

Top-Down Proteomics Mass Spectrometry

University of Florence, March 19, 2010

“Bottom-Up” of proteins :

Before MS, slash, bash, smash into peptides.

2010: MS Proteomics >90% bottom-up

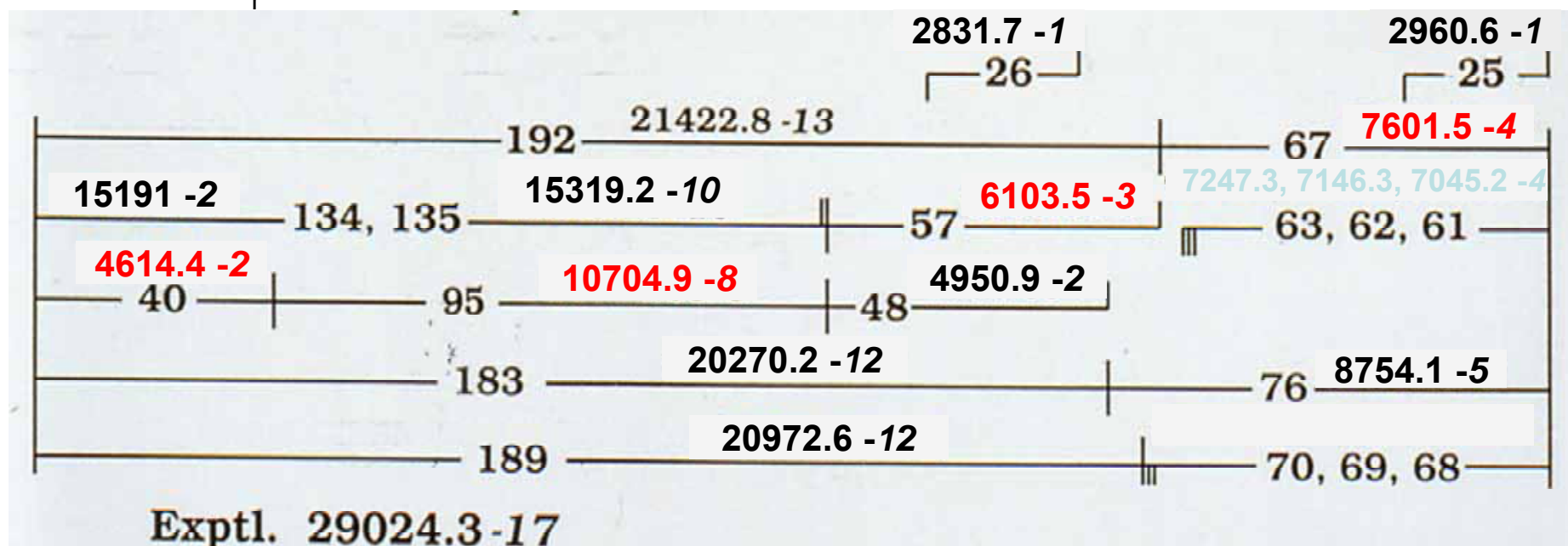
Fred W. McLafferty, Cornell University

Top-Down versus Bottom-Up Protein *Characterization* by Tandem High-Resolution Mass Spectrometry

Kelleher, N. L, Lin, H. Y, Valaskovic, G. A, Aaserud, D. J, Fridriksson, E. K, McLafferty, F. W.
J. Am. Chem. Soc. **1999**, 121, 806-812

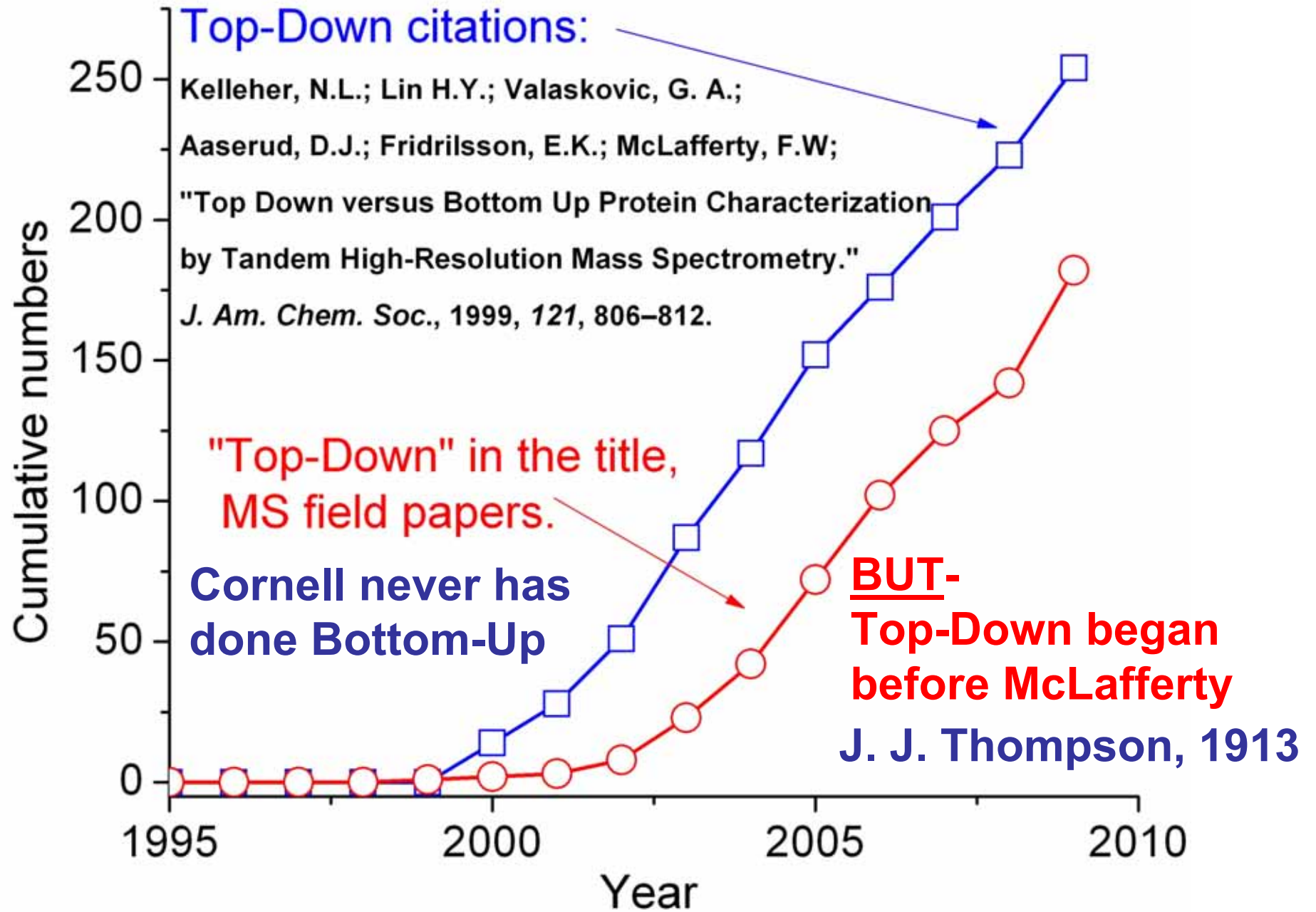
Carbonic Anhydrase

Calculated $M_r = 29023.7-17$



21422.8 -13	4614.4 -2	15319.2 -10
7601.5 -4	10704.9 -8	4950.9 -2
29024.3 -17	6103.5 -3	29024.2 -17
	7601.5 -4	
	29024.3 -17	

$b_{135} - b_{134}$, 128.01 : Q, -0.05 Da
$y_{63} - y_{62}$, 101.04 : T, -0.01
$y_{62} - y_{61}$, 101.11 : T, +0.06



MS of Linear Molecules (1958)

Mycoserolic acid (1 μg) from tubercle bacilli

Showing four methyl branch positions

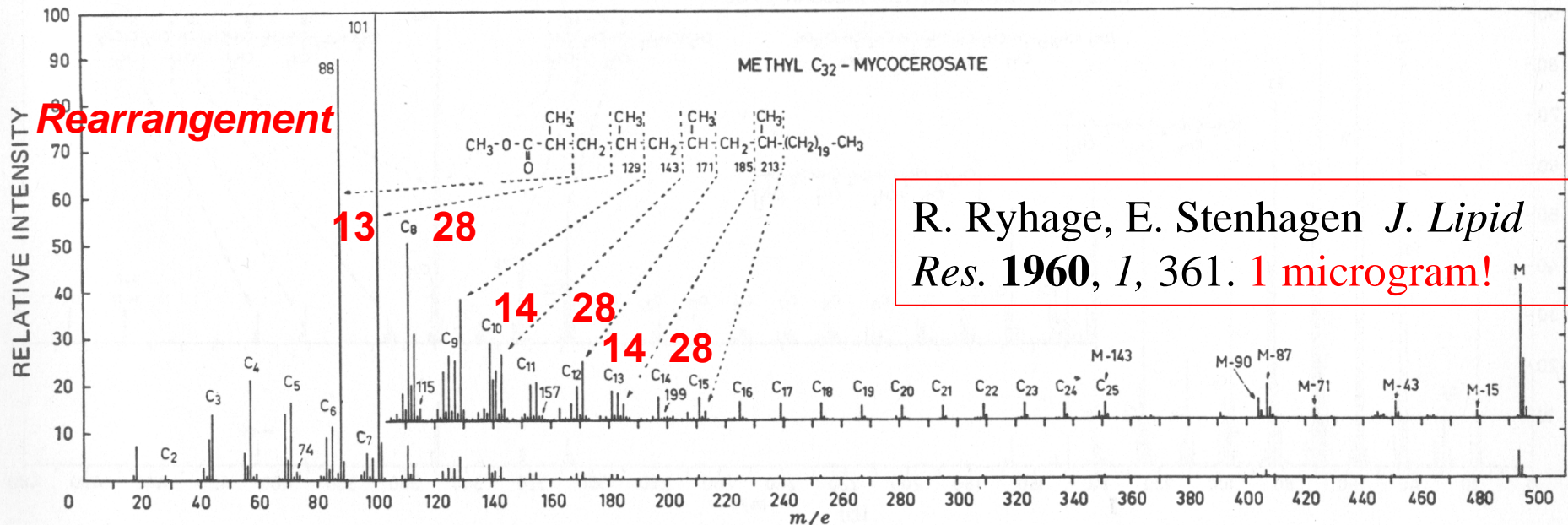


Figure 1-10. Mass spectrum of methyl C_{32} -mycocerosate (18).

Top-Down Units: CH_2 , 14 Da; $\text{CH}(\text{CH}_3)$, 28 Da

Problem: MS of Nonlinear Molecules

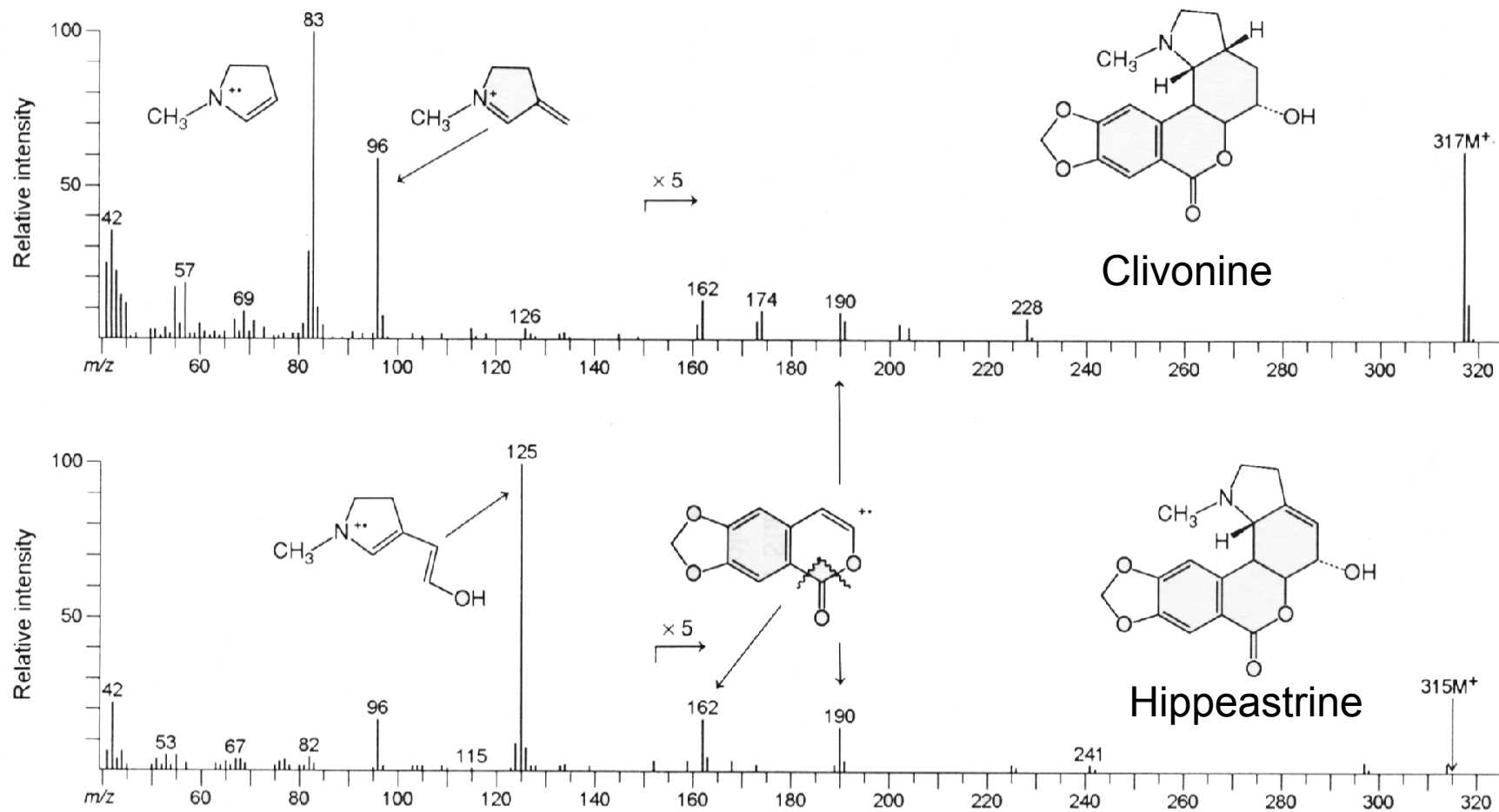


Figure 8.1. The EI mass spectra of clivonine and hippeastrine (see Equations 8.68, 8.69).

Solution #1: Study Klaus Biemann's papers

Not a Linear Molecule? Rearrangement?

Databases of Electron Ionization Mass Spectra

Wiley 1969 *Registry of Mass Spectral Data*, 6.8K spectra
Stenhagen, Abrahamsson, McLafferty, Editors

Wiley 2009 *Registry*, 9th Edition, 660K spectra

NIST 2008 Edition, 220K spectra

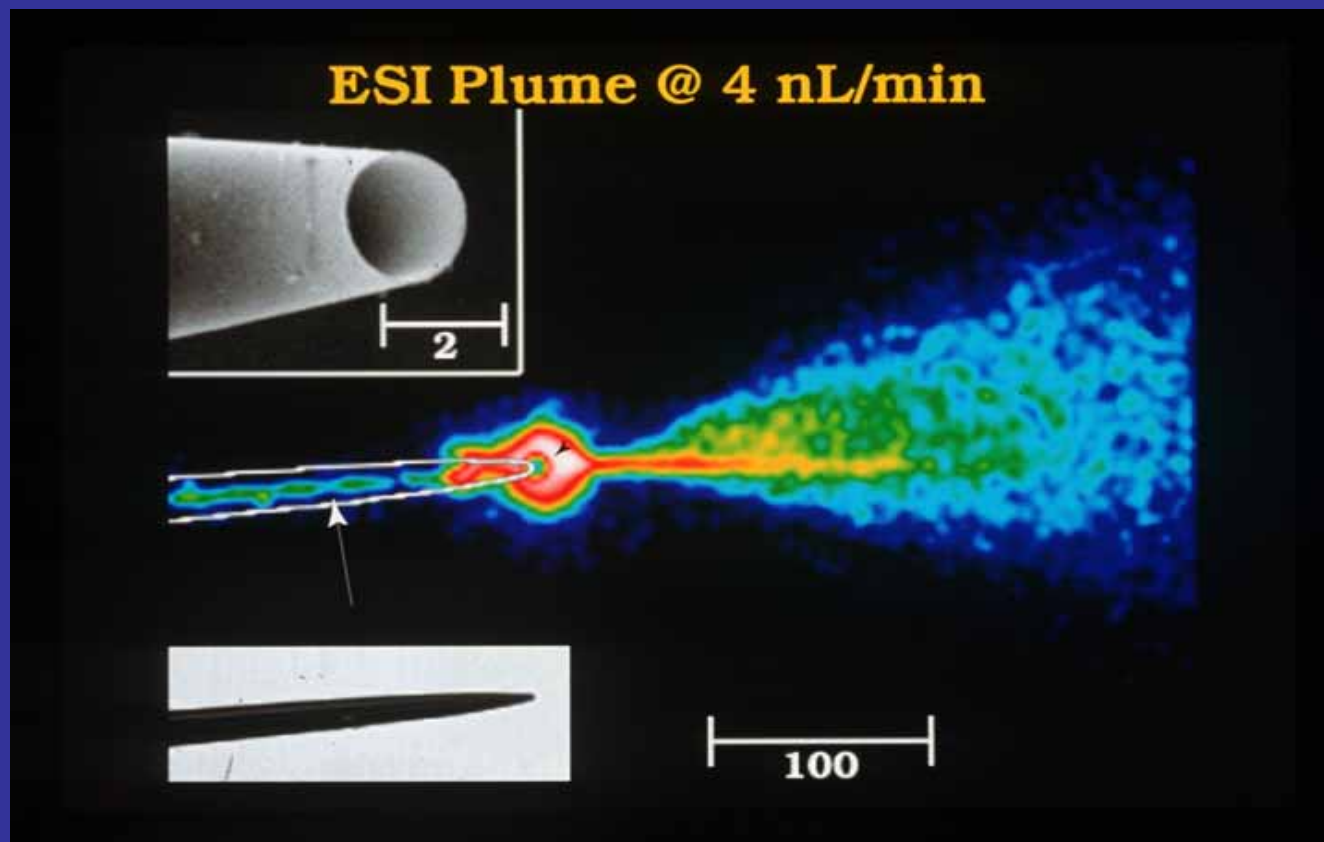
Wiley 2009 *Registry with NIST*, 9th Edition, 796K
spectra

