Introduction to Mass Spectrometry

W.M. Keck Biomedical Mass Spectrometry Lab

Moore Health Sciences Library Rooms 1335 & 1337

May 18, 2010

The Keck Mass Spectrometry Lab of the Biomolecular Resource Facility

- Jordan Hall Rooms 1105 and 1034
- 434 924-0070

- Nicholas E. Sherman, Ph.D., Director
- Jim Farmar, Ph.D., Assistant Director
- Carla Castro, Ph.D., Postdoctoral Fellow
- Rachel Reuther, M.S., Laboratory Technician
- John Shannon, Ph.D., Director, Shared Instrumentation Core Facility

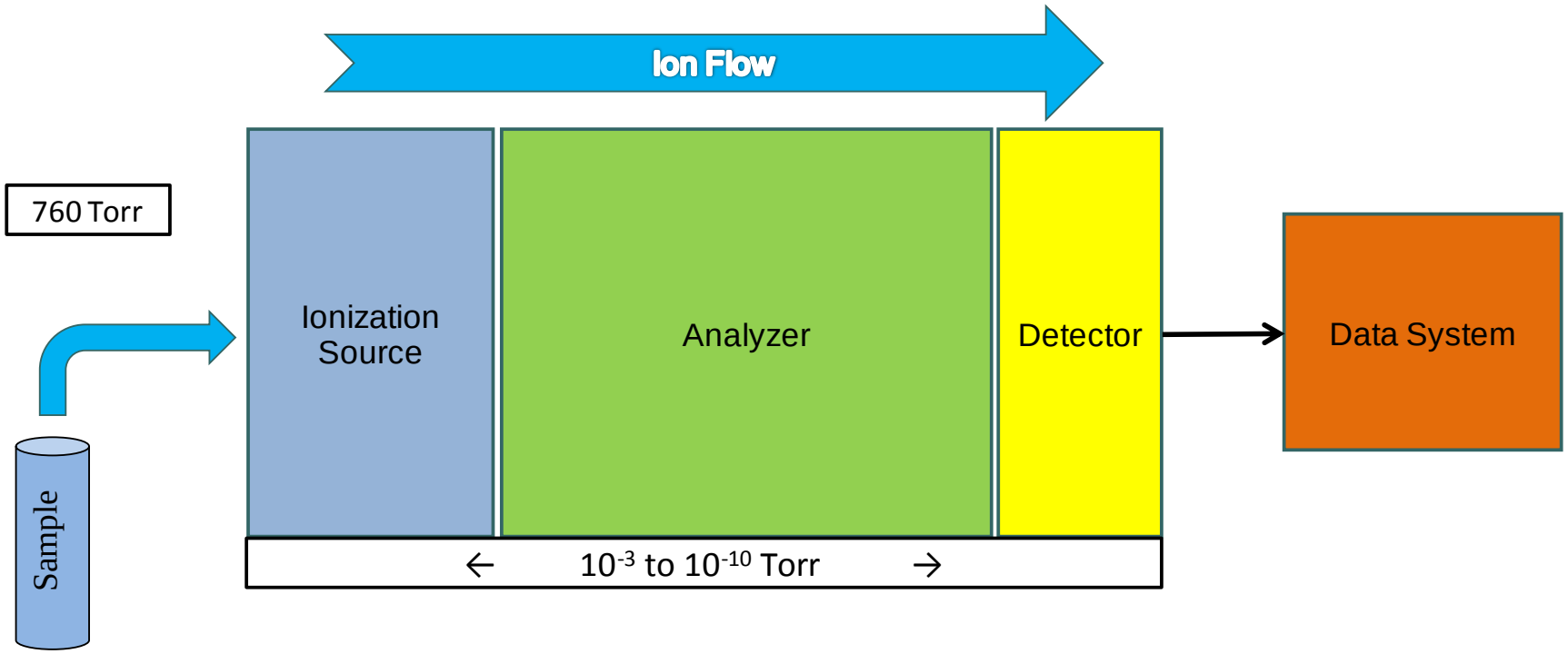
Course Objectives

- How a Mass Spectrometer Works.
- What a Mass Spectrometer Can Tell You About Your Biomolecule.
- Types of Mass Spectrometers Available in the Keck MS Lab.
- Services Available in the Keck MS Lab.
- How to Work With the Keck MS Lab to Maximize Your Research Success.
- Examples

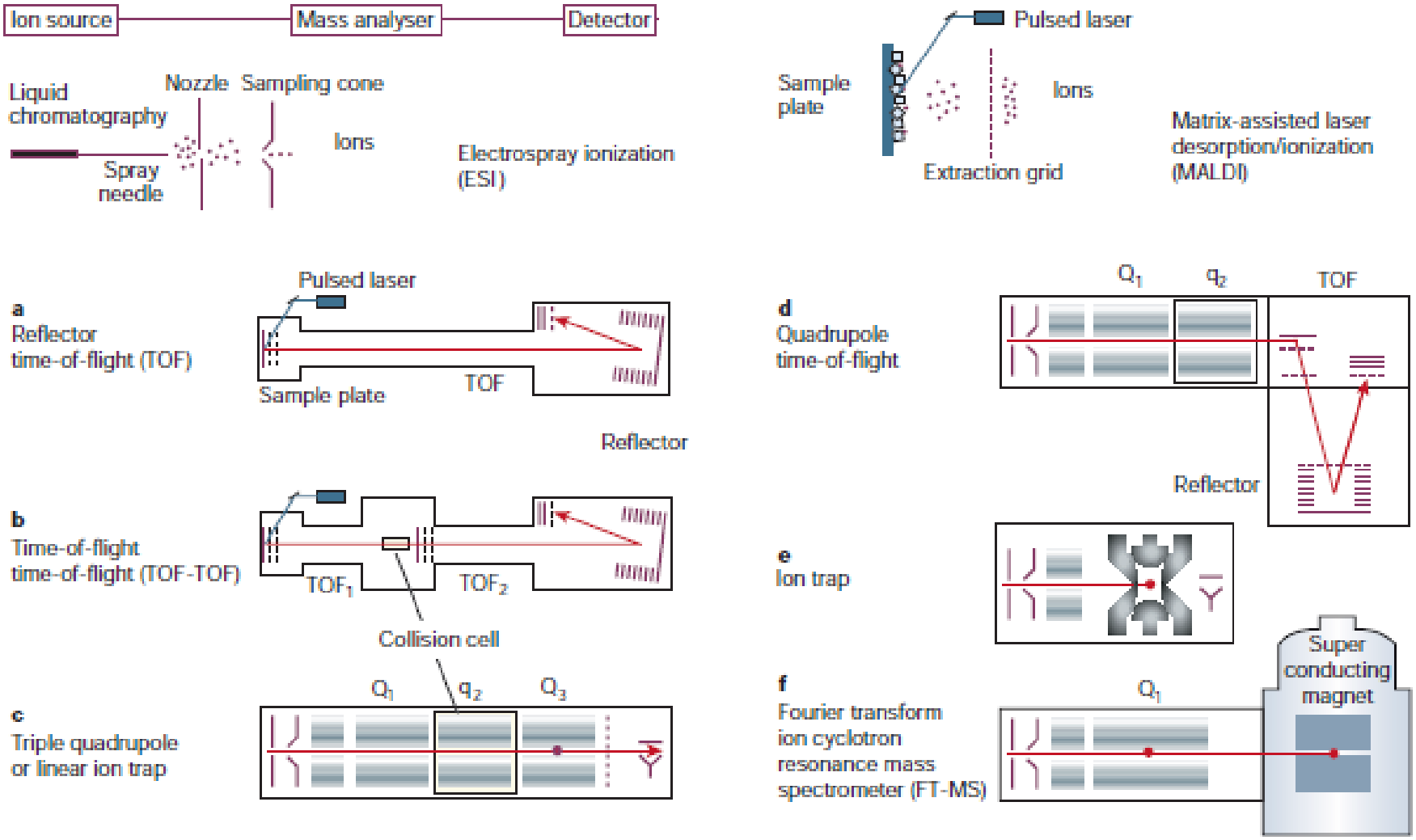
How Does a Mass Spectrometer Work?

1. Forms Ions from Molecules
2. Analyzes Ions by mass to charge (m/z)
3. Detects the Separated Ions
4. Collects the Data

How Does a Mass Spectrometer Work?



Mass Spectrometer Varieties



Aebersold & Mann Nature 2003, 198

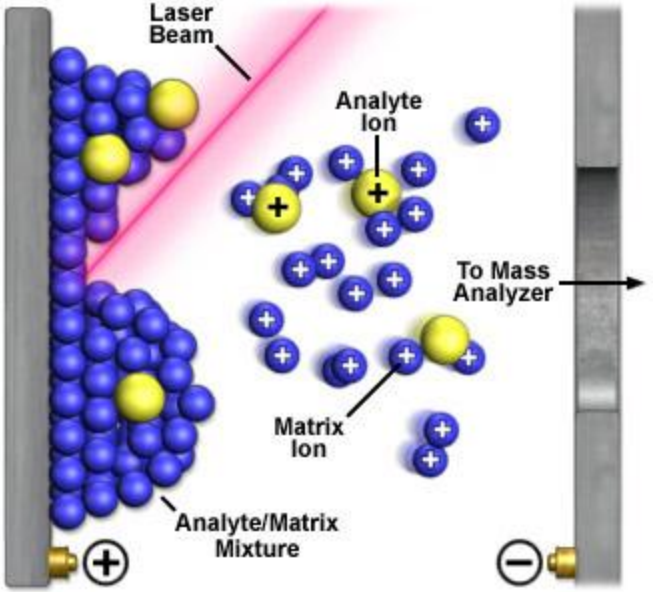
Forming Ions: MALDI (Matrix-Assisted Laser Desorption Ionization)

MALDI

- Laser energy strikes co-crystallized analyte/matrix under vacuum and sputtering produces a plume/explosion of analyte and matrix ions
- Singly Charged Ions (usually)
- Usually Paired with a Time of Flight Analyzer.

Ions

- Formed from molecules: H^+ or H^- or H^{++} or higher
- Reactive, so keep under vacuum.
- Moved in the MS by electromagnetic fields.
- Ions can be made to react with other species in the MS.



