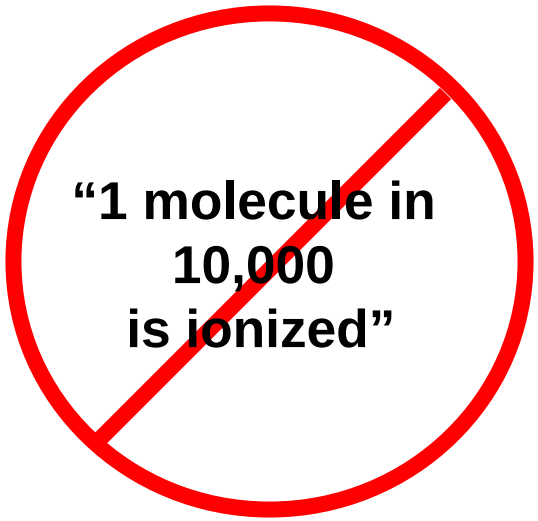
MALDI of PS 5050



There must be an appropriate cationization reagent present in the MALDI sample preparation

Questions (ionization) to consider:

- What is the ionization efficiency of the MALDI experiment?



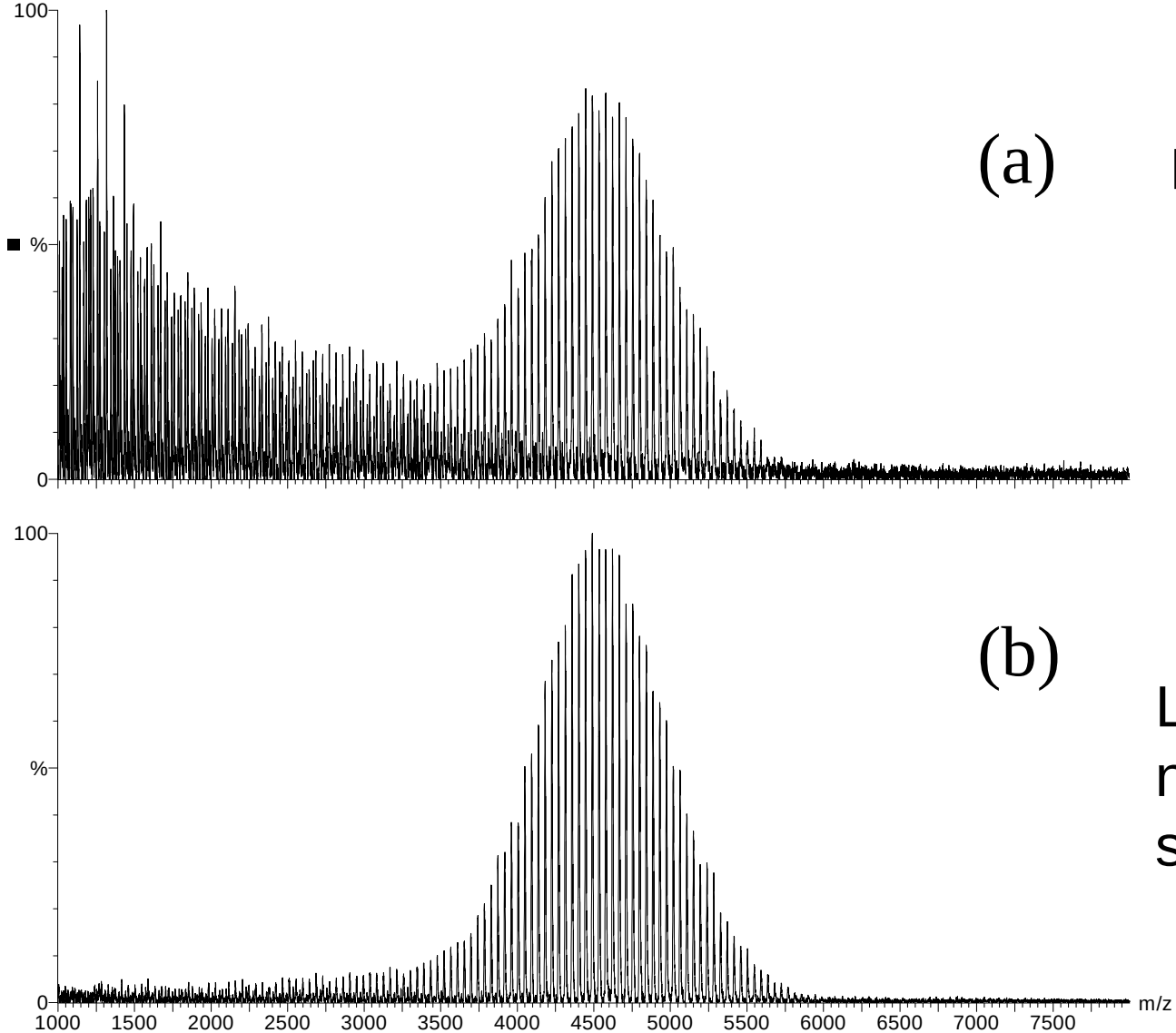
The ionization efficiency can vary by several (many?) orders of magnitude depending on the choice of matrix and analyte.

