NIJ National Institute of Justice *Technology Transition Workshop*

Introduction to Biological Mass Spectrometry
(Mass Spectrometry 101)

Steven A. Hofstadler, Ph.D.

Ibis Biosciences, Inc.

Disclaimer

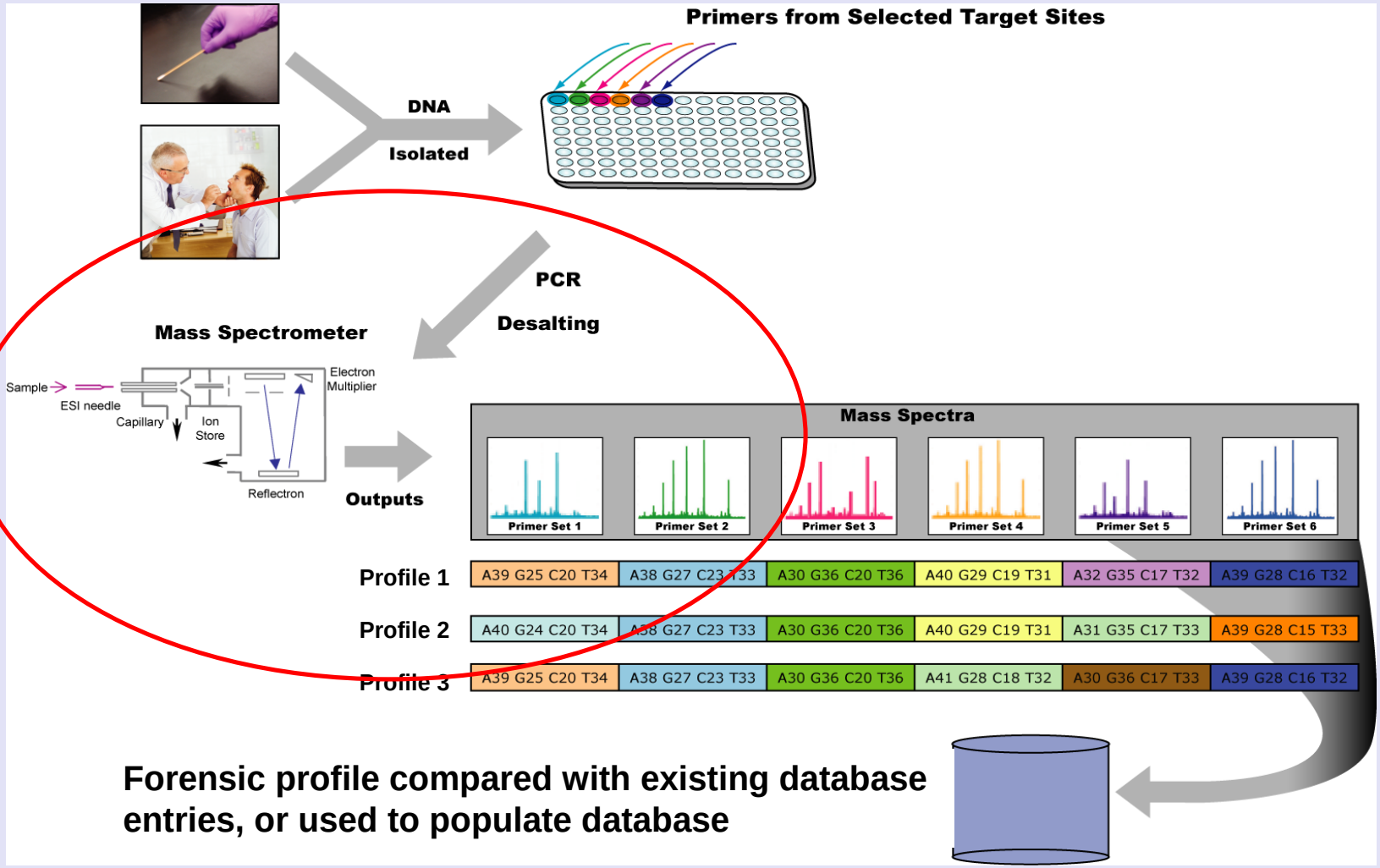
- **This presentation covers the basic concepts of mass spectrometry**
- **The material is not specifically required to operate the Ibis T5000™**
- **Users are not expected to tune and/or optimize the mass spectrometer**
- **The goal of this presentation is to give the user a basic understanding of where/how mass spectrometry fits into the Ibis workflow**

Overview

- **Introduction – what is Mass Spectrometry?**
  - **Mass Spectrometry (MS) and Ibis T5000™**
- **Brief History**
- **General Components**
- **The “Mass” Spectrum**
  - **Definitions and Nomenclature**
- **Ionization Sources**
  - **Matrix Assisted Laser Desorption Ionization (MALDI)**
  - **Electrospray Ionization (ESI)**
  - **Others**
- **Time-of-Flight (TOF) Mass Analyzers**
- **ESI-TOF of nucleic acids**

Technology Transition Workshop

Mass Spectrometry and Ibis platform



Forensic profile compared with existing database entries, or used to populate database

IMAGE COURTESY OF IBIS BIOSCIENCES, INC.

NIJ National Institute of Justice *Technology Transition Workshop*

Mass Spectrometry of Nucleic Acids?

- **Information content**
  - **From precise mass measurements unambiguous base compositions are derived [A10 G23 C32 T17] = [10 23 32 17]**
- **Speed**
  - **≤ 1 minute/sample**
- **Applicability to mixtures**
  - **Dynamic range is around 100:1**
  - **MS succeeds where sequencing fails (e.g. mixtures)**
- **Automation**
  - **End-to-end process is highly automated (including spectral processing/interpretation)**
- **Sensitivity**
  - **Single copy detection demonstrated with PCR front-end**

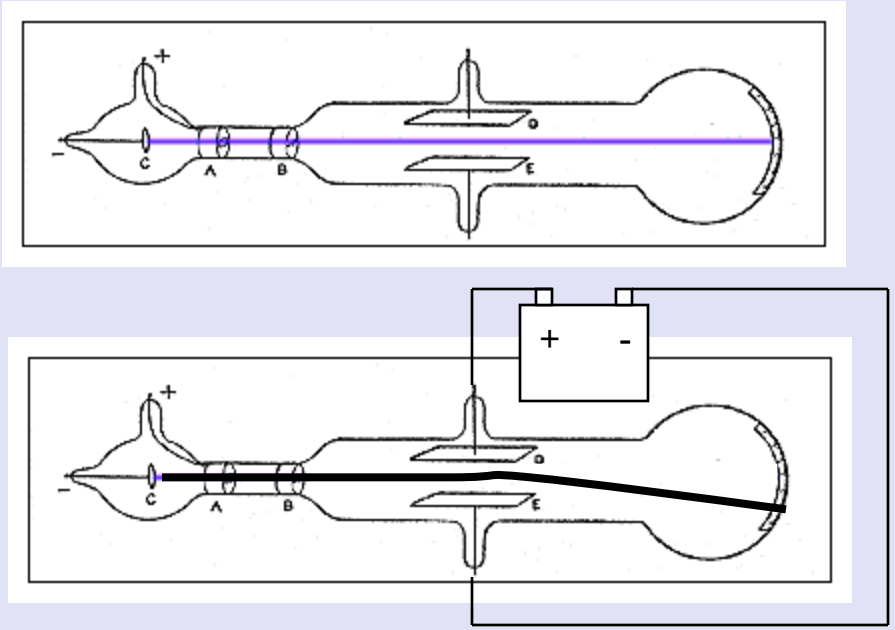
What is a Mass Spectrometer?

- **An instrument which measures the mass-to-charge ratio (*m/z*) of ionized analyte based on its response to applied electric and/or magnetic fields**
  - **atoms, molecules, clusters, and macromolecular complexes**
- **The *m/z* measurement is converted to a mass measurement**
  - ***m* is in atomic mass units or Daltons (Da)**
    - **1 Da = 1/12 the mass of a single atom of ^{12}C**
    - **1 Da = 1.66 x 10^{-24} grams**
  - ***z* is an integer multiplier of the fundamental unit of charge (q)**
    - **q = 1.602 x 10^{-19} Coulombs**
  - **Mass = *m*/z X z**
- **A mass spectrometer is essentially a "molecular (or atomic) scale" that "weighs" analytes of interest**

Brief History

- **1897 J.J. Thompson announced the presence of electrons or "corpuscles" based on the deflection of cathode rays by electric and magnetic fields**
- **He later used this "beam-deflection device" to measure the mass of the electron (1906 Nobel Prize)**

IMAGE COURTESY OF:
http://www.manep.ch/img/photo/challenges/nanotubes/thompson.jpg



IMAGES COURTESY OF STEVEN A. HOFSTADLER, PH.D.

Brief History (cont.)

- **1919 F. W. Aston used Thompson's mass spectrometer to measure the atomic masses of 30 gaseous elements and prove the existence of multiple isotopes. Relative abundance measurements were made by recording isotope lines on film. "Mass spectroscopy" *Nature* 1919; 104; 393. (1922 Nobel Prize)**
  - Design principles are the basis of modern electric and magnetic sector instruments

Aston's original "Positive-Ray Mass Spectrograph"

IMAGE COURTESY OF SCIENCE MUSEUM/SCIENCE & SOCIETY PICTURE LIBRARY:
http://www.ingenious.org.uk/site.asp?s=S2&DCID=1927-1085

Basic Components

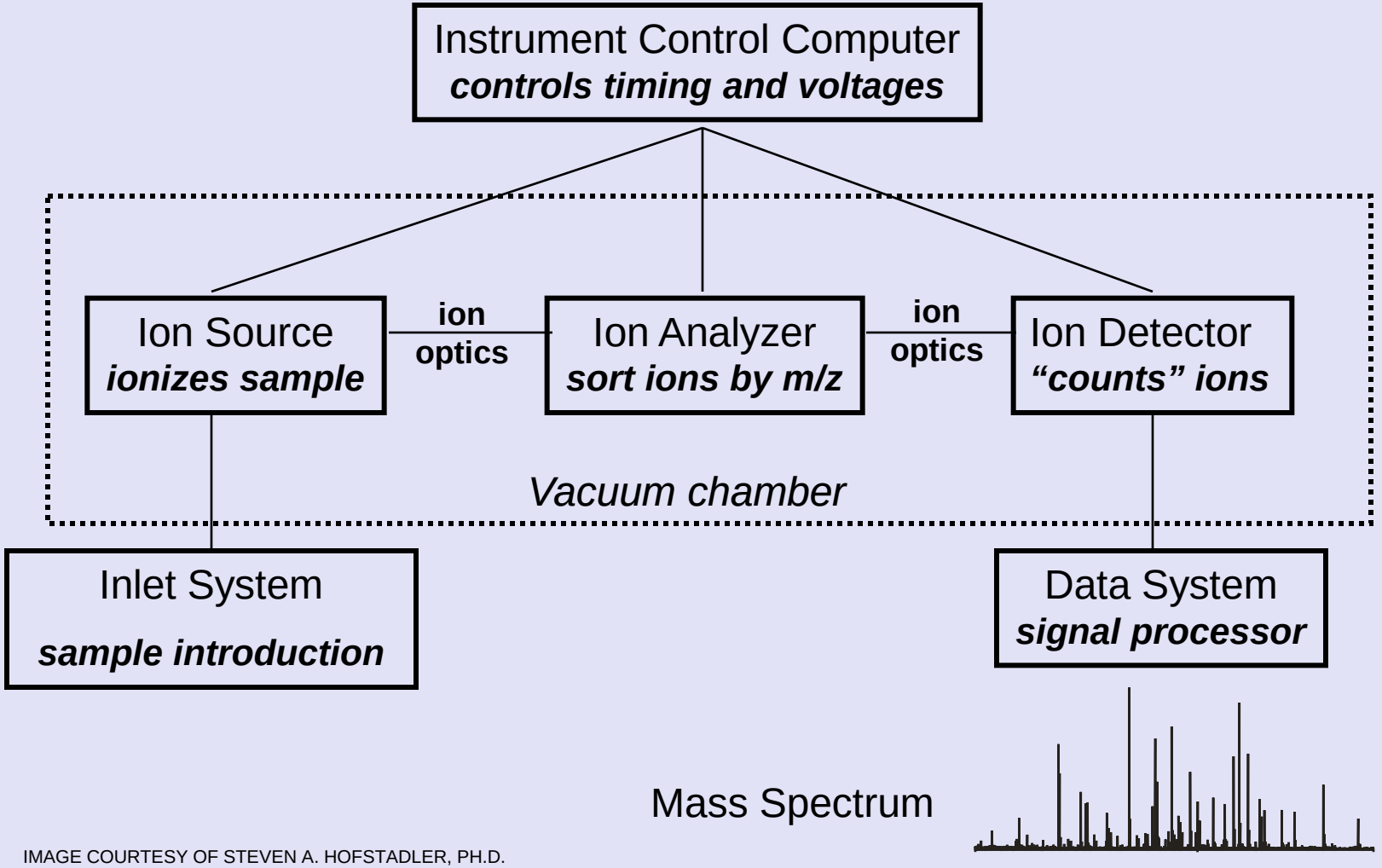


IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

The “Mass” Spectrum

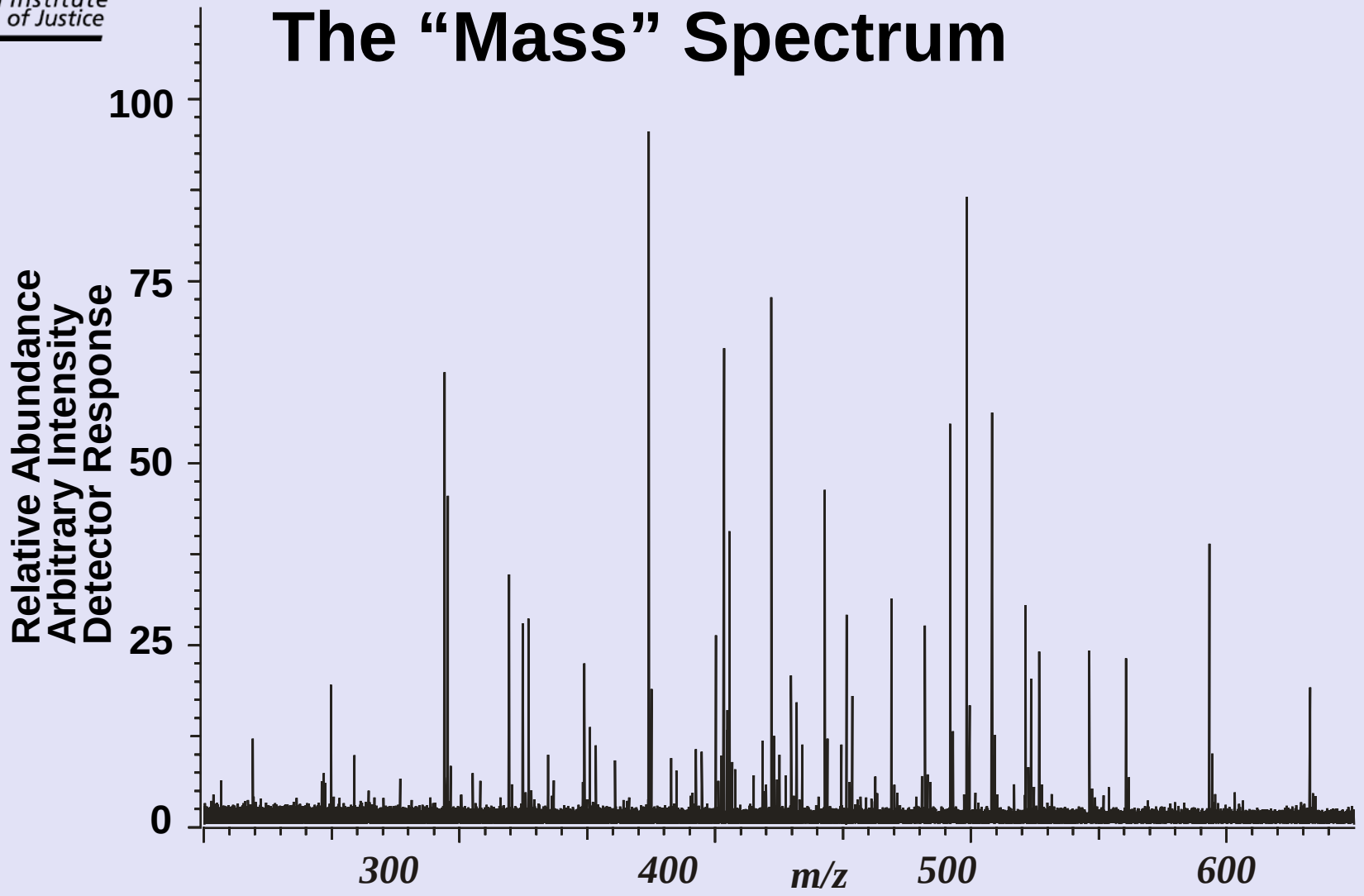
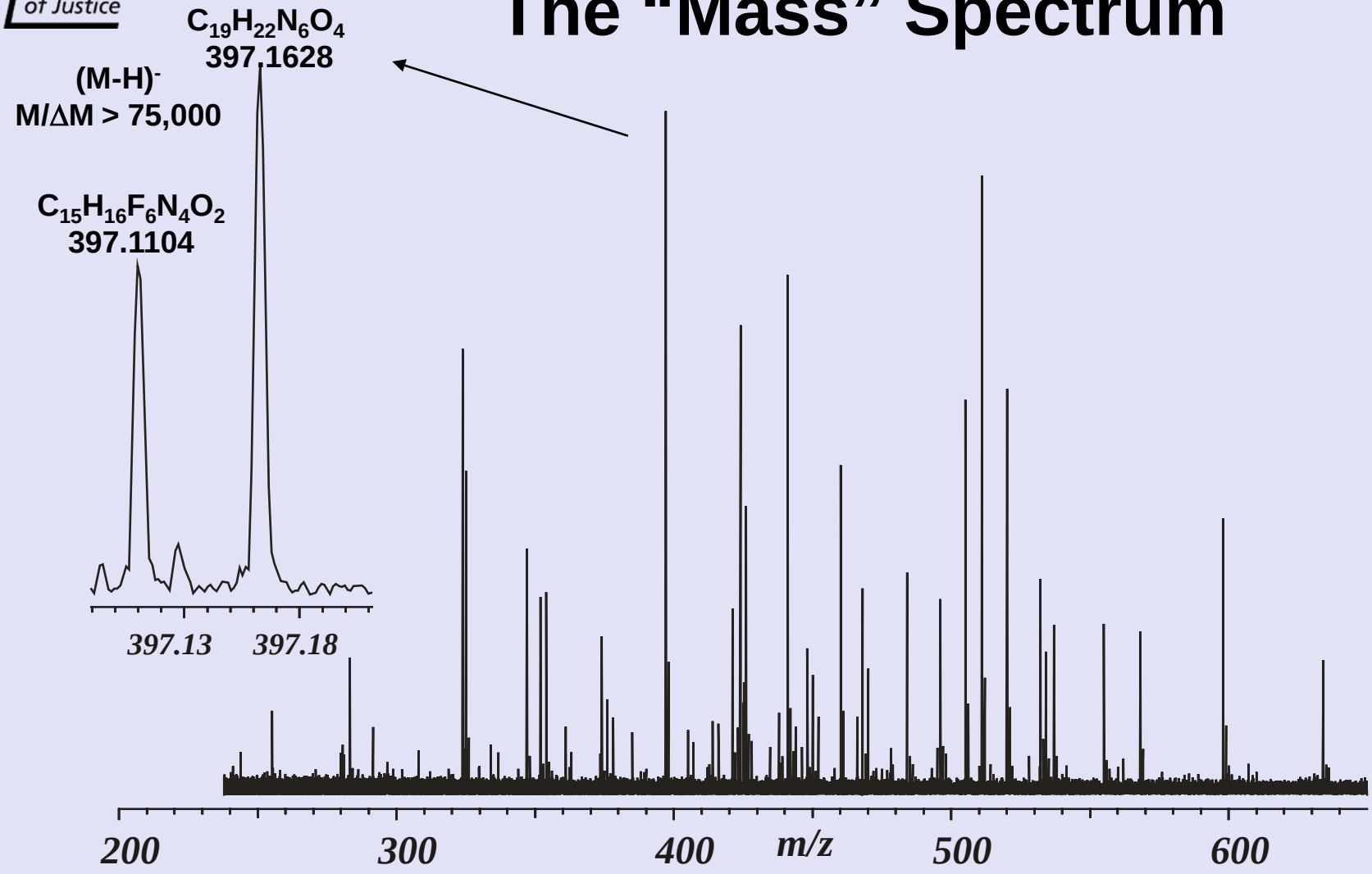


IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

The “Mass” Spectrum



IMAGES COURTESY OF STEVEN A. HOFSTADLER, PH.D.

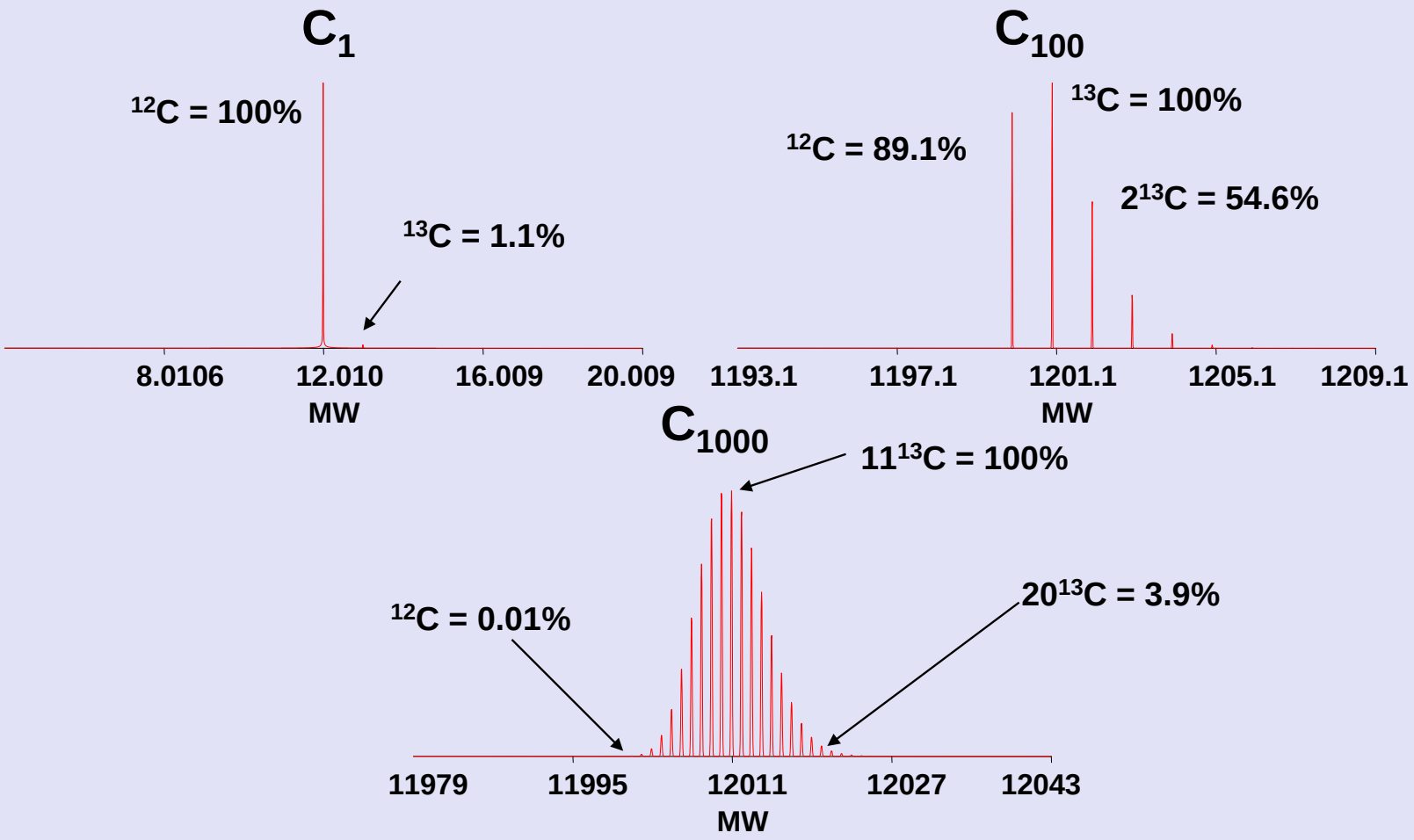
The Isotopic Envelope

- **Most elements have more than 1 isotope**
- **For a given atom type, different isotopes have different numbers of neutrons**
  - **e.g. an atom of ^{12}C has 6 neutrons, 6 protons, and 6 electrons**
  - **an atom of ^{13}C has 7 neutrons, 6 protons, and 6 electrons**
- **The mass of a neutron is 1.00867 Da**
- **Each element has different numbers and relative abundances of other isotopes:**
  - **^{12}C = 98.90% ^{13}C = 1.10%**
  - **^{35}Cl = 75.77% ^{37}Cl = 24.23%**
  - **^{19}F = 100%**

The Isotopic Envelope

- **Unless a molecule is composed of only monoisotopic elements, there is a finite probability that it will contain one or more heavy isotopes**
- **The relative abundance of the monoisotopic peak decreases with increasing mass**
- **Observed distribution is the sum of isotopic contributions from all hetero-isotopes**
- **Except in a few cases, "isotopic fine structure" cannot be resolved**
  - **e.g. for an N+2 peak the contributions from 2 ^{13}C and 1 ^{18}O cannot be resolved**
- **Consider carbon clusters:**

Isotope Distributions of Carbon



IMAGES COURTESY OF STEVEN A. HOFSTADLER, PH.D.

Definitions and Nomenclature

- **Resolution : M/ΔM**
  - **actually (m/z)/Δ(m/z)**
  - **Δ(m/z) : peak width at full width half maximum (FWHM)**

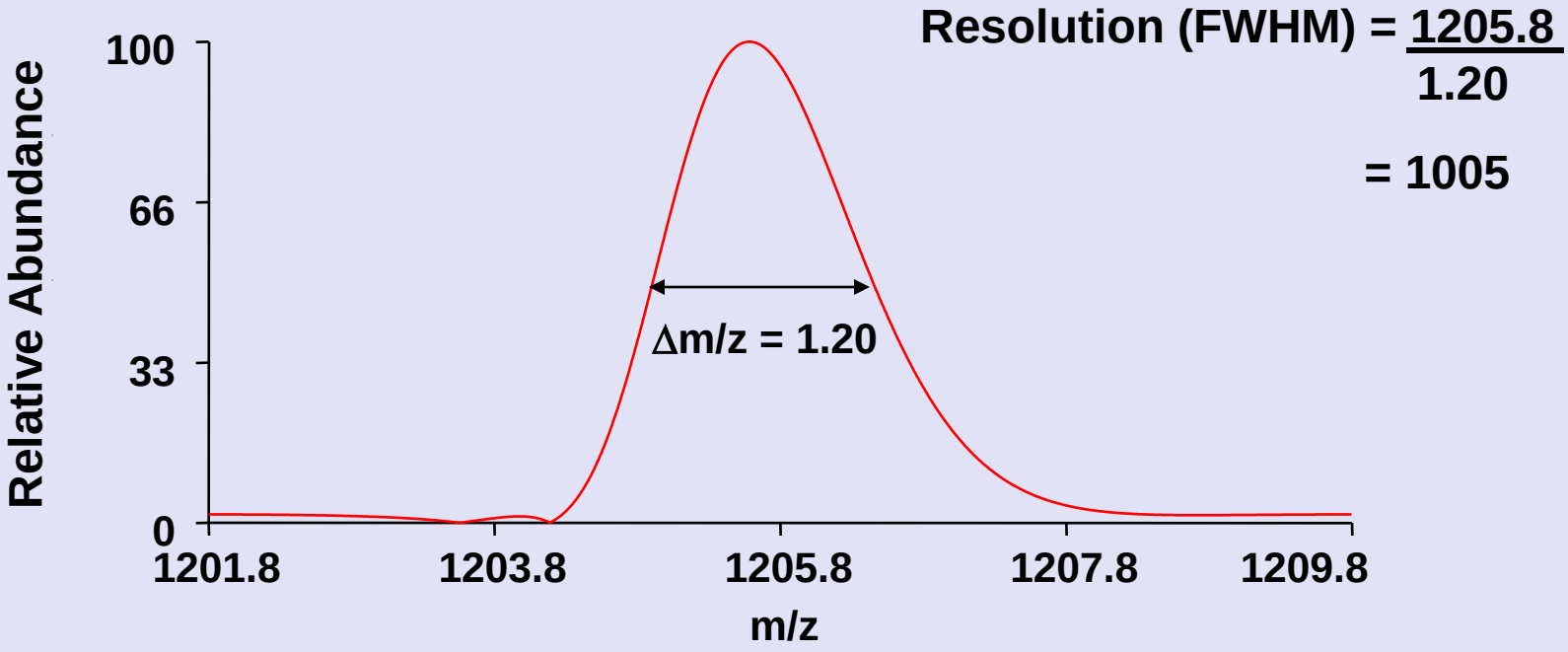


IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

Definitions and Nomenclature

- **Resolution (cont.)**
  - **can be limited by the inherent width of the isotope envelope**
  - **step function to isotopic resolution**
    - **need M/ΔM > molecular weight for isotopic resolution**

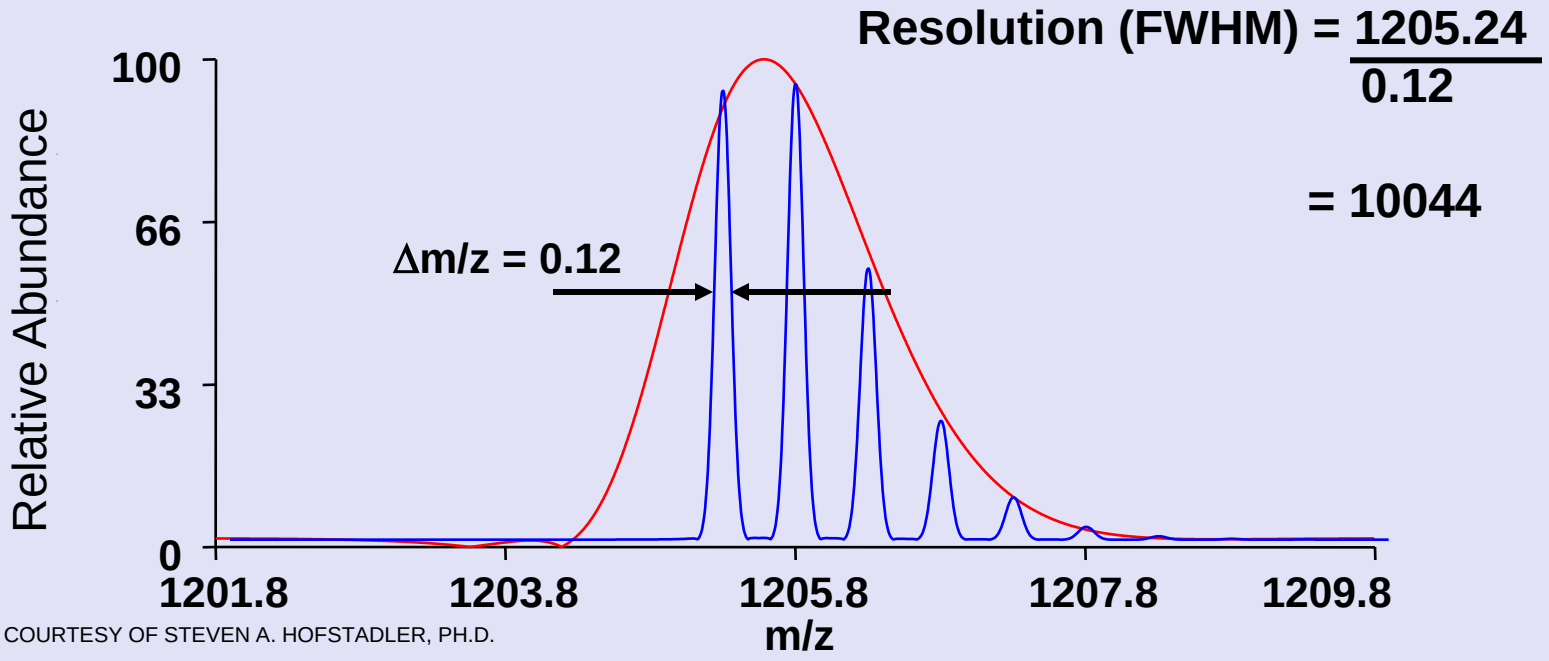
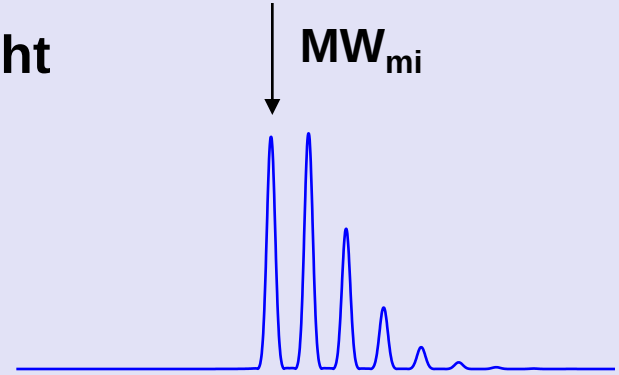


IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

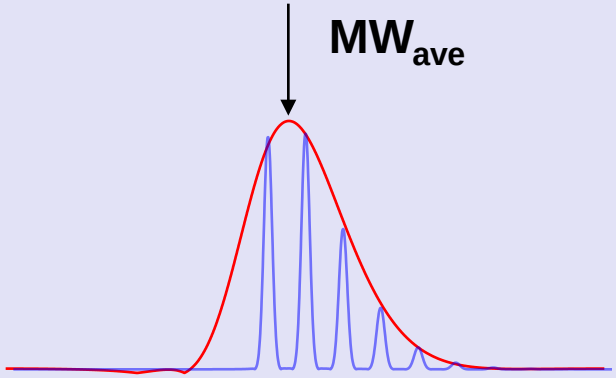
Definitions and Nomenclature: Mass Measurements

3 ways to specify molecular weight

- **Monoisotopic Molecular Weight**
  - **All ^{12}C, ^{14}N, ^{16}O, etc.**
  - **most accurate method for low MW species**
  - **monoisotopic peak is base peak (i.e. most abundant peak) up to about 2 kDa**



- **Average Molecular Weight**
  - **most commonly used**
  - **few MS platforms can resolve isotopes for analytes > 5 kDa**
    - **Δ between monoisotopic and average increases with increasing MW**



IMAGES COURTESY OF STEVEN A. HOFSTADLER, PH.D.

Definitions and Nomenclature: Mass Measurements (cont.)

