



Probing Structural Changes Due to Oxidative Damage in Proteins

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Dr. Joshua Sharp

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- 2003: PhD in Genome Science and Technology at the University of Tennessee, Knoxville and Oak Ridge National Laboratory
- currently: postdoctoral fellow with Dr. Kenneth Tomer at the National Institute of Environmental Health Sciences, part of the National Institutes of Health





Outline

- Mass spectrometry
- Oxidative protein-footprinting approach
- Example: CaM





Mass spectrometry

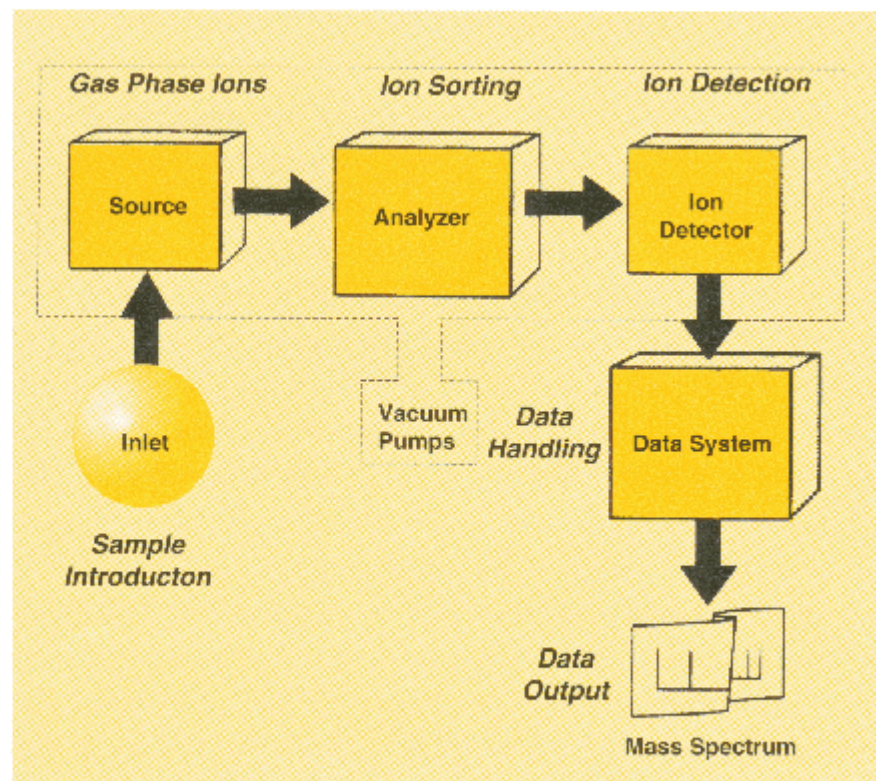
- Method for determining the mass/charge ratio of ions
- In practice used to determine the composition of a certain substance of interest