Problem... The matrix compounds interfere, but the analytes are labile (i.e., we need assistance because LD is too harsh)

Solution:

- Instead of using a matrix, modify the substrate surface to facilitate easier/gentler desorption for LD
  - DIOS
  - Modify the hydrophobicity of surfaces
  - Use a nanoparticle “matrix”
- Important considerations
  - Absorption of laser energy
  - **Surface energy/surface tension**

Surface Effects in LD



LD on SS

LD on nonporous silicon

Andrew Hoteling, PhD Thesis, 2004.

Too many problems at once... How do we analyze mixtures?

For synthetic polymers:

- Molar mass discrimination
  - Wide-polydispersity samples (Pd>1.2)
- Structural discrimination
  - Qualitative analysis
    - Co-polymer analysis- block effects
  - Quantitative relationships
    - Effect of end-group on detectability
    - Effect of polymer chain length on detectability

Solutions:

- Combine a separations technique and MALDI (either on-line or off-line)
  - SEC-MALDI
  - LC-MALDI

- But, you can also use discrimination to your advantage
  - Modify the sample preparation conditions to express the components of interest and “suppress” other components

Problem... How do we analyze “insoluble materials” or solvent sensitive materials

Solution:

- Solid sample preparation

Questions to consider:

- How does the solid phase sample preparation technique really work?
- Is the technique completely general?

Problem… Now that we know what is there, can we reliably know how much is there?

Questions to consider:

- Can MALDI be quantitative?
- How does MALDI compare to UV/visible spectroscopy?

Solutions:

- Use of an internal standard (AQUA peptides)
- ICAT or iTRAQ labeling

Problem… When difficulties occur, how do we know which problems are due to the MALDI process (science) and which are due to the MALDI instrument (technology)?

Question to consider:

- Is there any effect of the instrument operating parameters on the MALDI result?
  - delayed extraction time
  - atmospheric pressure versus vacuum MALDI
  - low voltage versus high voltage acceleration
  - grid effects

Problem… How do you choose the right tool (analysis method) for the problem at hand

We apply the technique we have at hand.

Need to understand the principles (fundamentals, mechanism) of the MALDI process.