746K searchable chemical structures

667K different chemical compounds

Probability Based Matching: 796K in <1 s

Electrospray Ionization - John Fenn



1-3 kV

ESI is Really Gentle - BUT

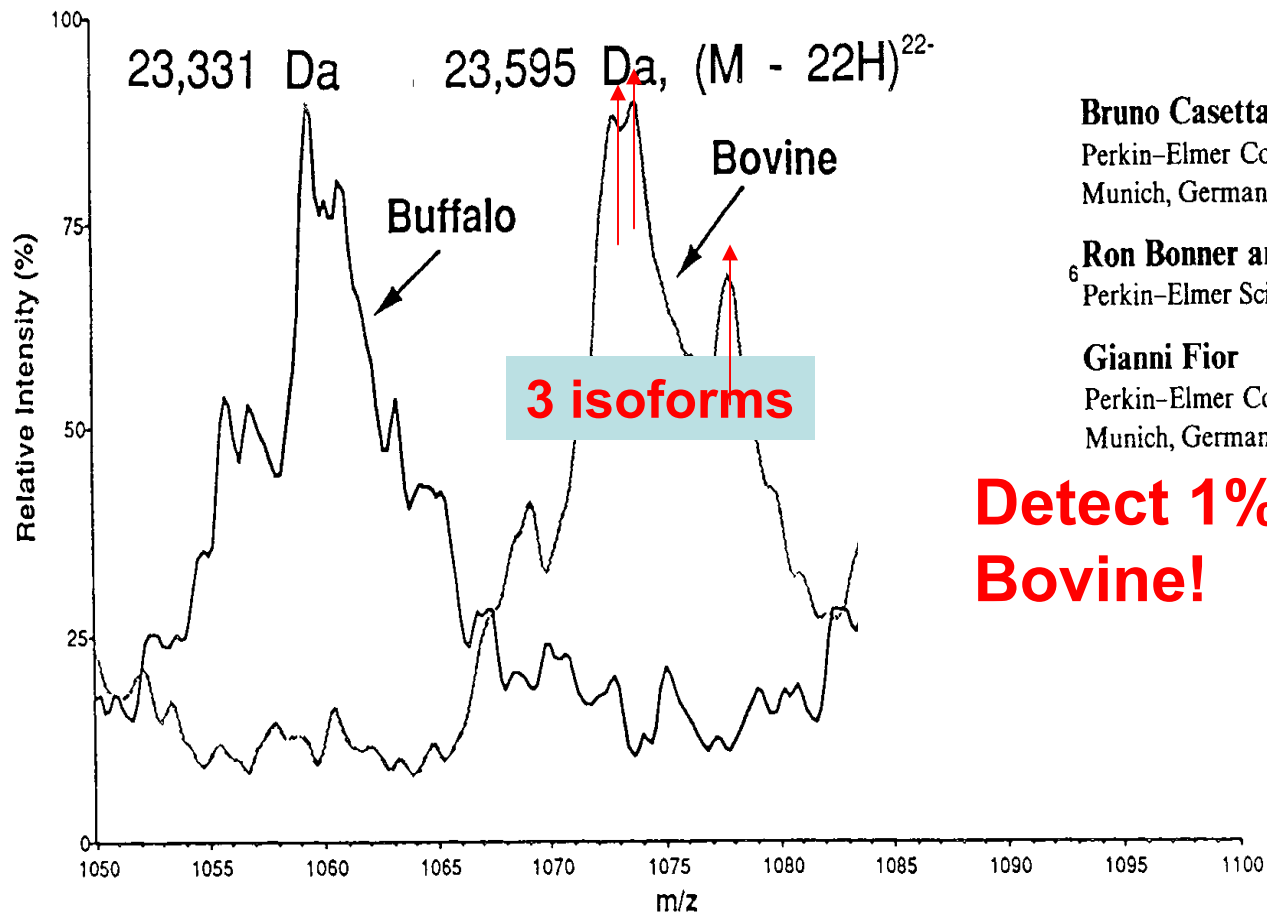
Is the native conformation retained?

Molecular Weight, Specificity

Bovine Casein in Mozzarella Cheese

Legal limit 5%, used “Tasters”

Electrospray Ionization MS, 1989



Bruno Casetta

Perkin-Elmer Corporation

Munich, Germany

Ron Bonner and Bori Shushan

Perkin-Elmer Sciex, Toronto, Canada

Gianni Fior

Perkin-Elmer Corporation

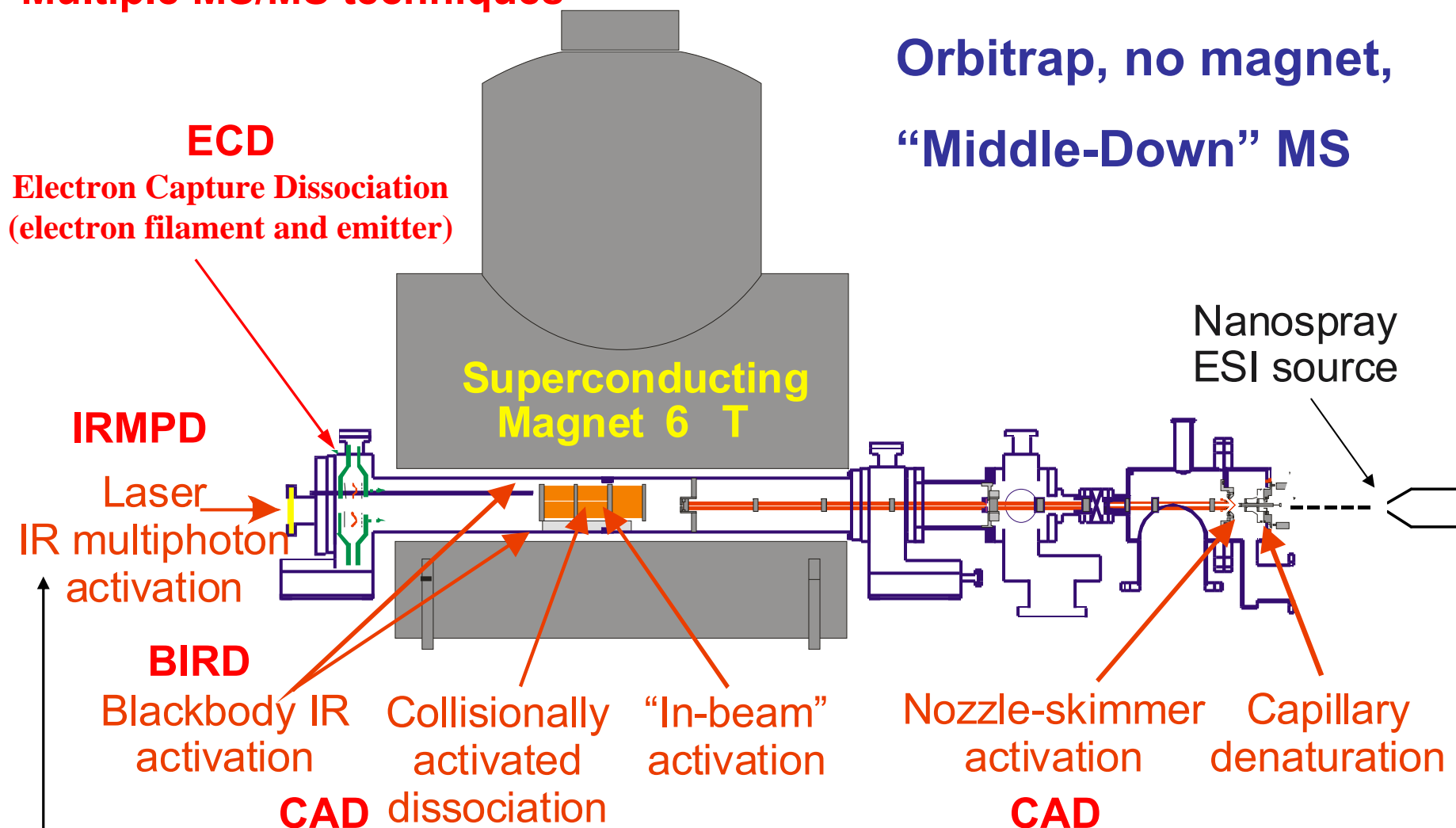
Munich, Germany

**Detect 1%
Bovine!**

Fourier-Transform Ion Cyclotron Resonance Mass Spectrometer Comisarow and Marshall

Multiple MS/MS techniques

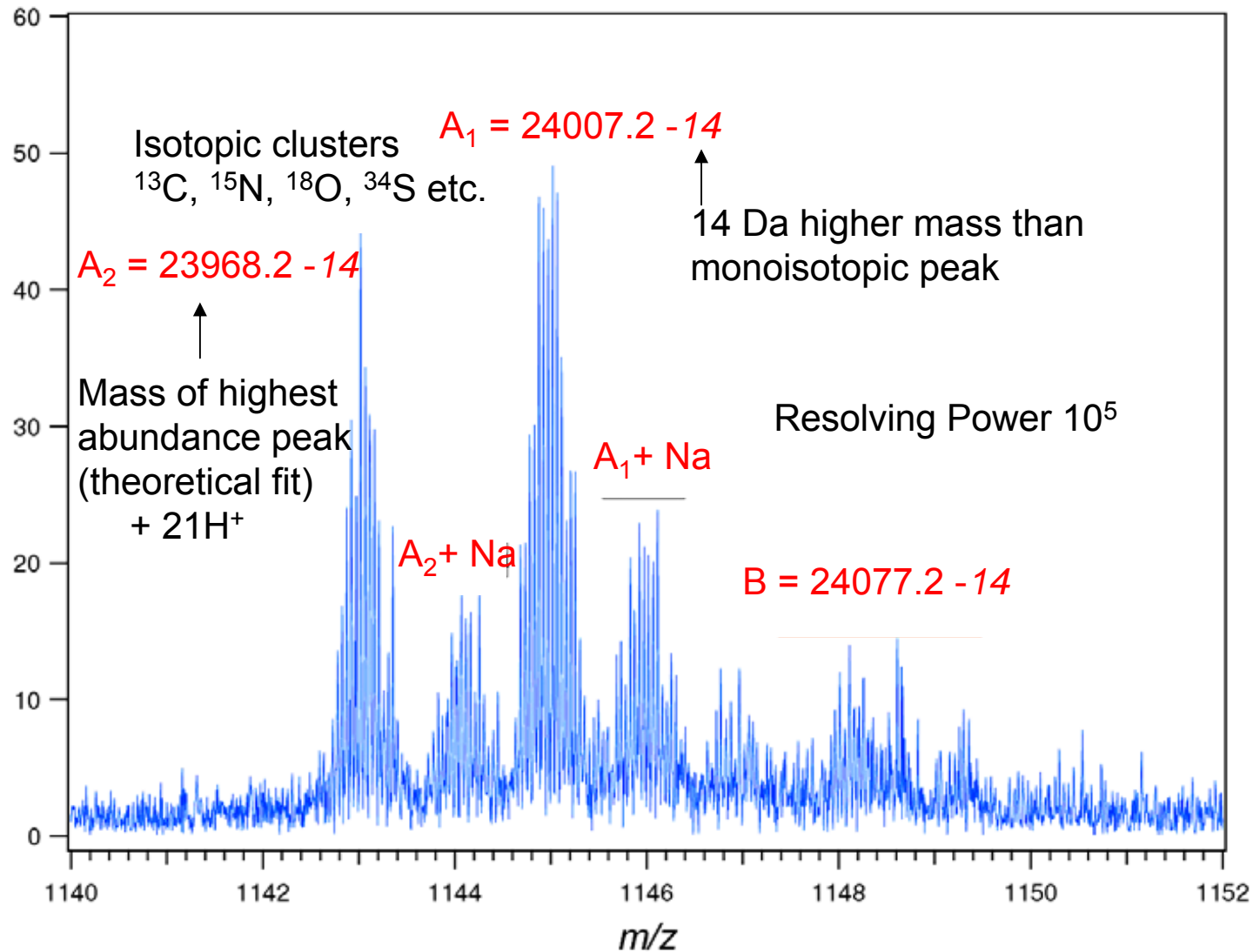
Orbitrap, no magnet,
“Middle-Down” MS



Also Tunable IR Laser for IR Photofragment Spectroscopy

Electrospray Ionization of Bovine β -Casein

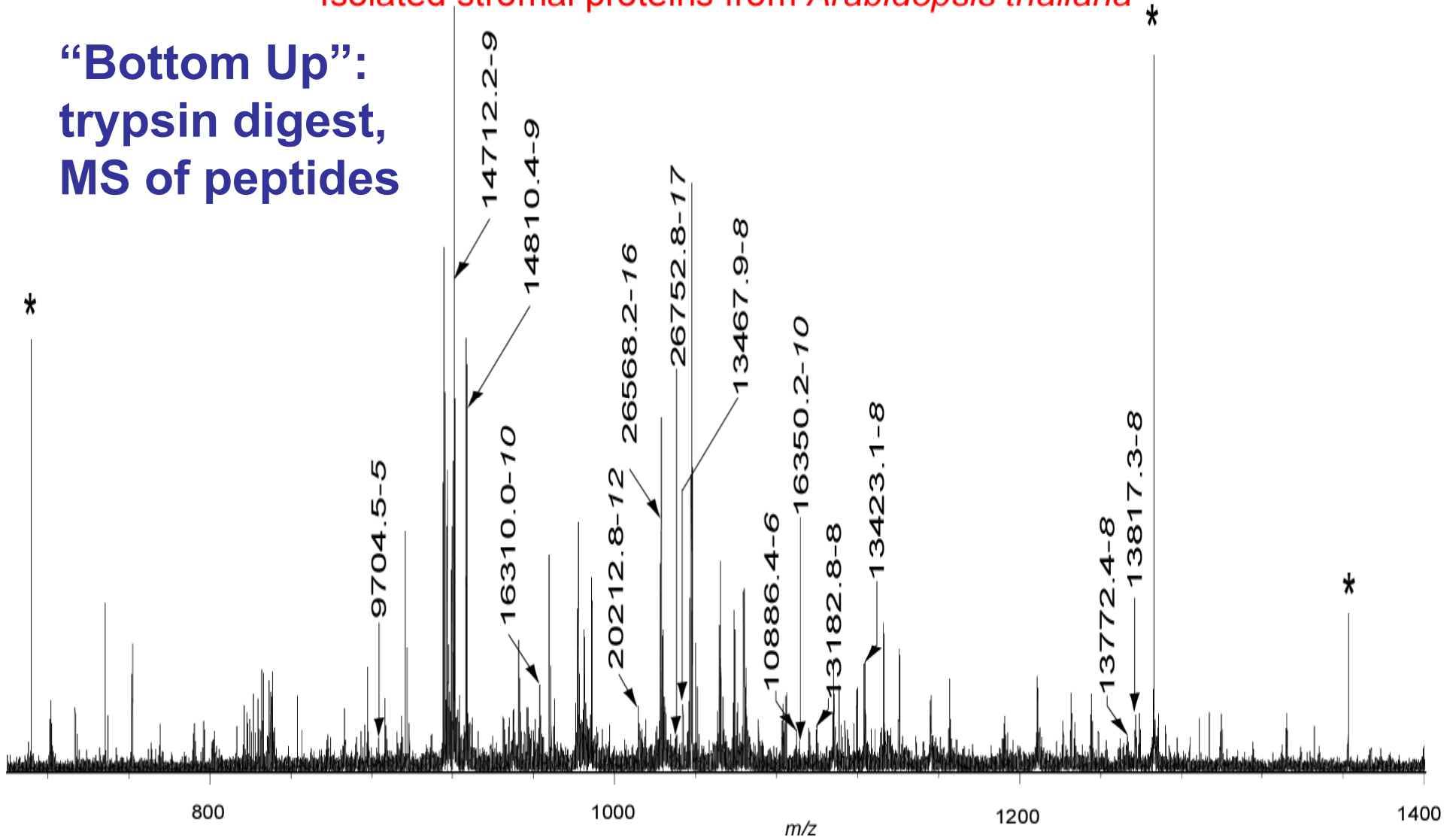
3 Variants, 5 Phosphorylations



Electrospray Ionization Top Down Visualization of a Protein Mixture

Isolated stromal proteins from *Arabidopsis thaliana*

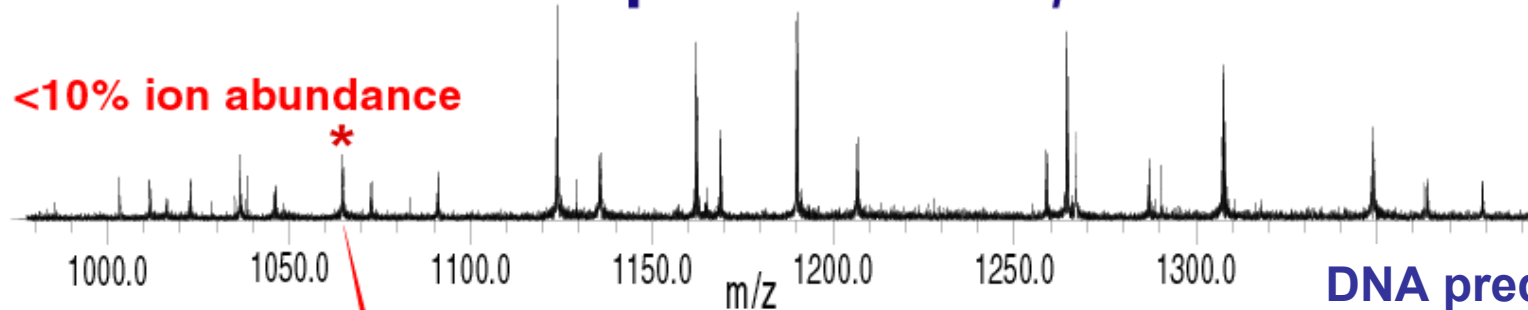
“Bottom Up”:
trypsin digest,
MS of peptides



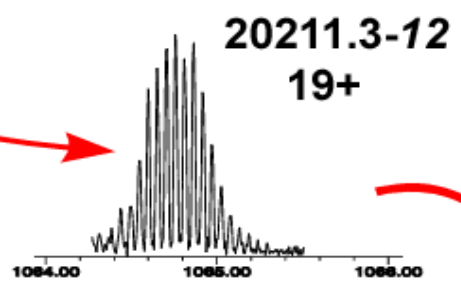
V Zabrouskov, L Giacomelli, KJ van Wijk, and FW McLafferty,
Molec. Cellular Proteomics **2003**, 2, 1253-1260.