www.mc.vanderbilt.edu/msrc



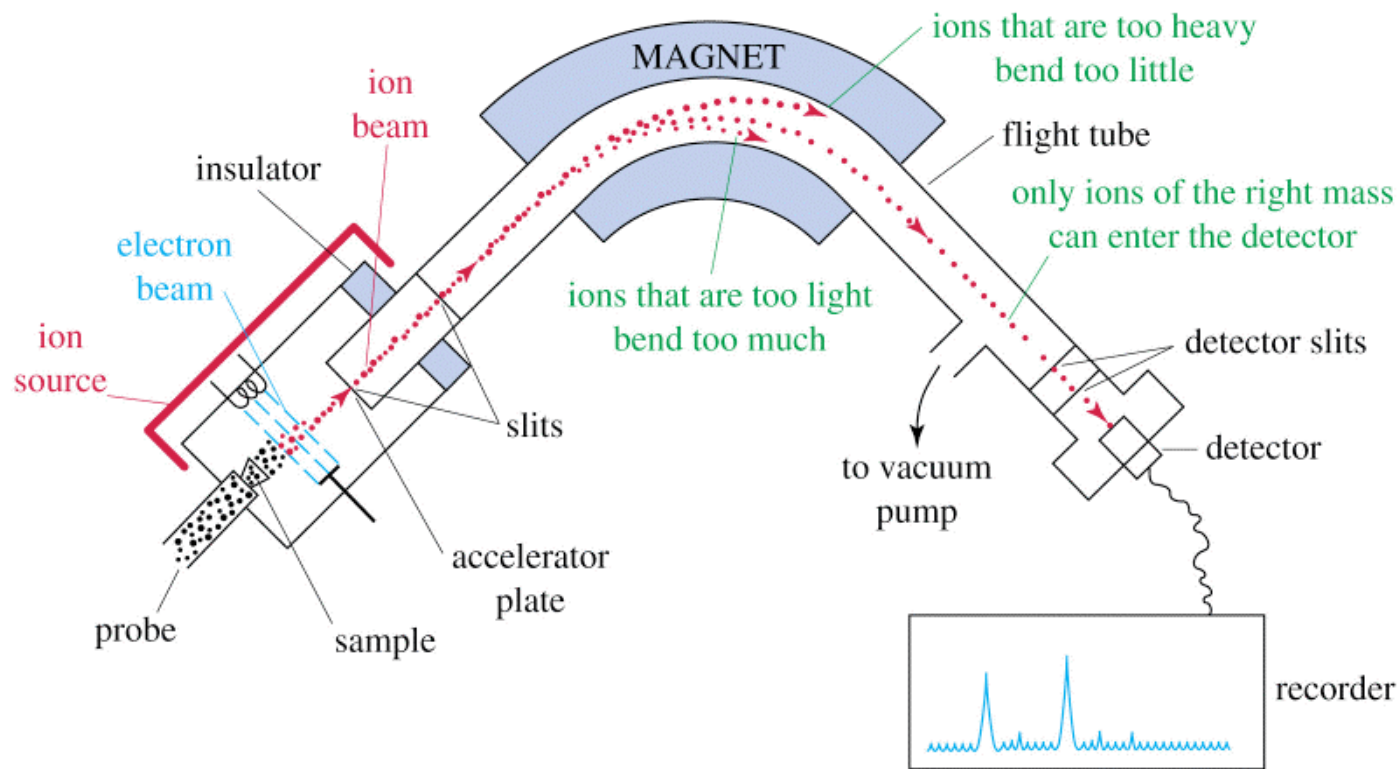
MS – basic principle



www.asms.org



MS - working principle



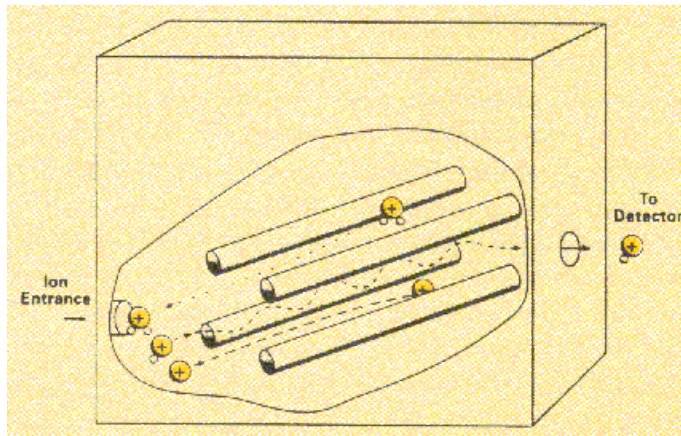
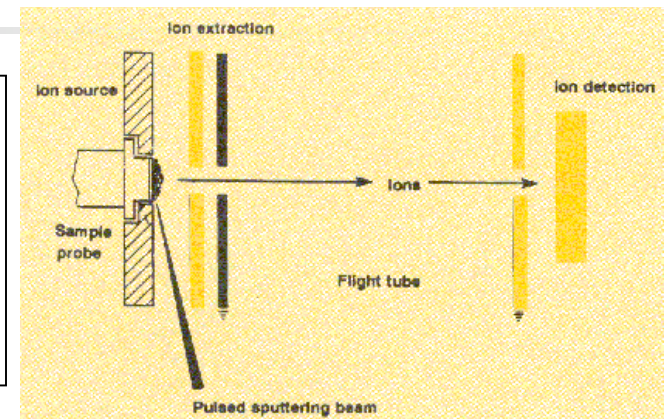
www.atmosphere.mpg.de



Different mass analyzers

Time-of-flight

- purely electric method
- ionization during very short interval
- acceleration in el. field depends on mass and charge
- measure time for way from source to detector