3 ways to specify molecular weight

- **Most Abundant Isotope Molecular Weight**
  - **not widely used**
  - **convenient for high MW, isotopically resolved species**

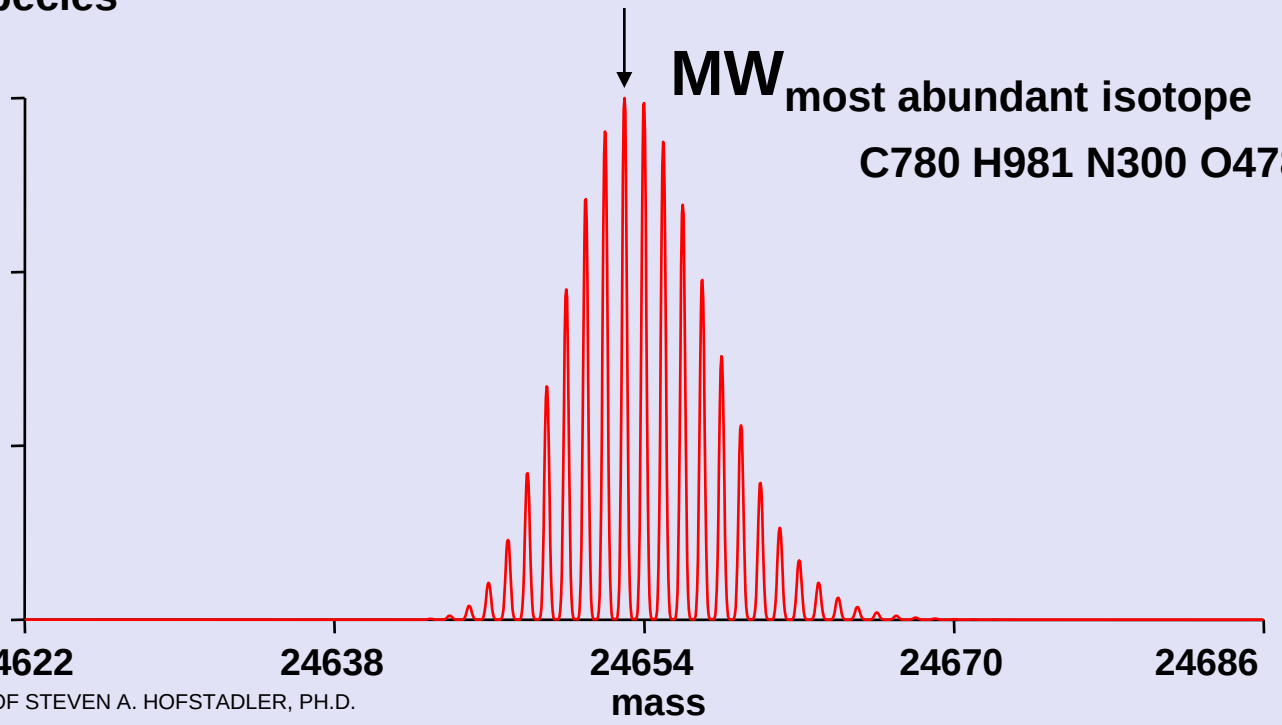
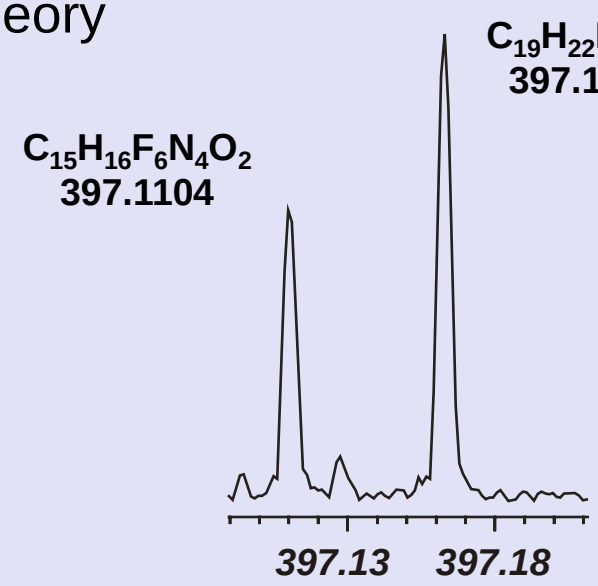


IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

Definitions and Nomenclature

- Mass Measurement Accuracy

$$\frac{|(m/z)\text{theory} - (m/z)\text{measured}|}{(m/z)\text{theory}} = \textbf{Mass Measurement Error (ppm)}$$



(M-H)$^-$
M/ΔM > 75,000

cmpd	M_{calc}	M_{meas}	Δm	ppm
$C_{15}H_{16}F_6N_4O_2$	397.1105	397.1104	0.0001	0.25
$C_{19}H_{22}N_6O_4$	397.1630	397.1628	0.0002	0.50

IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

Mass Spectrometry Nuts and Bolts

- Ionization Sources
- Mass Analyzers

Ionization Sources

- **Ionization is the process by which analytes are "charged"**
  - **Adding or removing electrons (e^-) (MW = 0.0006 Da)**
  - **Adding or removing protons (H^+) (MW = 1.0078 Da)**

- **Several very effective methods for ionizing low molecular weight and/or volatile compounds. Limited MS to analytes with molecular weights under ~1 kDa**

- **In the 1980's, two ionization methods developed for ionizing high molecular weight analytes**
  - **MALDI & ESI**

Ionization Sources for Low MW Analytes

- **APCI Atmospheric Pressure Chemical Ionization**
  - formation of analyte ions through charge exchange with ionized carrier gas
- **EI Electron Ionization**
  - generation of ions by bombarding gas phase molecules with high energy electrons
  - analyte must be volatile
  - ionization energy dictates extent of fragmentation
  - still widely used w/ GC

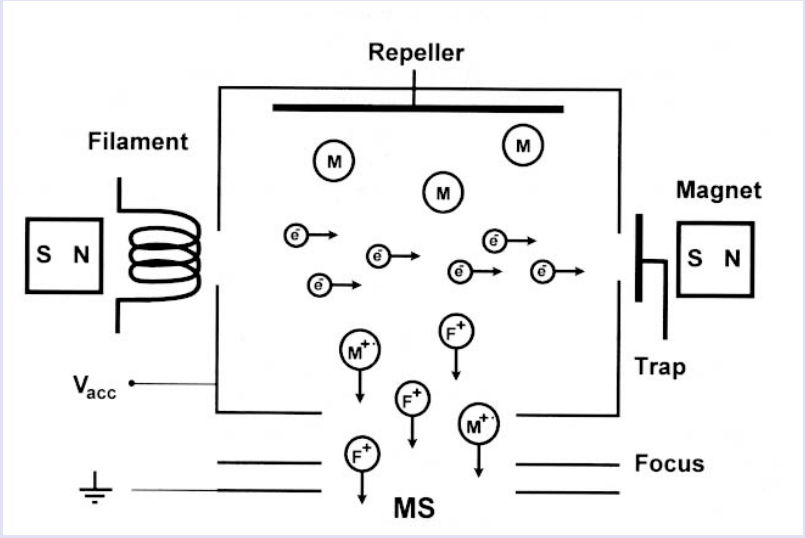
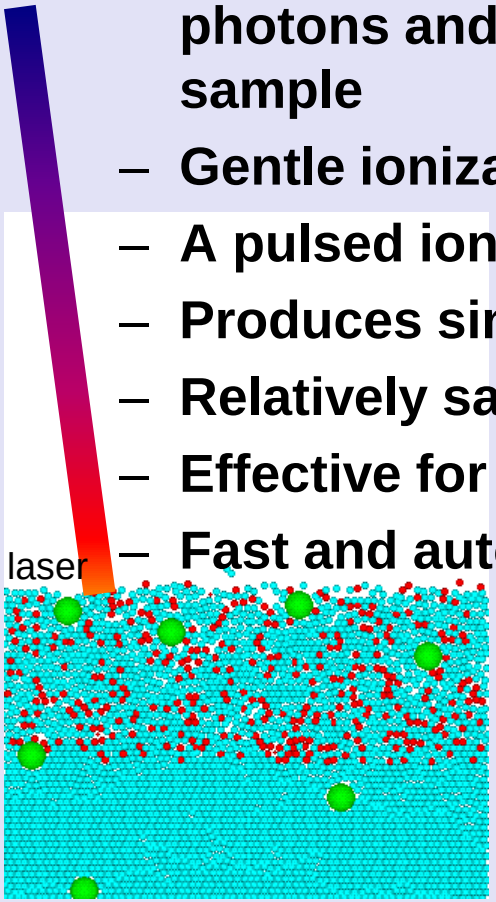
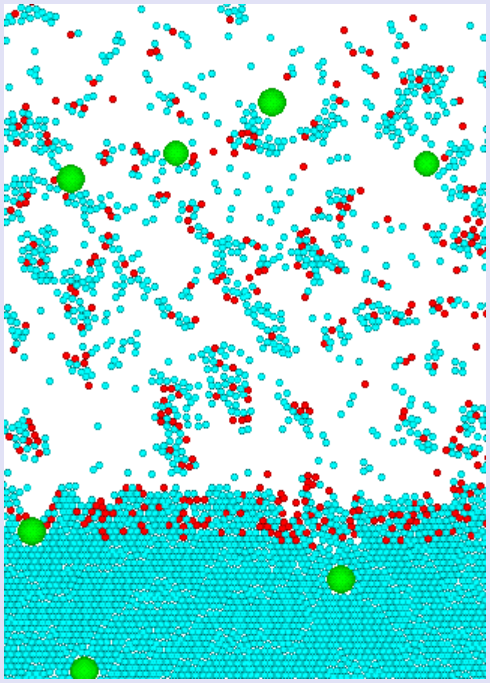


IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

Ionization Sources - MALDI

- **Matrix Assisted Laser Desorption Ionization**
  - **Sample is co-crystallized with a matrix which absorbs photons and creates a desorption plume that ionizes the sample**
  - **Gentle ionization technique (harsher than ESI)**
  - **A pulsed ion source**
  - **Produces singly charge ions**
  - **Relatively salt tolerant**
  - **Effective for wide range of MW's**
  - **Fast and automatable**





IMAGES COURTESY OF STEVEN A. HOFSTADLER, PH.D.

Ionization Sources - ESI

- Electrospray Ionization
  - Ions are desolvated/desorbed from highly charged liquid droplets
  - Generates multiple charge states of large analytes
    - results in “folded-over” spectra which can be recorded over narrower *m/z* range
  - Very soft ionization technique
    - applicable to labile molecules and noncovalent complexes
  - Low tolerance for nonvolatile salts, buffer additives, and detergents
    - rigorous sample clean-up required for some applications
  - High sensitivity
    - applicable to analyte concentrations < 100 nM

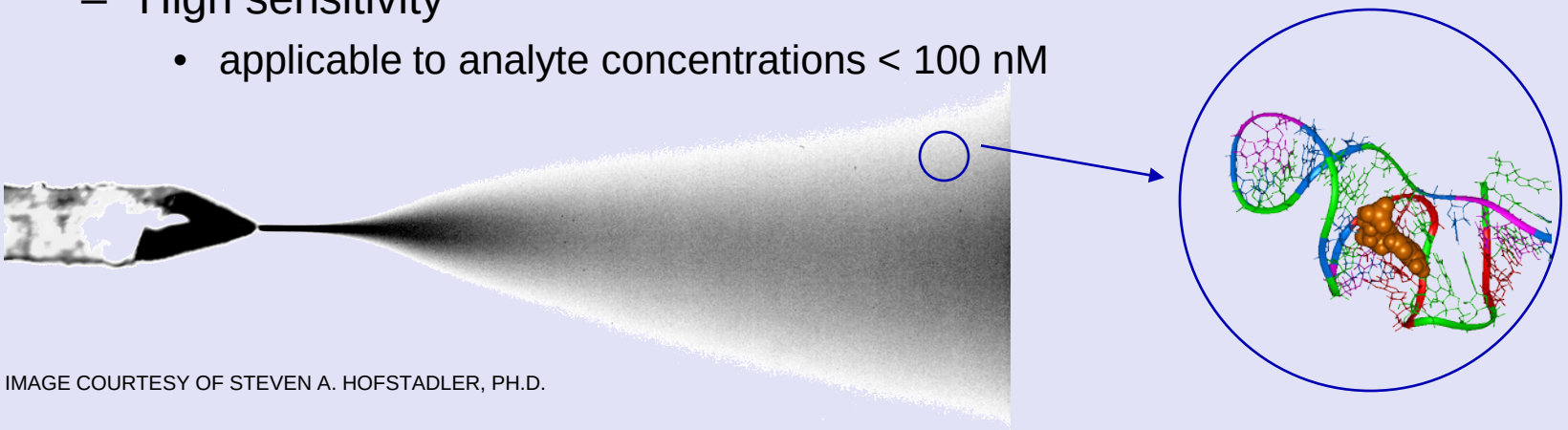


IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

A Negative Ionization Mode ESI Mass Spectrum of a Low MW Analyte: Singly Charged Spectrum

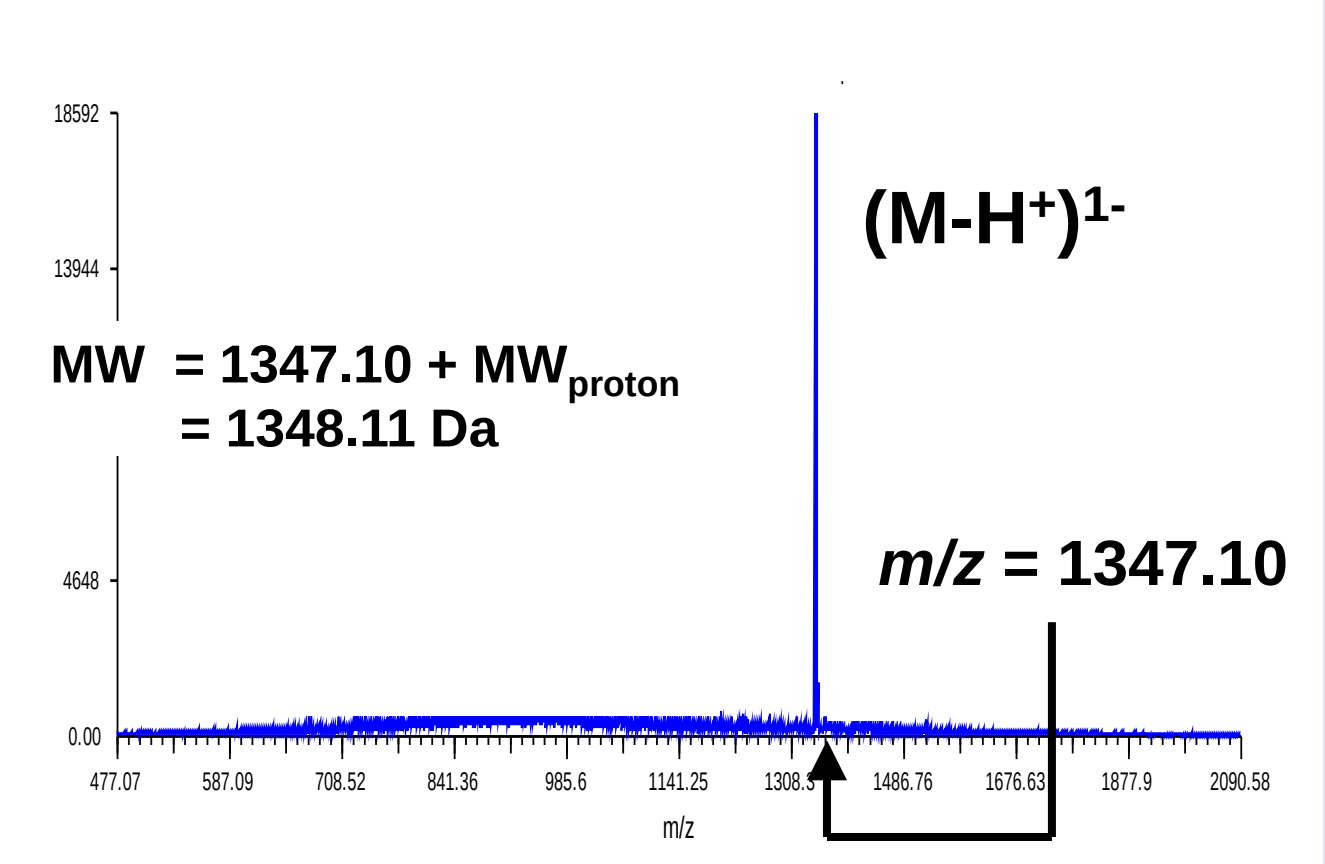
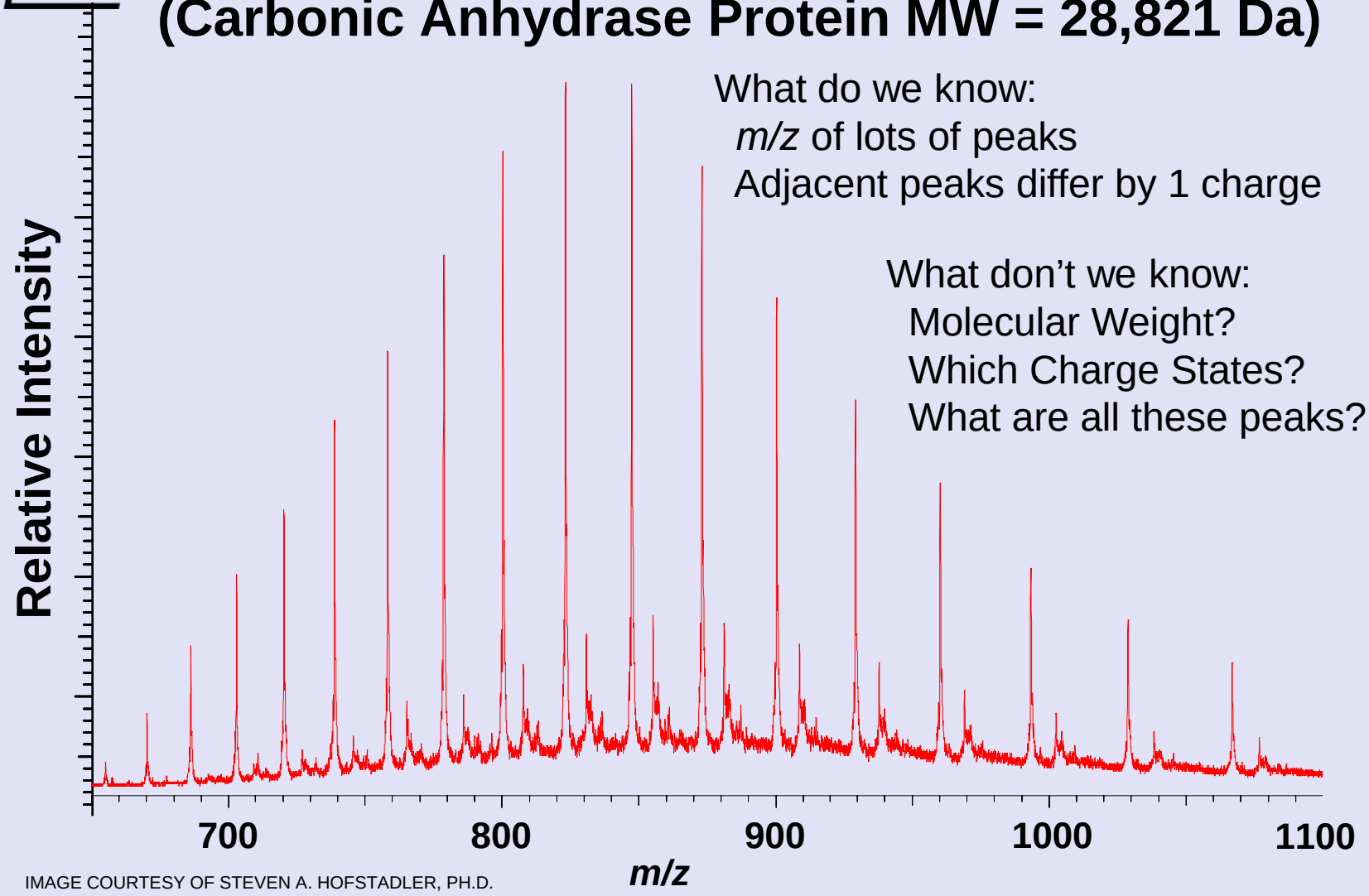


IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

An ESI (+) Mass Spectrum of a High MW Analyte (Carbonic Anhydrase Protein MW = 28,821 Da)



What do we know:
- *m/z* of lots of peaks
- Adjacent peaks differ by 1 charge

What don't we know:
- Molecular Weight?
- Which Charge States?
- What are all these peaks?

IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

Conventional "Deconvolution" of ESI-MS Spectrum

- From m/z of peaks and Δz, calculate z of one peak.

$$\frac{m/z_2}{m/z_1 - m/z_2} = z_1$$

$$\frac{994.8}{33.1} = 30 = z_1$$

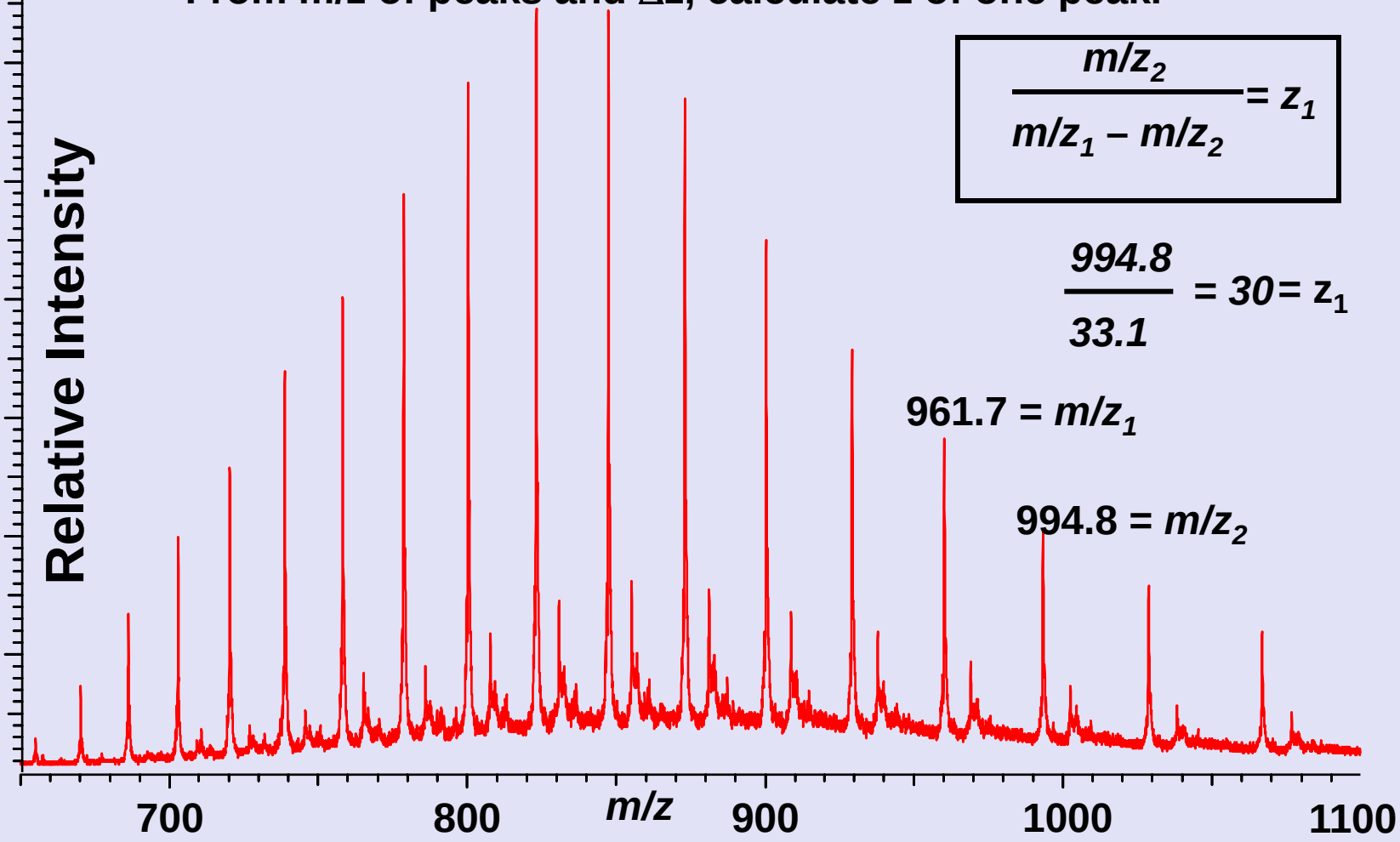


IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

Conventional "Deconvolution" of ESI-MS Spectrum

- Use *z* and *m/z* to calculate mass

MW = (961.7 x 30) – (30 x 1.0078)
= 28,821 Da

accuracy improved by averaging across multiple (all) charge states

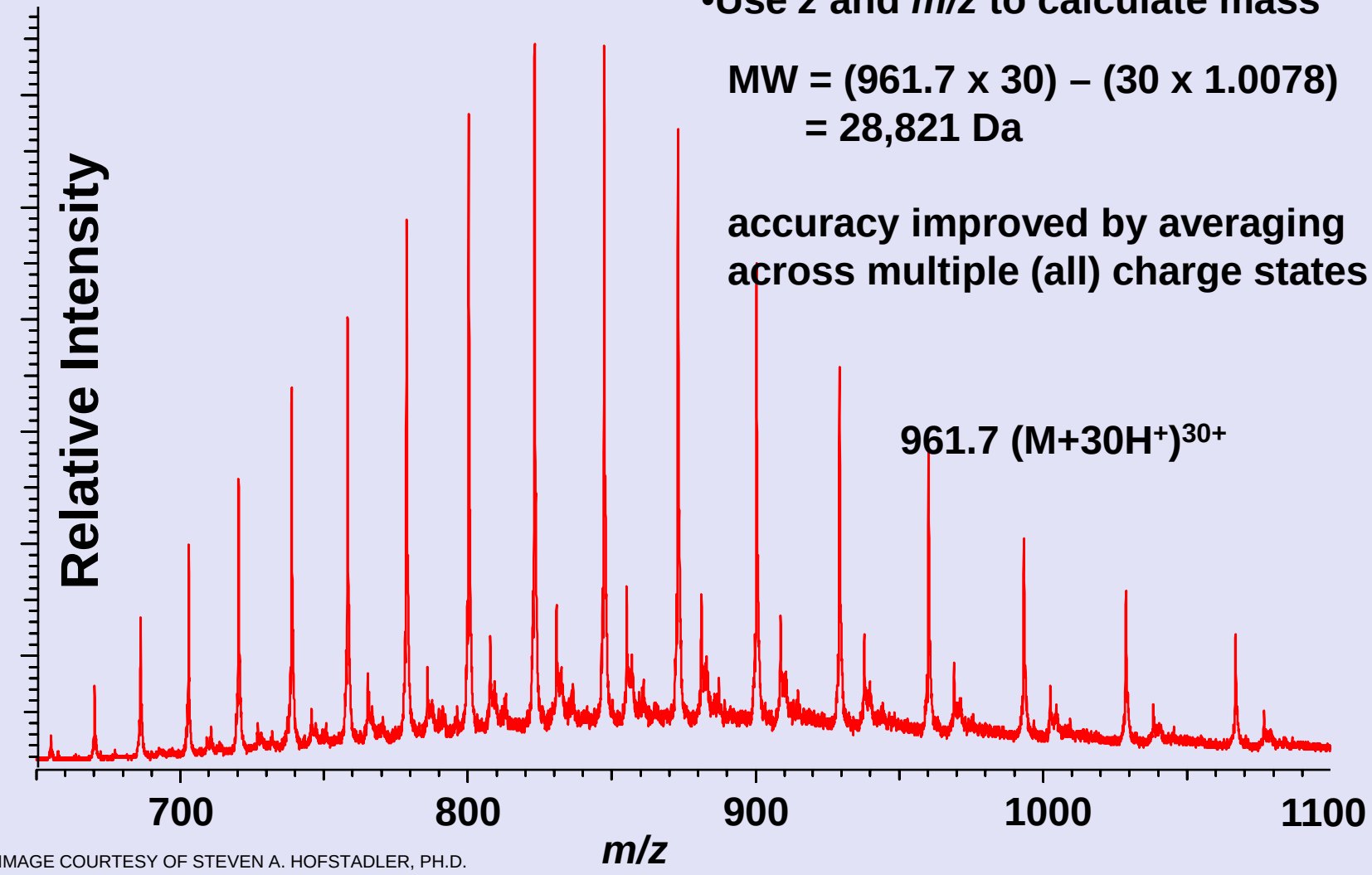


IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

ESI-MS of DNA

- Phosphodiester backbone is easily deprotonated at high pH
- ESI most effective in negative mode (in positive ionization mode basic groups on bases are protonated)
- Both backbone and nucleobase linkages to sugar are relatively labile
- We have optimized solution and interface conditions for DNA analysis by mass spectrometry over the past 10 years

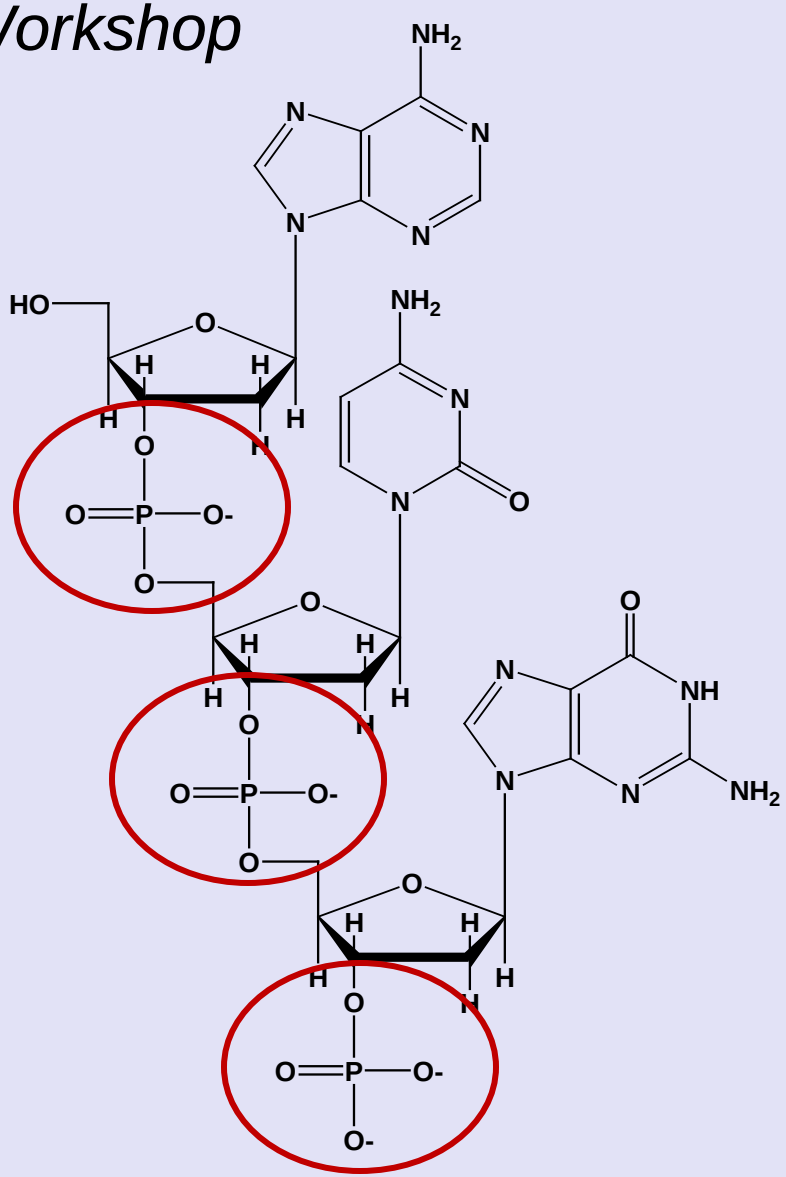
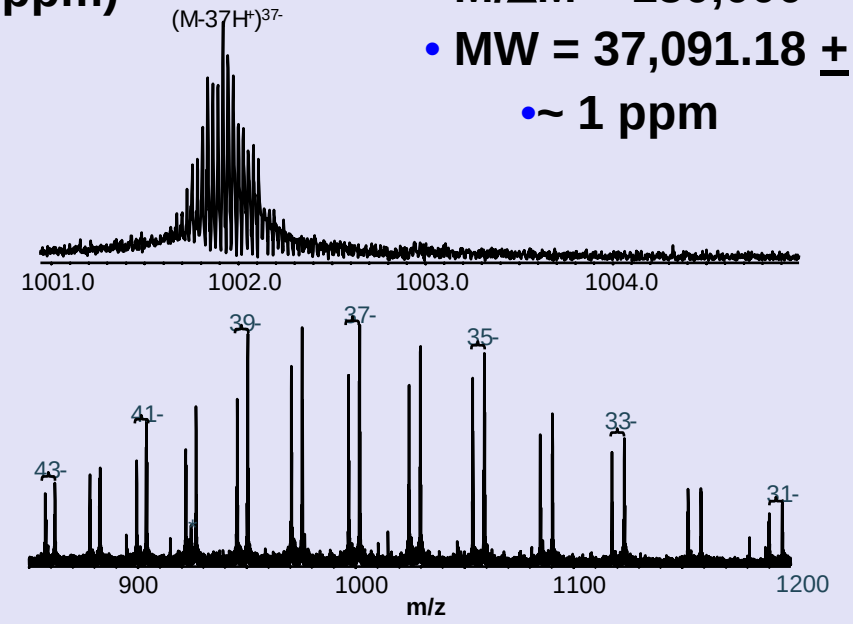


IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

Then and Now…

- **Then (1981)**
  - **Pre-contributions of Fenn, Tanaka, Hillenkamp/Karas**
  - **20-mer DNA**
  - **Cf^{252} desorption - TOF**
  - **M/ΔM ~ 25**
  - **MW = 6301 ± 5 (~ 800 ppm)**

- **Now**
  - **Additional contributions from Marshall, McLafferty, McLuckey, Smith and others**
  - **120-mer DNA acquired in fully automated modality**
  - **ESI-FTICR**
  - **M/ΔM = 150,000**
  - **MW = 37,091.18 ± 0.04**
    - **~ 1 ppm**



IMAGES COURTESY OF STEVEN A. HOFSTADLER, PH.D.