TOP-DOWN IDENTIFICATION

Chloroplast *Arabidopsis th*

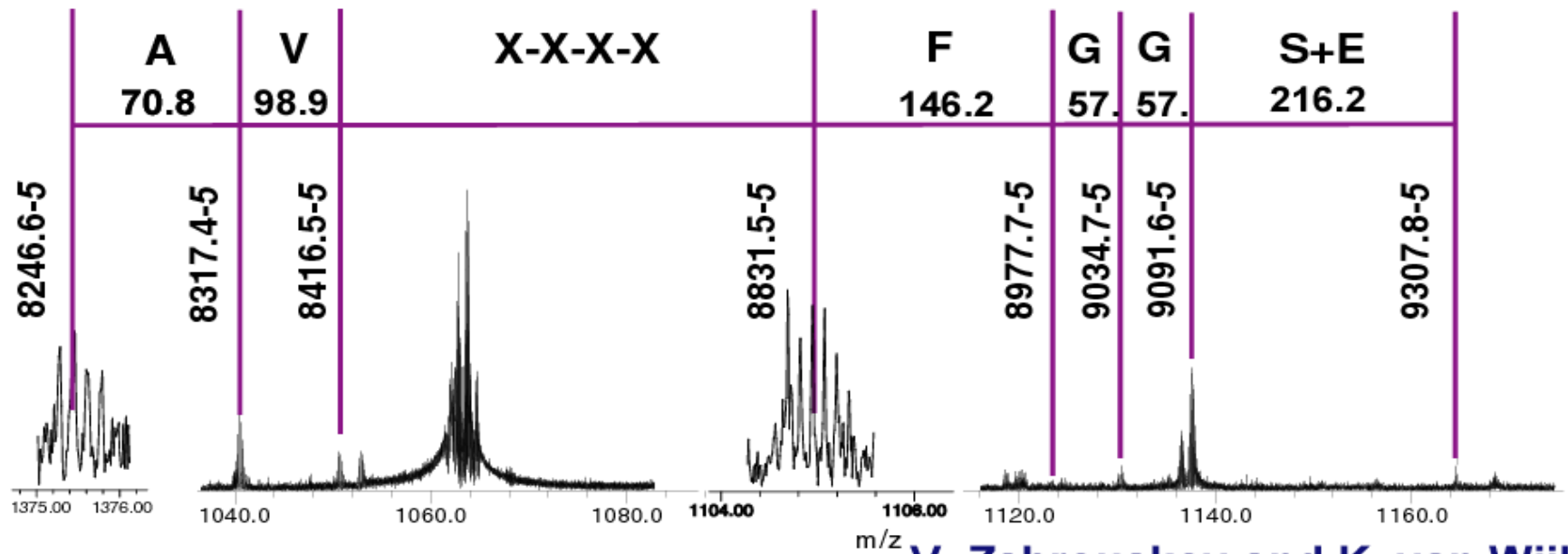


SWIFT



CAD
MS/MS

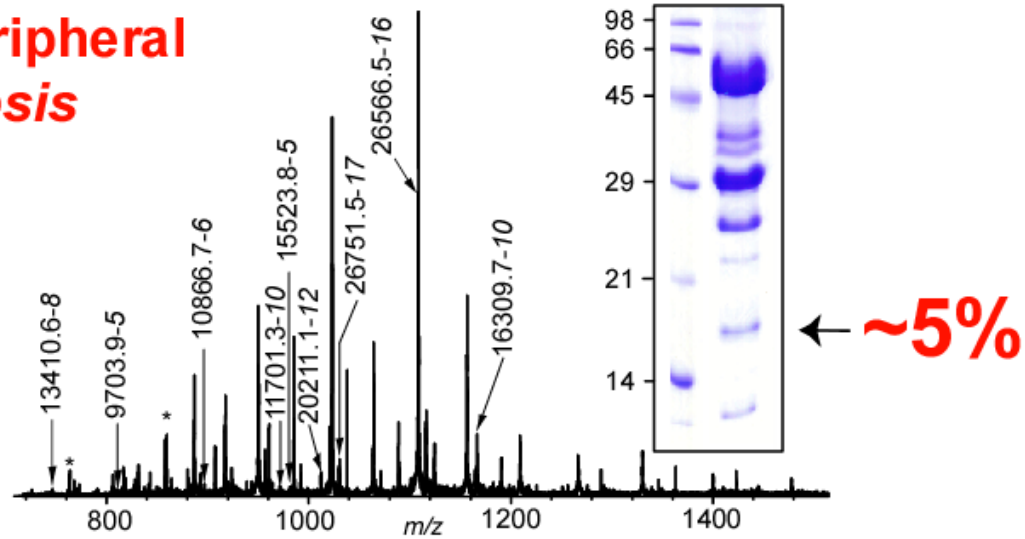
DNA predicted proteins:
Database search
At1g06680
M_r 20211.9



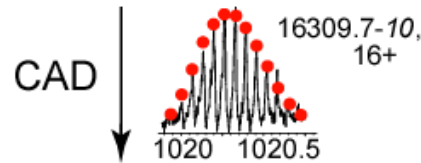
V. Zabrouskov and K. van Wijk

Characterization of a Protein $M_r = 16309.7$

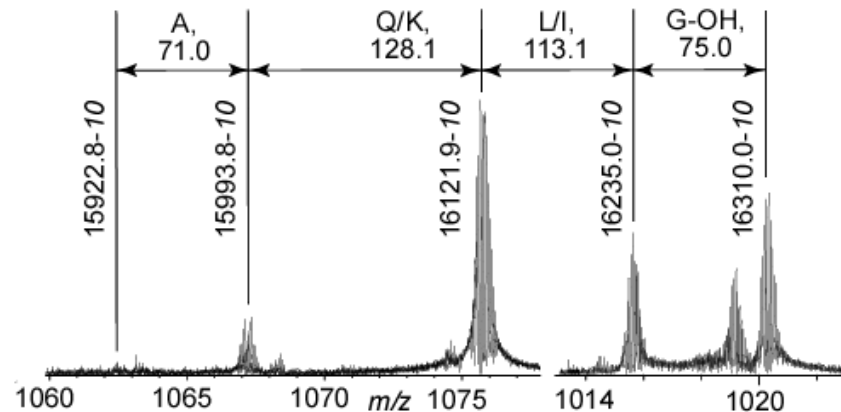
Thylakoid peripheral
Arabidopsis



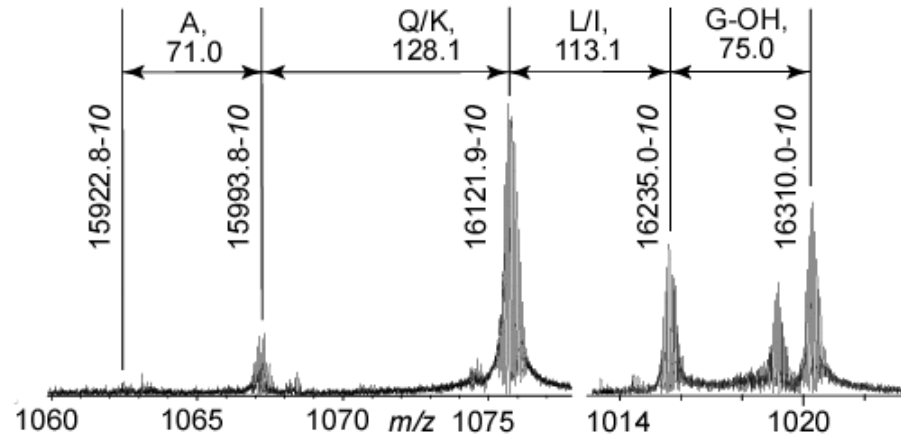
C-terminal sequence tag
matches At4g21280,
 $M_r = 16123.4$



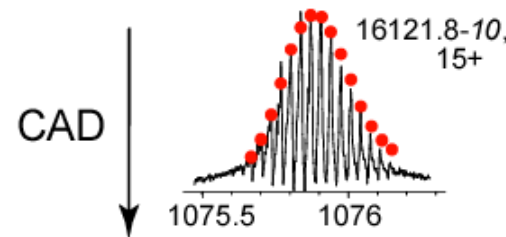
“Identification” by
Bottom Up



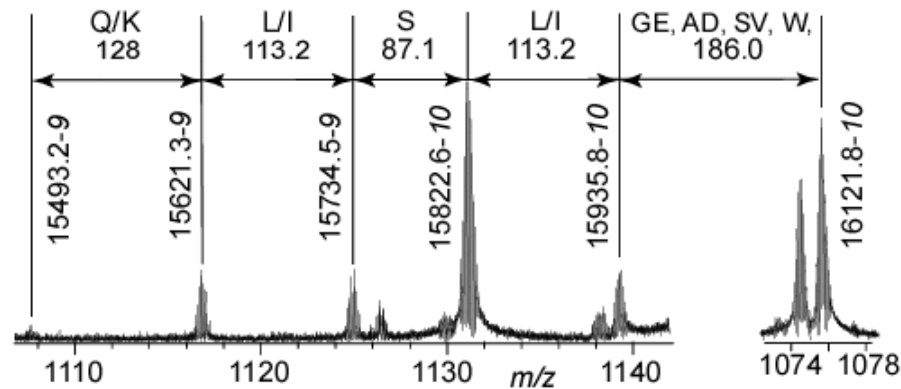
MS³ of 16122 Da Ions from M_r = 16310



**C-terminal sequence tag
also matches At4g21280,
M_r = 16123.4**

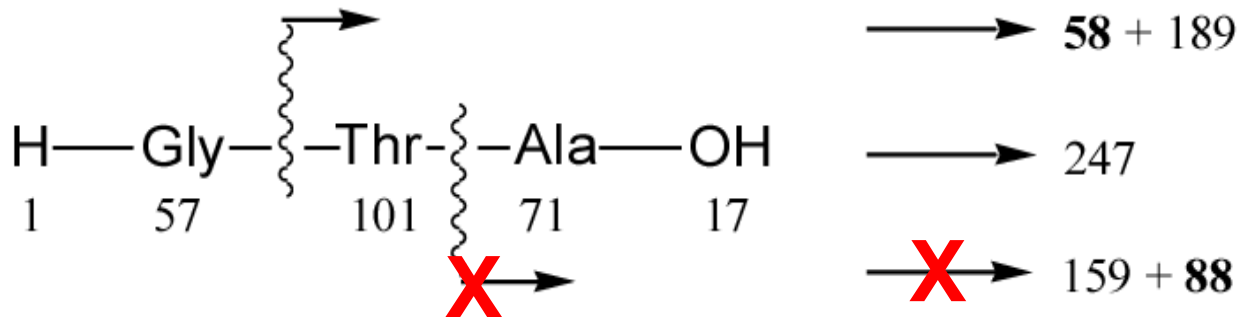
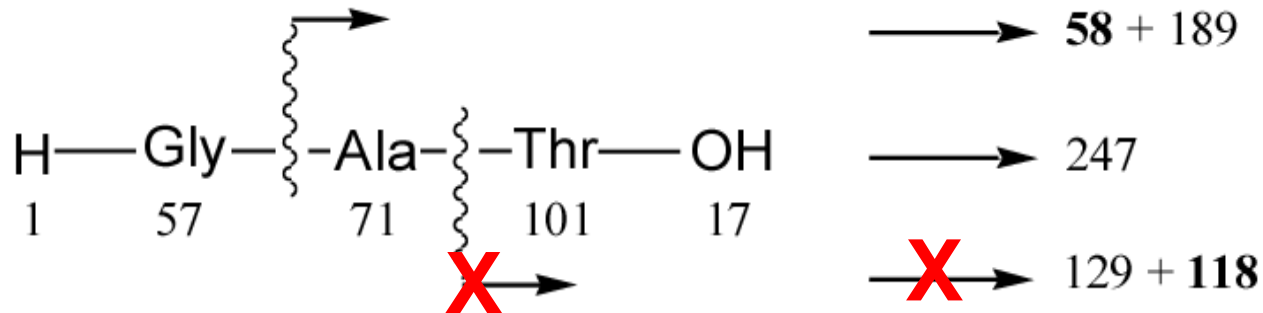


*“Characterization”
By Top Down*



**Top-Down: 1) Select protein by MS/MS.
2) Don't throw away connectivity info.**

Units: Amino Acids



**BUT complete sequence or exact PTM positioning demands a fragment mass from each interresidue bond cleavage –
and weakest bonds preferentially cleaved**

Chemistry of The Mass Spectrometry of Large Molecules

Electron ionization – requires vaporization
Removes an electron, yields **Odd-Electron Ion**
More easily dissociated, less rearrangement

MS requires charged species-

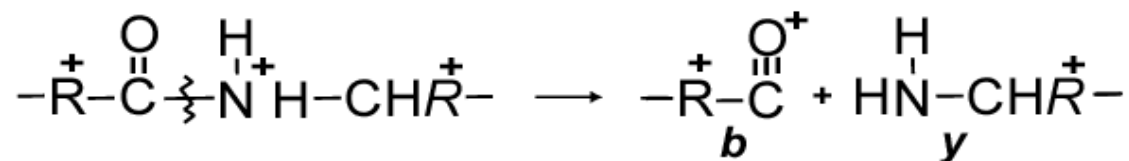
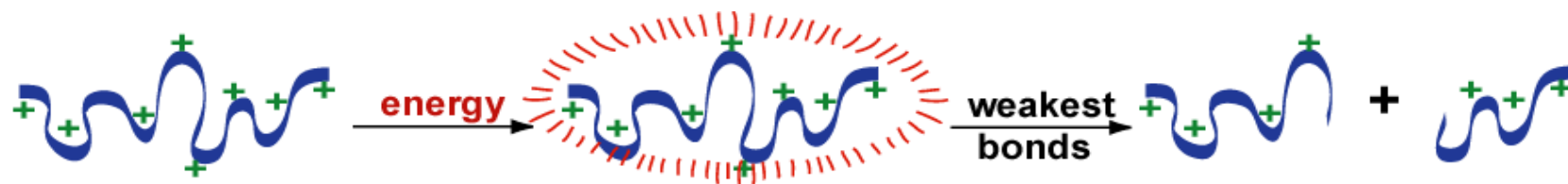
Chemical Ionization, Plasma Desorption, Fast
Atom Bombardment, Matrix Assisted Laser
Desorption Ionization, Electrospray Ionization

But – these produce mainly Even-Electron Ions!

New Chemical Reaction Needed



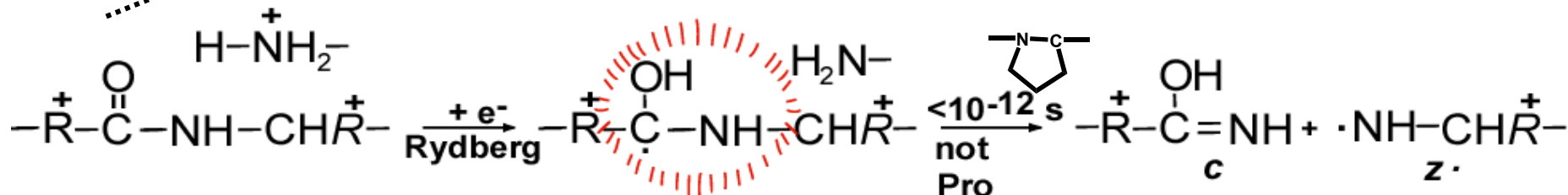
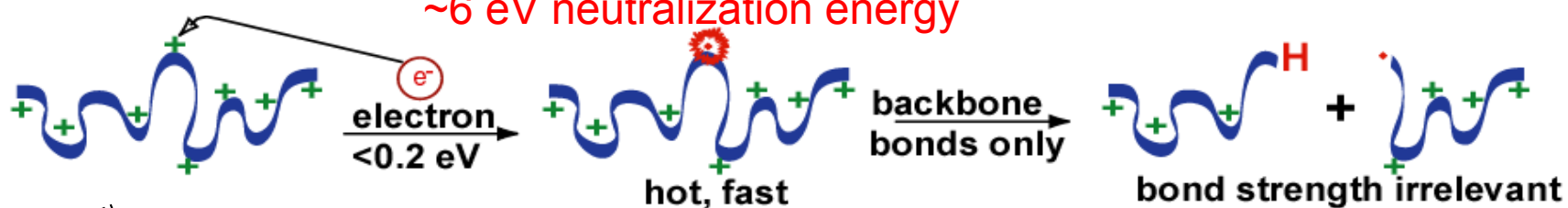
Energetic Dissociation: CAD, IRMPD, BIRD, SID



Electron Capture Dissociation (with FTMS)

Zubarev, R. A.; Kelleher, N. L.; McLafferty, F. W. *JACS*, 1998, 120, 3265

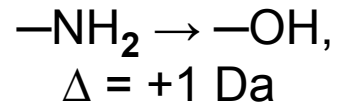
~6 eV neutralization energy



Electron Transfer Dissociation (**Bottom-Up!**)

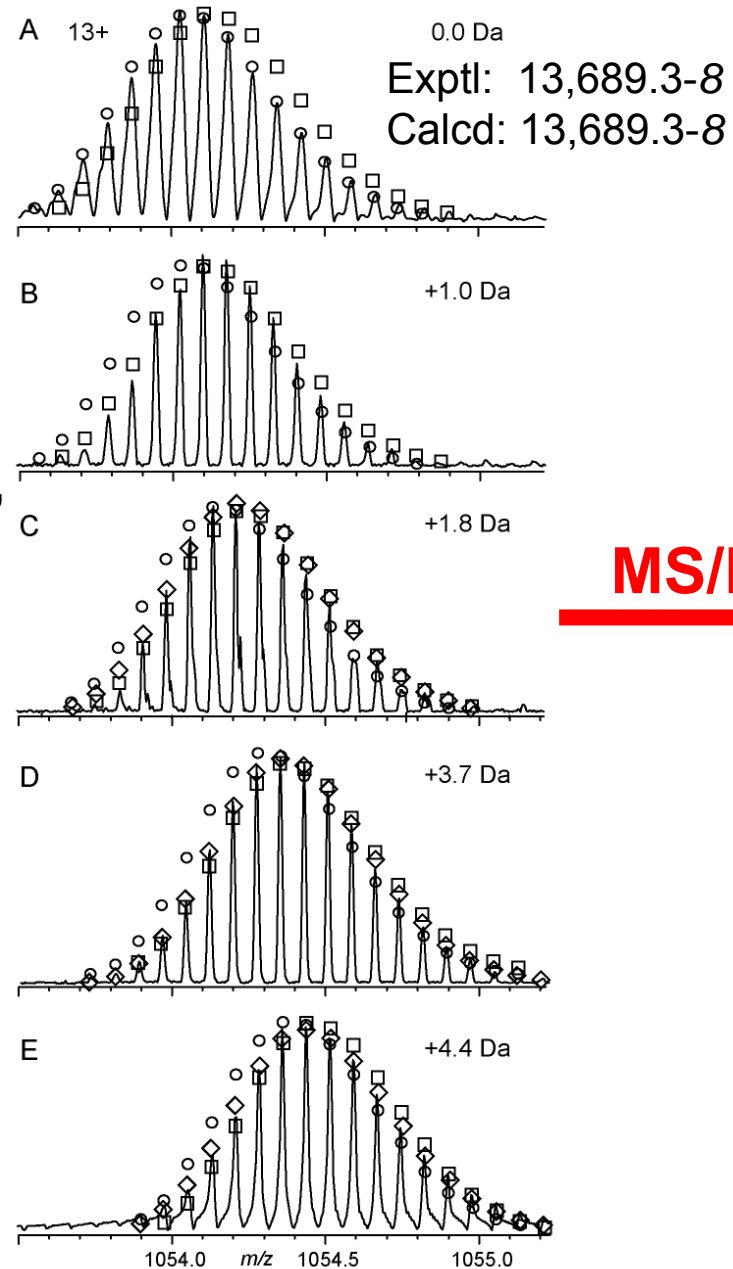
Syka, J. E. P.; Coon, J. J.; Schroeder, M. J.; Shabanowitz, J.; Hunt, D. F. *PNAS*, 2004, 101, 9528.