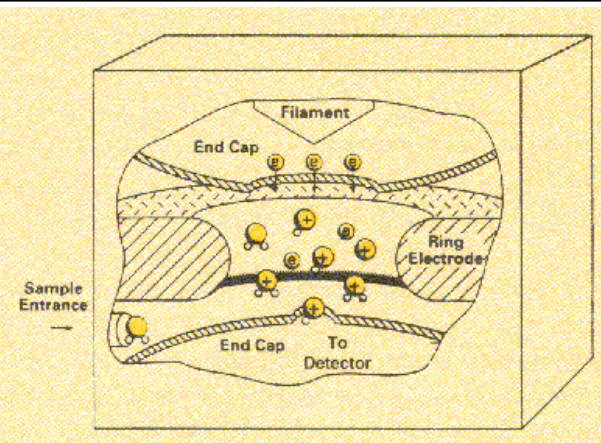


Quadrupole

- consists of 4 parallel rods
- apply oscillating electric fields
- depending on frequency and field strength only certain m/z value are transmitted

Ion trap

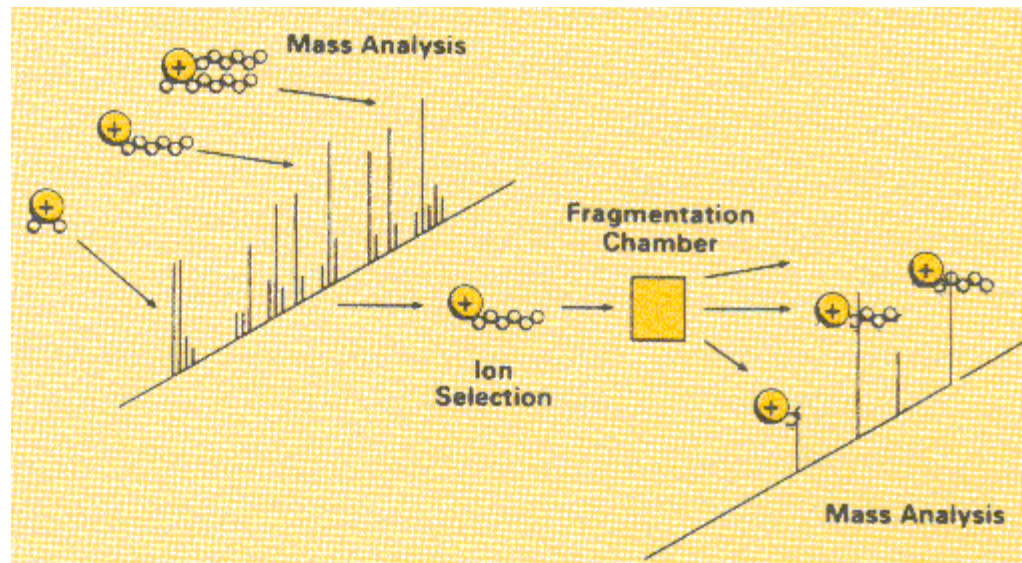
- mostly in conjunction with quadrupole
- not a filter, traps ions inside





MS/MS

- two stage MS process
- first step selects ions of certain m/z ratio
- these are then fragmented and the fragments are analyzed in a second stage of mass spectrometry