National Magnet Lab
www.magnet.fsu.edu

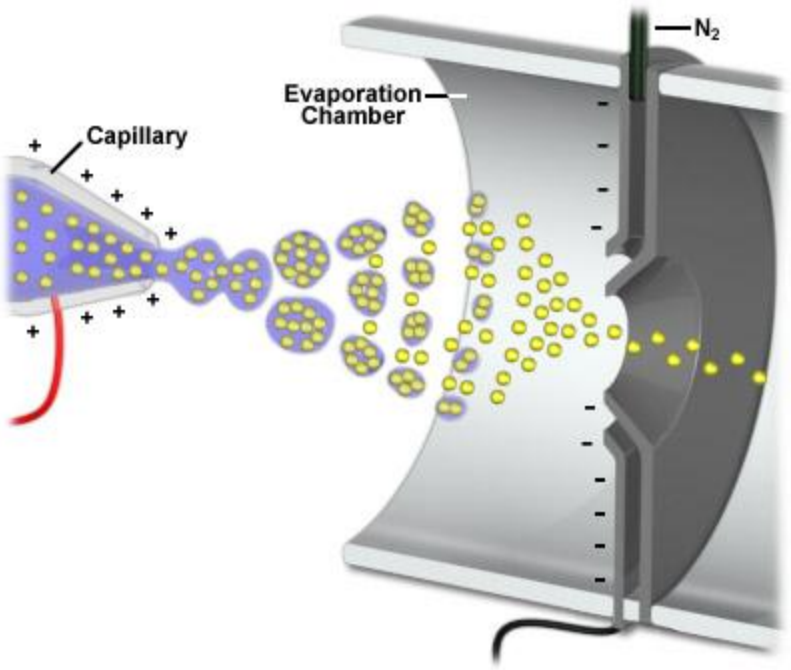
Forming Ions: ESI ElectroSpray Ionization

ESI

- Molecules separated via HPLC.
- Jet of highly charged droplets emerge from capillary tip
- Solvent is evaporated.
- Multiply Charged Ions enter the analyzer.
- Paired with Quadrupoles, Ion Traps, FTs and Orbitraps Analyzers.

Ions

- Formed from molecules: H^+ or H^- or H^{++} or higher
- Reactive, so keep under vacuum.
- Moved in the MS by electromagnetic fields.
- Ions can be made to react with other species in the MS.



National Magnet Lab
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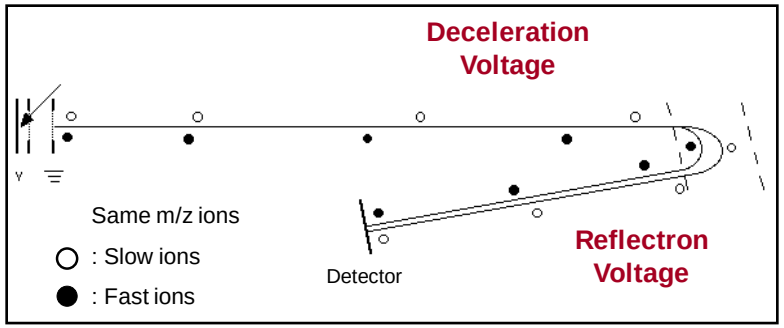
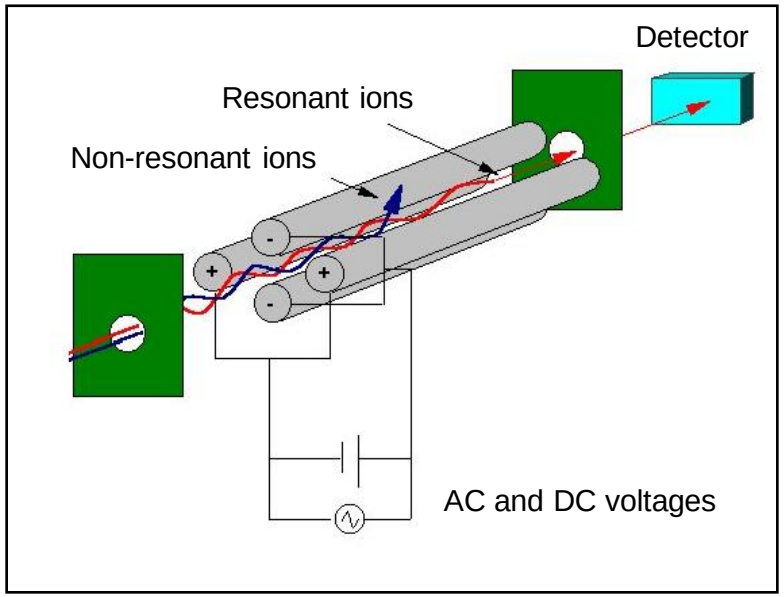
Analyzing Ions: Analyzer Types



Time of Flight

- Ions fly along a long tube
- Diagram shows a reflector
- Smaller ions get to the detector 1st
- Analogous to a gel

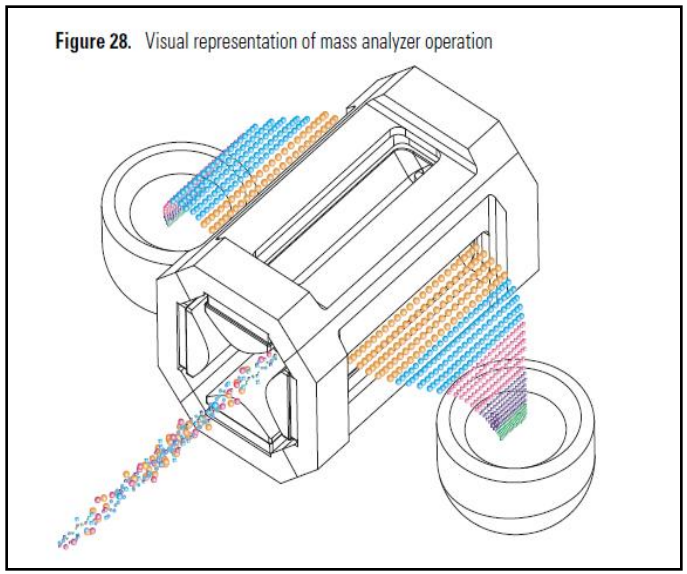


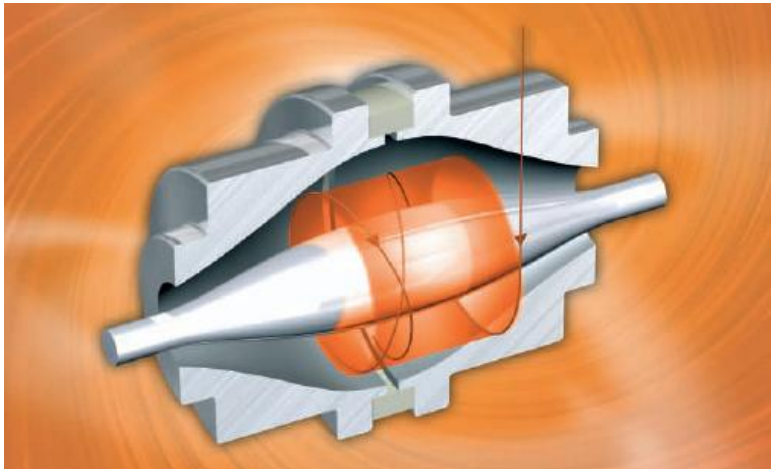
Quadrupole

- Voltages on 4 rods are scanned as ions enter from source.
- For a certain voltage, only a narrow range of m/z will pass.
- Other m/z's are rejected.
- Result: a Scanning Mass Filter
- Usually used in a series of 3

www.chemistry.adelaide.edu.au
Angeletti, Einstein CoM 2007

Analyzing Ions: Analyzer Types





Ion Trap

- Traps ions in a 2D field
- Both MS and MS/MS analyses can occur.
- Ions are sequentially ejected to the detector
- High sensitivity, Good range
- Low resolution, Low accuracy

Orbitrap (FT)

- Ions are trapped between a central & an outer electrode
- Ions oscillate to produce a current
- Current is transformed into a m/z
- High sensitivity, good range
- Very High Resolution & Accuracy

Thermo Fisher

Detecting Ions: How?

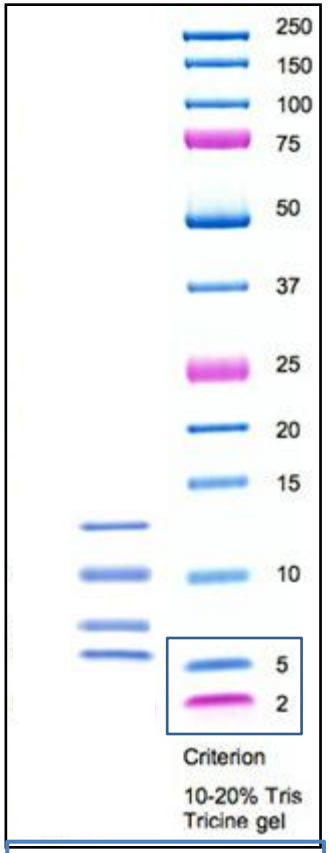
- Plate and Ion Multiplier Type
  - For ToF, Quadrupole and Ion Trap
  - Conversion Dynode: ions produce 2^0 particles
  - Electron Multiplier: 2^0 particles generate multiple electrons
  - High sensitivity and High range
- ICR Type
  - For FT and Orbitrap
  - Measures the current induced by ions rotating in a magnetic field or around an electrode.
  - High mass accuracy, high sensitivity, & good range
- Detectors can only count

Your Biomolecule(s): What Information Do You Want?

- Molecular Mass
- Structure
- Sequence
- Identity
- Quantity

Key Concept:
Telling One Biomolecule from Another

SDS PAGE



13 kDa?

10 kDa?

7 kDa?
5 kDa?

Separation by MW and Charge

Detection: Sensitivity vs. Specificity

- Stain: sensitive but non-specific; weak dynamic range
- Ab: very sensitive & specific for an epitope

Resolution:

- Distinguish one biomolecule from another of similar properties
- 2000 Da +/- 500
- Smaller MWs, Better Resolution

Accuracy:

- Compare Standard Proteins to Sample Proteins.
- How accurate?
- What are the MWs of the sample Bands?

Bio-Rad

Key Concept:
MS of Peptides not the Protein

- Problem:
  - Proteins are too big;
  - Small but important differences in the MS aren't seen and are difficult to locate
- Solution:
  - Digest the protein to produce peptides
- Trypsin is the preferred enzyme:
  - Very well-characterized
  - Efficient
  - Produces good-sized peptides (10 amino acid avg length)
  - Cleaves between KX or RX except if X is Pro

Key Concept: Mass Accuracy Is Important

- Measured Value vs. True Value.
- Increase Mass Accuracy: Decrease Number of Matching Molecules from the Protein Databases.
- Modern MS Accuracy: within 10 ppm.
- Practical Limits to instrument accuracy.

