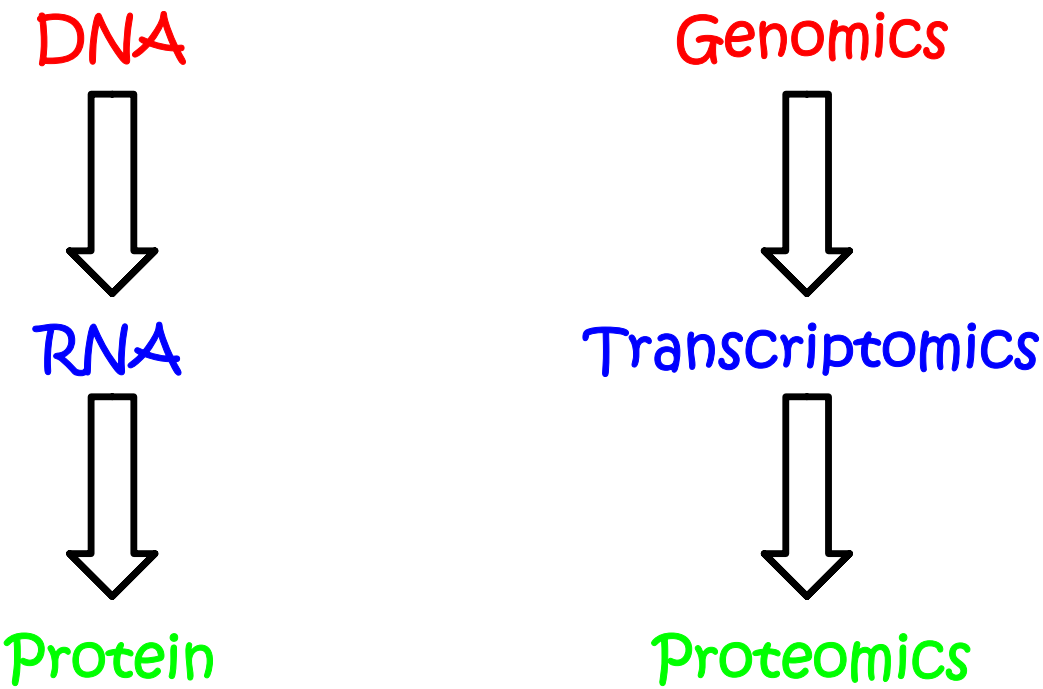
Chemical Approaches for Quantitative and Functional Proteomics