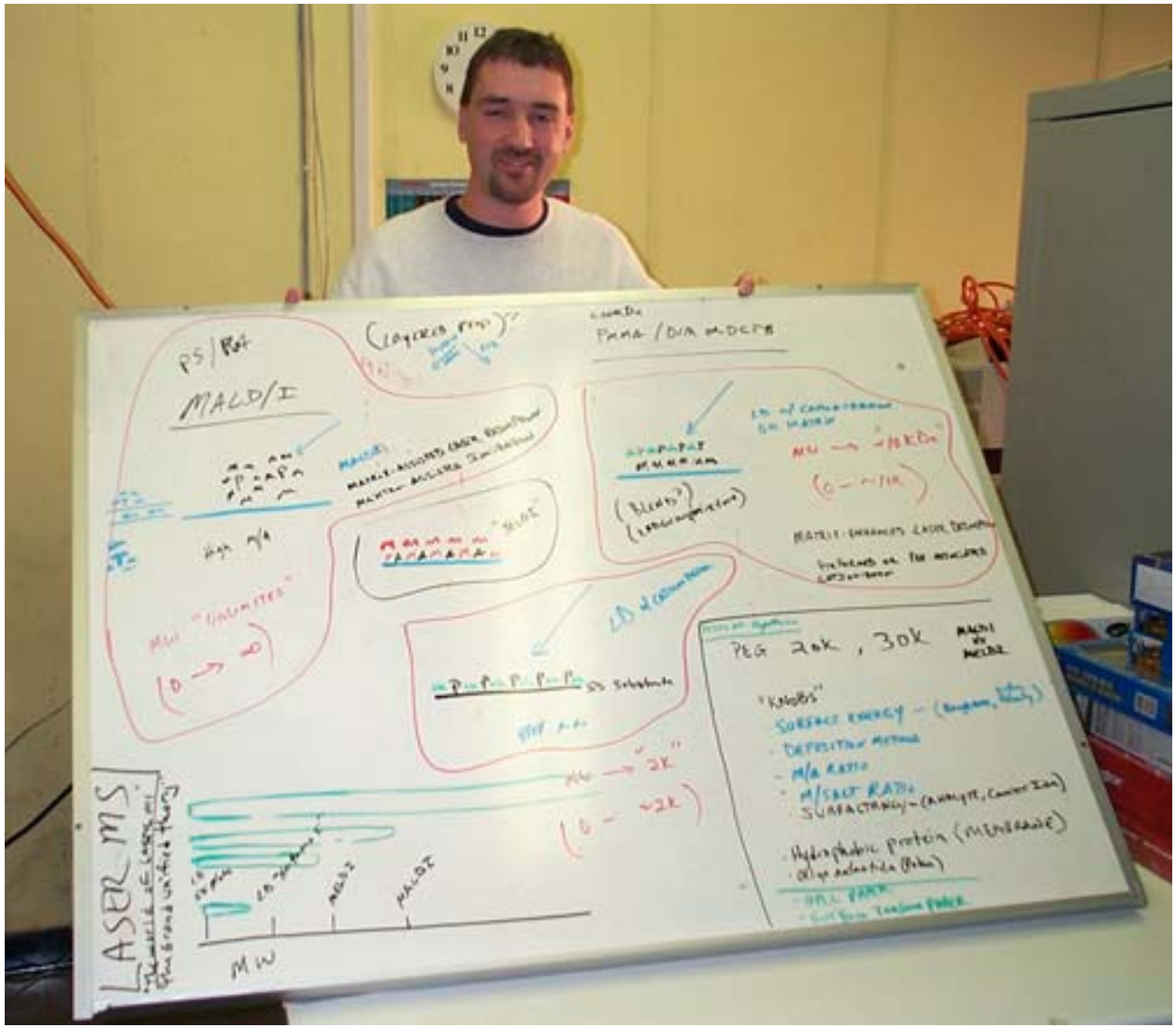
When something doesn’t work, then it starts getting interesting.

Look deeply for similarities between individual (disparate) techniques or technologies.

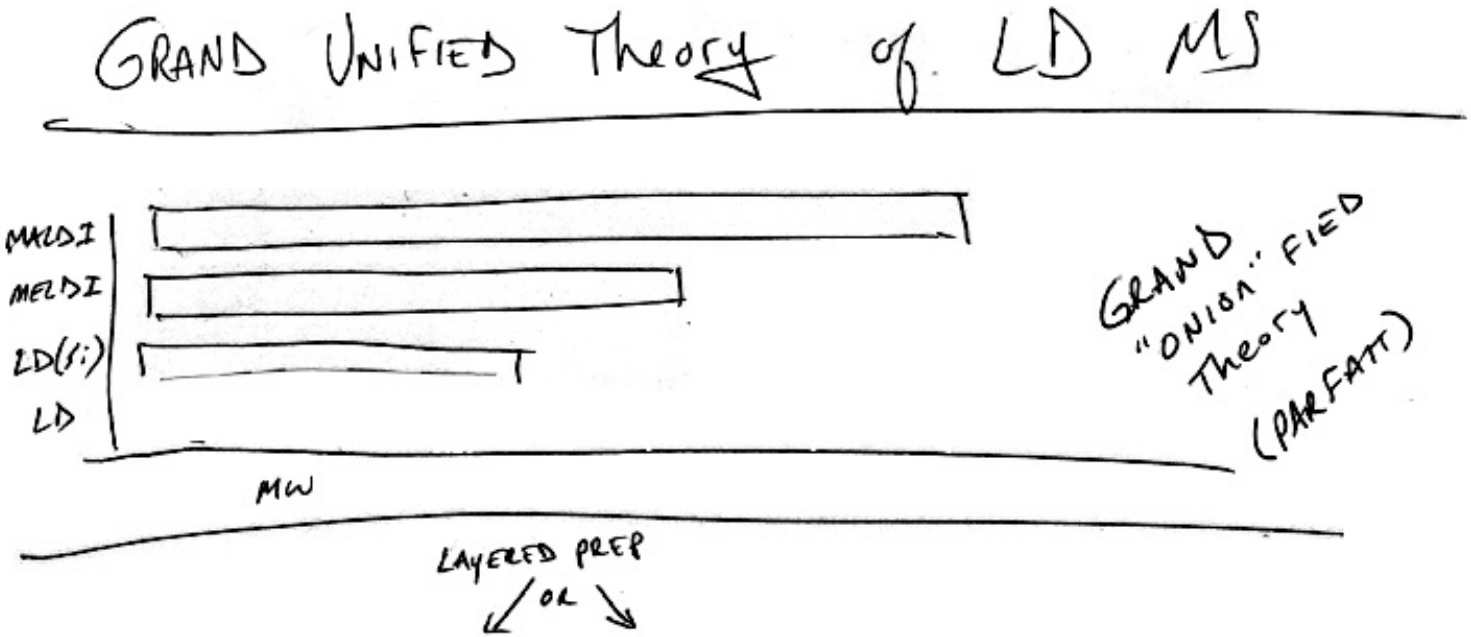
How similar are the different laser desorption techniques?