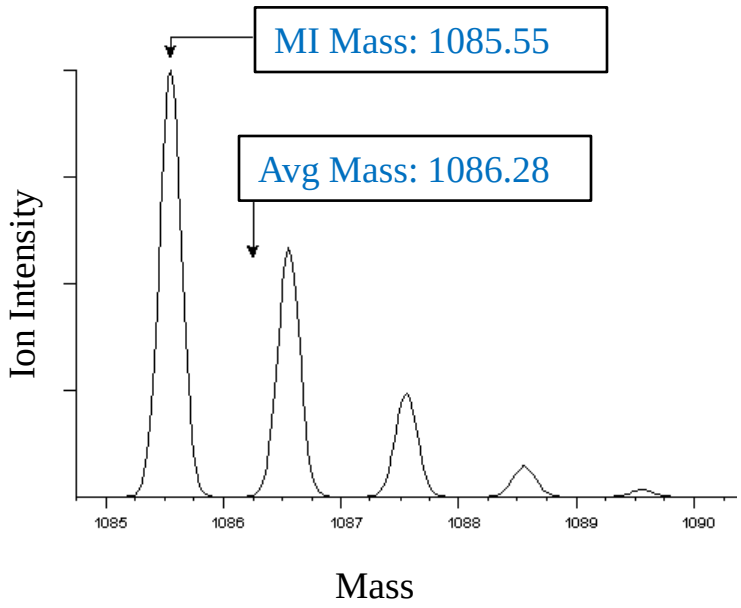
Effect of Mass Accuracy/Tolerance on Peptide Mass Fingerprinting Result

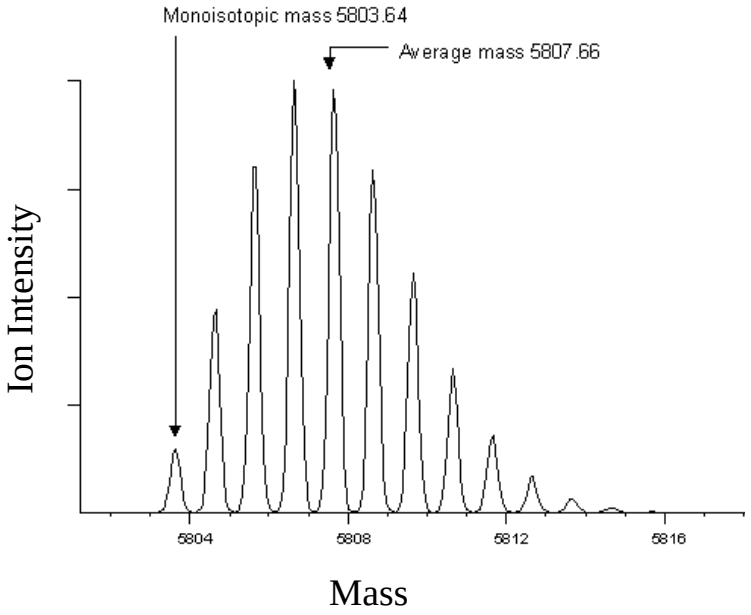
Search m/z	Mass Tolerance (Da)	# Hits
1529	1	478
1529.7	0.1	164
1529.73	0.01	25
1529.734	0.001	4
1529.7348	0.0001	2

Liebler, 2002

Key Concept: Isotopes

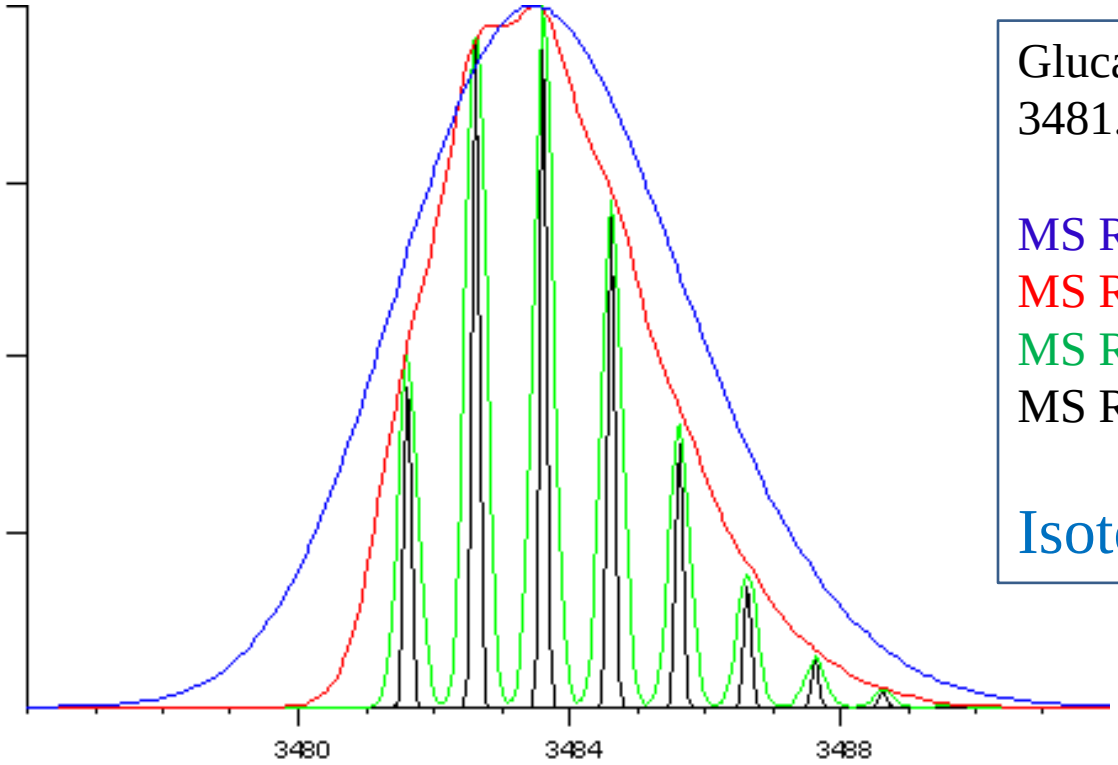
- Natural Abundance:
  - ^{12}C: 98.93%
  - ^{13}C: 1.07%
- Use Monoisotopic Mass (all ^{12}C molecule) For High Mass Accuracy





Key Concept:
Resolution for Mass Spectrometers

The ability of a MS to distinguish one ion from another of very similar m/z's.



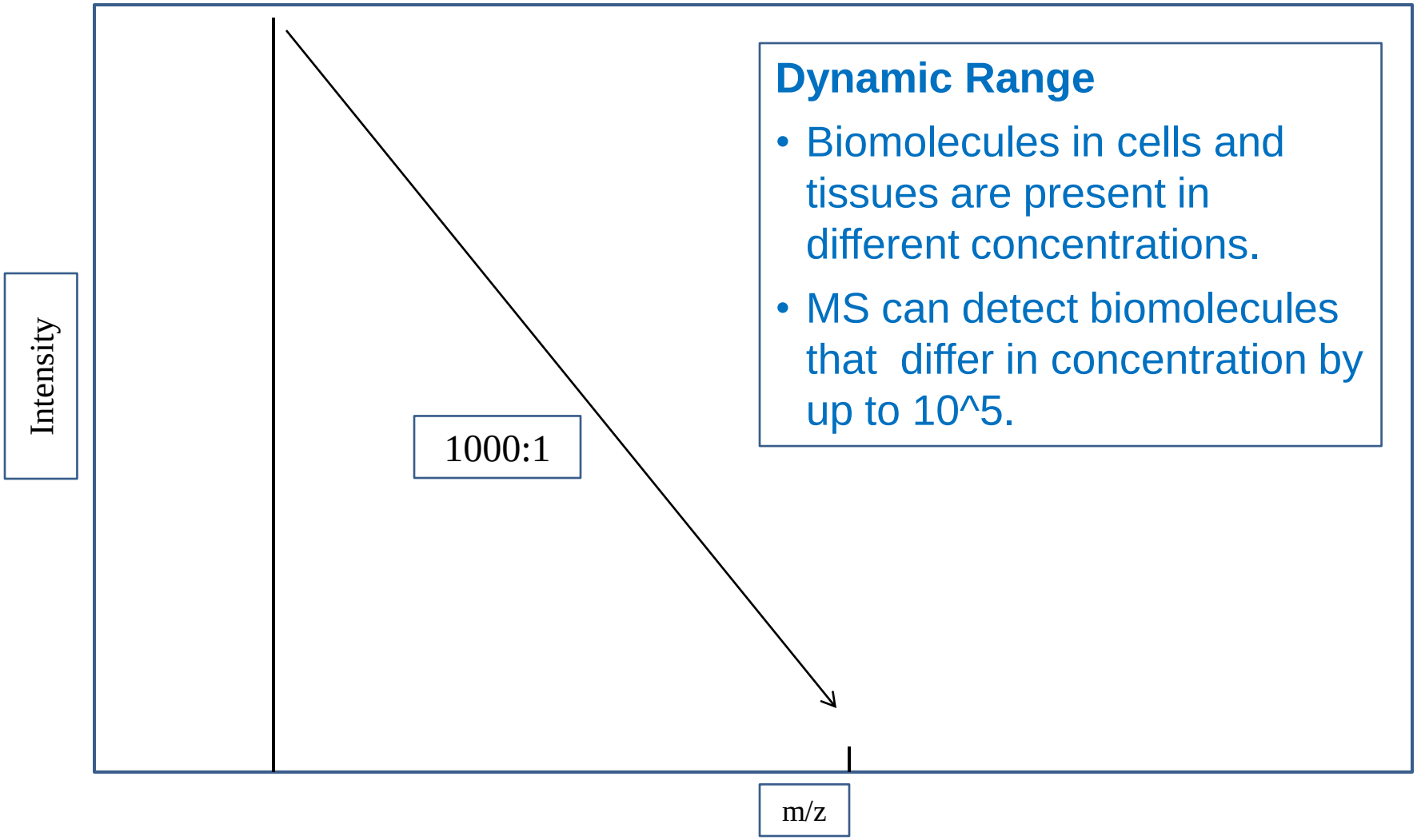
Glucagon at Different Resolutions
3481.62 MH^+

MS Resolution @ 1,000
MS Resolution @ 3,000
MS Resolution @ 10,000
MS Resolution @ 30,000

Isotopes Resolved!

Matrix Science

Key Concept:
Dynamic Range of Mass Spectrometers



Dynamic Range

- Biomolecules in cells and tissues are present in different concentrations.
- MS can detect biomolecules that differ in concentration by up to 10^5.

Key Concept:
Advantages of a Mass Spectrometer

- Accuracy: >10 part per million
- Resolution: up to 10^6
- Range: greater than 10^5 difference in concentration
- MS as a Universal Detector gives
  - Sensitivity &
  - Specificity.