Carmen F. Drahl

Organic Supergroup Meeting

5 May 2004

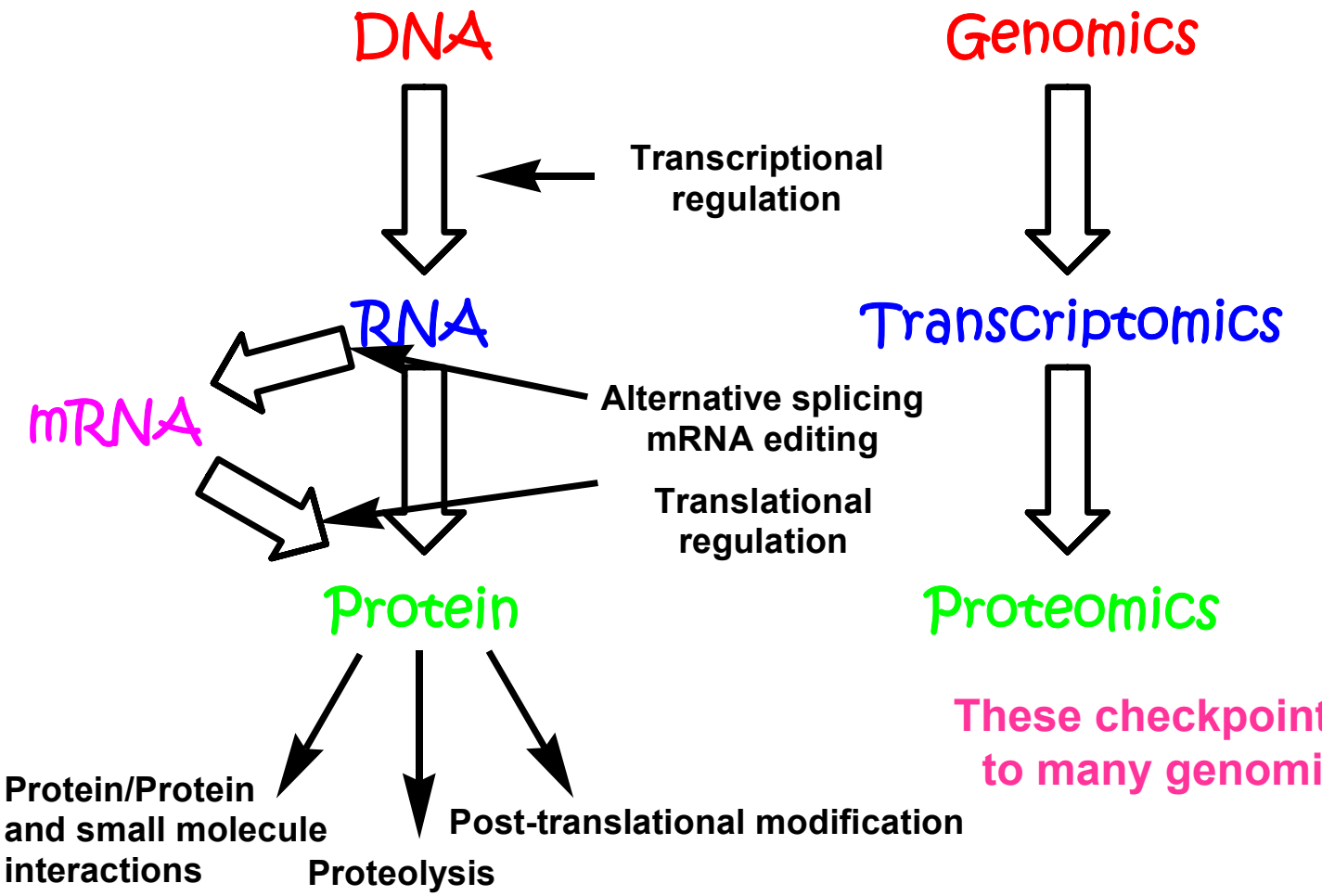
“Central Dogma” Revisited



Francis Crick, 1958

Mass Spec. Rev. **20**, 1-57.

“Central Dogma” Revisited



These checkpoints are INVISIBLE to many genomics techniques!

Microb. Mol. Biol. Rev. **66**, 39-63.

Why Not Genomics?

- Science and medicine need to derive *meaning* from extensive genome data.
- One gene ≠ one protein
- Genome = static, Proteome = dynamic
- At the molecular level, function is most closely associated with the biochemical activities of proteins.

Function → Protein Activity

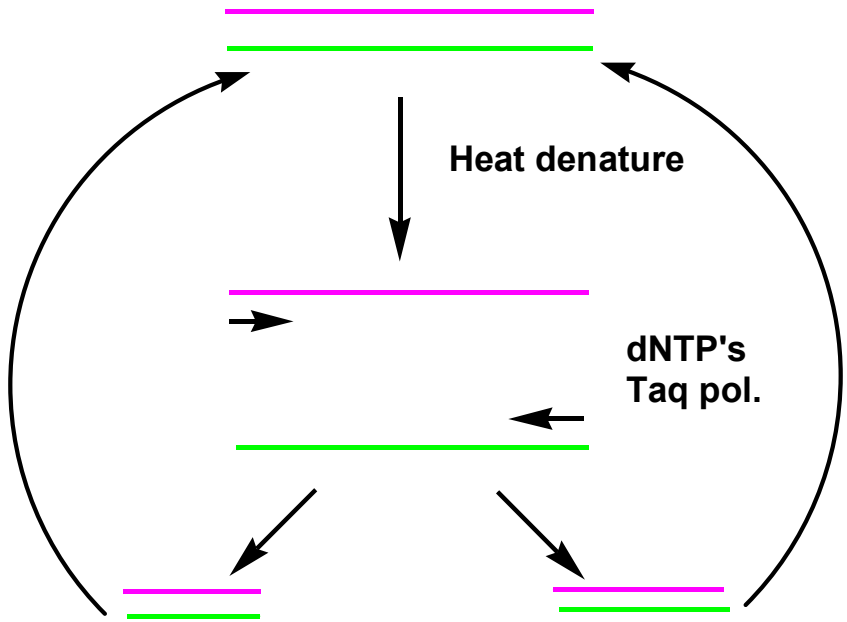
- Most drugs target proteins, yet disease profiling is at present dominated by DNA microarrays.