An ESI Mass Spectrum of a PCR Product

doublet peaks at each charge state correspond to forward and reverse strands of amplicon

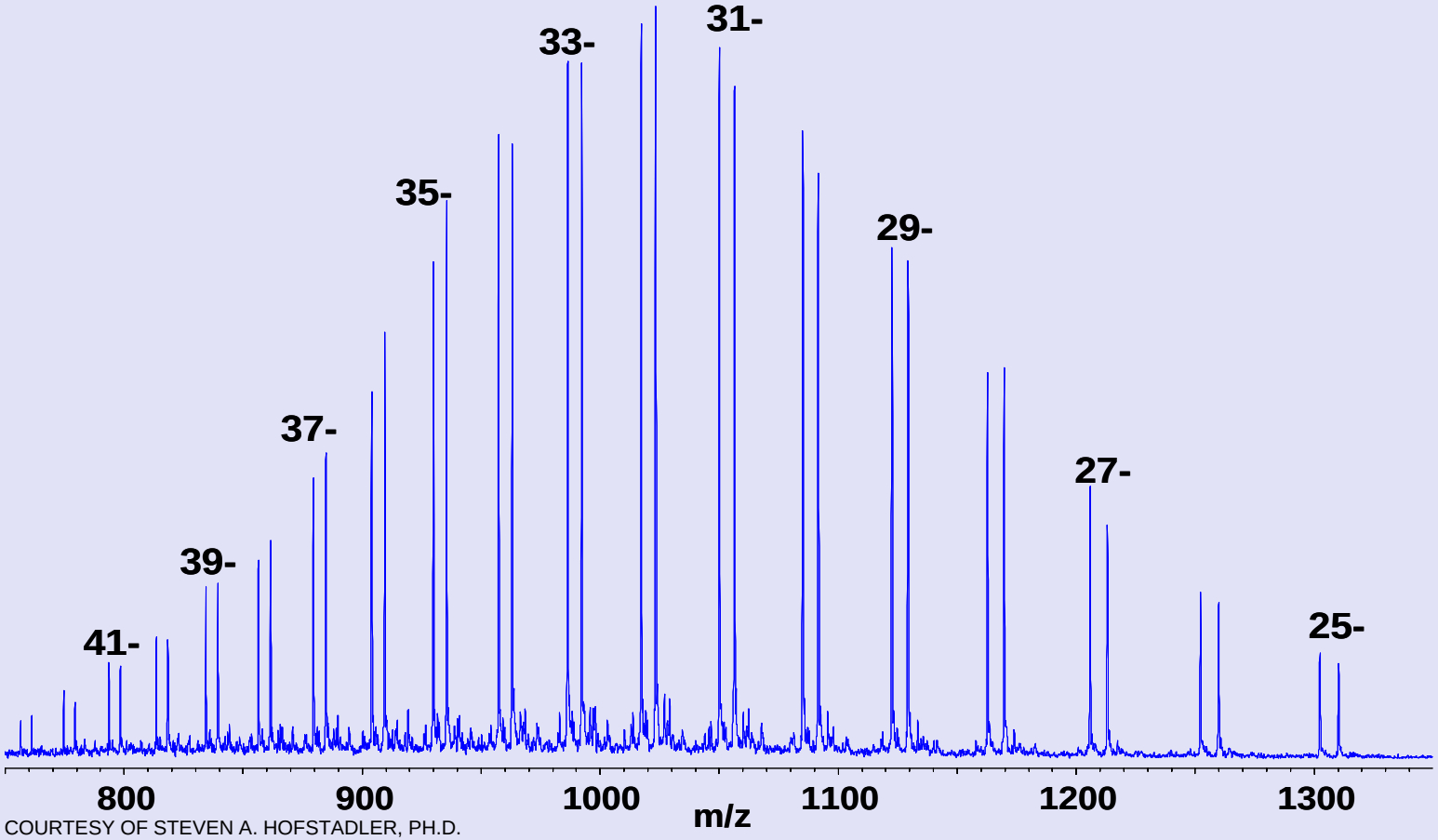


IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

"Raw" ESI-MS spectrum from 3-plex PCR mix

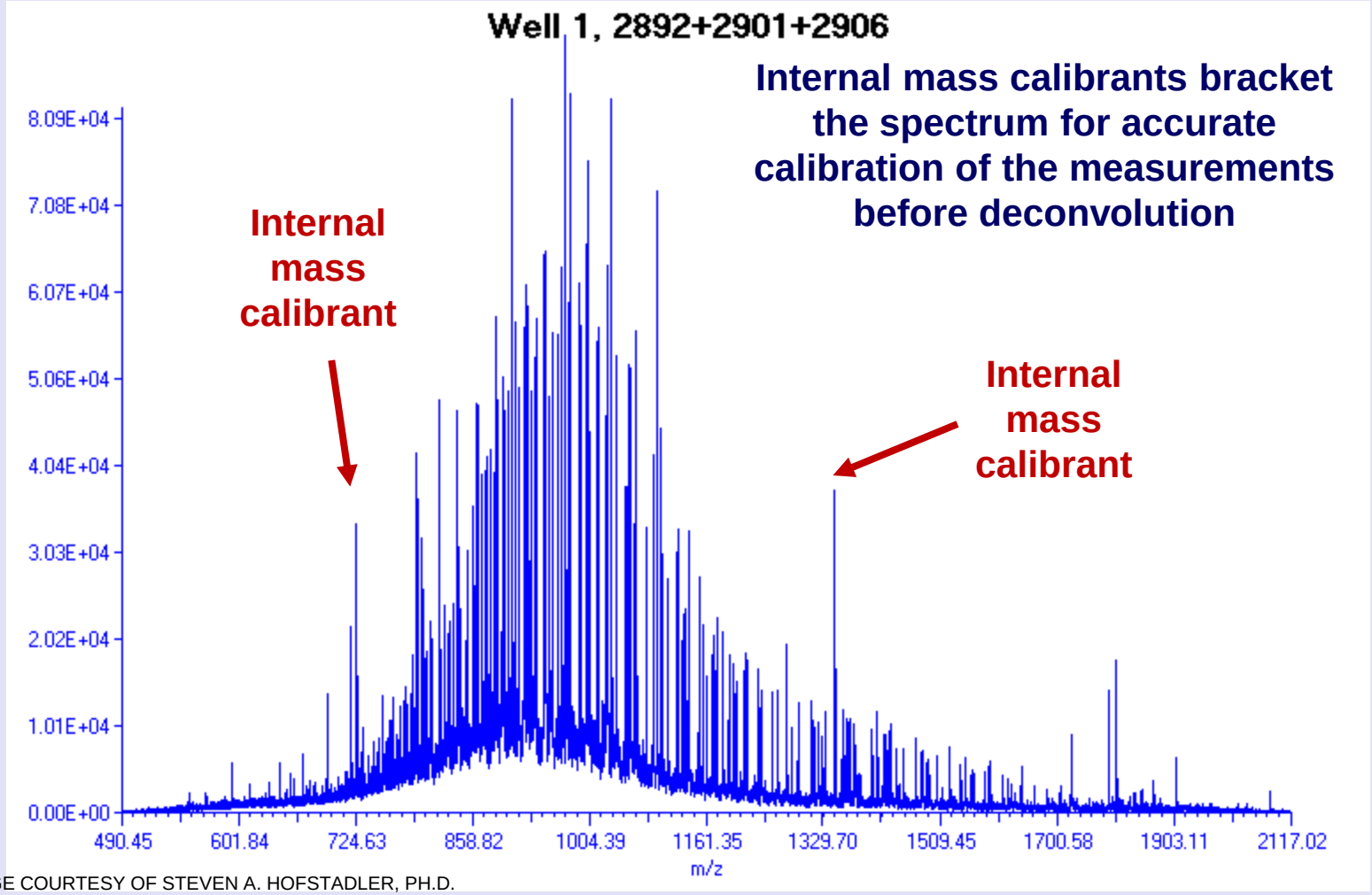


IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

Deconvolved ESI-MS spectrum from 3-plex PCR

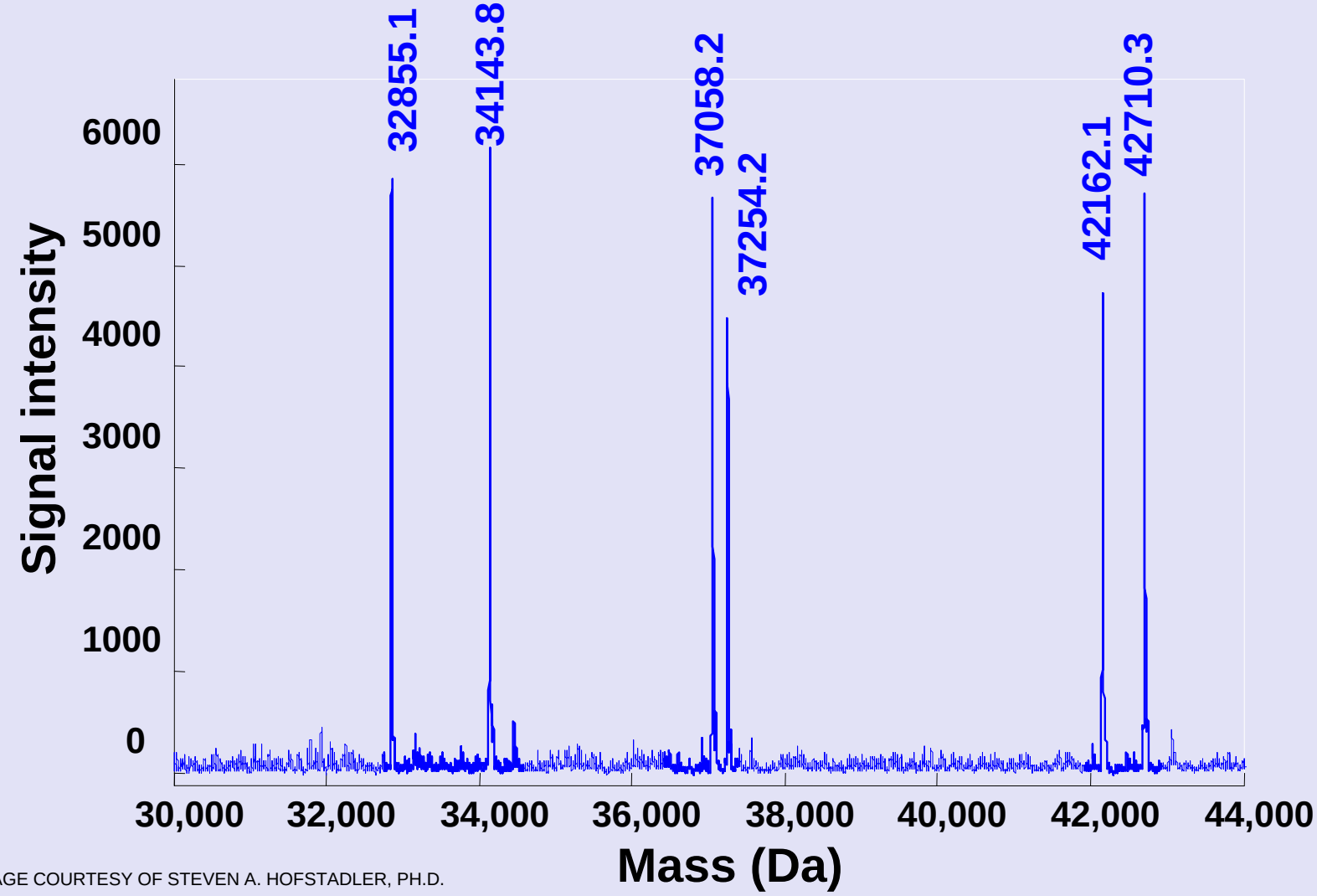


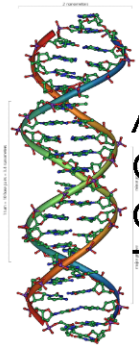
IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

Masses to Base Composition

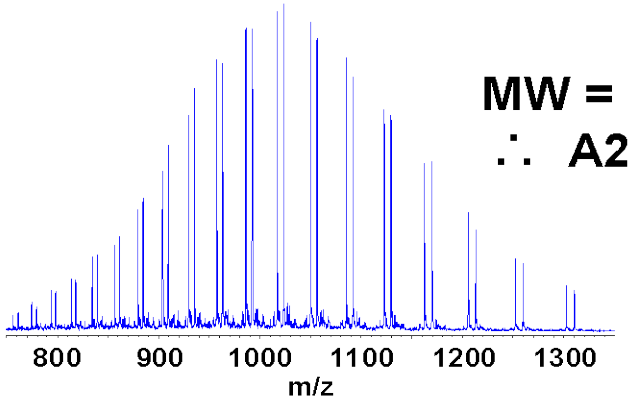
Penny = 2.500 g
Nickel = 3.950 g
Dime = 2.268 g
Quarter = 5.670 g

Scale

Weight = 4.6 grams
∴ 2 dimes



A = 313.0576 amu
G = 329.0526 amu
C = 289.0464 amu
T = 304.0461 amu



Mass spectrum

MW = 32,588.90 amu
∴ A28 G29 C25 T24

Requires 25 ppm mass measurement error

Math takes into account Watson-Crick base pairing

IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

Masses to Base Composition

- **Require masses of both strands and fact that the strands are complimentary to determine base composition**

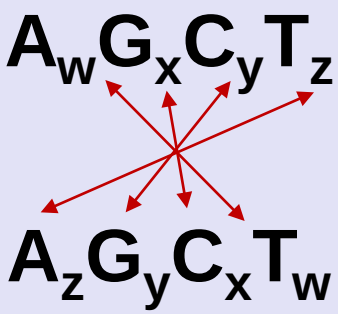
Single Strand: 32889.450 Da
(± 25 ppm or 0.75 Da): 928 base comps
(± 1 ppm or 0.03 Da): 82 base comps

Single Strand: 33071.462 Da
(± 25 ppm or 0.75 Da): 948 base comps
(± 1 ppm or 0.03 Da): 95 base comps

Da: Dalton (atomic mass unit) **ppm: part per million**

Exact Mass Measurements of Both Strands Facilitates Unambiguous Base Composition Determination

ppm	# comp pairs
0-25	1
50	13
100	66
250	378
500	1447



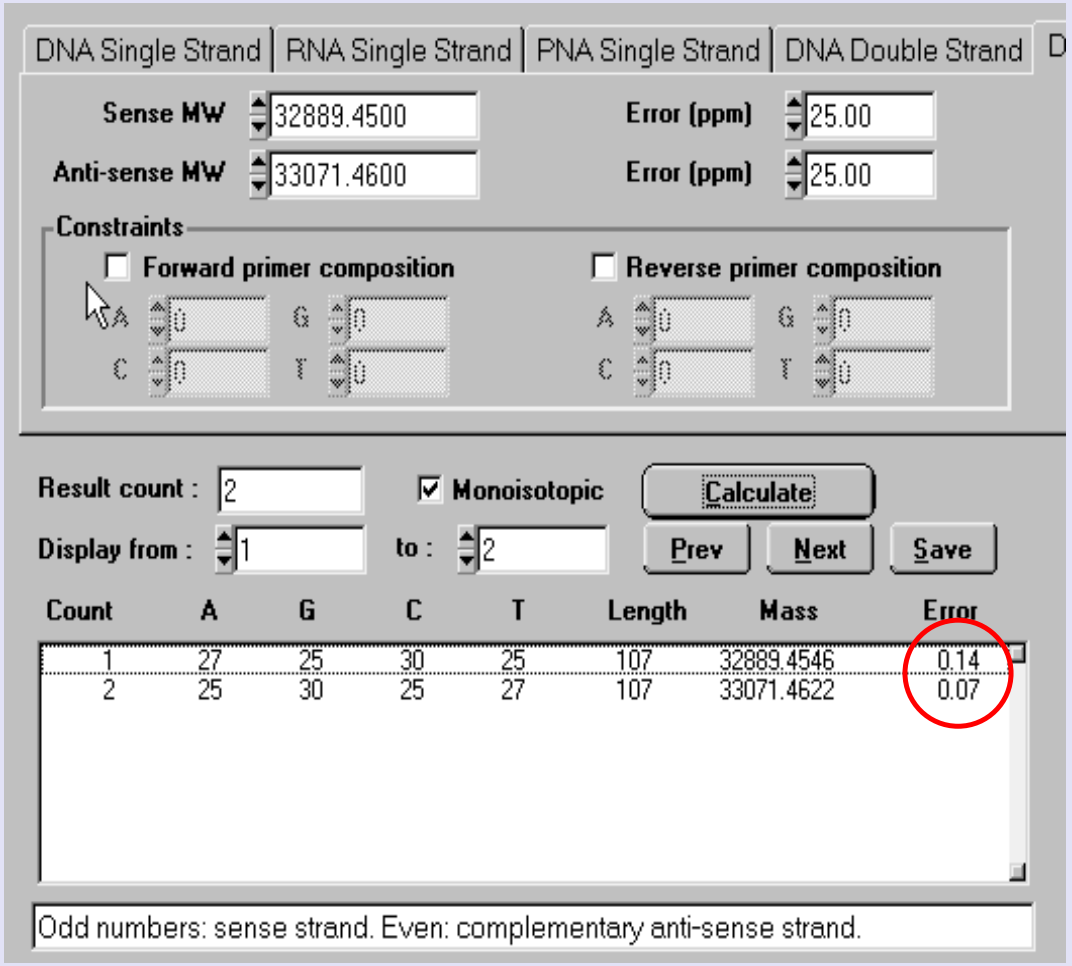
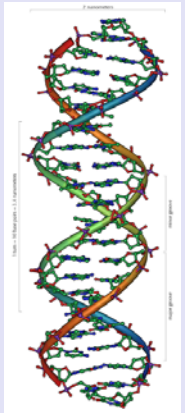


IMAGE COURTESY OF IBIS BIOSCIENCES, INC.

Mass Measurement and the "Canadian Nickel"

Penny = 2.500 g
Nickel* = 3.950 g
Dime = 2.268 g
Quarter = 5.670 g

- **The "Coins and Scale" analogy doesn't work if using all US coins as a US Nickel weighs 5.000 g**
  - **Thus 5 g could be two pennies or one nickel**
- **Interesting parallel to nucleobases with mass measurement error**
  - **A mass shift of 15 ± 1 Da could be a A -> G or a C -> T**
  - **A double SNP A -> G and T-> C would result in a 1 Dalton difference**
  - **A one Dalton uncertainty is consistent with two base compositions**



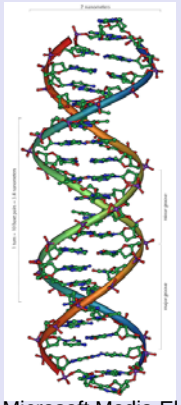
A = 313.0576 amu
G = 329.0526 amu
C = 289.0464 amu
T = 304.0461 amu

Technology Transition Workshop

Mass Measurement and the "Canadian Nickel"

Penny = 2.500 g
Nickel* = 3.950 g
Dime = 2.268 g
Quarter = 5.670 g

- The "Coins and Scale" analogy doesn't work if using all US coins as a US Nickel weighs 5.000 g
  - Thus 5 g could be two pennies or one nickel
- Interesting parallel to nucleobases with mass measurement error
  - A mass shift of 15 ± 1 Da could be a A -> G or a C -> T
  - A double SNP A -> G and T-> C would result in a 1 Dalton difference
  - A one Dalton uncertainty is consistent with two base compositions



A = 313.0576 amu
G = 339.1662 amu
C = 289.0464 amu
T = 304.0461 amu

- **We have a "Canadian Nickel" nucleobase**
  - **^{13}C labeled guanosine shifts the mass by 10 Da per incorporation**
  - **No confusion over which SNP is present**
  - **No uncertainty as to whether the A/G T/C double SNP is present**

High mass precision and mass tag combine to provide unambiguous base compositions in routine operation

Some double SNPs cause small mass differences

Without mass tag:

Product strands differ by 1 Da for two products that differ by a G→A and C→T SNP at the same time.