Key Concept: Tandem MS or MS/MS

- To get more information about an ion:
  - Fragment the ion into Product ions.
  - Obtain the MS of the fragments.
  - From the fragments, deduce how they fit together.
- Tandem MS uses 2 or more Analyzers in series.
- For Peptides: Sequence Information

Trp-Ser-Val-Lys

519.2926 (MH^+)

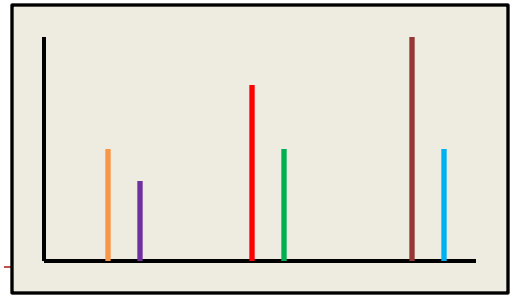
y2: 246.18

y3: 333.21

y1: 147.11

b3: 373.19

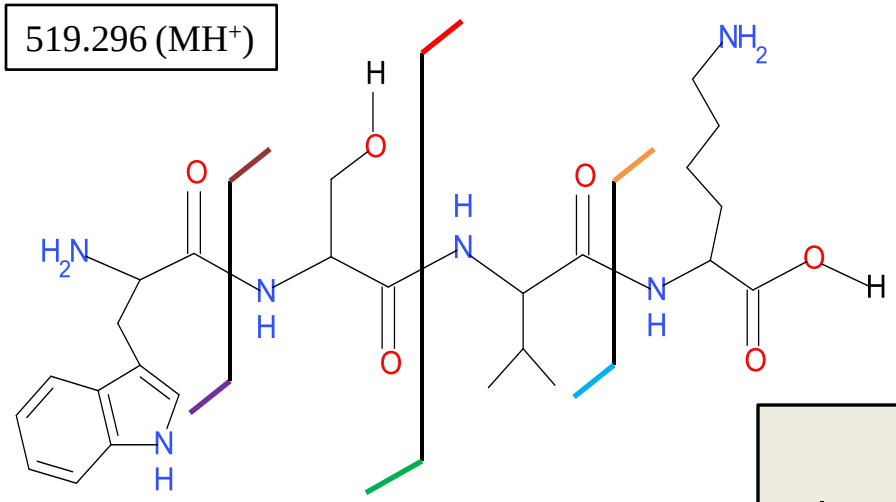
b2: 274.12



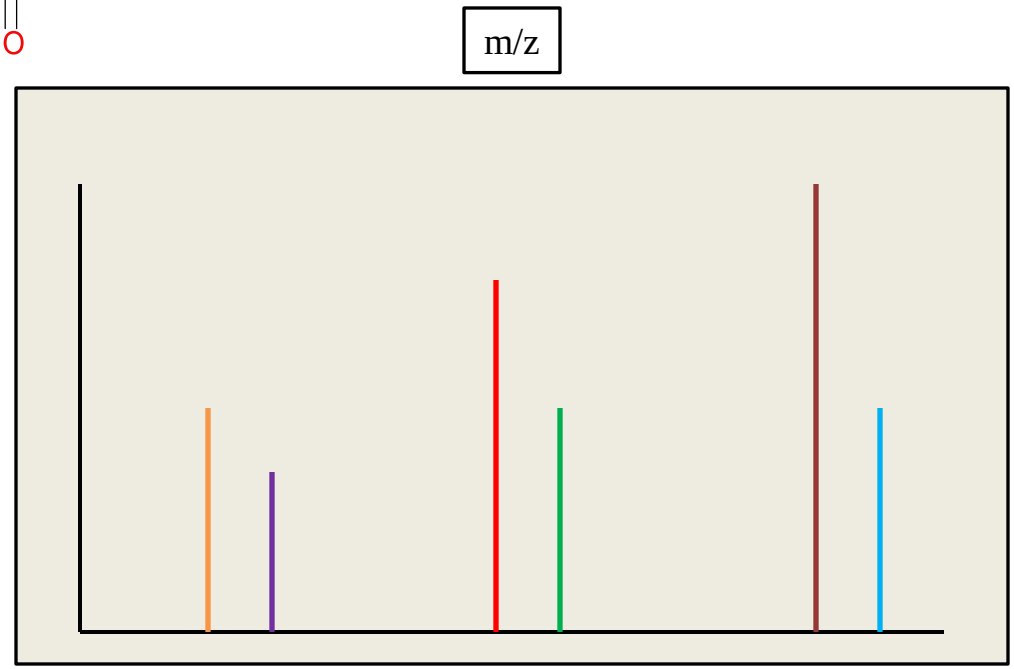
m/z

Trp-Ser-Val-Lys

519.296 (MH^+)



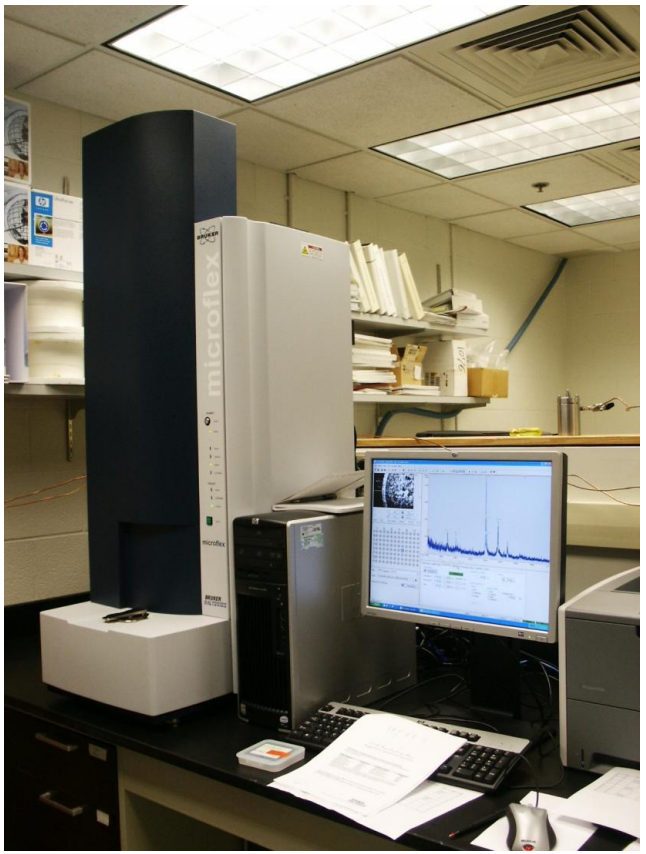
b^+ Ions		y+ Ions
Trp-Ser-Val	+	Lys
Trp-Ser	+	Val-Lys
Trp	+	Ser-Val-Lys



Keck MS Lab: Normal Resolution MS

- Bruker MALDI-ToF MS
  - Available for Training & Use.
  - Confirmation of mass for a biomolecule (500-150,000 Da).
  - Protein confirmation via Peptide Mass Fingerprinting (PMF).
  - Isotopic Resolution
  - Accuracy can be 50 ppm.
  - Sensitive.
- Limits:
  - Cannot determine the sequence of a peptide
  - Not good for complicated mixtures
  - No quantification.

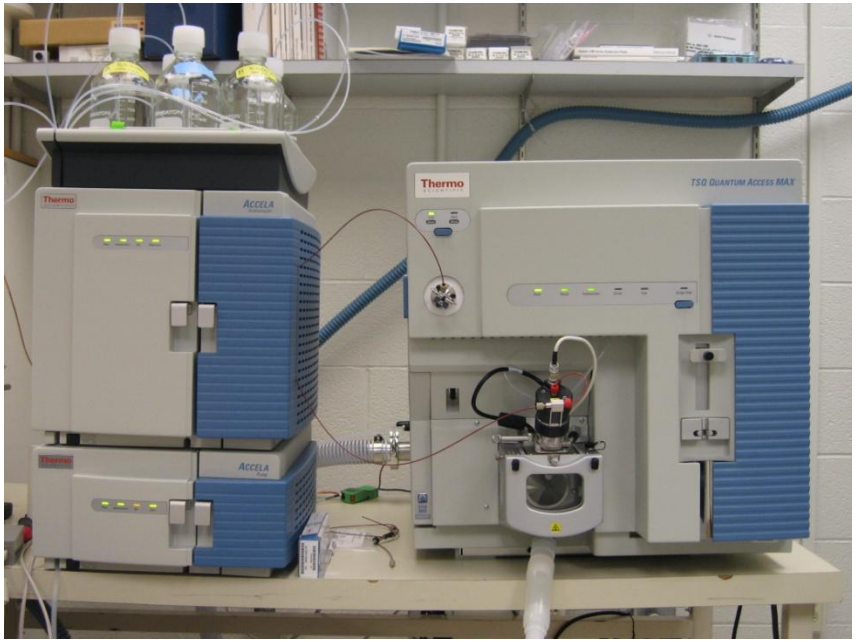
Bruker MALDI ToF



Keck MS Lab: Normal Resolution MS

- Three Quadrupoles in Series
- Quantification of proteins, peptides and metabolites
- Selected Reaction Monitoring (SRM, aka MRM) measures intensity of a specific transition for each peptide
  - Parent ion to Product Ion
- High Sensitivity
- Resolution: 1000

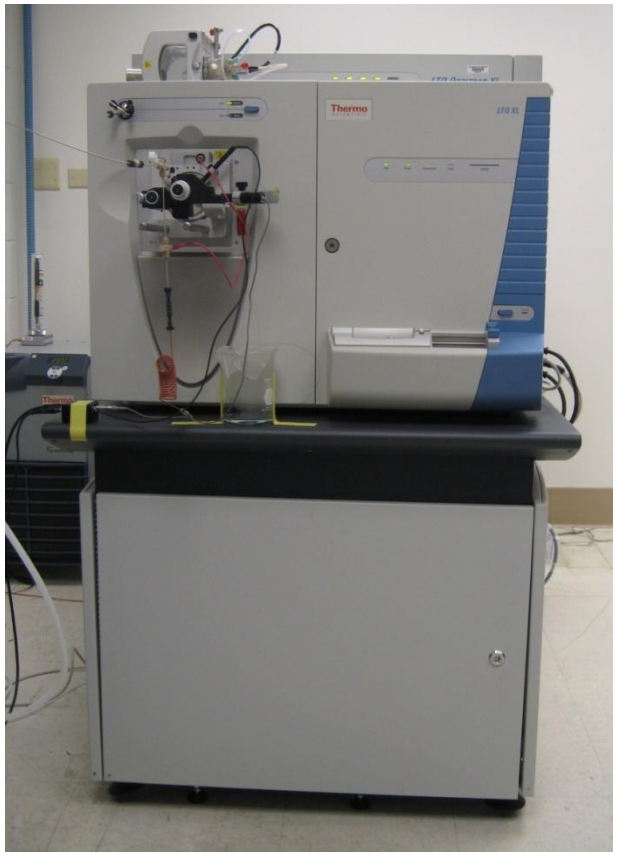
Thermo TSQ Triple Quad



Our Lab: High Resolution MS

- Double Ion Trap & Orbitrap
  - Resolution: 1:400,000 (max)
  - Mass Accuracy: 1 ppm
  - Very fast
- Tandem MS for:
  - Sequence of peptides
  - Locates the PTM to a specific amino acid in the peptide
- Quantitation by chemical labeling of tryptic digests.
- Global proteomics

Thermo Orbitrap Velos



Our Lab: High Resolution MS

- Thermo LTQ ICR FT:
  - Ion Trap (LTQ)
  - Ion Cyclotron Resonance Fourier Transform MS
  - Resolution: >1:400,000
  - Mass Accuracy: 1 ppm
- Tandem MS for:
  - Sequence of peptides
- Global proteomics

Thermo FT ICR

Keck MS Lab: MS Services

1. Confirmation of peptide identity by MALDI mass measurements.
2. Protein identification from gel or solution by Peptide Mass Fingerprinting (PMF).
3. Analysis of protein mixtures from tissue and media .
4. Absolute quantitation of proteins using labeled peptides and selective reaction monitoring.
5. ID of post-translational modifications (PO_4, Ac, Methylation, etc.,) & sites.