Drug Name	*Protein Target*	*2003 Global Sales ($bil)*
Lipitor	HMG-CoA reductase	$10.3
Viagra	Phosphodiesterase-5	$1.7
Zyprexa	Serotonin and dopamine receptors	$4.3

Comb. Chem. and H.T.S. **7**, 1-9.
http://www.forbes.com

Difficulties Inherent in Proteomics

- Amplification: No PCR equivalent exists for protein.
- A paradigm shift from identifying individual players to characterizing interacting networks and fluxes must occur in biology.



PCR reaction cycle

Proteomics Technologies Must..

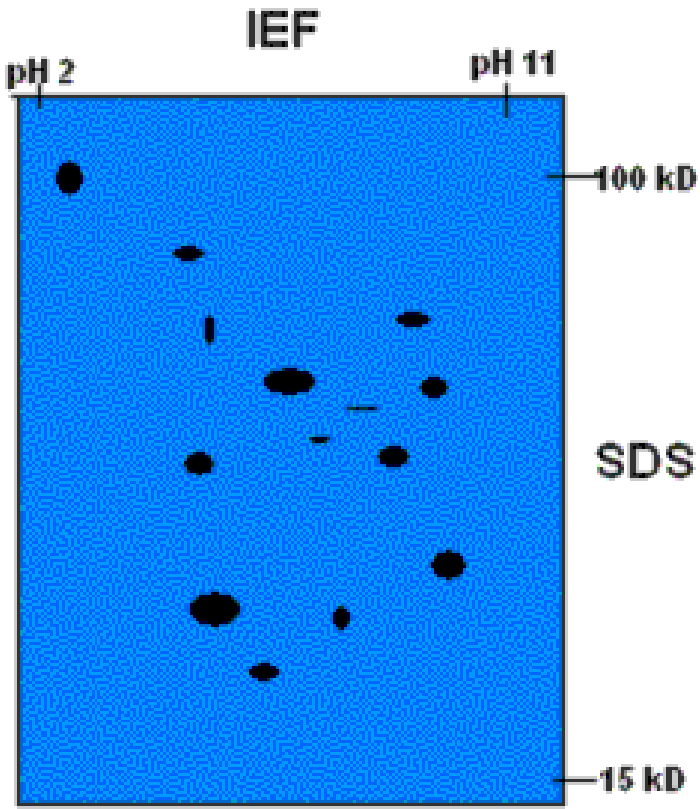
- Display/ Separate/ Isolate
- Identify

and

- Quantify

large numbers of proteins
from complex mixtures.

Two Dimensional Electrophoresis



- First developed in 1975
- Detection by staining
  - Coomassie blue
  - Silver
  - Sypro
- Mature proteomics technology together with mass spec.

J. Biol. Chem. **250**, 4007-4021.
http://www.aber.ac.uk/parasitology/Proteome/Tut_2D.html

Applications for 2DE-MS

- Total Protein
  - Global identification of “all” proteins in a sample
    - Full proteome
    - Fractionated tissue
    - Can detect post-translational modifications

- Differential Expression
  - Compare 2 or more samples and find differences in their protein expression
    - Overlaid on same gel (DIGE)
    - Compare two or more gels

Anal. Chem. **75**, 2891-2905.
Mass. Spec. Rev. **20**, 1-57.
Drug Disc. Today **4**, 55-62.

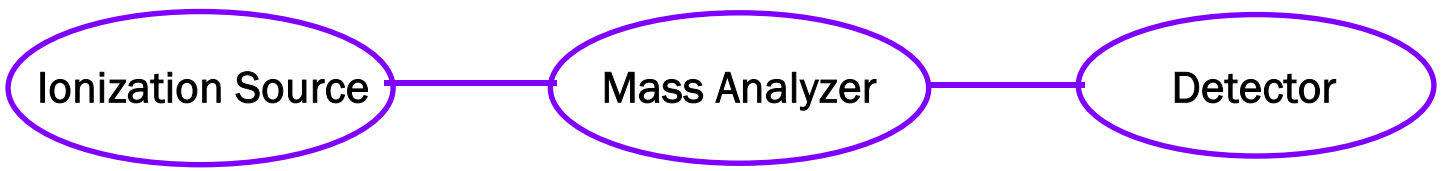
Address 2DE Technical Concerns

- Dynamic range of detection method
  - Solution? multi-photon detection
- Overlapping proteins on gel
- Reproducibility
  - Solution? Immobilized pH gradients
- Detection limits of gel for extremes of pI and MW, low abundance proteins

Mass Spec. Rev. **20**, 1-57.