Deamidation of Reduced Ribonuclease A



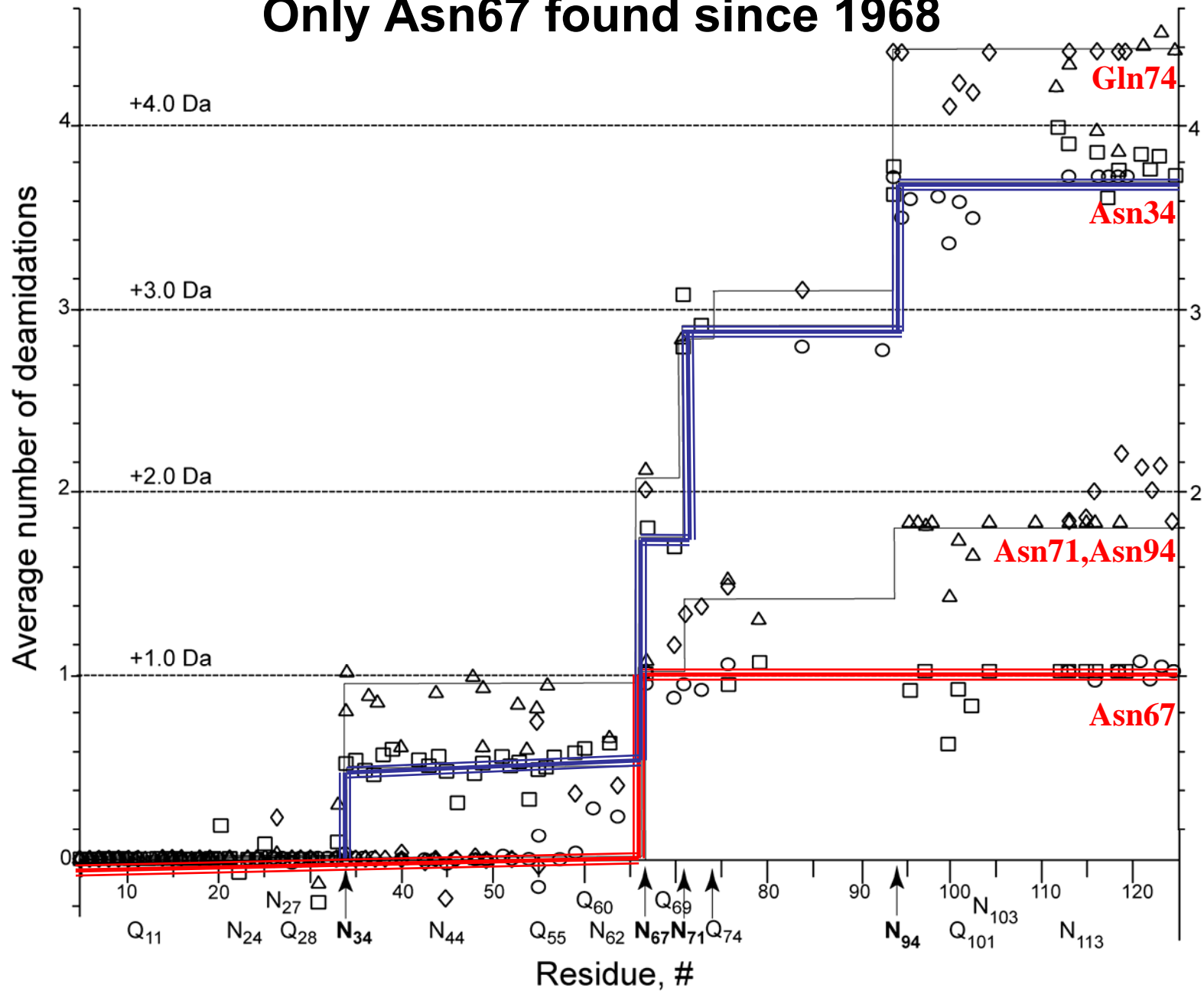
Zabrouskov, Han, Welker,
Zhai, Lin, van Wijk, Scheraga,
McLafferty, *Biochemistry* **2006**,
45, 987-992.

**Method to
measure age?**



Deamidation of RNase A

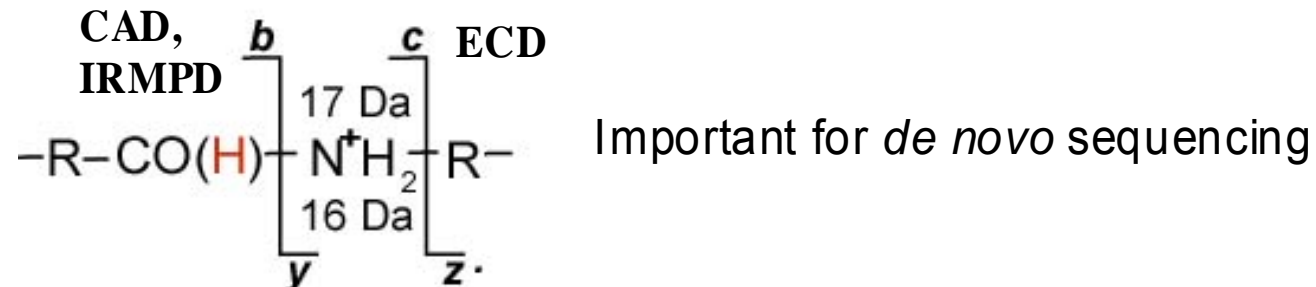
Only Asn67 found since 1968



Electron Capture Nonergodic Dissociation

$<10^{-12}$ s, before energy randomization

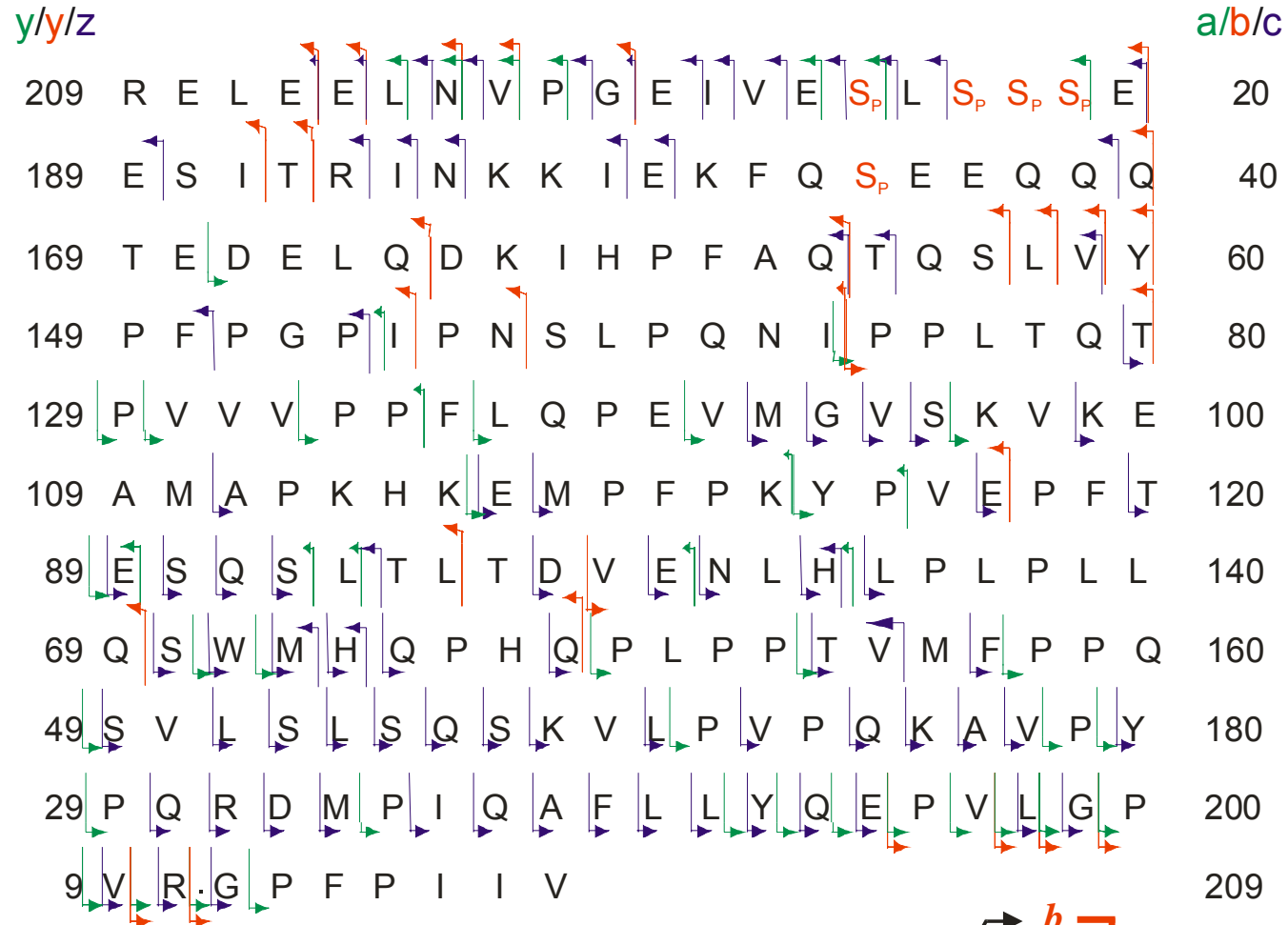
- Negligible time for rearrangement
stops H/D scrambling (D atoms on exposed conformers)
- Most interresidue bonds can be cleaved (250/258)
 e^- neutralization energy \gg bond dissociation energy
- Fragment ion has N- or C-terminus, identifiable by



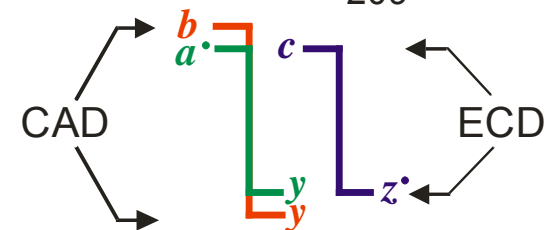
- **Side chain posttranslational modifications are stable**

26 Phosphorylation Sites in β -Casein by Plasma-ECD

126/208 interresidue cleavages



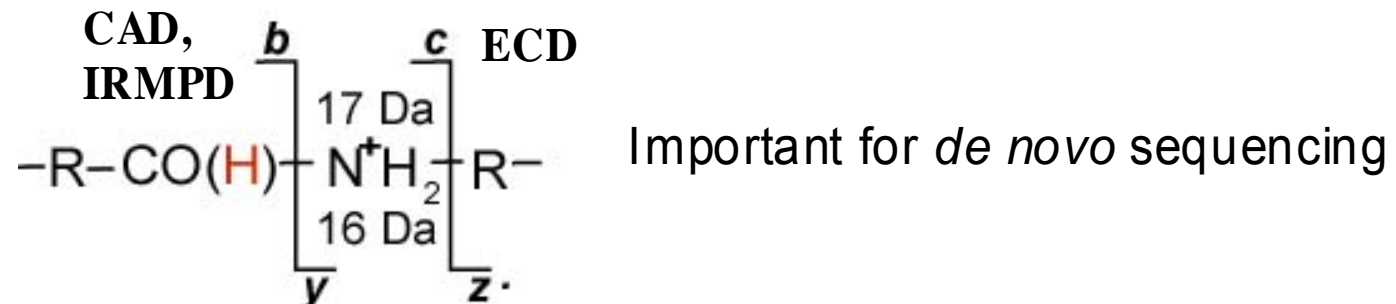
Sze, S. K.; Ge, Y.; Oh, H. B.; McLafferty, F. W.
Anal. Chem. **2003**, 75, 1599-1603.



Electron Capture Nonergodic Dissociation

$<10^{-12}$ s, before energy randomization

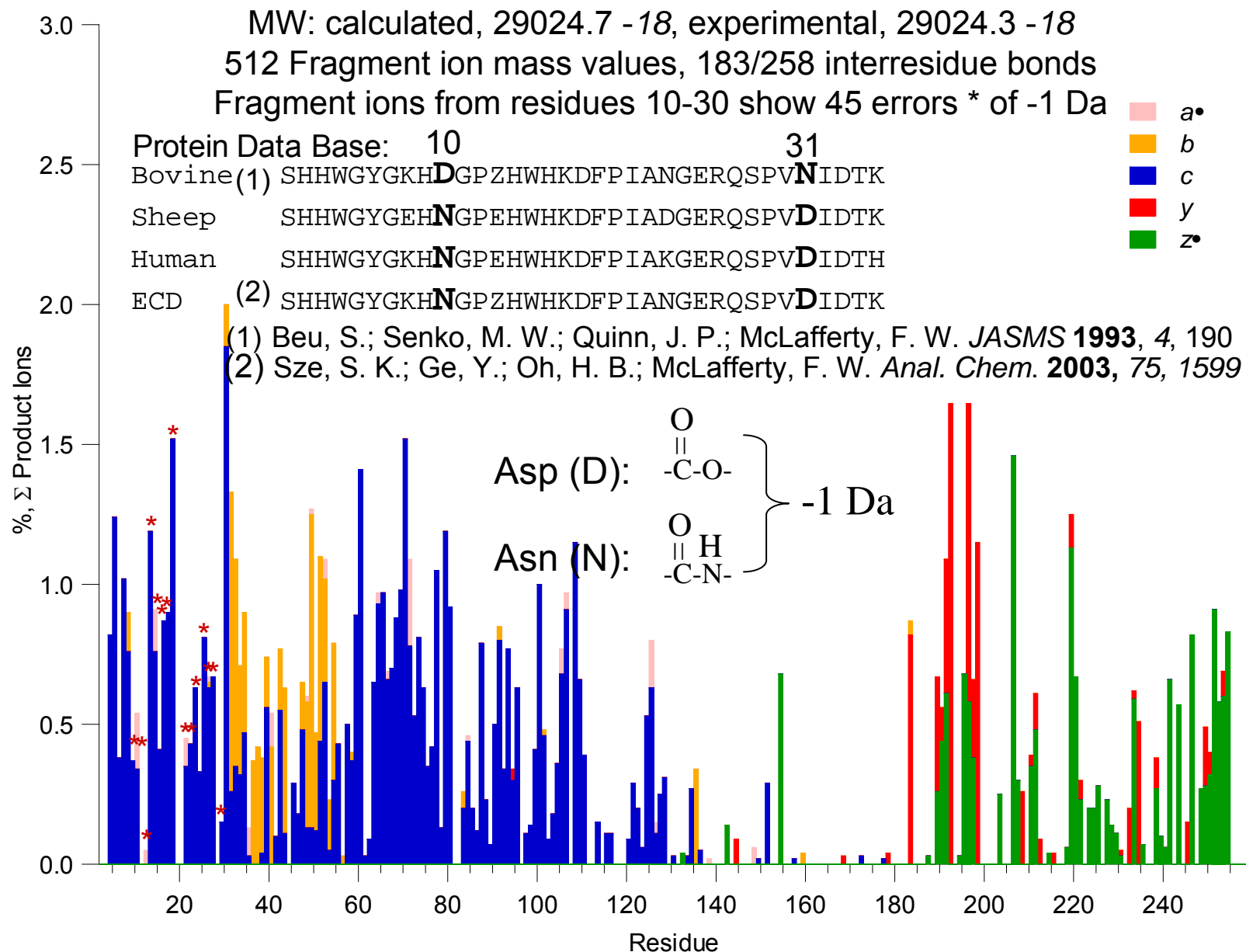
- Negligible time for rearrangement
stops H/D scrambling (D atoms on exposed conformers)
- Most interresidue bonds can be cleaved (250/258)
 e^- neutralization energy \gg bond dissociation energy
- Fragment ion has N- or C-terminus, identifiable by



- Side chain posttranslational modifications are stable
- **Tertiary noncovalent bonds not cleaved – ECD**
cleavage sites depend on conformation!

Horn, Ge, McLafferty, Activated Ion Electron Capture Dissociation for Mass Spectral Sequencing of Larger (42 kDa) Proteins, *Anal. Chem.* **2000**, 72, 4778-4784.

Top Down Characterization of Carbonic Anhydrase



Automated MS Proteomics

Bottom-Up:

7000 proteins identified, M. Mann, *et al.*, Nat. Methods, **2009**, 6, 359.

Top-Down: Neil Kelleher, University of Illinois

Off Line Separation: 1D Gel, RP LC, collect fractions

MS: ESI, Octopole ion concentration, exact mass FTMS

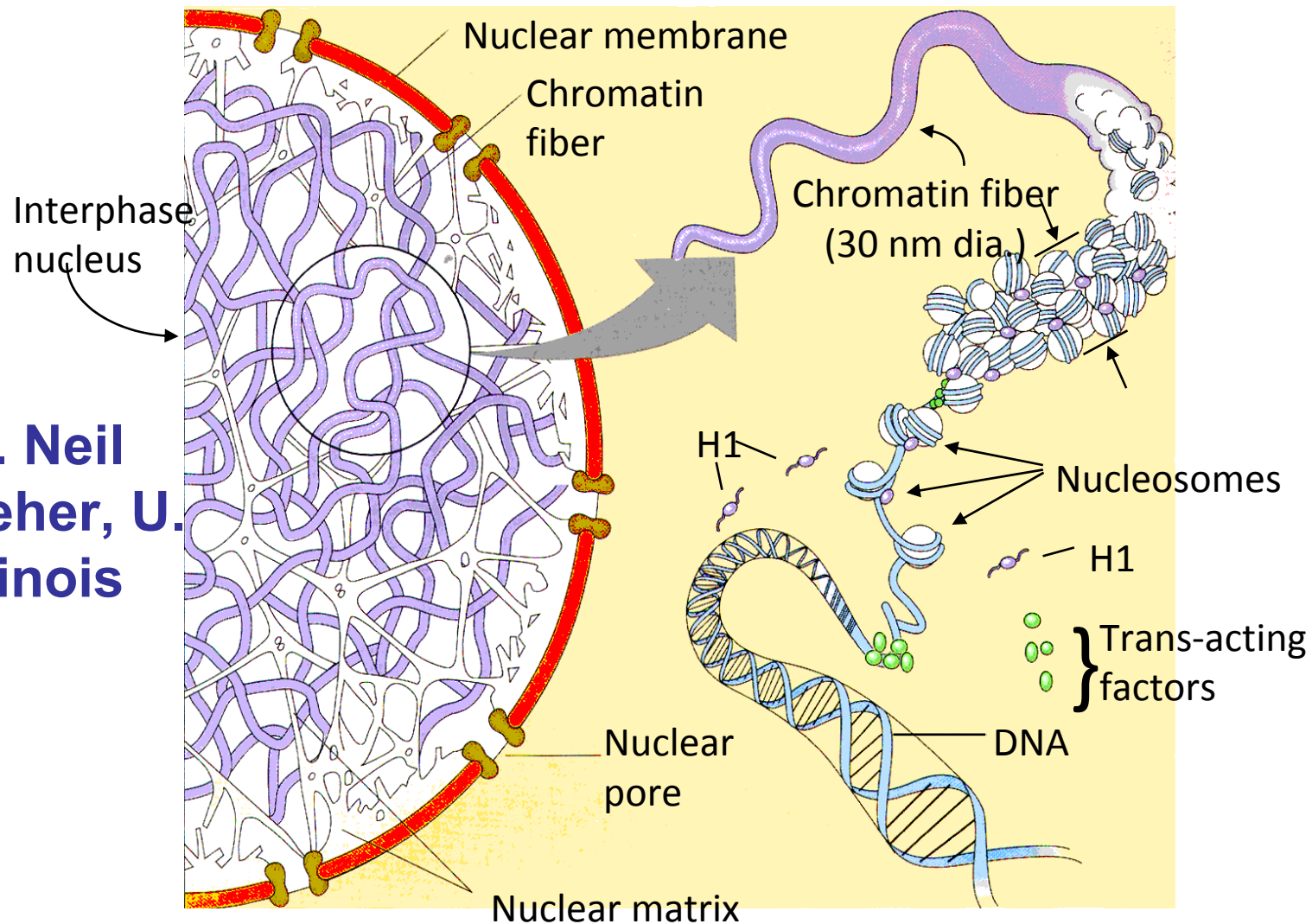
MS/MS: CAD, ECD

Data Reduction: THRASH, ProSight PTM vs. predicted proteins

Anal. Chem. **2004**, 76, 197A-203A.