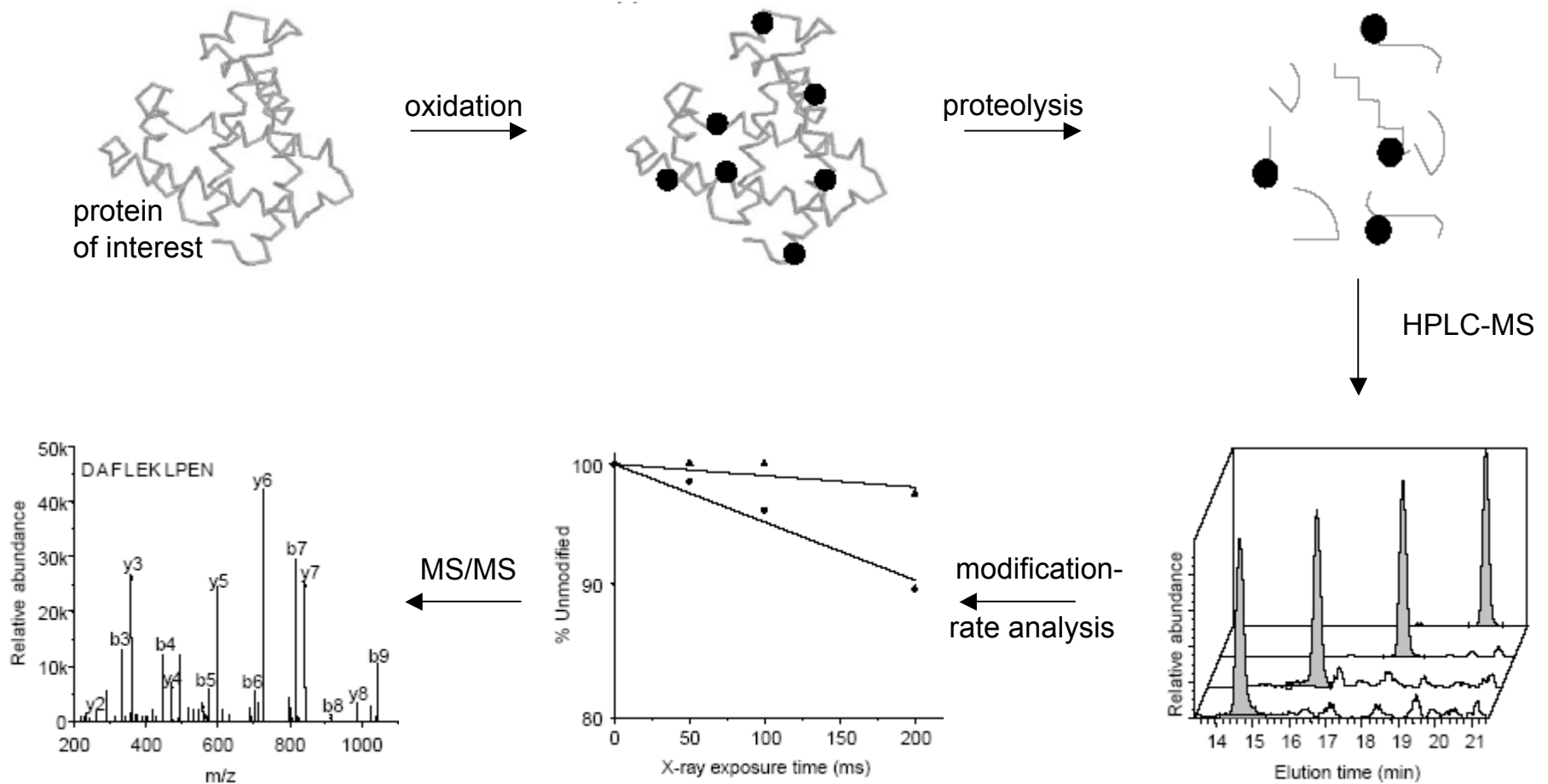


Oxidative protein-footprinting approaches

- Aim: understand composition and structure of proteins
- Alternative to crystallography and NMR
- Uses mass spectrometry

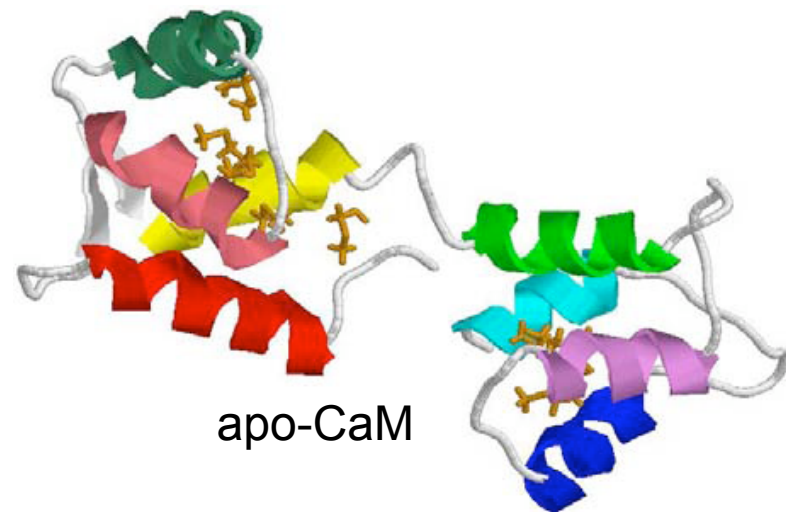
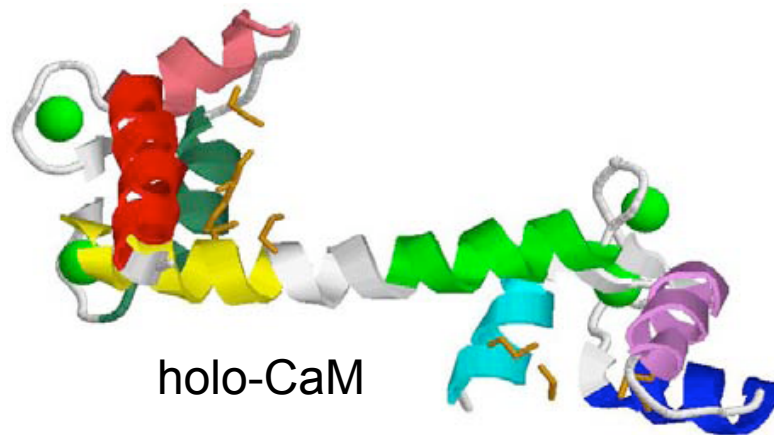