Mass Spectrometry

- MS allows rapid, sensitive (1 pM or below) identification of protein fragments in high complexity samples.
- MS may be used in tandem with separation techniques.
  - 2DE
  - LC
- The 2002 Nobel acknowledged the influence that improvements in protein MS have had on the proteomics field.



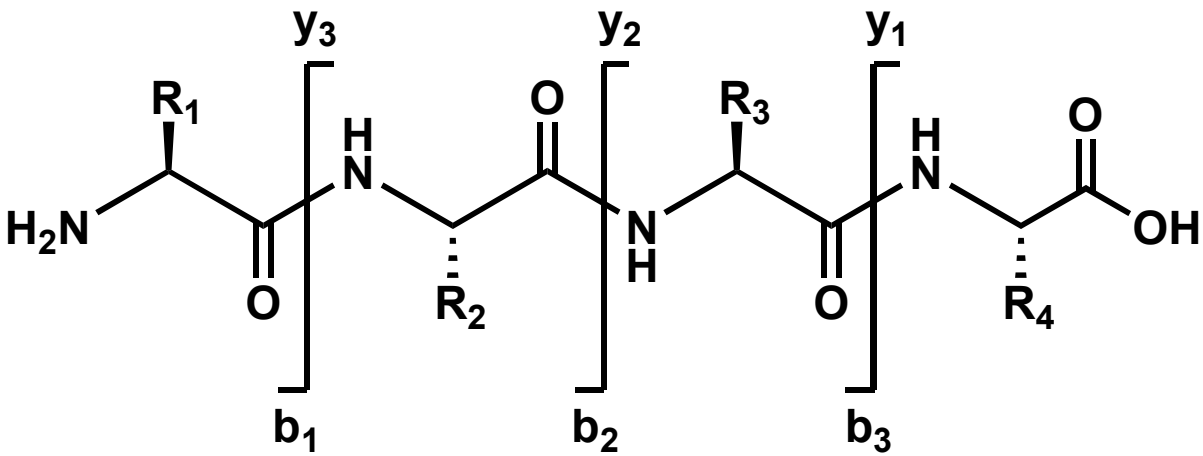
MS Ionization for Proteomics

- Soft Ionizations
- Electrospray Ionization (ESI)
- Matrix-assisted laser desorption/ionization (MALDI)

Nature **422**, 198-207.

Mass Spectrometry

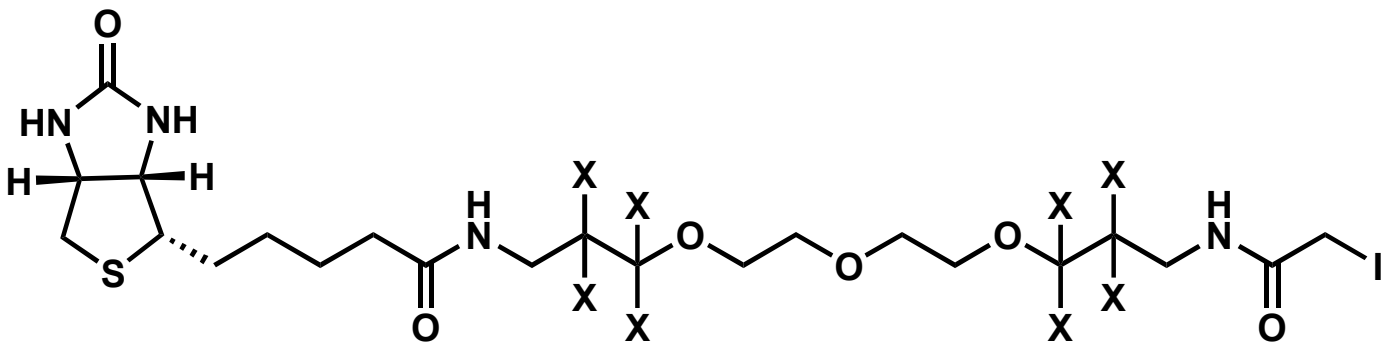
- MS Analysis of Proteins
  - Peptide mass analysis/ mass fingerprinting
  - Amino acid sequencing



PNAS **83**, 6233-6237.