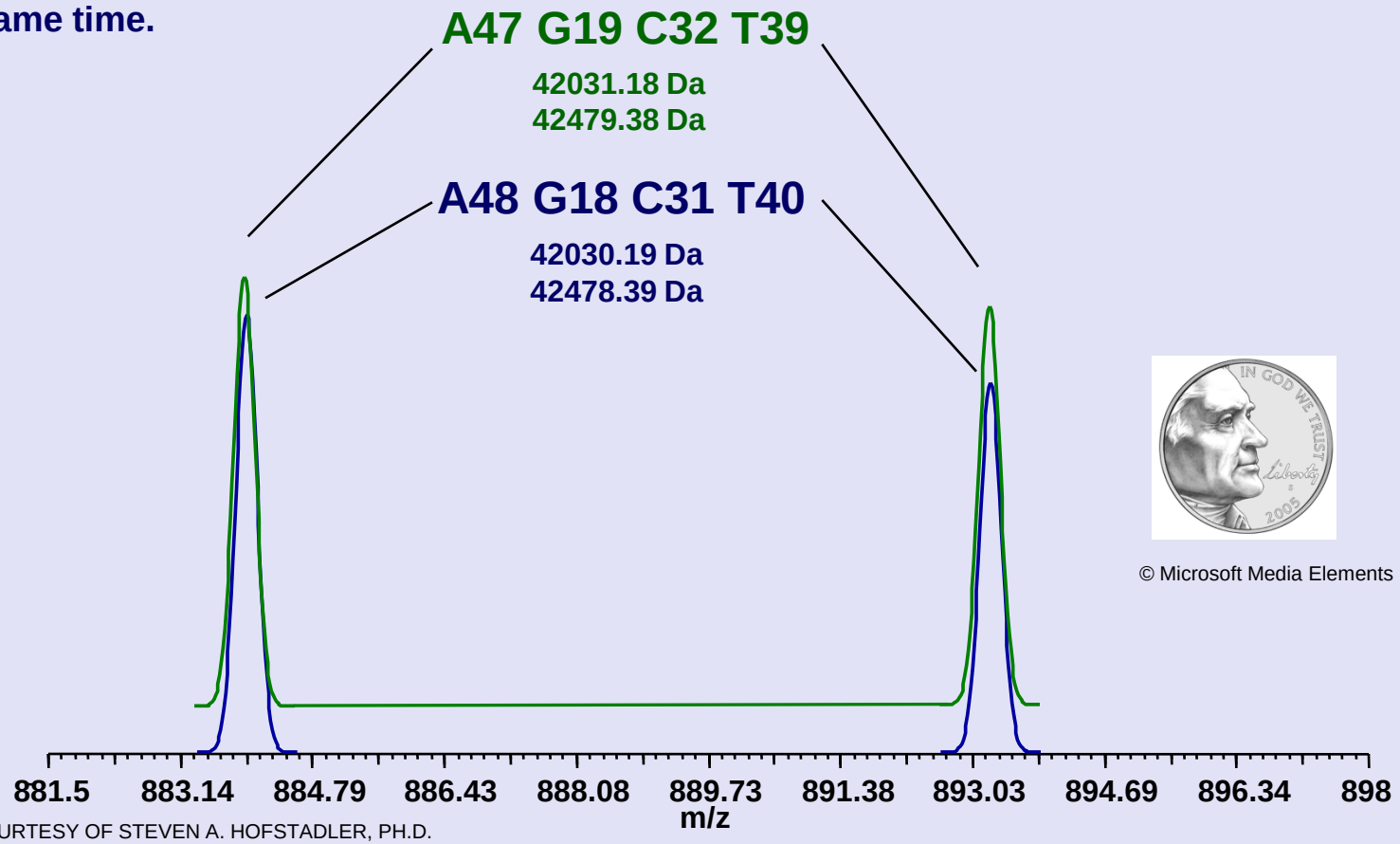


IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

Mass tag increases mass separation for these SNPs

With mass tag:

With ^{13}C-dGTP, the mass separation increases to ~10 Da for each strand

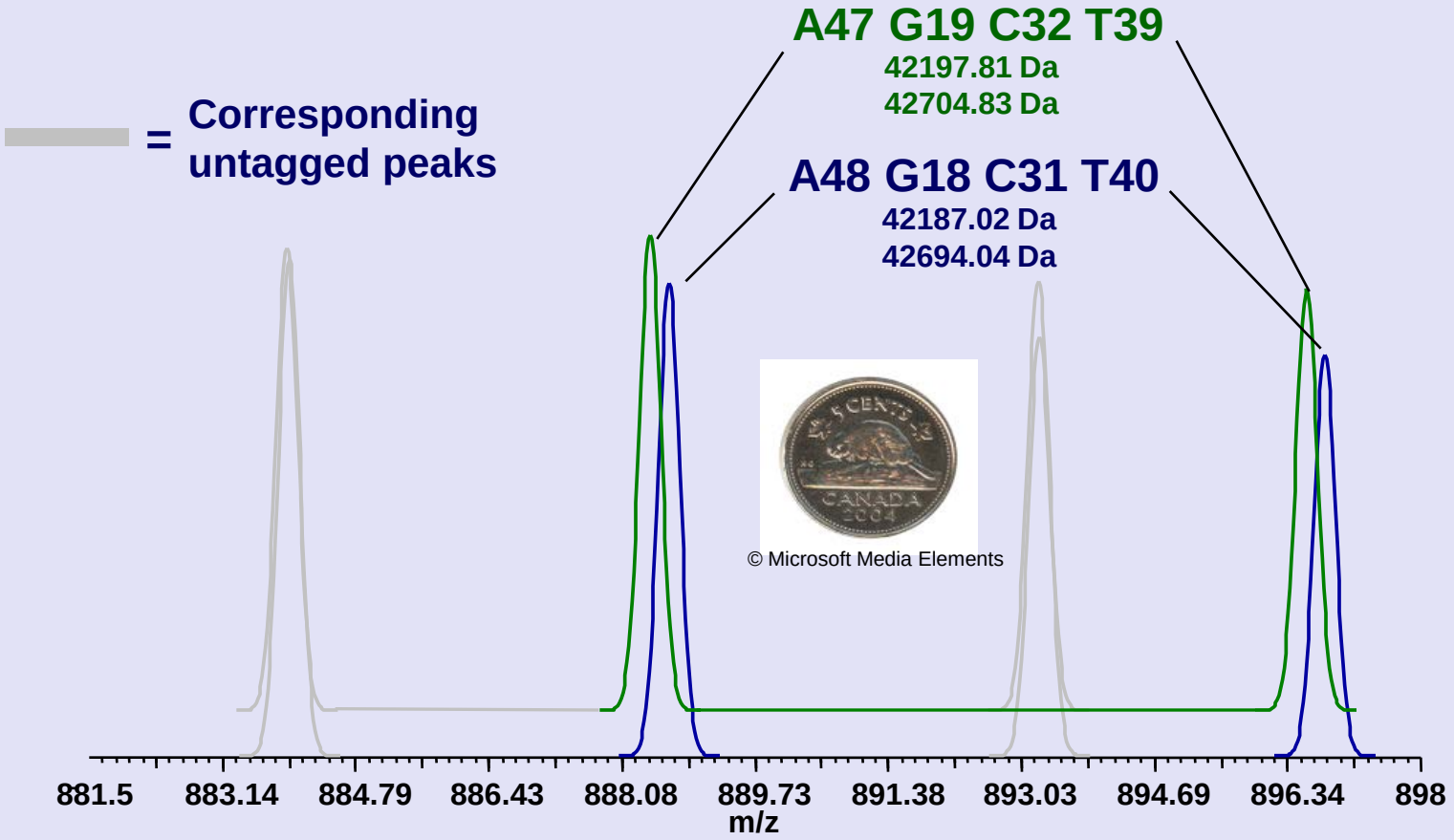


IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

Technology Transition Workshop

[A40 G9 C40 T19] [A47 G18 C25 T30] [A49 G17 C31 T40]

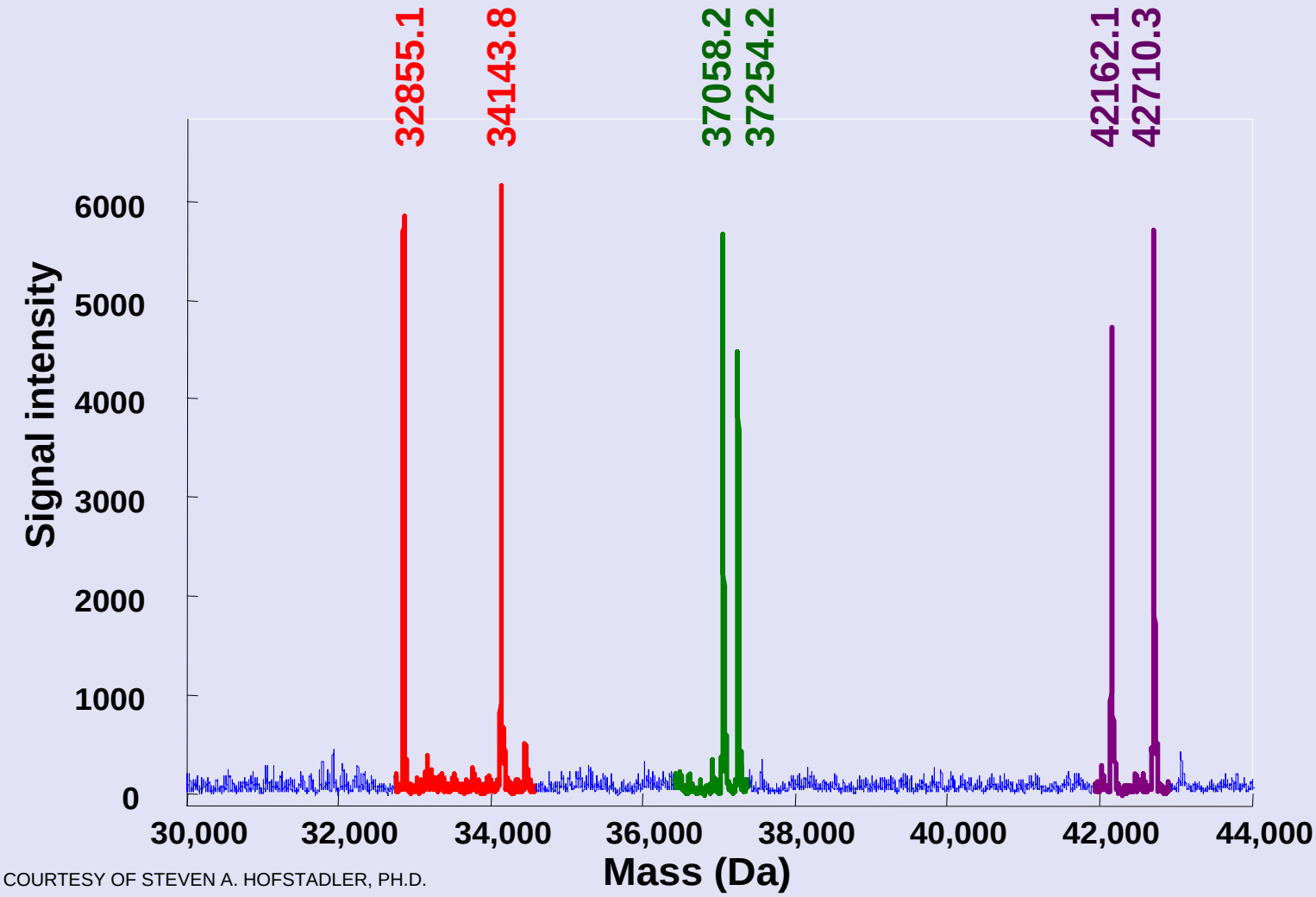


IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

Size Constraints

- **We generally characterize PCR products ≤ 150 bp (~47 kDa/strand)**
- **In general, 25 ppm mass measurement error or better will provide unambiguous base composition for double stranded products ≤ 150 bp**
- **Analysis of larger products is feasible, but information content is lower**
  - **Spectra more “congested”**
  - **Math not in our favor**

Technology Transition Workshop

299 bp PCR product on ESI-TOF (well 9/24)

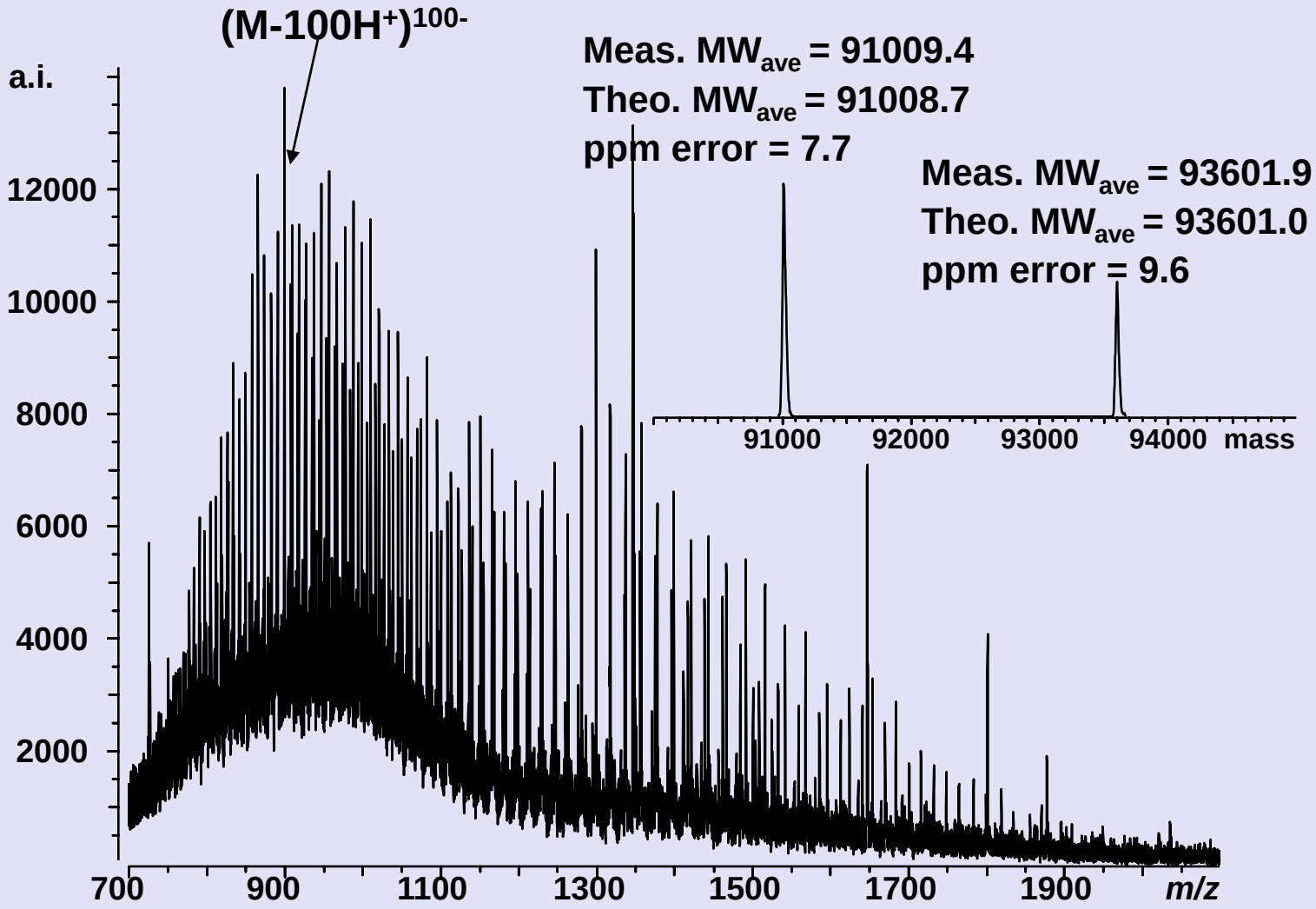


IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

Technology Transition Workshop

Number of possible base compositions as a function of ppm mass error and mass

ppm Error	SS1	SS2	DS
	MW_{ave}=91008.7	MW_{ave}=93601.0	
1	2563	2580	1
5	12846	14296	3
10	25809	29054	10
20 *	x	x	62
25	x	x	89
50	x	x	367

***Average ESI-TOF error over 24 replicates of 299-mer**

ESI Parameters

- Ion desolvation is controlled by several parameters
  - Temperature of desolvation gas
  - Capillary-skimmer potential difference
  - Pressure in capillary-skimmer interface region
- Excessive activation in the interface region can lead to dissociation
  - DNA is labile relative to proteins and one must use “gentle” interface conditions