Top Down in Chromatin Biology

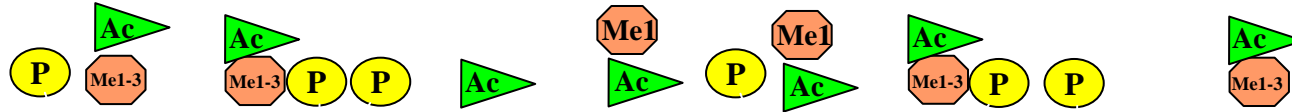
Prof. Neil Kelleher, U. of Illinois



Top Down has provided the definitive list of histone isoforms present in yeast and human cells.

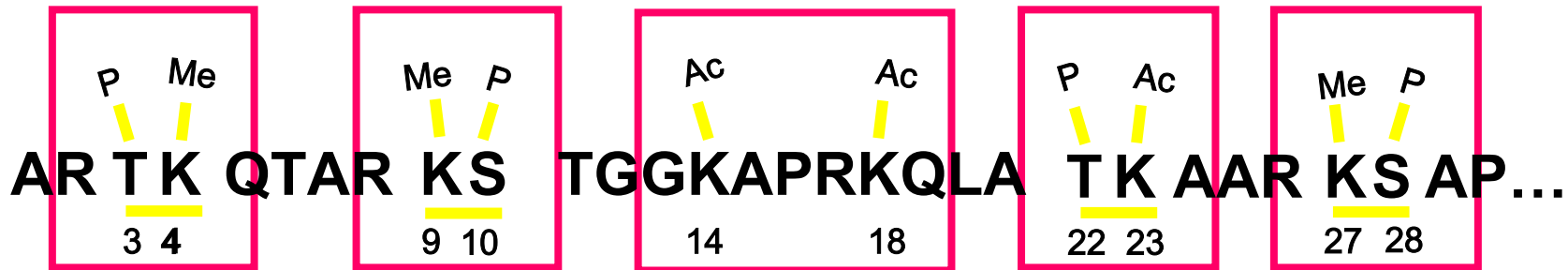
What about a possible combinatorial “Histone Code”?

Histone H3:



ARTKQTARKSTGGKAPRKQLATKAARKSAPSTGGVKKPH...

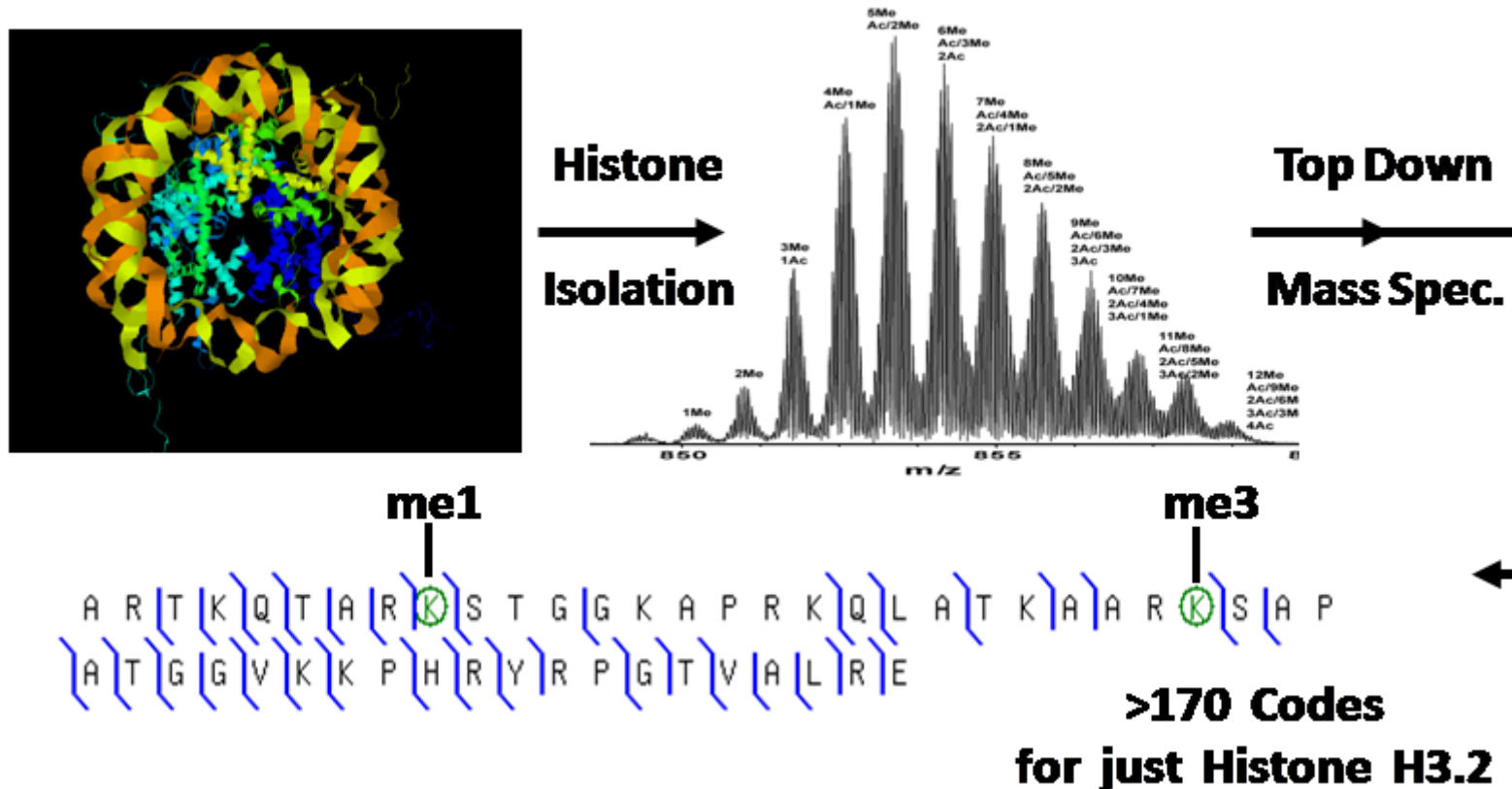
$K_{me2}S_{phos}TGGK_{ac}APR$ $TARKSTGGK_{ac}APRKQL$ $YRPGTVALR$
 $TK_{me2}QTAR$ $K_{ac}QLATK_{ac}AAR$ $K_{me3}SAPSTGGVK_{me1}KPHR$ $KAARK_{me1}SAPSTGGVKK$



Can we use mass spectrometry to identify modifications on Single Forms that occur concurrently?

High Resolution MS/MS on Histone H3

“Middle-Down” MS Proteomics



B.A. Garcia, J.J. Pesavento, C.A. Mizzen and N.L. Kelleher, *Nature Methods*, 2007, 4, 487.

M. Mann, N. L. Kelleher, *PNAS*, 2008, 105, 18132-18138..

Effect of Electrospray on Protein Conformation

Electrospray retains biological activity! Ions mass-separated in instrument, **ACTIVE. Virus:** Gary Siuzdak. **Soft landing:** Graham Cooks, Vicki Wysocki

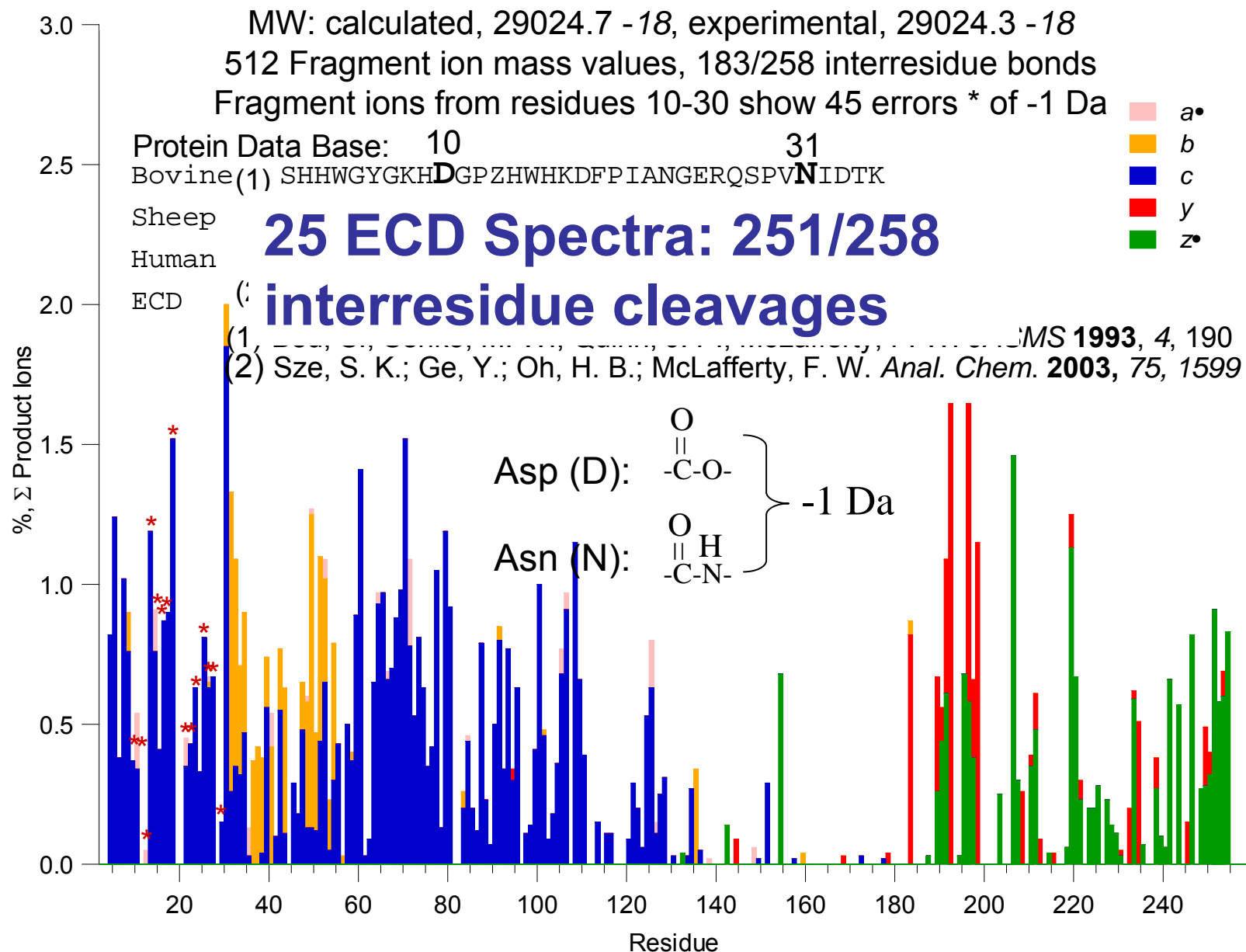
Large (>0.5 MDa) Protein Complexes: Carol Robinson: Intraprotein noncovalent bonds stable.

Protein/Ligand Noncovalent Complexes: Steve Hofstadler, Nathan Yates, etc.: Noncovalent retention critical

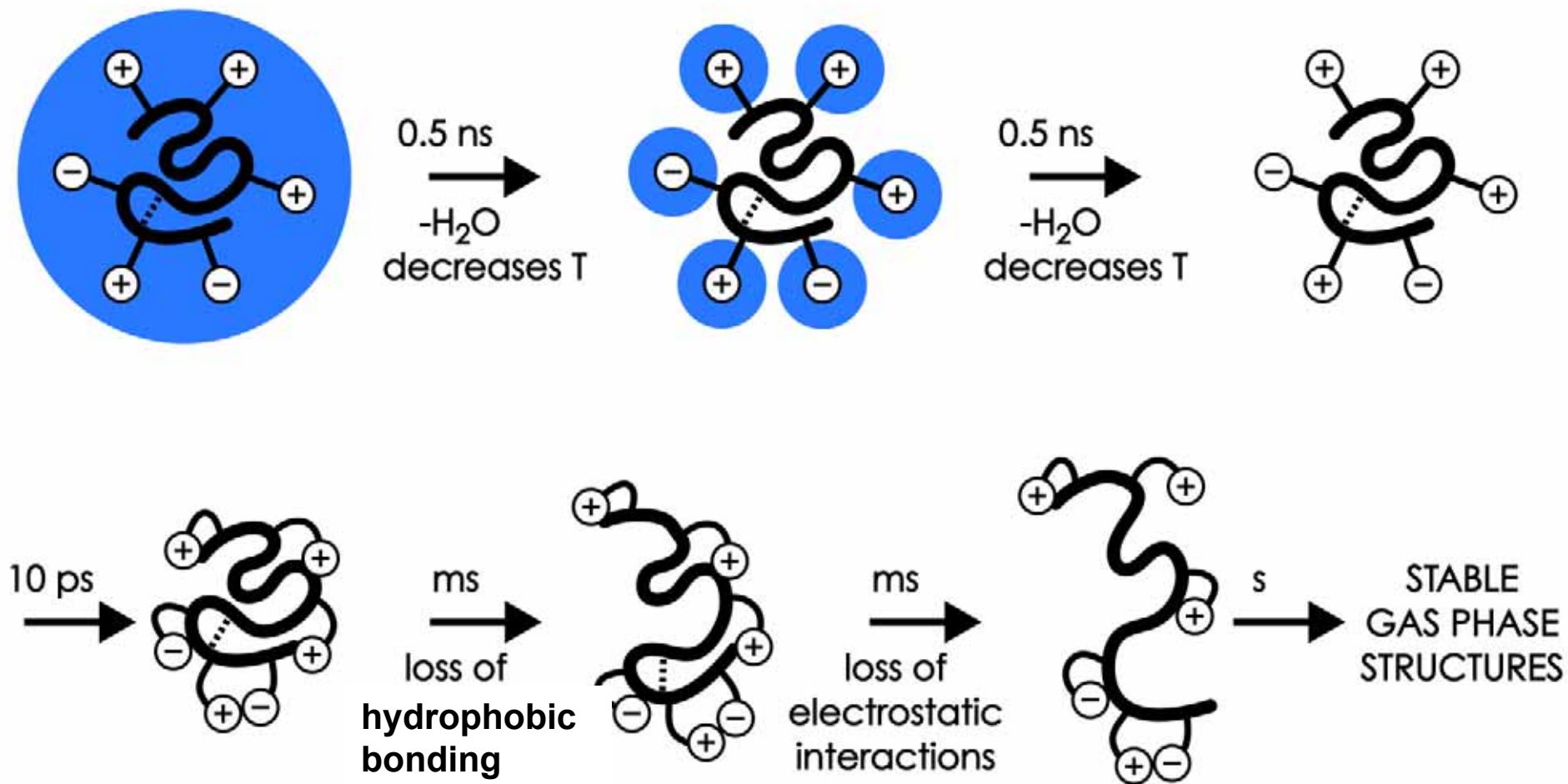
H/D Scrambling minimized by ECD: Cornell, Joe Loo

Top-Down of >50 kDa Proteins: “Activated ion” fails.