Keck MS Lab: MS Services

6. High resolution, High Mass Accuracy measurements of peptides and small proteins.
7. Protein identification and sequencing from gel or solution by ESI-LC/MS/MS.
8. Proteomics: comparison of proteins in samples.
9. *De Novo* (manual) sequence analysis of novel proteins to enable cloning.
10. Identification of binding partners.

Keck MS Lab: Sample Types Analyzed

- Gel Bands
  - Coomassie stain
  - Silver stain*
  - Fluorescent stains
- Proteins in solution
- Immunoprecipitations
- Tissue samples
- Biofluids
- Media

Your Sample: Keep in Mind

- Important: Talk with us before you begin your experiment.
- Use our online sample submission process (we can show you how).
- One band or one spot may contain several proteins and in varying amounts.
- Minimize contamination.

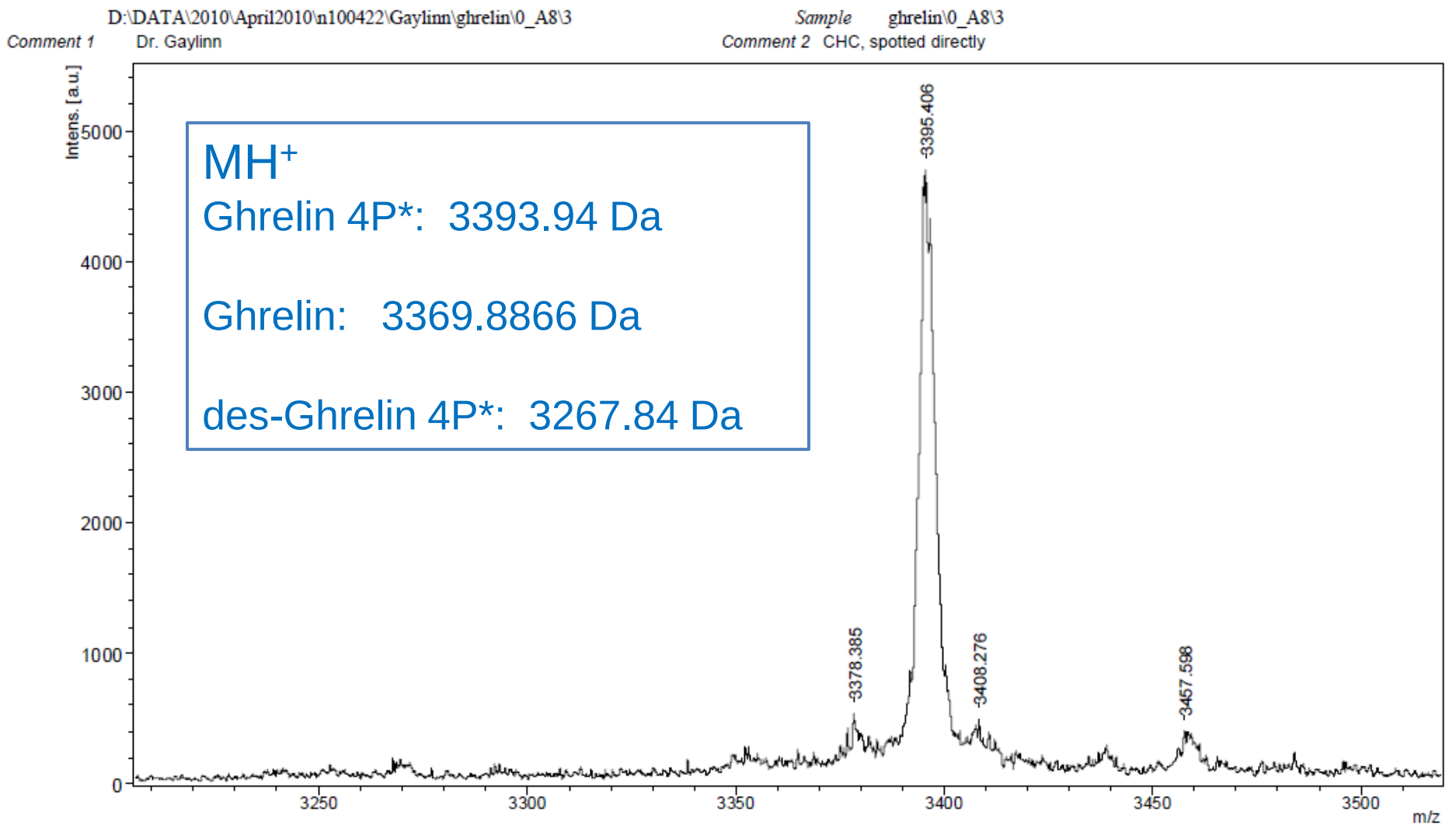
In the Keck MS Lab

- Sample Modification:
  - Cleanup
  - Chemistry
  - Digest
- Chromatography
- Mass Spectrometer
- Raw Data Analysis
- Results are reported to you as Protein Matches

Bruker MALDI TOF Experiment

- Was the correct version of Ghrelin synthesized?
- Wanted:
  - GSS(oct)FLSP*EHQRVQQRKESKKP*P*AKLQP*R
    - Octanoyl moiety adds 126.1 Da to Ghrelin ($C_8H_{14}O$).
    - Each Heavy Proline (P*) adds 6 Da for a total Δ of 24 Da.
- Are all Prolines made with heavy C and N atoms?
- Has the Ghrelin lost the octanoyl group to form des-Ghrelin?

Bruker MALDI ToF in Linear Mode

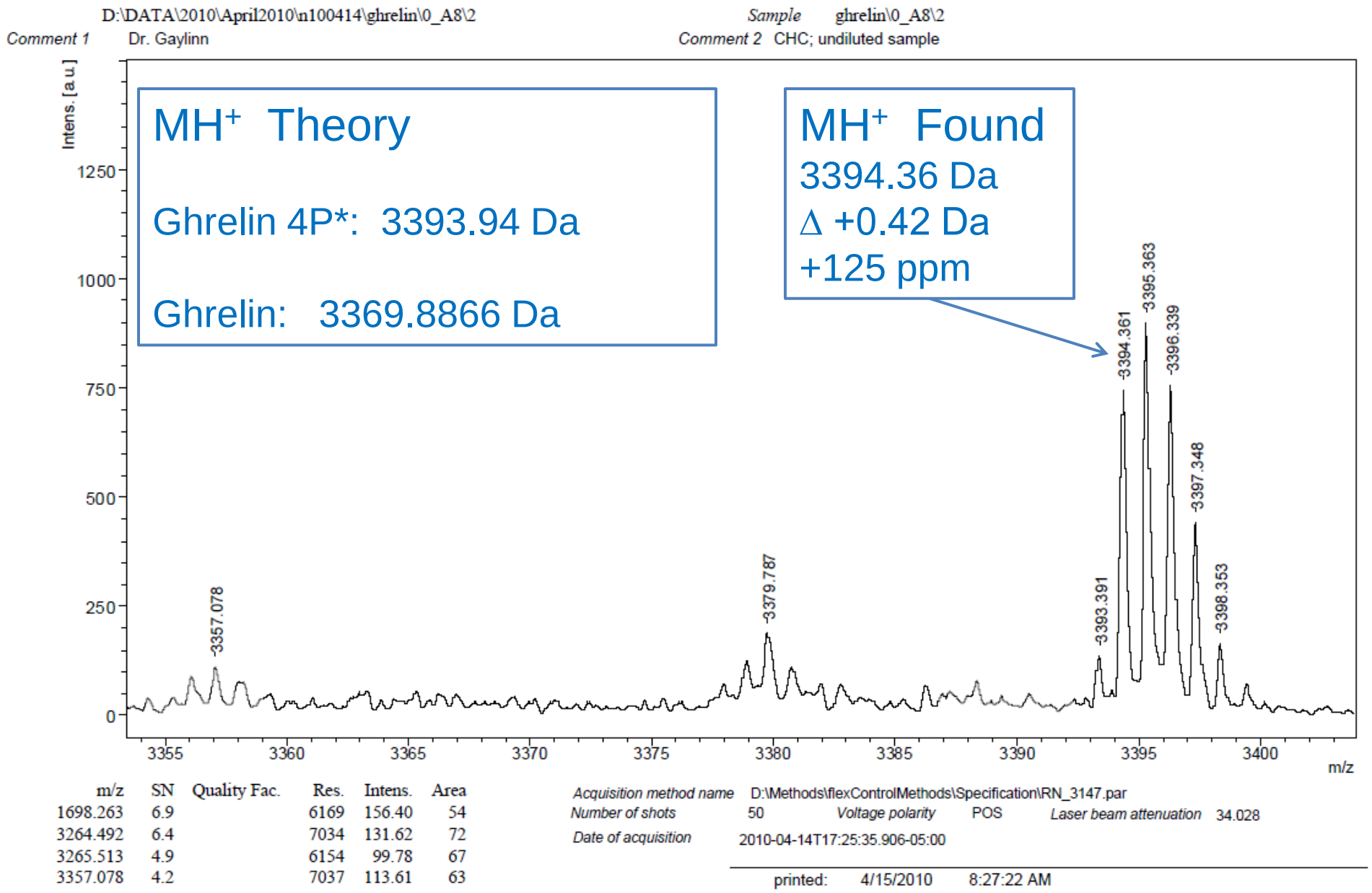


m/z	SN	Quality Fac.	Res.	Intens.	Area
1698.181	9.4		2701	235.37	51
3376.808	4.6		3611	405.60	334
3378.385	5.8		2398	518.57	562
3395.406	51.3		1566	4503.20	3735

Acquisition method name D:\Methods\flexControlMethods\Specification\LP_PepMix.par
Number of shots 50 *Voltage polarity* POS *Laser beam attenuation* 43.75
Date of acquisition 2010-04-22T09:50:39.250-05:00

printed: 4/22/2010 10:03:31 AM

Bruker MALDI ToF in Reflector Mode



m/z	SN	Quality Fac.	Res.	Intens.	Area
1698.263	6.9		6169	156.40	54
3264.492	6.4		7034	131.62	72
3265.513	4.9		6154	99.78	67
3357.078	4.2		7037	113.61	63

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Date of acquisition 2010-04-14T17:25:35.906-05:00