“For the man with a silver hammer, everything looks like a nail.” Mark Twain

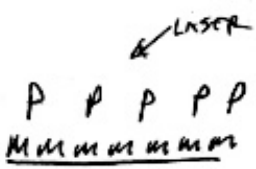
“Grand Unified Theory of Laser Desorption”



“Grand Unified Theory of Laser Desorption”



MELDI = MATRIX-ENHANCED LASER DESORPTION w/ Preformed or pre-ASSOCIATED CATION RATIO



Overarching Questions

- Is MALDI just a “special case” of LD?
- Is MALDI really that different from other MS “particle” bombardment techniques?
  - Fast Atom Bombardment (FAB)
  - Secondary Ion Mass Spectrometry (SIMS)
  - Cf-252 Plasma Desorption (PD)
- What makes a matrix a matrix?
  - What molecular properties are required for a molecule to be a successful matrix?

Conclusions

- Many of these “particle desorption” techniques are more similar than they are different: look for similarities in the underlying mechanisms of desorption and ionization: physical chemistry is physical chemistry.
- In MALDI, dispersion, desorption and ionization are separate processes that can be optimized independently.
- It is not necessary to find a single compound that performs all of the functions of a MALDI matrix- it may be a mixture rather than single compound. The “matrix” must do all jobs- act as dispersant, desorber, and ionization reagent.
- The energy to enable all of these processes to occur comes from the laser- the matrix must absorb the laser light.
- Need to use other techniques besides MALDI to understand the MALDI process.

“The most incomprehensible thing about the world is that it is comprehensible.”
Albert Einstein

Guide to the Players …

- **Co-workers**
  - Richard King (Merck, Inc.)
  - Yansan Xiong (Micromass, Inc.)
  - Robert Goldschmidt (US Dept. Agriculture)
  - Russell Hensel (Johnson & Johnson, Inc.)
  - Cynthia Chavez-Eng (Merck, Inc.)
  - Drew Hoteling (Eastman Kodak Co.)
  - Bill Erb
- **Collaborators**
  - Scott Hanton, Air Products & Chemicals
  - Bill Nichols, Kenji Kawaoka, Eastman Kodak Co.
  - Bill Simonsick, Jr., E.I. Dupont deNemours, Inc.

… and the Payers

- **Funding**
  - Air Products & Chemicals, Inc.
  - Eastman Kodak Company
  - Burle Electro-Optics, Inc.
  - Xerox Foundation
  - National Science Foundation (CRIF)
  - Commonwealth of Pennsylvania Ben Franklin Partnership Program
  - Drexel University
  - Rohm & Haas, Inc.

“Science is a wonderful thing if one does not have to earn one's living at it.”
Albert Einstein

Questions?

“I never said most of the things I said.” Yogi Berra