Top Down Characterization of Carbonic Anhydrase



STRUCTURAL EVOLUTION

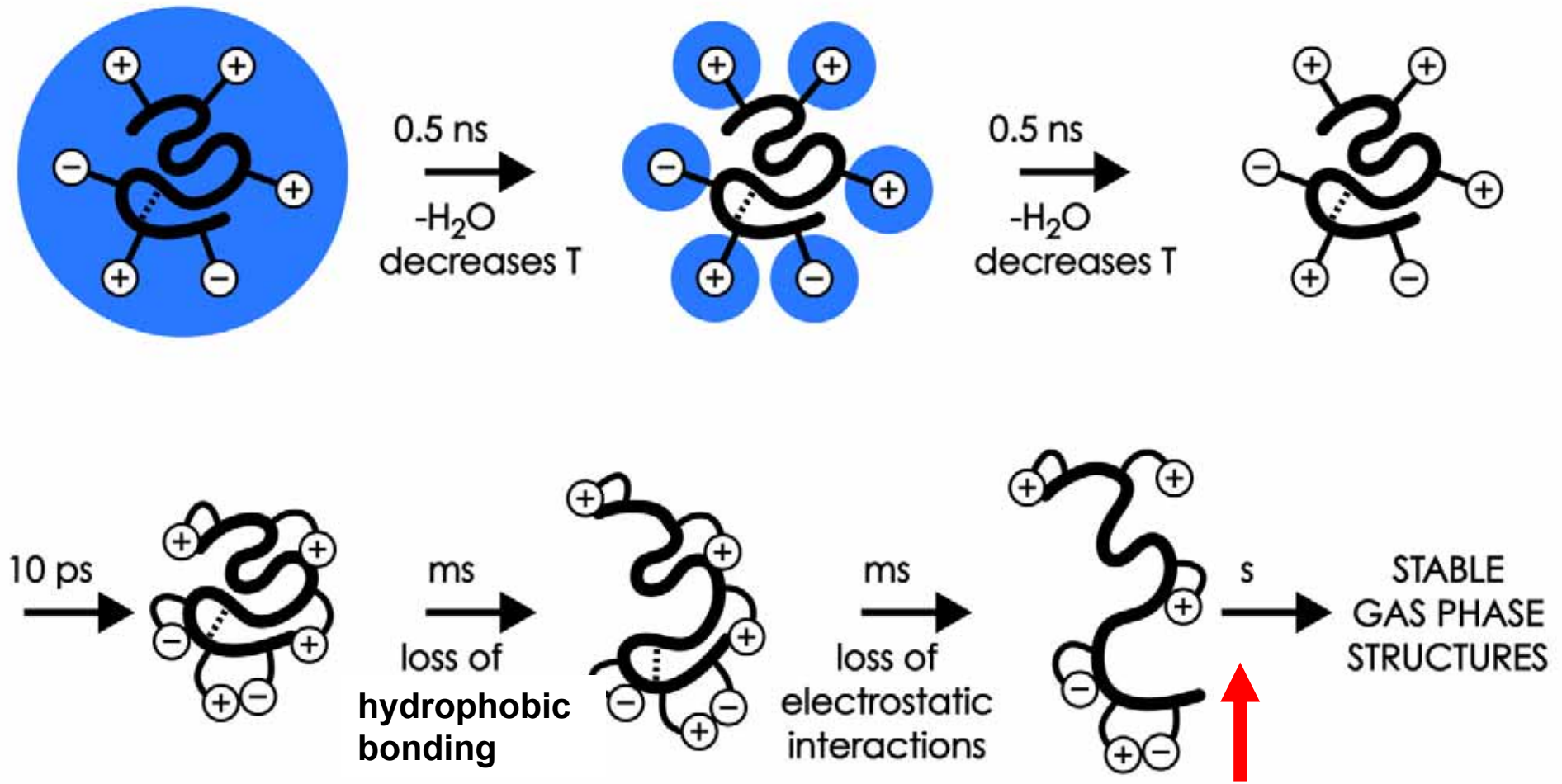


..... hydrogen bonding

Breuker, McLafferty PNAS, 2008, 105, 18151

STRUCTURAL EVOLUTION

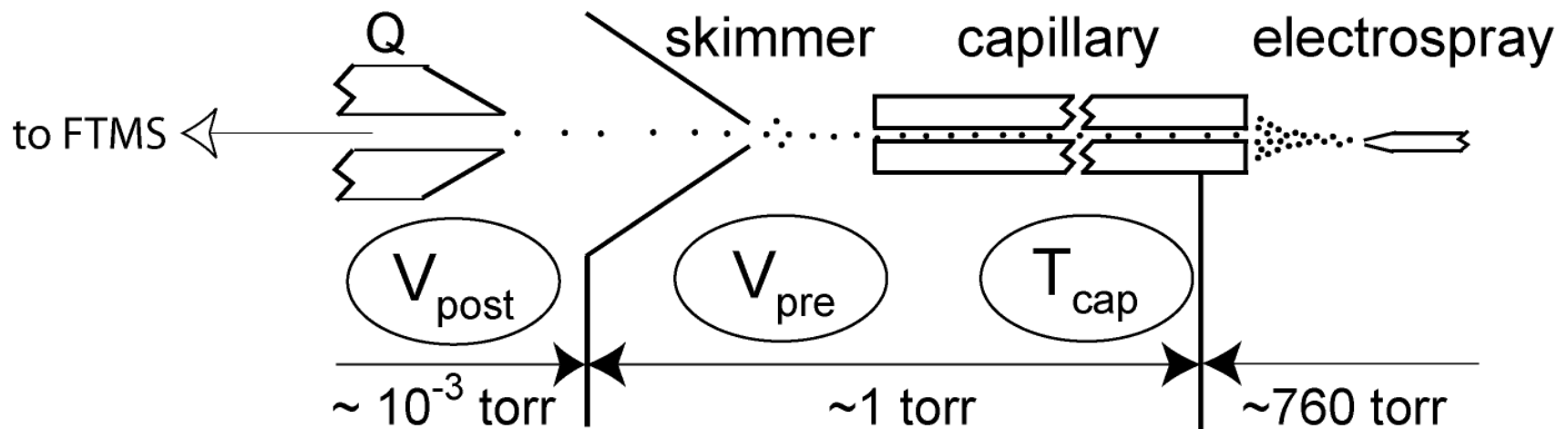
>75 kDa proteins, negligible topdown dissociation



“Prefolding Dissociation”

Prefolding Dissociation

Protein molecular ion intractability above 50 kDa

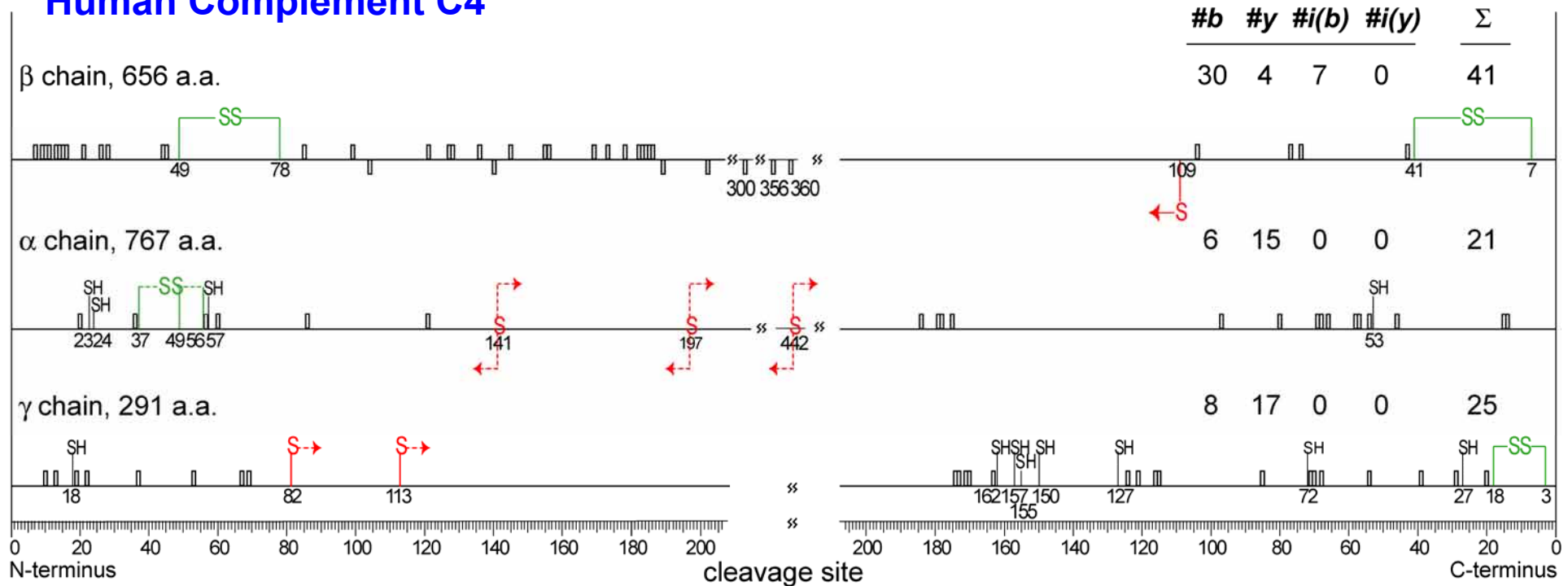


Han, Jin, Breuker, McLafferty *Science*, **2006**, 314, 109-112

Top Down PFD of a 200 kDa Protein

All 27 Cysteines assigned as S-H or S-S

Human Complement C4



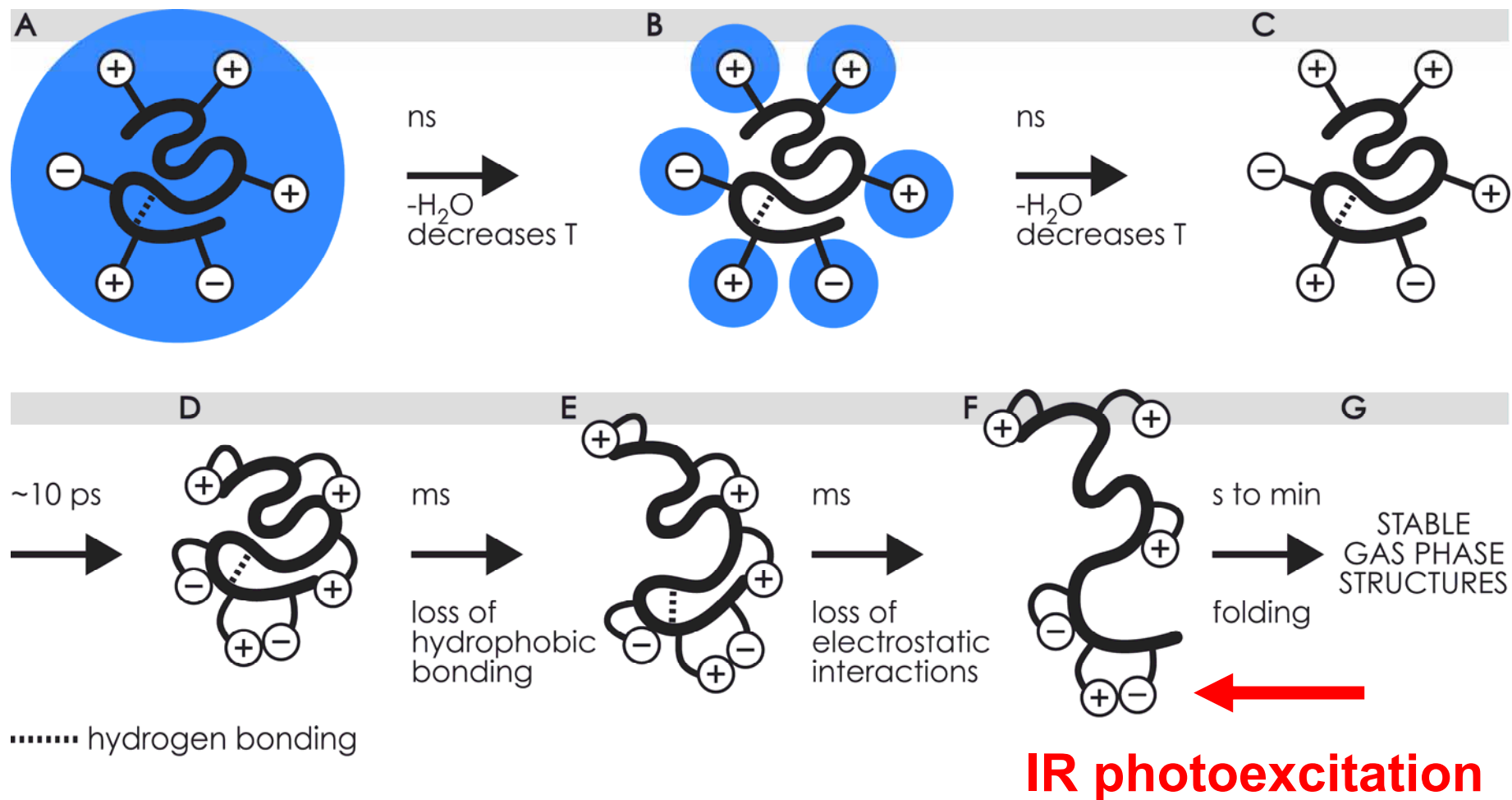
87 fragment ion masses
(no glycosylation)

57% overall sequence
coverage

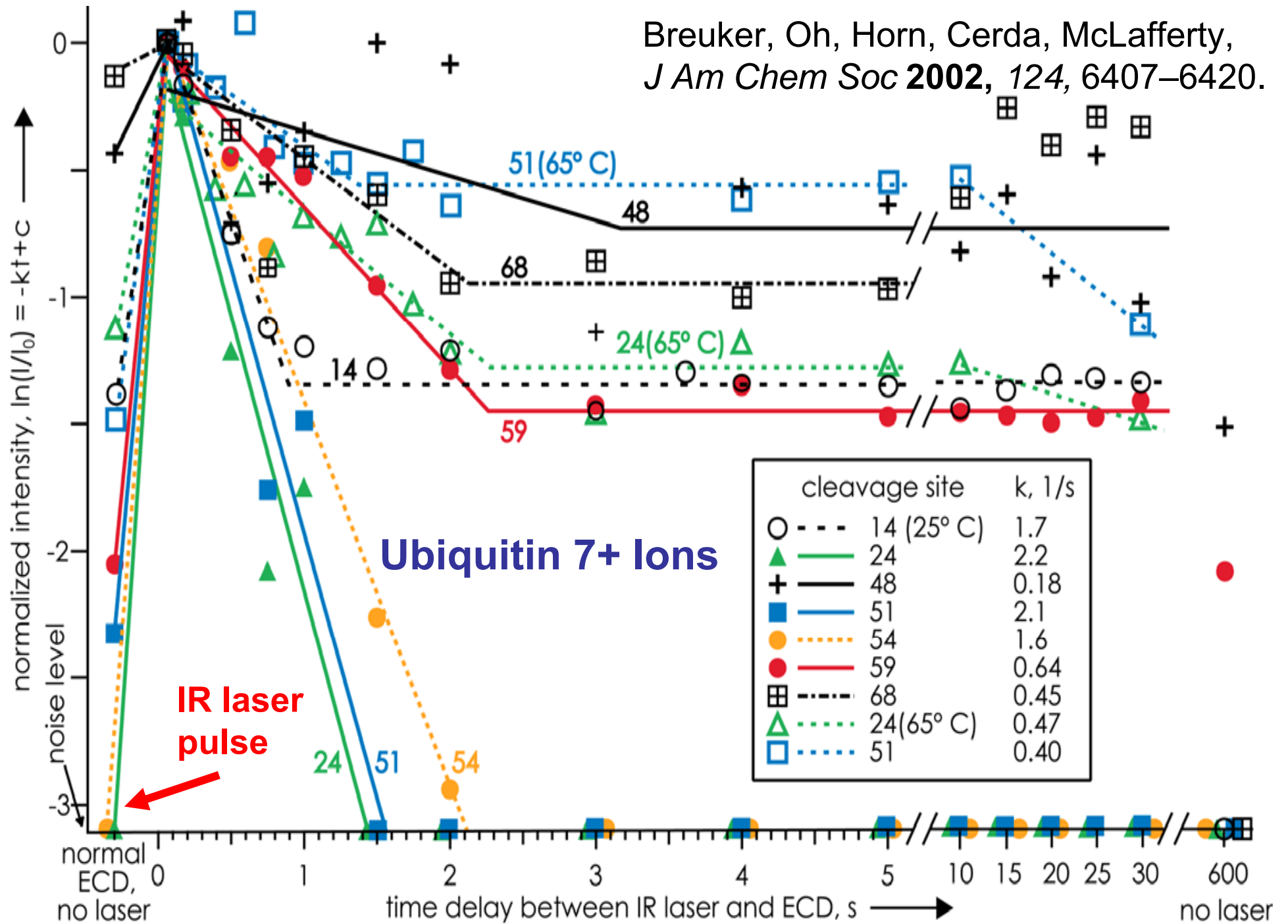
Specify 4 previously unidentified intrachain S-S bonds

Localize 6 Cys residues of three interchain S-S bonds

Denaturing Refolded Proteins in the Ion Cell



Breuker, Oh, Horn, Cerda, McLafferty,
J Am Chem Soc **2002**, 124, 6407–6420.



(Ser₈ + 8H)⁺: Prebiotic Chiral Selection?

S.C. Nanita, R.G. Cooks, *Angew. Chem. Int. Ed.* 2006, 45, 554-569.

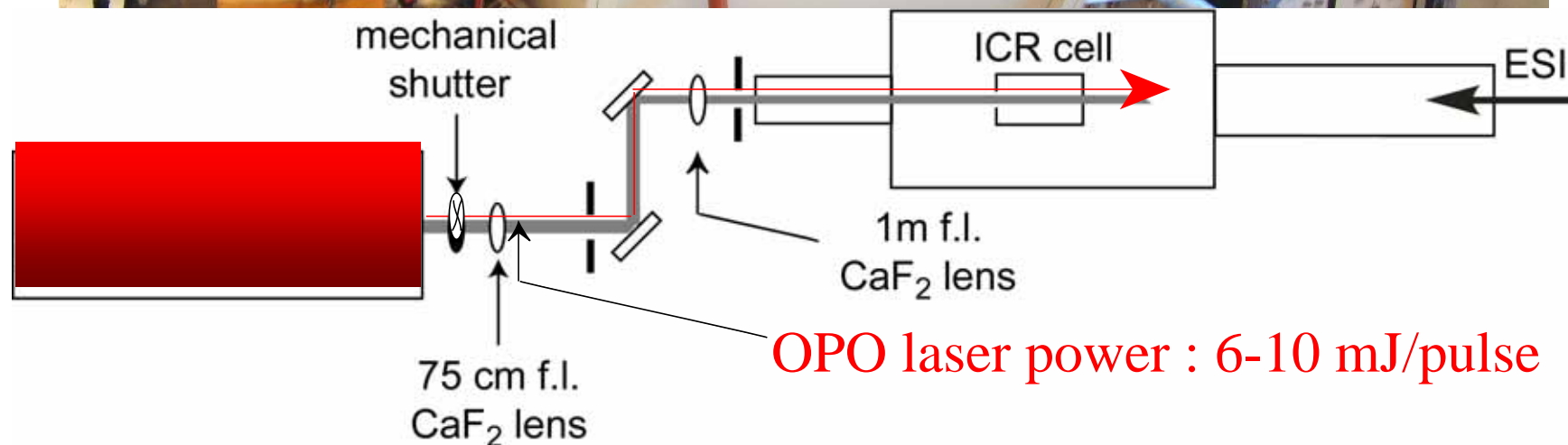
- D,L-Ser selectively yields (D-Ser₈ + H)⁺ and (L-Ser₈ + H)⁺.
- serine octamers are also selectively formed as neutrals and anions
- serine octamers are also selectively formed from solid and solution phases.

Electrospray retains the conformer structure?