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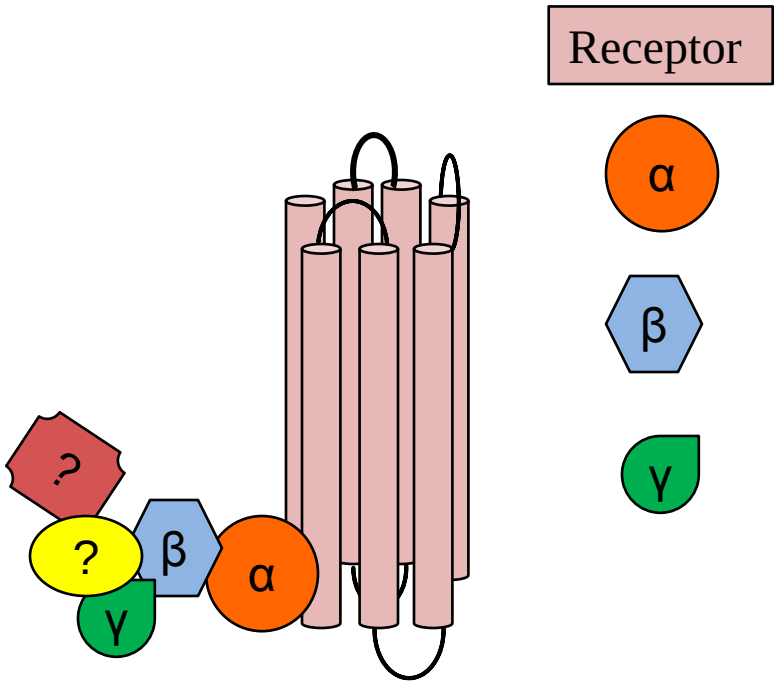
Orbitrap and FT MS Experiment

βγ Isoforms Binding to Adenosine A1 Receptor: Gαi1 Complex

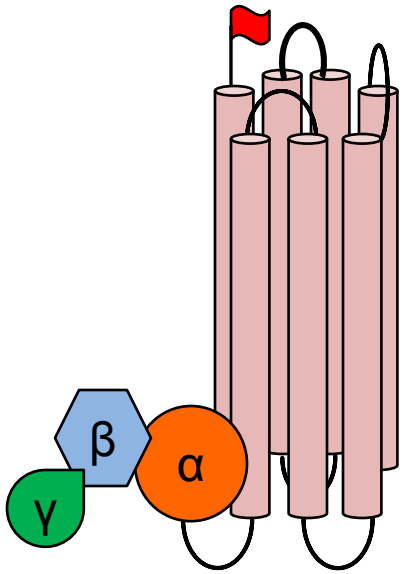
- Use Orbitrap Velos or FT MS
- Use SILAC:
  - Stable Isotope Labeling with Amino Acids in Cell Culture
  - For Relative Quantification of proteins from two cell cultures
  - Compares the tryptic peptides of proteins
    - From cells grown with Heavy Arg and/or Lys (^{13}C for +6 Da for each present).
    - To cells grown with Light Arg/Lys (^{12}C).

βγ Isoforms Binding to Adenosine A1 Receptor: Gαi1 Complex



- The **receptor:Gαβγ** ternary complex is involved in starting signaling cascades.
- G protein consists of αβγ trimer bound to a receptor.
- The **βγ dimer** binds to both receptor and the Gα subunit.
- The complex **regulates** many different signaling proteins **through the βγ dimer**.
- 20+ Gα isoforms.
- 5 Gβ Isoforms
- 12 Gγ isoforms.
- Do specific receptors choose particular combinations of α, β, and γ subunits?

βγ Isoforms Binding to Adenosine A_1 Receptor: $G\alpha_{i1}$ Complex Using SILAC



- Building a trap for βγ dimers
- Adenosine A_1 Receptor
- Fused with $G\alpha_{i1}$ & His-Flag Tag
- βγ dimers binds to receptor:$G\alpha_{i1}$ fusion protein
- Non-specific Ab to β for total quantitation.
- Which β and γ isoforms bound to Adenosine A_1:$G\alpha_{i1}$?

Quantification of βγ Isoforms in an R:G Complex

Recombinant Baculovirus in Sf9 Cells

Purify & Quantify

β

$\beta_1\gamma_2$
$\beta_2\gamma_2$
$\beta_4\gamma_2$

γ

$\beta_1\gamma_5$
$\beta_1\gamma_7$
$\beta_1\gamma_{10}$
$\beta_1\gamma_{11}$
$\beta_1\gamma_{12}$

Spike in 10 β equiv Each **βγ**

Total Amount of β Determined by a non-specific β Ab

SDS PAGE

β

γ

Trypsin Digest

HPLC

1. Select γ_5 Specific Peptides
2. Compare Areas Heavy vs. Light peptides
3. Calculate Relative Amount of γ_5

1. Repeat Steps 1-3 for each γ Isoform
2. Calculate % of Each γ Isoform vs. Total γ Isoforms

Adenosine A1:Gα Fusion Protein human HEK Cells Heavy Lys and Arg

1 β equiv Total for all **βγ**

Lyse

Ppt

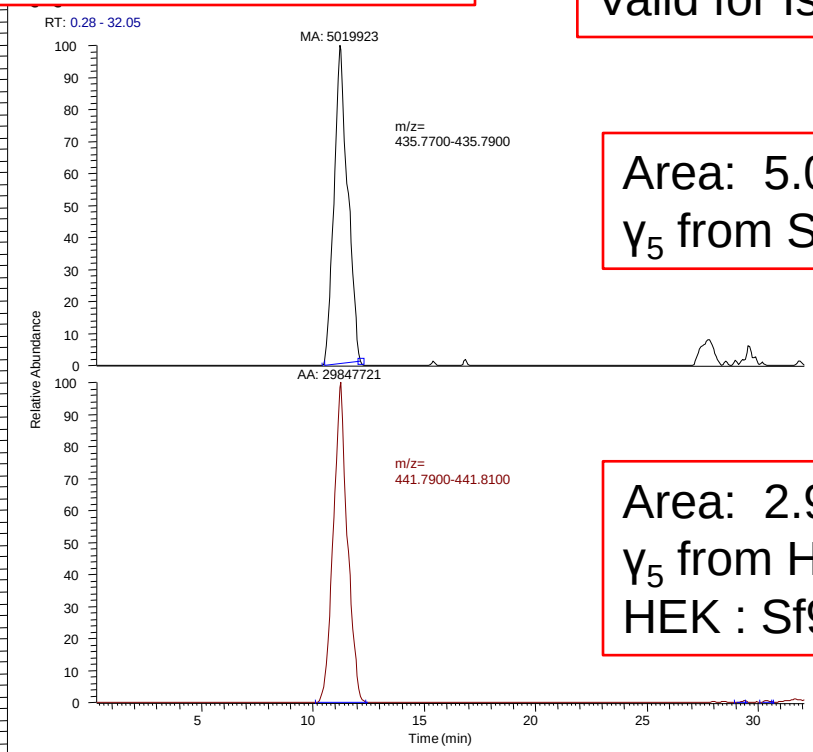
FLAG M2

Wash

Elute

γ_5 Peptide: KVVQQLR

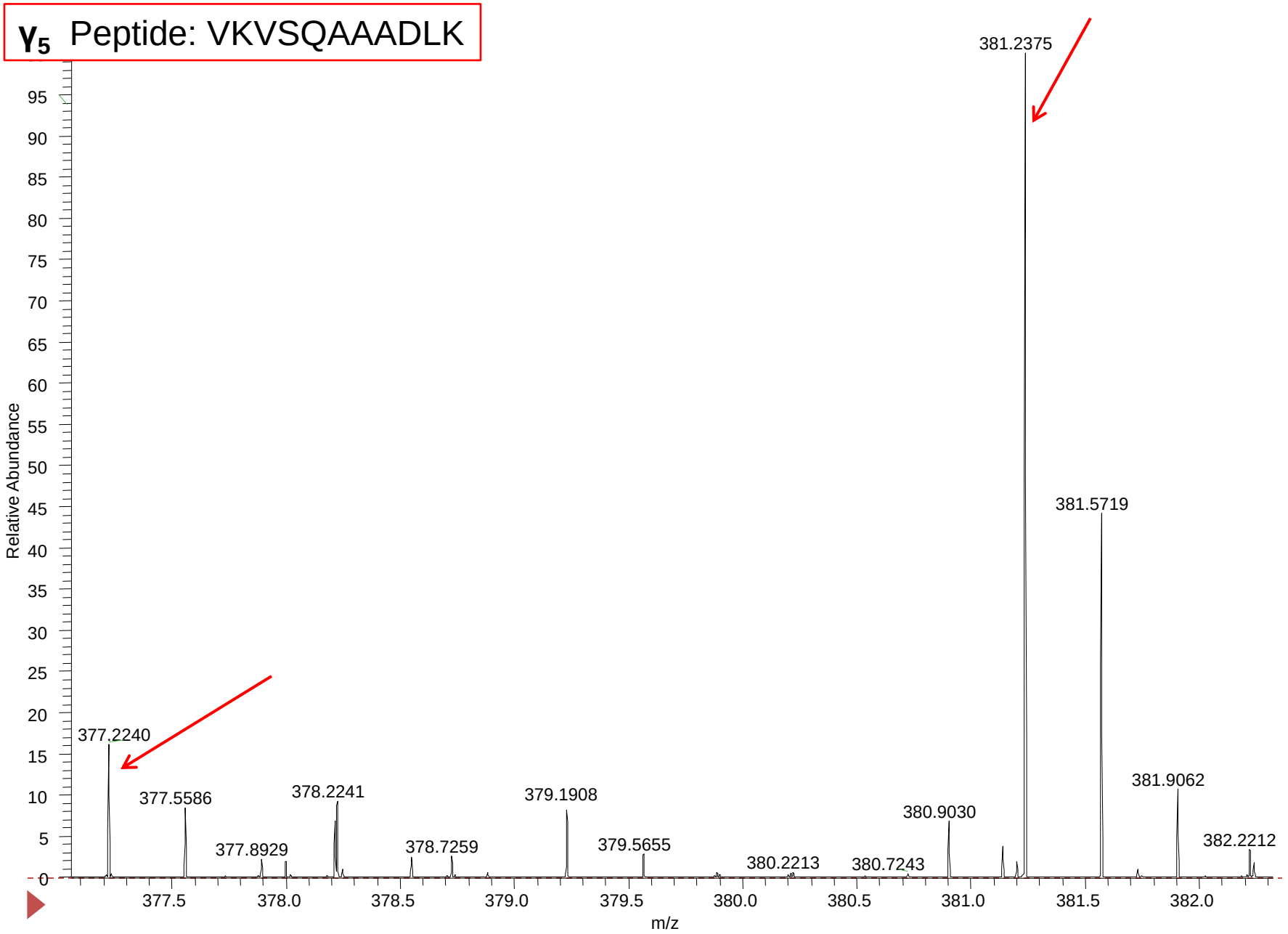
Area Comparisons Are Valid for Isotopic Peptides