Oxidative protein-footprinting with MS



Calmodulin example

- Calmodulin (CaM) is important signaling protein in all eukaryotes
- is found in two conformations:
 - holo-CaM (with Ca^{2+} bound)
 - apo-CaM (without Ca^{2+} bound)



CaM is an oxidative stress sensor

- ROS in cell accumulate
- “oxidative stress”

external stress mechanisms

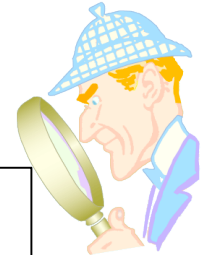
- Met144 and Met145 in CaM become oxidized
- CaM inhibits Ca²⁺-ATPase

- lower rate of metabolism
- lower rate of ROS production



- normal cellular metabolism
- normal CaM function

- cellular repair system repairs oxidative damage (including CaM)





oxidative surface-mapping of CaM

- biophysical characterization of CaM oxidation products
- not possible with crystallography or NMR
- constant, low concentration of hydroxyl radicals in solution
- oxidation of amino acid side chains depends on:
 - inherent reactivity of amino acid
 - accessibility of amino acid to the radical
- you can use this information to infer the conformational changes



the experiment

- monitor all 9 Met's and 3 non-Met residues in CaM (holo and apo)
- increase dose of radicals
- kinetics of oxidation are followed by mass spectrometry
- oxidation sites are determined by MS/MS (see spectrum next slide)
- see changes in the rate constant of oxidation after increased exposure → indicates conformational changes as a function of time





results

- holo-CaM
 - most sites rapidly become less exposed to hydroxyl radicals as the protein accumulates oxidative damage
 - this suggests a closing of the hydrophobic pockets in the N- and C-terminal lobes
- apo-CaM
 - many sites rapidly become more exposed until they resemble the solvent accessibility of holo-CaM in the native structure
 - then they rapidly become more buried, mimicking the conformational changes of holo-CaM



literature

- **Sharp, J.S.**, Sullivan, D.M., Cavanagh, J., and Tomer, K.B. (2006) Measurement of multi-site oxidation kinetics reveals an active site conformational change in Spo0F as a result of protein oxidation. *Biochemistry* 45, 6260-6266.
- **Sharp, J.S.** and Tomer, K.B. Analysis of the oxidative damage-induced conformational changes of apo- and holo-calmodulin by dose dependant protein oxidative surface mapping. *Biophys J* (In press).
- Guan, J.Q. and Chance, M.R. (2005) Structural proteomics of macromolecular assemblies using oxidative footprinting and mass spectrometry. *Trends Biochem Sci* 30, 583-592.
- Maleknia, S.D., Wong, J.W., and Downard, K.M. (2004) Photochemical and electrophysical production of radicals on millisecond timescales to probe the structure, dynamics and interactions of proteins. *Photochem Photobiol Sci* 3, 741-748
- **Sharp, J.S.**, Guo, J., Uchiki, T., Xu, Y., Dealwis, C., and Hettich, R.L (2005). Photochemical surface mapping of C14S-Sml1p for constrained computational modeling of protein structure. *Analytical Biochemistry* 340, 201-212.
- **Sharp, J.S.**, Becker, J.M., and Hettich, R.L. (2004) Analysis of protein solvent accessible surfaces by photochemical oxidation and mass spectrometry. *Analytical Chemistry* 76, 672-683.
- **Sharp, J.S.**, Becker, J.M., and Hettich, R.L. (2002) Protein surface mapping by chemical oxidation: structural analysis by mass spectrometry. *Analytical Biochemistry* 313, 216-225.



Thank you!



Happy Holidays!