Infrared Photodissociation Spectroscopy

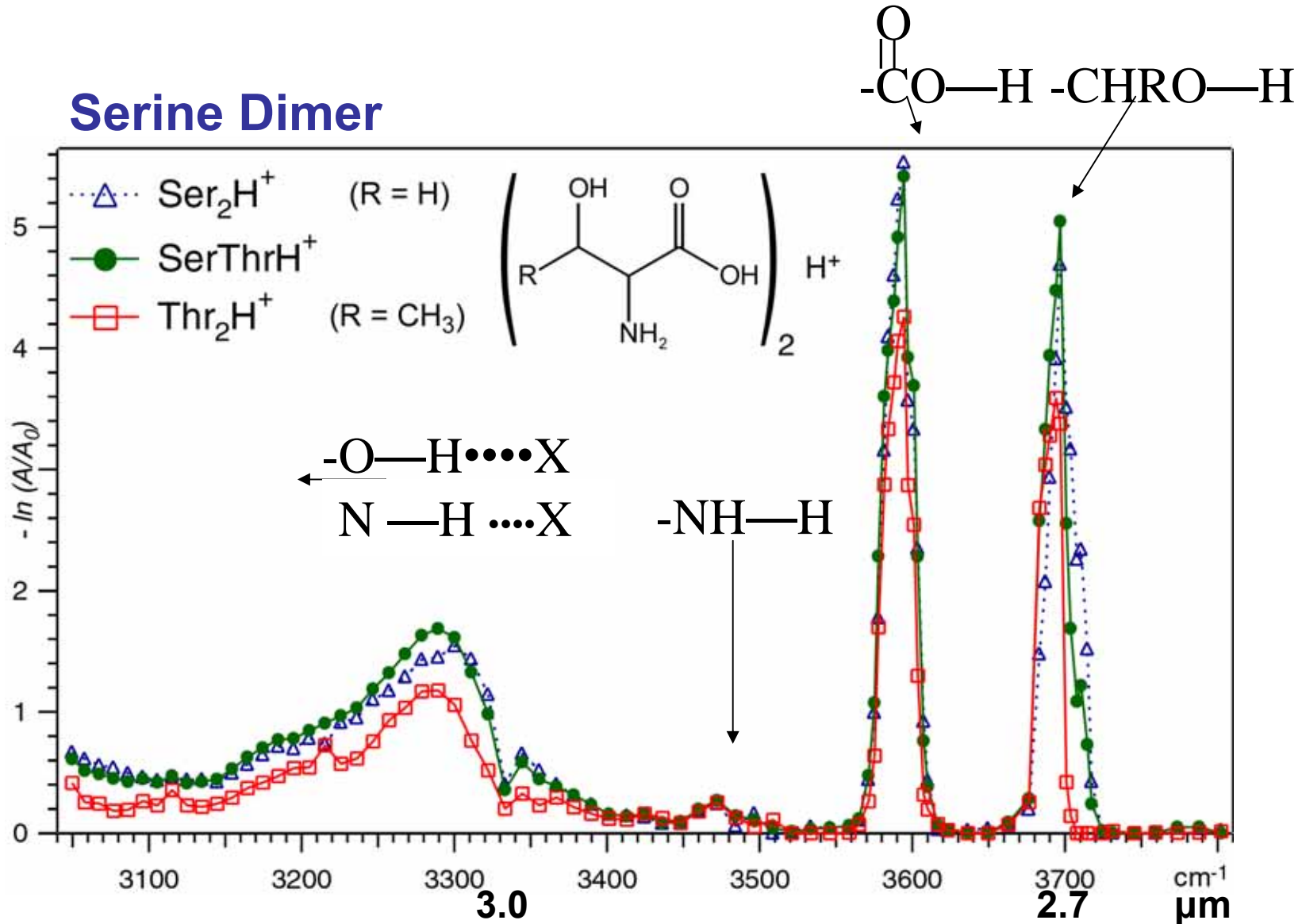
Oh, Breuker, Sze, Ge, Carpenter, McLafferty *Proc. Natl. Acad. Sci. USA* 2002, 99, 15863

Oh, Lin, Hwang, Zhai, Breuker, Zabrouskov, Carpenter, McLafferty, *JACS*, 2005, 127, 4076

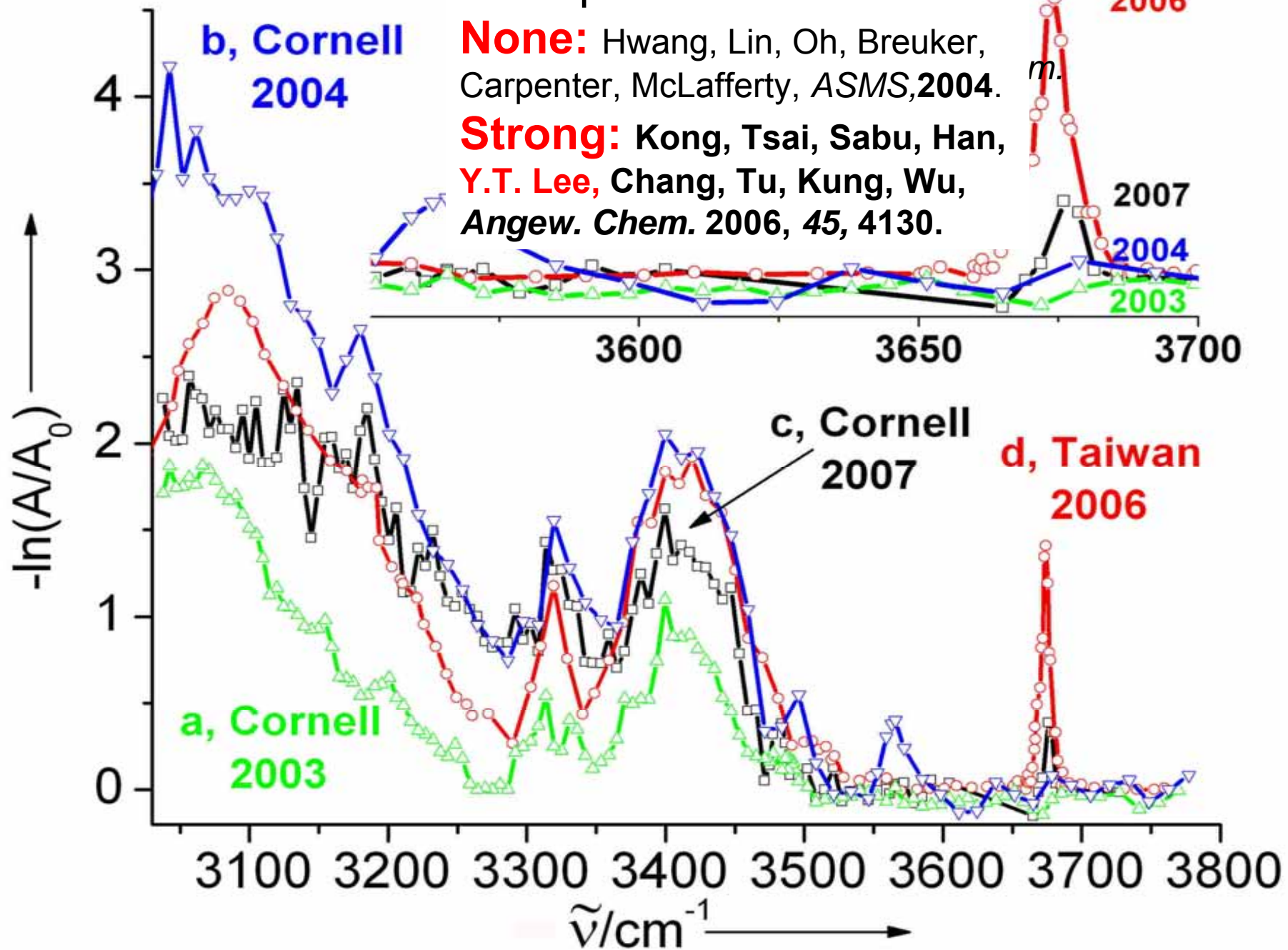


IRPDS Peak Assignments

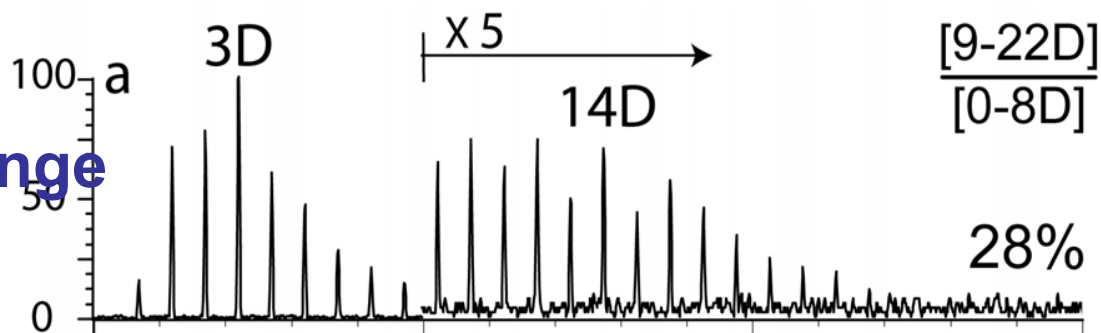
Oh, Lin, Hwang, Zhai, Breuker, Zabrouskov, Carpenter, McLafferty, *JACS*, 2005, 127, 4076-4083



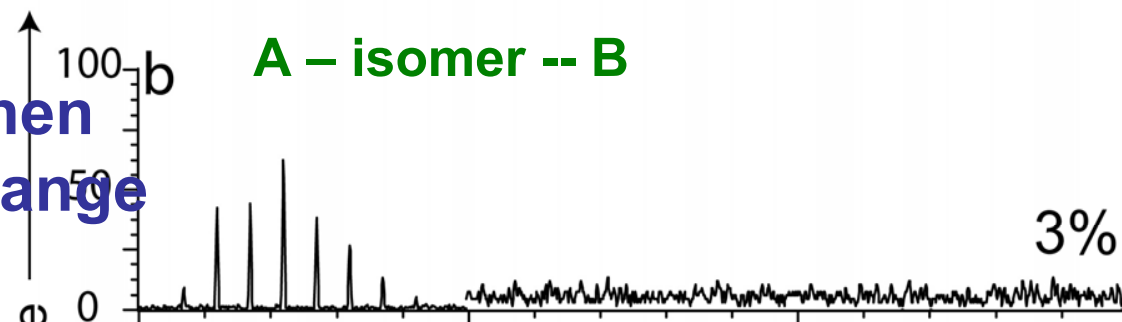
Absorption at 3675 cm⁻¹:



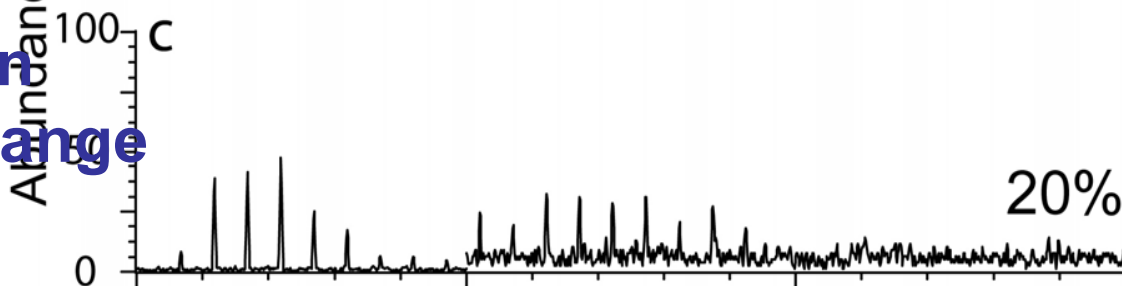
H/D exchange



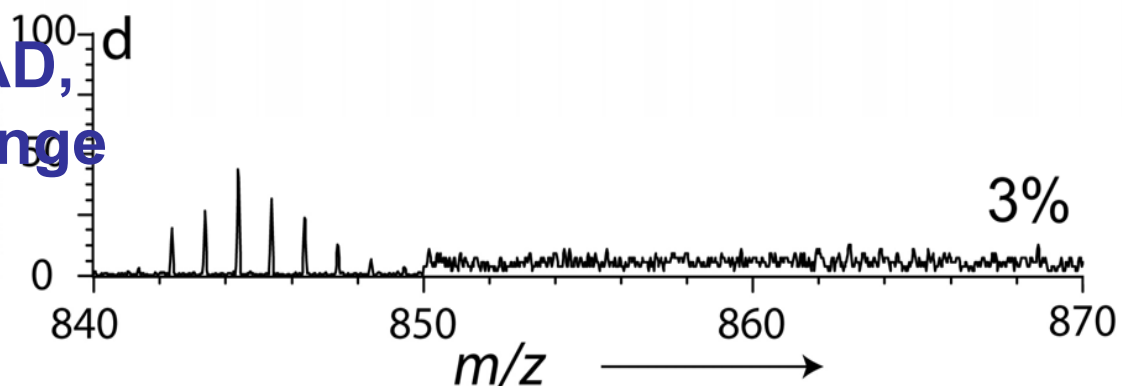
SWIFT, then
H/D exchange



CAD, then
H/D exchange

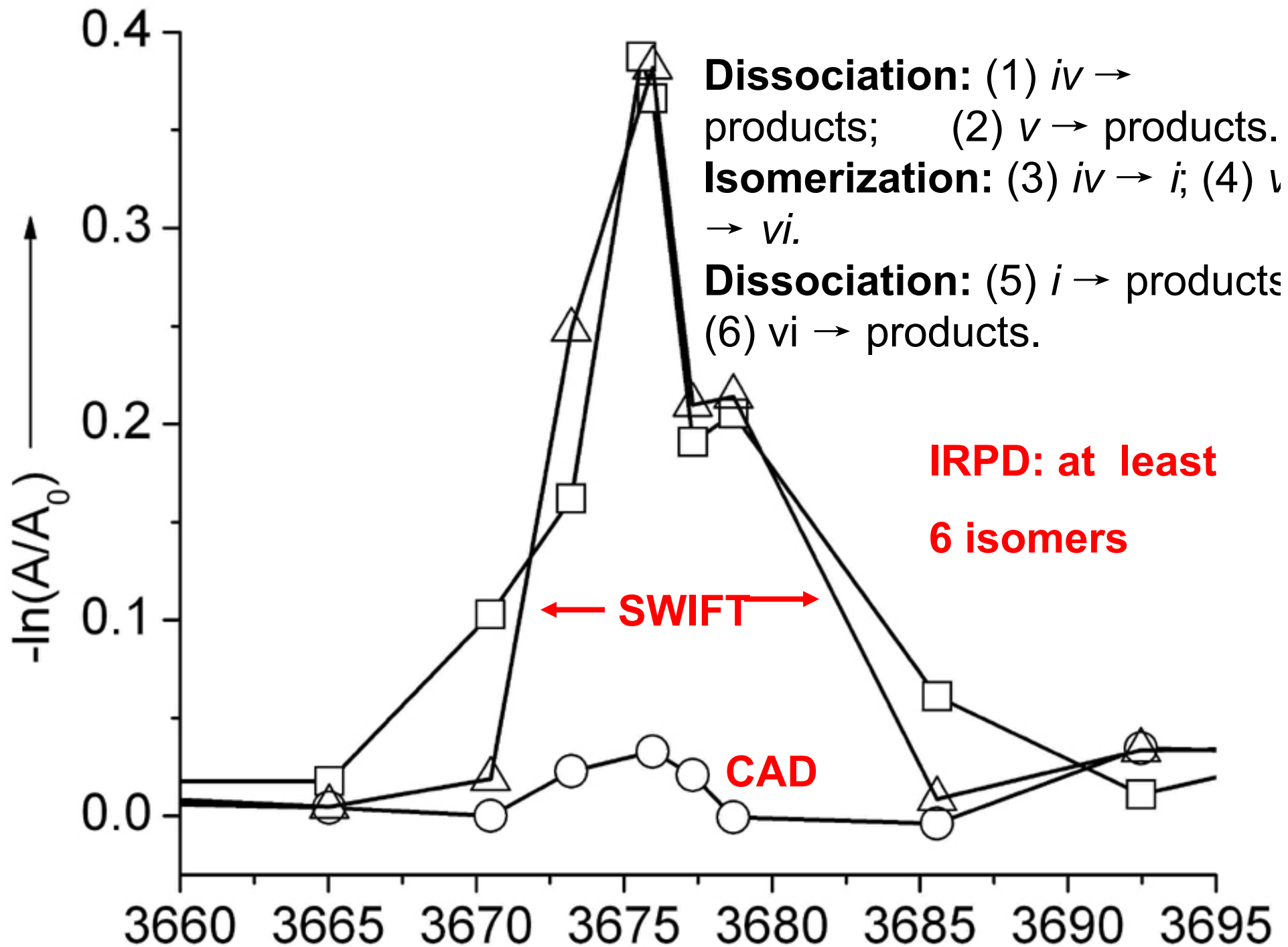


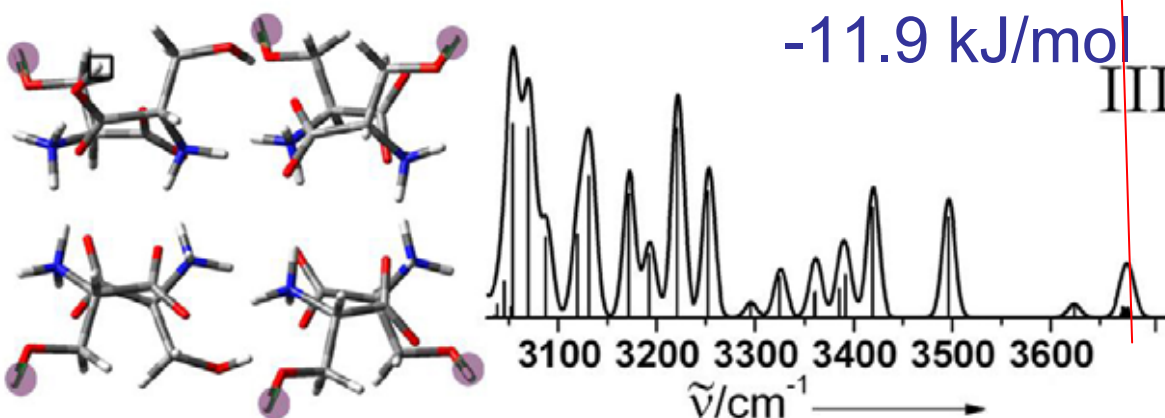
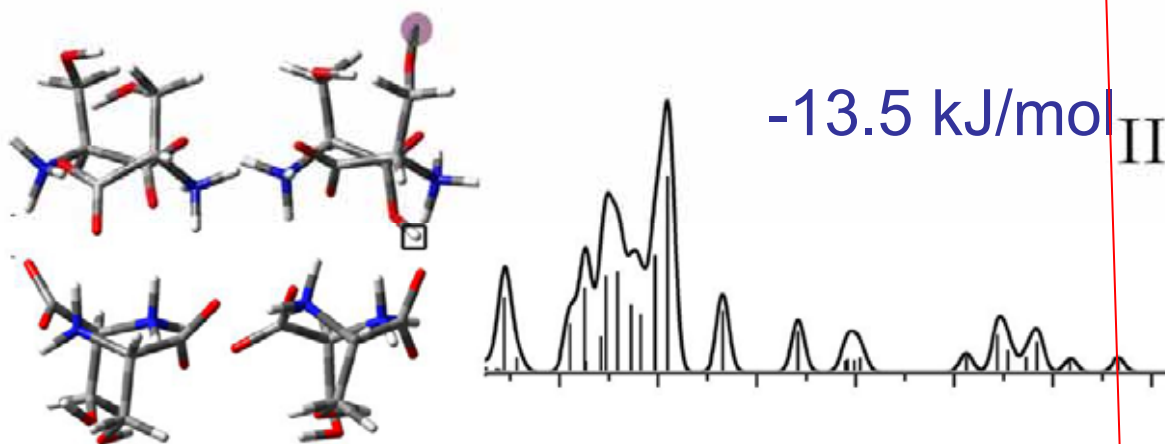
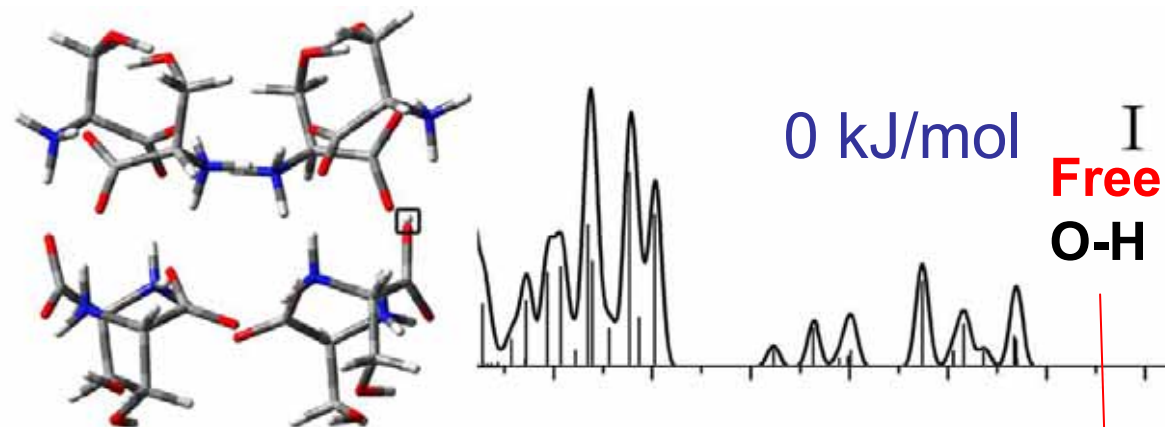
SWIFT, CAD,
H/D exchange



Mazurek, Geller,
Lifshitz, McFarland,
Marshall, Reuben,
J. Phys. Chem. A
2005, 109, 2107.