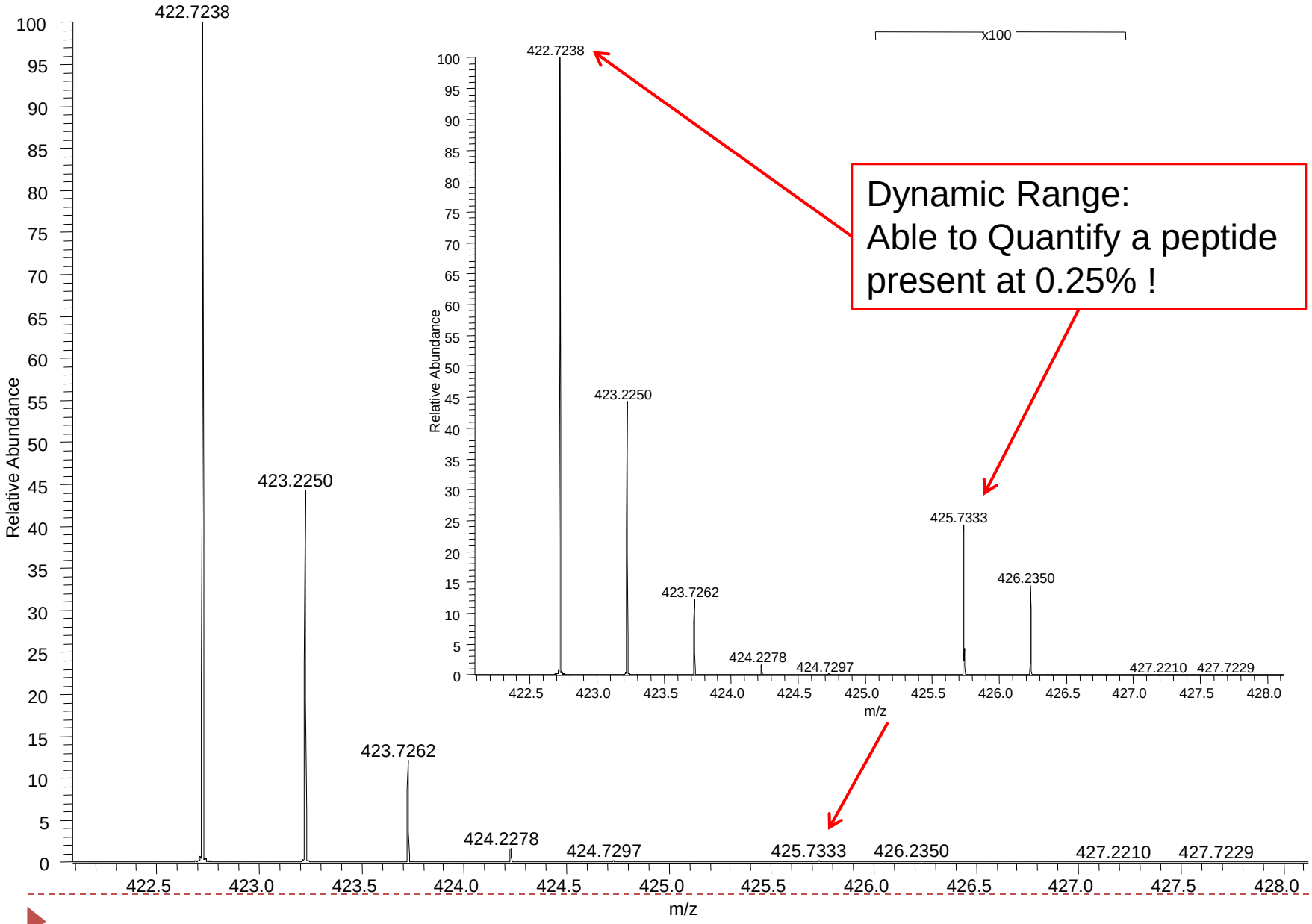
Area: 5.02 x10^6
γ_5 from Sf9 (light)

Area: 2.98 x10^7
γ_5 from HEK (Heavy)
HEK : Sf9 = 6:1

Resolution:
435.7796 vs.
435.7263 m/z


γ5 Peptide: VKVSQAAADLK
Relative Abundance
m/z
381.2375
381.5719
381.9062
382.2212
380.9030
380.7243
380.2213
379.5655
379.1908
378.7259
378.2241
377.8929
377.5586
377.2240
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
377.5
378.0
378.5
379.0
379.5
380.0
380.5
381.0
381.5
382.0

γ_{11} Peptide: SGEDPLVK



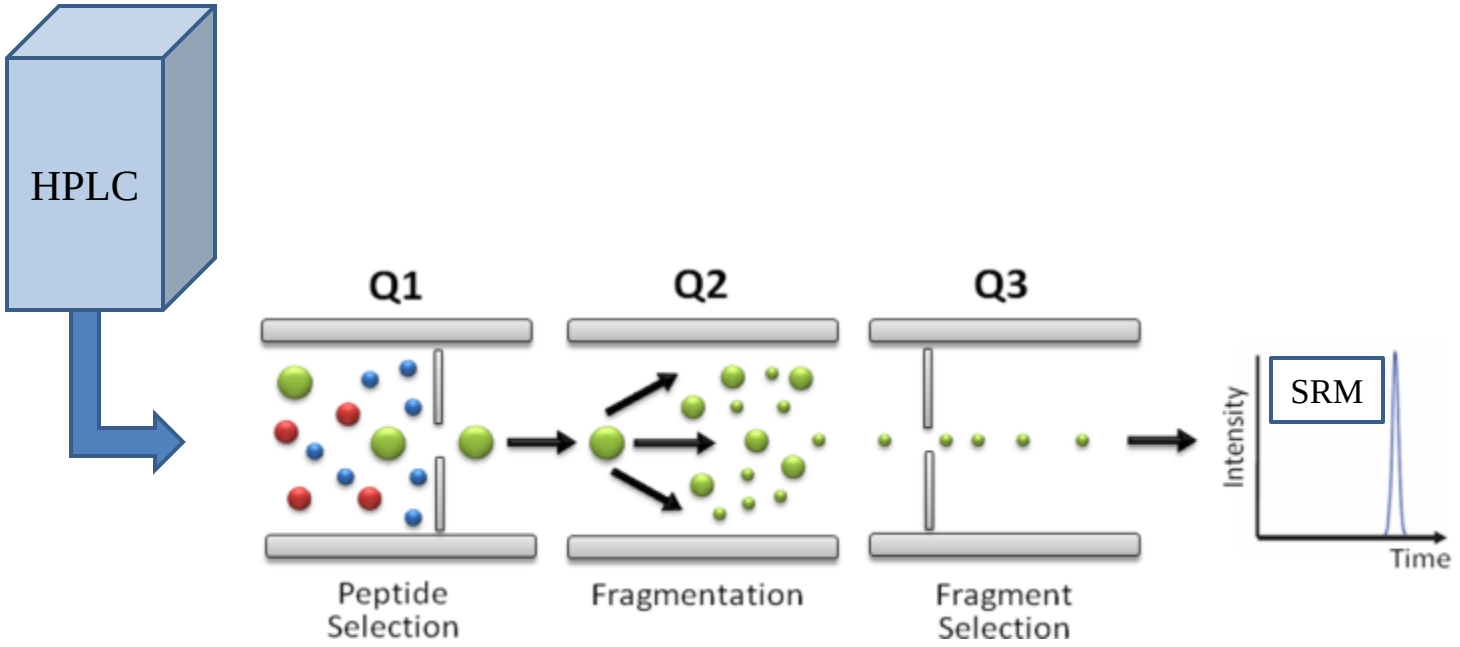
βγ Isoforms Binding to Adenosine A_1 Receptor: $G\alpha_{i1}$ Complex

γ Isoform	% Total γ	+/-
γ_2	6.3	0.9
γ_5	77.5	2.1
γ_7	2.3	0.1
γ_{10}	2.1	0.2
γ_{11}	0.03	0.01
γ_{12}	11.9	1.2

Thermo Triple Quad MS Experiment

- Quantitation by SRM
  - Selected Reaction Monitoring (SRM) or Multiple Reaction Monitoring (MRM).
  - Proteins, peptides, metabolites and PTMs of proteins.
  - No Ab needed
  - Variants easily detected.
  - Biomolecule are separated by chromatography.
  - Measures the fragment ion intensities of:
    - A biomolecule.
    - An isotopic (Heavy) version of the biomolecule which was added in a known amount.
  - Compares the two intensities to arrive at a relative amount for the natural biomolecule.