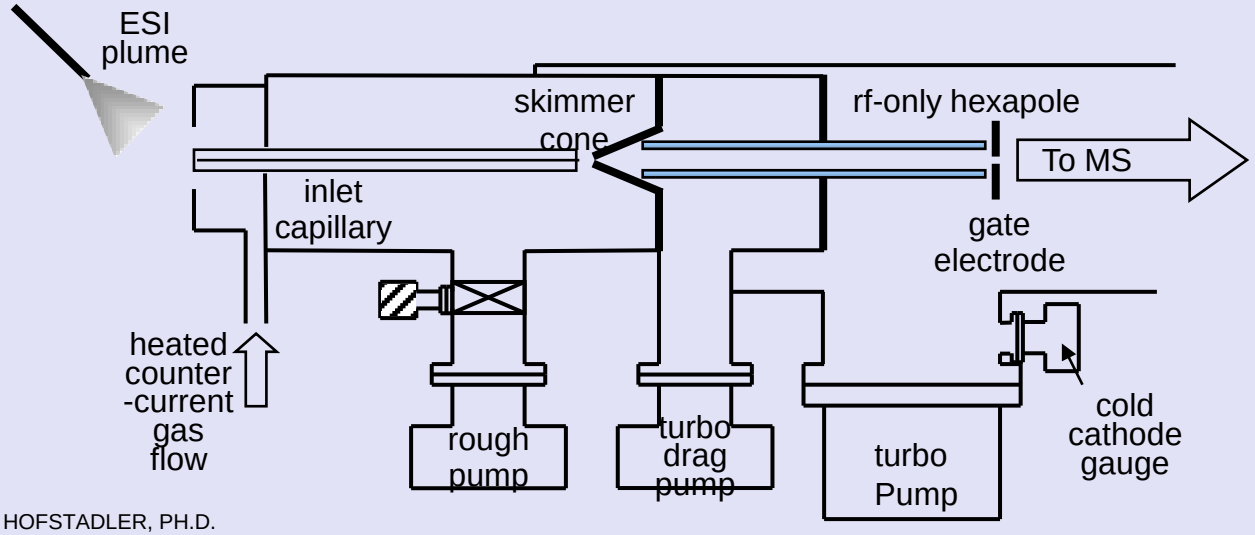
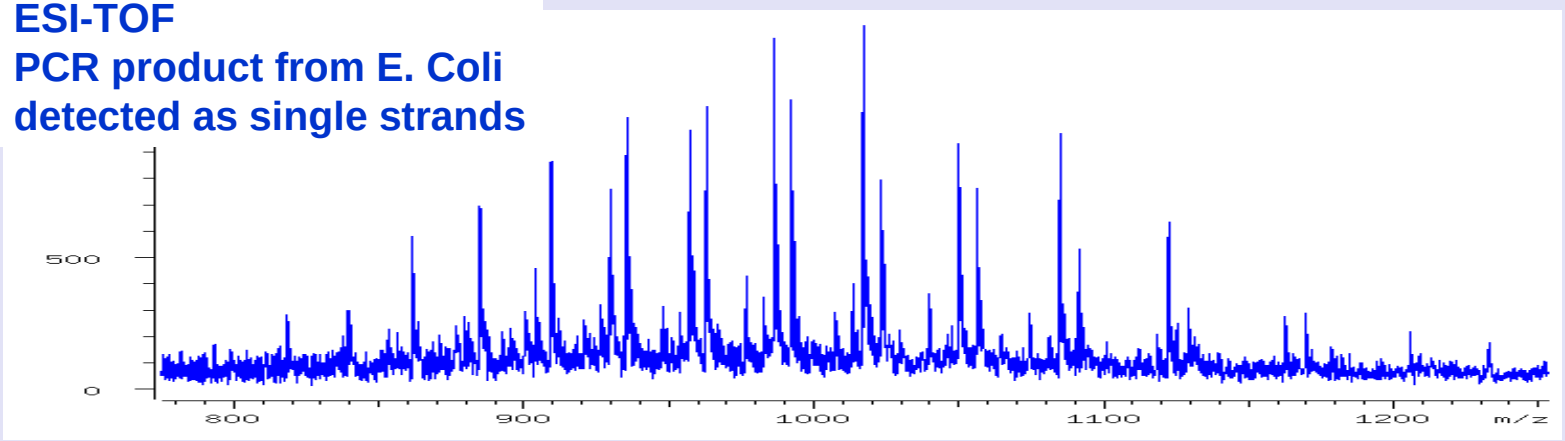


IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

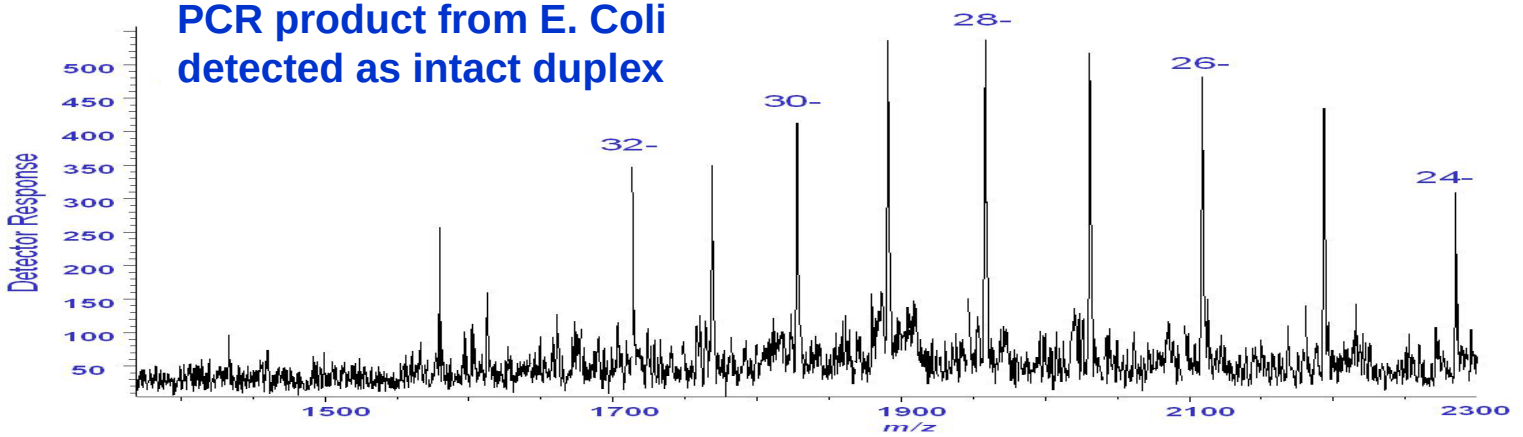
Solution/Desolvation Conditions

(same sample analyzed under different source conditions)

ESI-TOF
PCR product from E. Coli
detected as single strands

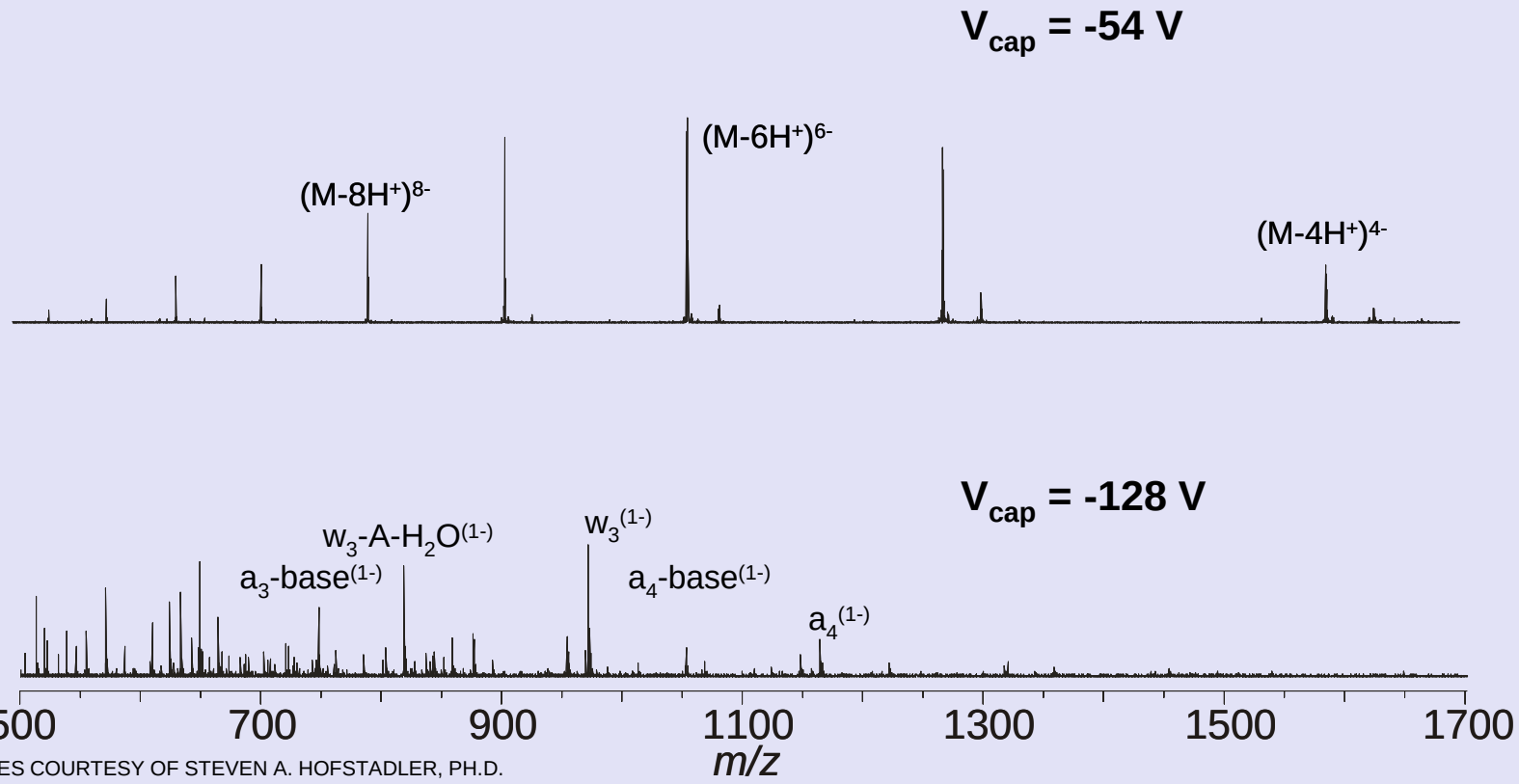


ESI-TOF
PCR product from E. Coli
detected as intact duplex



IMAGES COURTESY OF STEVEN A. HOFSTADLER, PH.D.

Effect of Capillary-Skimmer Potential Difference 20-mer phosphorothioate oligonucleotide



IMAGES COURTESY OF STEVEN A. HOFSTADLER, PH.D.

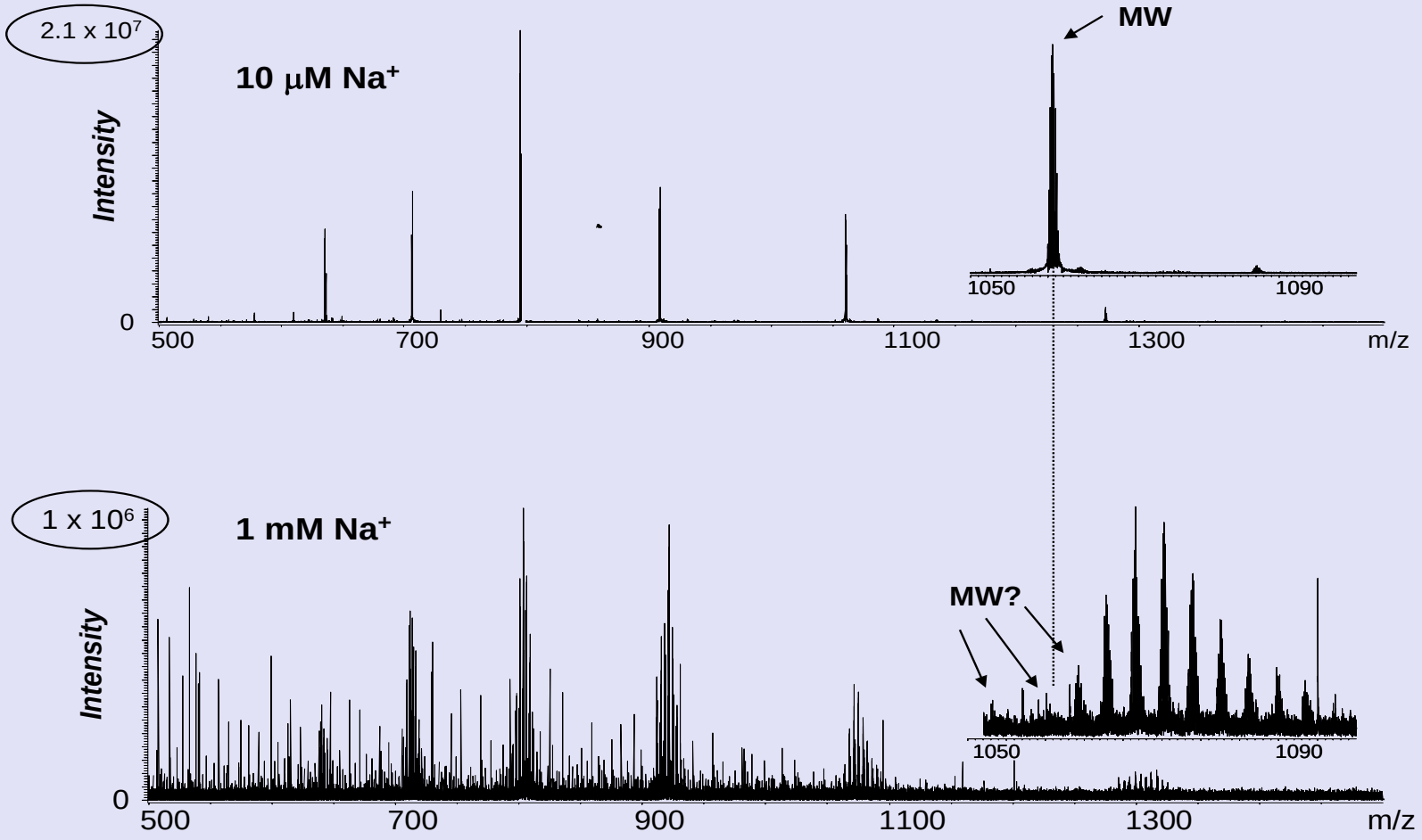
Salt is a Killer…

- **Nonvolatile counterions (e.g. Na^+, K^+, Mg^{2+}, etc) are not removed during desolvation**
  - **High concentrations can preclude the generation of a stable ESI plume**
- **Oligonucleotides are more vulnerable to contamination than proteins**
  - **Phosphodiester backbone is highly anionic**
  - **Larger oligonucleotides more salt intolerant than smaller ones**
- **Effects of salt can be partially mitigated by choice of buffers**
  - **See Griffey et al. RCMS 1995; 9; 97-102.**

Technology Transition Workshop

Salt is a Killer…

ESI-MS of 20-mer phosphorothioate oligonucleotide

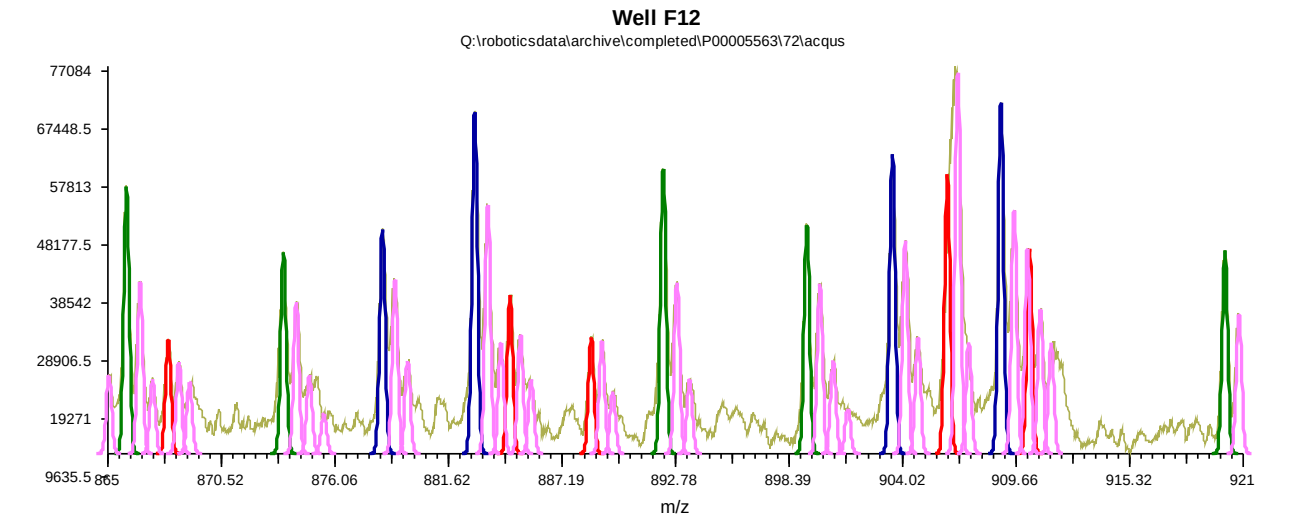


IMAGES COURTESY OF STEVEN A. HOFSTADLER, PH.D.

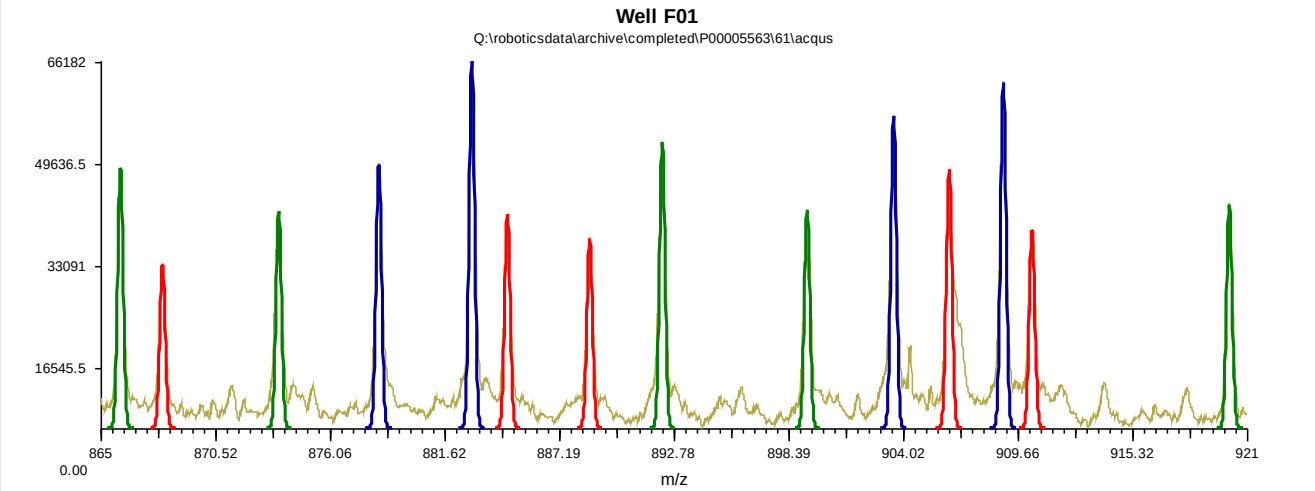
Technology Transition Workshop

Comparison of raw data for adducted vs. non-adducted mass spectrum

Adducted



Not adducted



IMAGES COURTESY OF STEVEN A. HOFSTADLER, PH.D.