Isomer B
chirally
inactive



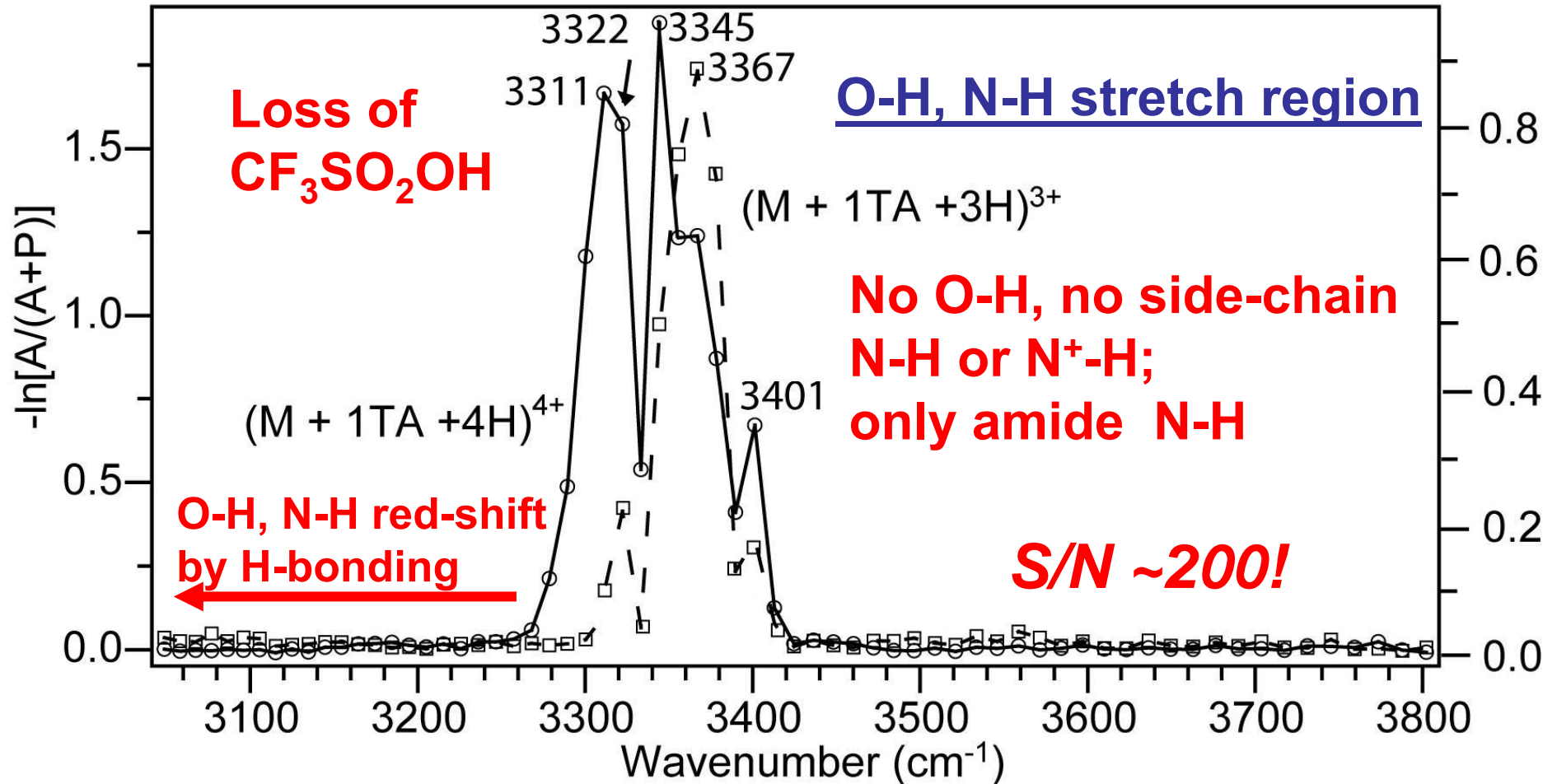


CAD of II yields I

Xianglei Kong, Cheng Lin,
Giuseppe Infusini, Han-
Bin Oh, Honghai Jiang,
Kathrin Breuker, Chih-Che
Wu, Oleg P. Charkin,
Huan-Cheng Chang, and
Fred W. McLafferty*,
ChemPhysChem, **2009**.

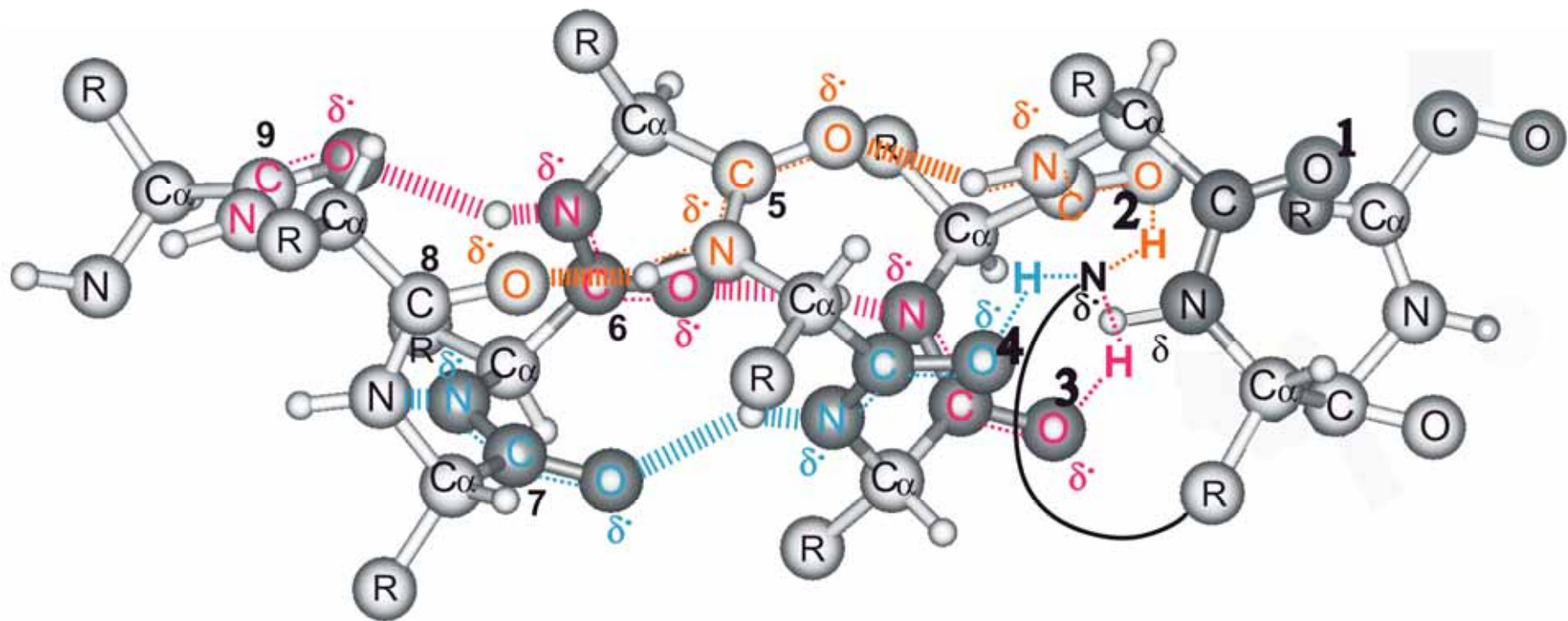
IRPD Spectra of Protein Ions

CF₃SO₂OH adduct of Mellitin, 2.7 kDa



**Similar spectra from 8.6, 12.3, and 13.7 kDa proteins -
-----AND MOST CHARGE STATES**

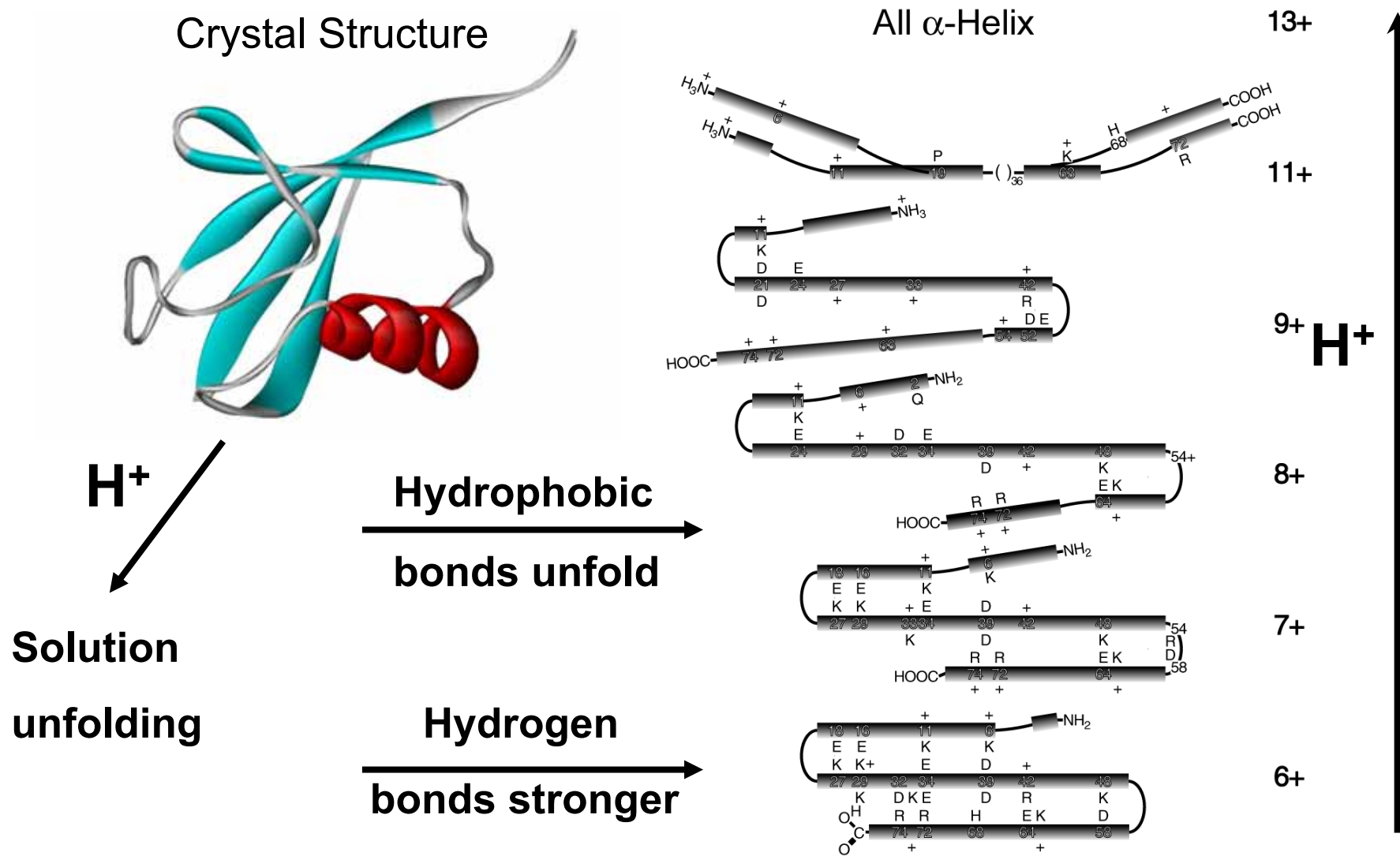
PROPOSED HELICAL STRUCTURE



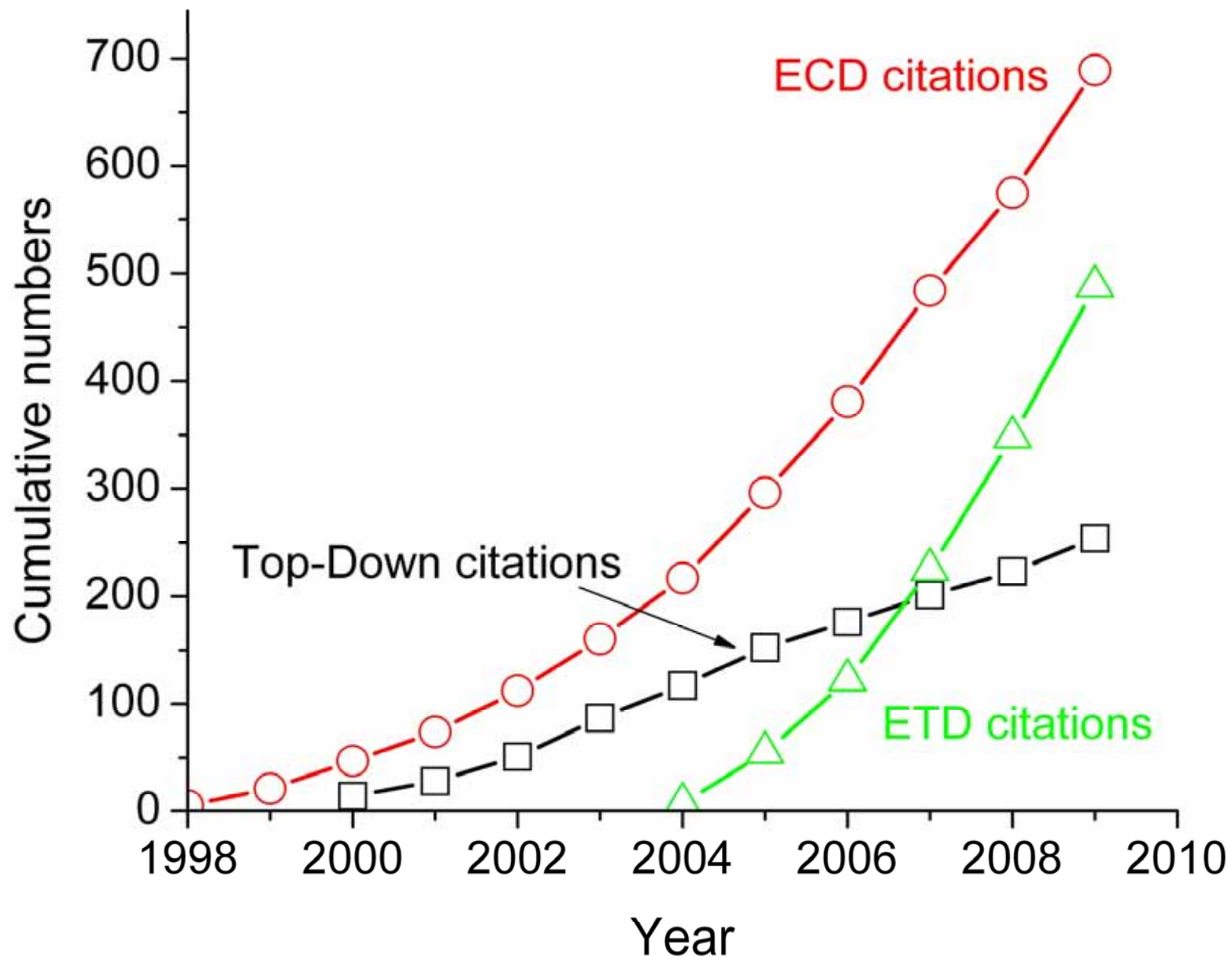
Breuker, K.; Oh, H. B.; Lin, C.; Carpenter, B. K.; McLafferty, F. W. *Proc. Natl. Acad. Sci. USA* 2004, 101, 14011-14016.

Tertiary Structures of Gaseous Ubiquitin Ions

K. Breuker, H.-B. Oh, D. M. Horn, B. A. Cerda, F. W. McLafferty *JACS* **2002**, *124*, 6407-6020.



Gaseous conformers, per ECD



What Does the Future Hold for Top Down Mass Spectrometry?

Benjamin A. Garcia^{a,b}

^a Department of Molecular Biology, Princeton University, Princeton, New Jersey, USA

^b Department of Chemistry, Princeton University, Princeton, New Jersey, USA

J Am Soc Mass Spectrom 2010, 21, 193–202

Conclusions

As all of these Top- or Middle Down methods continue through their growing pains similar to what small peptide MS went through several years ago, large molecule proteomics will evolve into techniques that will become more accessible to all types of scientists and will play pivotal roles in determining the biological structures of many proteins, protein complexes, including quantitatively characterizing PTMs and their influence on protein activity.

Acknowledgments

Kent Henry, Evan Williams, Bing Wang, Jorge Furlong, John Quinn, Joe Loo, Mike Senko, Steve Beu, Russ Chorush, Dan Little, Neil Kelleher, Paul Speir, Troy Wood, Peter O'Connor, Z. Guan, Gary Valaskovic, Dave Aaserud, Roman Zubarev, Dave Horn, Einar Fridriksson, Nate Kruger, Mark Lewis, Petia Shipkova, Blas Cerda, Ying Ge, Kathrin Breuker, HanBin Oh, Newman Sze, Huili Zhai, Mariam ElNaggar, Vlad Zabrouskov, Harold Hwang, Cheng Lin, Xuemei Han, Guiseppa Infusini, Honghai Jiang, Xianglei Kong, Mahmud Hossain, Sergio Castro

Tadhg Begley, Barbara Baird, Barry Carpenter, Harold Scheraga, Klaas van Wijk, Floyd Davis

**General Medical Institute,
National Institutes of Health**