Thermo Triple Quad MS: SRM or MRM

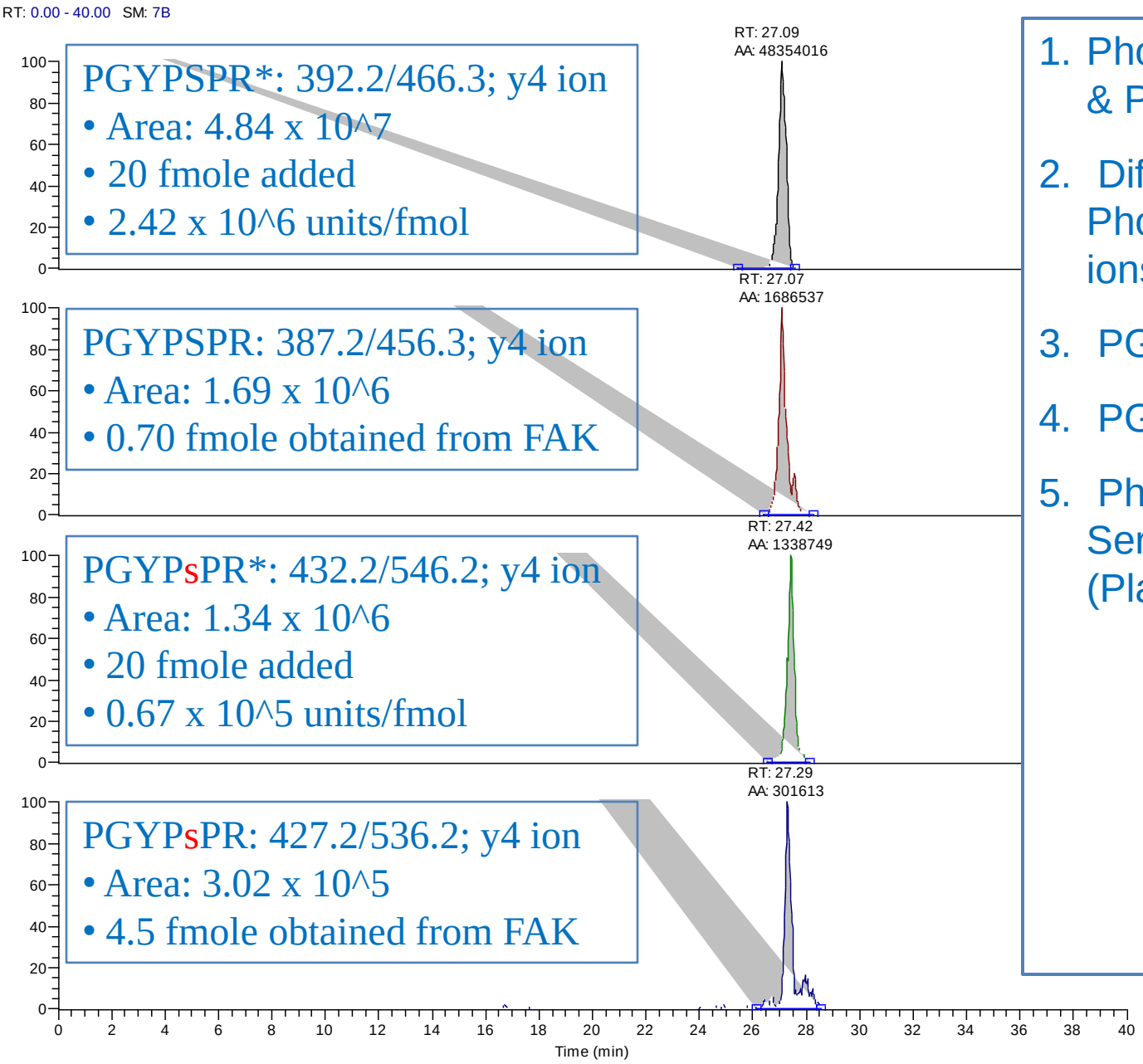


A. Schmidt, P. Picotti and R. Aebersold

FAK: Phosphorylation at Ser, Thr and Tyr

- Focal Adhesion Kinase I: 1052 Amino Acids
  - Involved in cell motility, proliferation and apoptosis;
  - Phosphorylation of Ser, Thr and Tyr controls interactions with other proteins.
- What Amount of Ser 722 is Phosphorylated?
- Experiment Design:
  1. Digest FAK to produce natural or “Light” peptides.
  2. Add a known amount of each “Heavy” tryptic peptide. (synthesized with $^{13}C/^{15}N$ Arg).
  3. Run Spiked digest on LC-MSMS (triple quad).
  4. For each Heavy/Light peptide, Compare area from a transition: MS ion (precursor)/MSMS ion (product ion).

SRM MS of +/- Phosphorylated PGYPSPR



PGYPSPR*: 392.2/466.3; y4 ion
- Area: 4.84 x 10^7
- 20 fmole added
- 2.42 x 10^6 units/fmol

PGYPSPR: 387.2/456.3; y4 ion
- Area: 1.69 x 10^6
- 0.70 fmole obtained from FAK

PGYPsPR*: 432.2/546.2; y4 ion
- Area: 1.34 x 10^6
- 20 fmole added
- 0.67 x 10^5 units/fmol

PGYPsPR: 427.2/536.2; y4 ion
- Area: 3.02 x 10^5
- 4.5 fmole obtained from FAK

1. Phosphorylated PGYPsPR & PGYPSPR co-elute.
2. Different intensities for Phosphorylated vs. Non-Pi ions.
3. PGYPSPR: 0.7 fmol
4. PGYPsPR: 4.5 fmol
5. Phosphorylation at FAK Ser722: 87% or 1:6.4 (Plain/Pi)

Summary Points

- A Mass Spectrometer makes ions and measures them accurately.
- A Mass Spectrometer Can Give:
  - Molecular Mass
  - Sequence & Structure
  - Identity
  - Quantity
- Different Mass Spectrometers for Different Information.
- The Keck MS Lab Services include:
  - Confirmation
  - Discovery
  - Quantification
- Talk to Us about Your Research Goals

Supplementary Slides to Introduction to Mass Spectrometry (May 18, 2010)

Mass Spectrometer Parameters

Table 1. Characteristics and performances of commonly used types of mass spectrometers. Check marks indicate available, check marks in parentheses indicate optional. +, ++, and +++ indicate possible or moderate, good or high, and excellent or very high, respectively. Seq., sequential.

	IT-LIT	Q-Q-ToF	ToF-ToF	FT-ICR	Q-Q-Q	QQ-LIT
Mass accuracy	Low	Good	Good	Excellent	Medium	Medium
Resolving power	Low	Good	High	Very high	Low	Low
Sensitivity (LOD)	Good		High	Medium	High	High
Dynamic range	Low	Medium	Medium	Medium	High	High
ESI	✓	✓		✓	✓	✓
MALDI	(✓)	(✓)	✓			
MS/MS capabilities	✓	✓	✓	✓	✓	✓
Additional capabilities	Seq. MS/MS			Precursor, Neutral loss, MRM		
Identification	++	++	++	+++	+	+
Quantification	+	+++	++	++	+++	+++
Throughput	+++	++	+++	++	++	++
Detection of modifications	+	+	+	+		+++

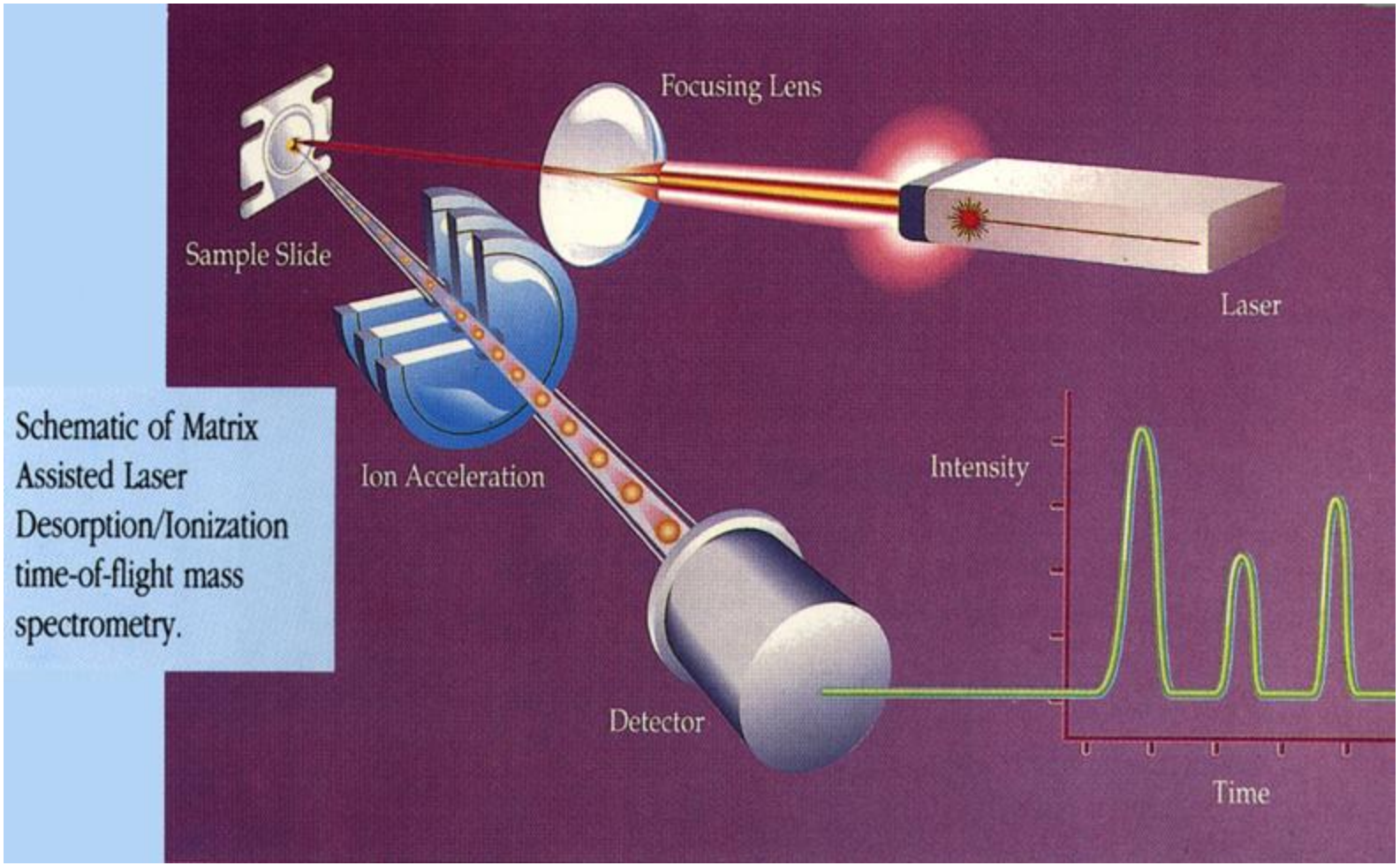
Domon & Mann Science 2006

Comparison of Mass Analyzers

	TOF	mass filter	ion-trap	sector	FT-ICR
mass resolving power	10^3-10^4	10^2-10^4	10^3-10^4	10^2-10^5	10^4-10^6
mass accuracy	5-50 ppm	100 ppm	50-100 ppm	1-5 ppm	1-5 ppm
mass range	>10^5	10^4	1.5 × 10^5	10^4	>10^4
linear dynamic range	10^2-10^6	10^7	10^2-10^5	10^9	10^2-10^5
precision	0.1-1%	0.1-5%	0.2-5%	0.01-1%	0.3-5%
abundance sensitivity	up to10^6	10^4-10^6	10^3	10^6-10^9	10^2-10^5
efficiency (transmission × duty cycle)	1-100%	<1-95%	<1-95%	<1% (scanning)	<1-95%
speed	10^1-10^4 Hz	1-20 Hz	1-30 Hz	0.1-20 Hz	0.001-10 Hz
compatibility with ionizer	pulsed & continuous	continuous	pulsed & continuous	continuous	pulsed & continuous
cost	moderate to high	relatively low	low to moderate	moderate to high	moderate to high
size/weight/utility requirements	benchtop	benchtop	benchtop	lab instruments	lab instrument

Angeletti , Eistein CoM 2007

Schematic of a MALDI Tof MS



Schematic of Matrix Assisted Laser Desorption/Ionization time-of-flight mass spectrometry.

http://kenickbiochem09.

Mass Spectrometry References

- UVa: Search “mass spectrometry AND Sherman”
- Google: Search “Siuzdak AND Scripps”
- Google: Search “ASMS”
- Google: “IonSource”
- National Magnet Lab/FSU:
  - www.magnet.fsu.edu/education/tutorials/tools/
- Books:
  - Introduction to Proteomics, Liebler
  - Protein Sequencing and Identification Using Tandem MS, Kinter & Sherman
  - Computational Methods for MS Proteomics, Eidhammer, et al.,

Terms

- m/z : mass of an ion divided by the number of charges
- Dalton:
- MW: Molecular Weight
  - Angiotensin I = 1296.48 g/mole
- MH^+ (mono): MonoIsotopic
  - Angiotensin I = 1296.6848 m/z (z = 1)
- MH^+ (avg): Average
  - Angiotensin I = 1297.5115 m/z
- MH^{+3} (mono): MonoIsotopic
  - Angiotensin I = 432.8998 m/z (z = 3)