Mass Analyzers

- All work by measuring the response of charged particles to electric and/or magnetic fields

- All work at reduced pressure to reduce ion-neutral collisions
  - Want to minimize scatter and/or neutralization
  - Typical operating pressures
    - Linear quadrupoles ~ 5×10^{-5} torr
    - FTICR < 10^{-9} torr
    - TOF 10^{-5} – 10^{-7} torr

Highlights of TOF-MS

- **Advantages:**
  - **Simple and rugged benchtop construction**
  - **Theoretically unlimited mass range**
  - **Adaptable to many ionization sources**
  - **Fast acquisition - signal averaging to improve S/N**
  - **Mass accuracy rivals that of FTICR**
- **Disadvantages:**
  - **Limited resolution**
    - **Theoretically limited to detection electronics**
    - **Practically limited by energy and spatial spreads in ions**
  - **TOF is inherently pulsed**
    - **Must wait for longest flight time ions before sending next packet of ions (Hz to kHz typical repetition rates)**
    - **Cannot simultaneously measure all *m/z* values**
      - **This is mitigated by external ion accumulation**

Time-of-flight (TOF) Mass Analyzers

- Ions are accelerated by electric field (V/d)
- Ions then drift at their final velocity for a fixed distance
- Ions impact a detector and their flight time is recorded
  - flight time is
    - proportional to velocity
    - proportional to the square root of *m/z*

$$K.E. = \frac{1}{2}mv^2 \Rightarrow \frac{1}{2}\left(\frac{m}{z}\right)\vec{v}^{\,2} = \vec{V}/d$$

where ν is velocity, V/d is field strength

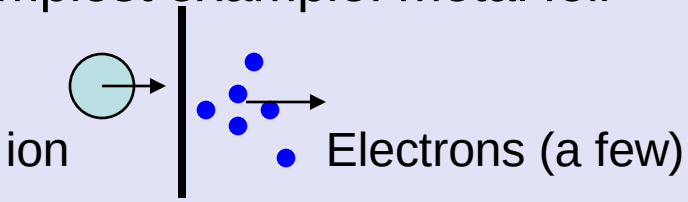
$$t = L/\nu = L\sqrt{m/2zV}$$

t: sec L: meters ν: velocity m: kg *z*: Coulombs V: volts
lower *m/z* ions reach higher velocity than higher *m/z* ions

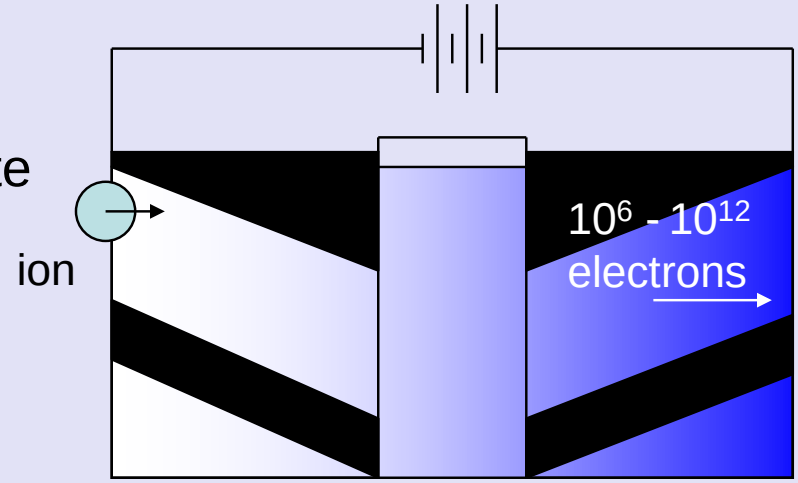
TOF-MS Detection Schemes

- "Particle impact, electron generation, and detection"
  - Electron Multiplier
  - Microchannel Plate
  - Hybrids or Other particle detectors

- Simplest example: metal foil



- Most common: microchannel plate
  - Array of tilted glass channels
  - 2-10 microns
  - Electron cascade=gain
  - Also used in night vision



IMAGES COURTESY OF STEVEN A. HOFSTADLER, PH.D.

Mass Analyzers - TOF

- **Linear Geometry**
  - **Ions drift in field-free region, but energy spread (+ΔE) leads to time spread (-ΔT) (more energy gives shorter TOF)**

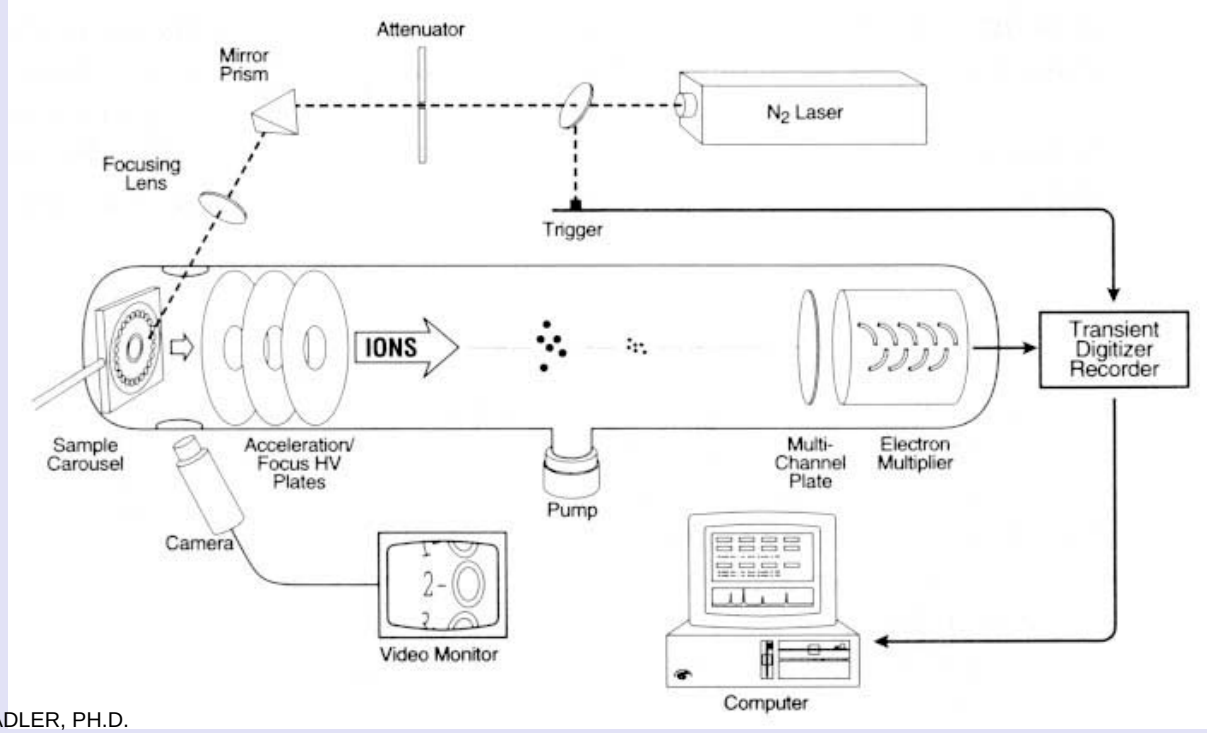


IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

Mass Analyzers - TOF

- **Reflectron**
  - **Ions drift, but at ion mirror they turn around**
  - **+ΔE (energy spread) leads to deeper penetration in ion mirror**
  - **Linear config: +ΔE leads to -ΔT**
  - **Reflectron config: +ΔE leads to -ΔT+ΔT (=0; energy spread eliminated at detector)**

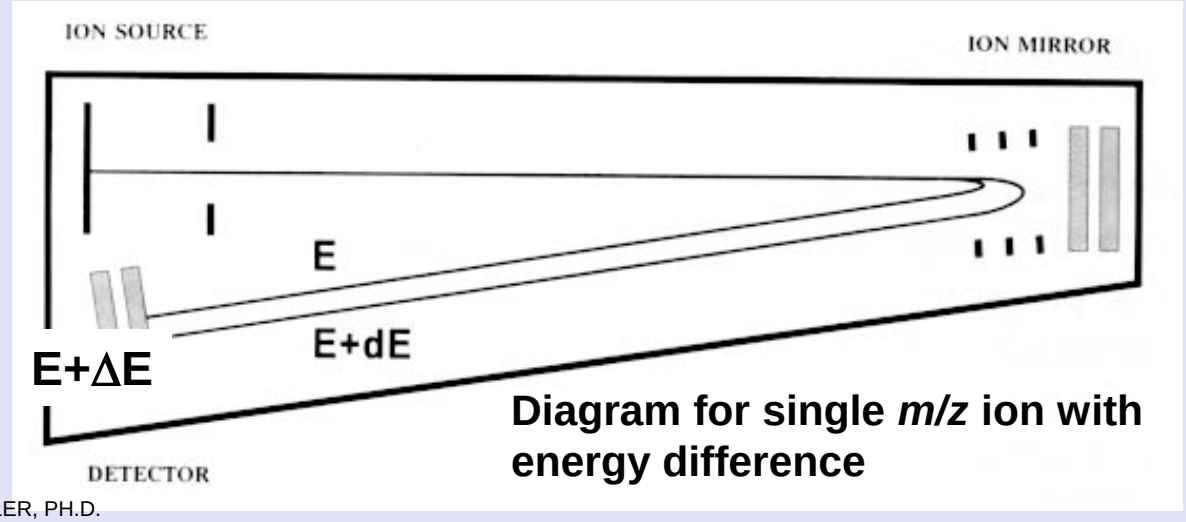


Diagram for single *m/z* ion with energy difference

IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

ESI with an External Ion Reservoir

- **ESI is a continuous ionization source while most MS platforms are most effectively coupled to pulsed sources**
- **Couple external ion reservoir with ESI to make a pulsed ionization source**
- **Nearly 100% ionization duty cycle**
  - **Ions are externally accumulated while others are being mass analyzed**

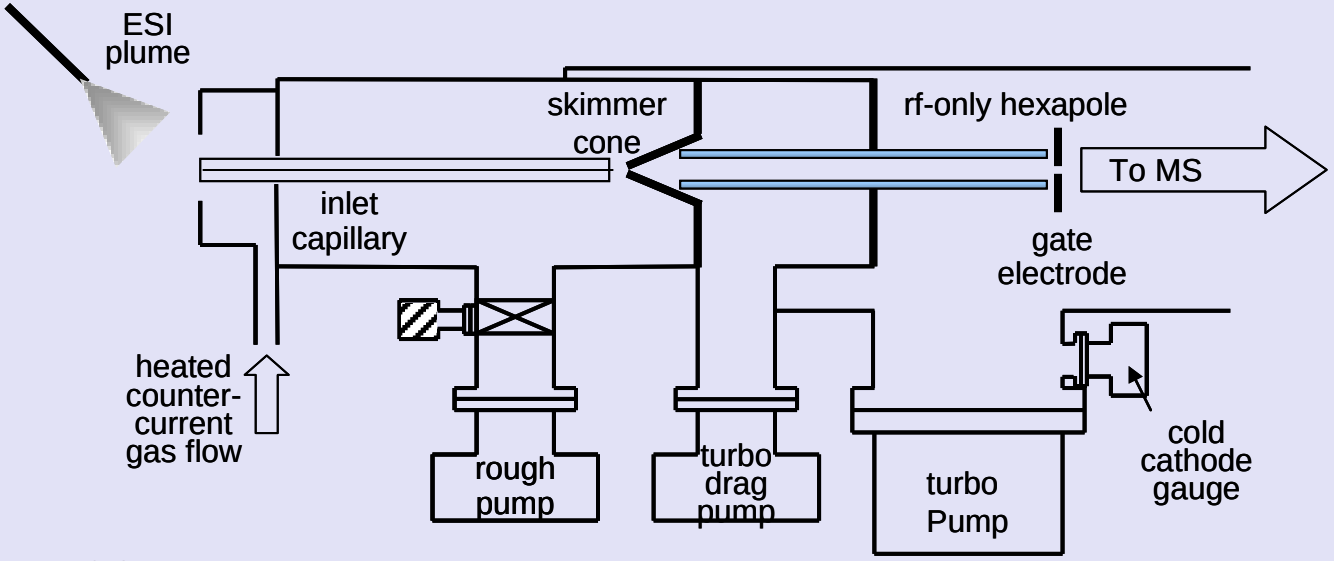
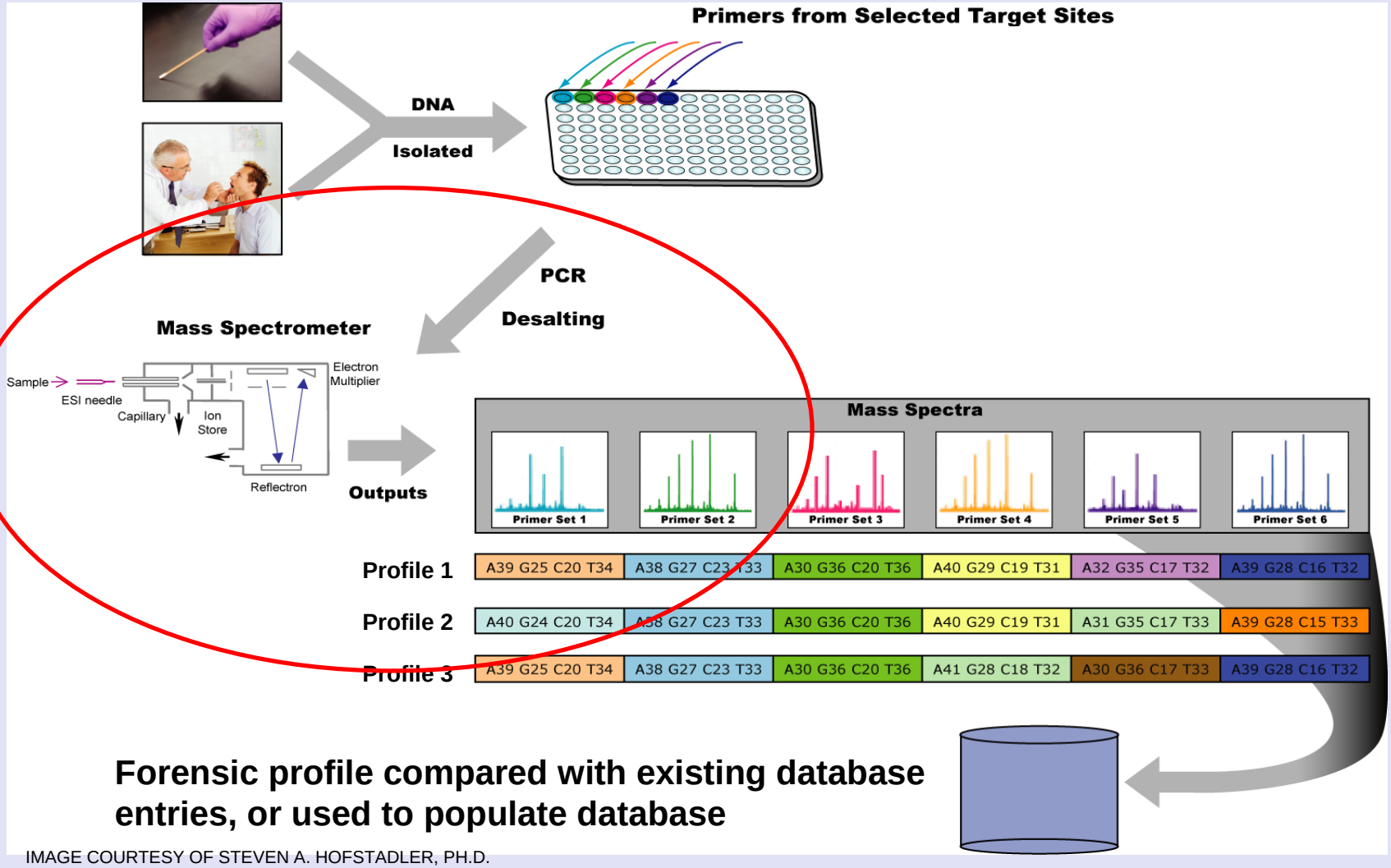


IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

NIJ National Institute of Justice

Technology Transition Workshop

Mass Spectrometry and T5000



Forensic profile compared with existing database entries, or used to populate database

IMAGE COURTESY OF STEVEN A. HOFSTADLER, PH.D.

Conclusions

- **In general, mass spectrometry is used to “weigh” molecular analytes of interest**
- **Electrospray ionization is employed as it can promote large, intact oligonucleotides into the gas phase**
- **Time-of-Flight mass spectrometry is used as it provides accurate molecular weight measurements in a robust, benchtop, instrument format**
- **As part of the Ibis process, amplified DNA is “weighed” with enough accuracy to unambiguously determine base composition [AGCT]**
- **Base composition profiles can be compared to other profiles and/or databases**

NIJ National Institute of Justice

Technology Transition Workshop

Abbreviations and Jargon

APCI	**atmospheric pressure chemical ionization**
bp	**base pair(s)**
CAD	**collisionally activated dissociation**
Da	**Dalton = atomic mass unit**
DNA	**deoxyribonucleic acid**
Ds	**double stranded (DNA)**
EI	**electron impact (ionization)**
ESI	**electrospray ionization**
FAB	**fast atom bombardment**
FD	**field desorption (ionization)**
FI	**field ionization**
FTICR	**Fourier transform ion cyclotron resonance**
FTMS	**Fourier transform mass spectrometry**
FWHM	**full width half maximum (used to specify resolution)**
GC	**gas chromatography**
Hz	**Hertz (cycles/second)**
IRMPD	**infrared multiphoton dissociation**

kDa	kilo Dalton(s)
m/Δm	mass divided by peak width (mass resolution)
m/z	mass to charge ratio
MALDI	matrix assisted laser desorption ionization
MSAD	multipole storage assisted dissociation
mtDNA	mitochondrial deoxyribonucleic acid
MW	molecular weight
PCR	polymerase chain reaction
PD	plasma desorption
ppm	parts per million
QIT	quadrupole ion trap
Q-TOF	quadrupole-time-of-flight
rf	radio frequency
SIMS	secondary ion mass spectrometry
ss	single stranded (DNA)
TOF	time-of-flight
TSP	thermospray (ionization)

Contact Information:

Steven A. Hofstadler, Ph.D.

Ibis Biosciences, Inc.

Email: shofstadler@ibisbio.com