Quantitative Proteomics

- Isotope-coded Affinity Tag (ICAT)
- Stable isotopes as internal standards
- Gel-free
- 1 experiment → ID and *relative* abundance



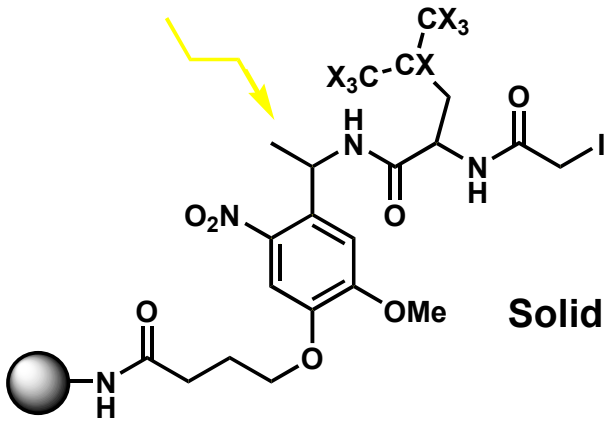
Biotin affinity group | **Isotopically labeled linker X = H or X = D** | **Cys-specific reactive group**

Nat. Biotech. **17**, 994-999.

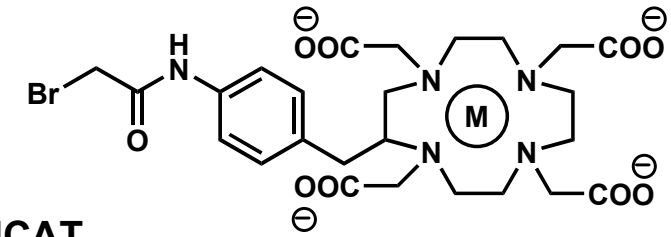
ICAT Applications

- Powerful method for global analysis of protein *abundance*
  - Different expression pattern following perturbation
    - *Nat. Biotech.* **19**, 946-951. (12-phorbol 13-myristate acid)
    - *Electrophoresis* **23**, 1591-8. (camptothecin)
- Systematic strategy for identifying candidate drug targets
  - ID proteins with binding selectivity for a drug candidate over a structurally related analog
    - *Anal. Chem.* **75**, 2159-2165

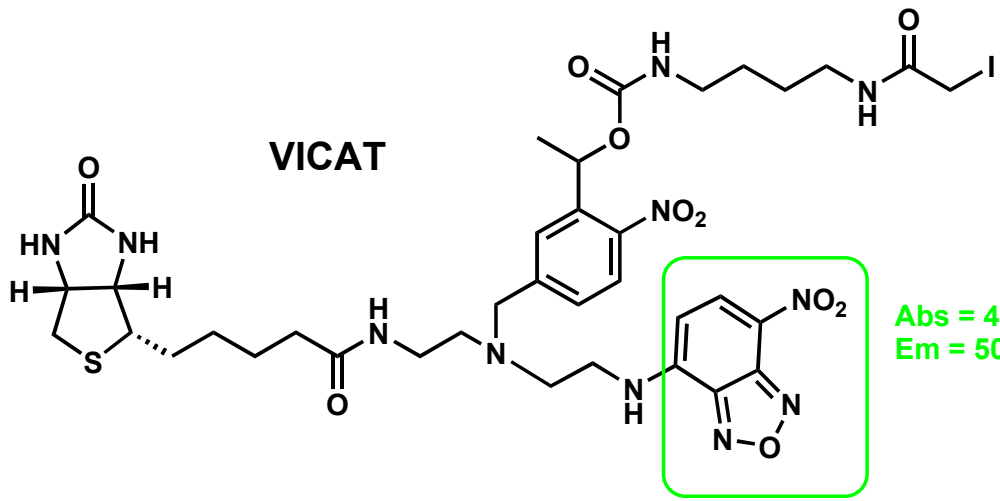
ICAT Variants



Solid Phase ICAT



ECAT



VICAT

Abs = 493 nm
Em = 503 nm

Nat. Biotech. **20**, 512-515.
Bioconj. Chem. **15**, 3-6.
Bioconj. Chem. **15**, 380-388.

Disadvantages of ICAT

- Not every protein contains Cys
- Incomplete reactivity of tag with proteomes
- Complex mass spec. arising from fragmentation of bulky tag, multiple Cys's, partial incorporation
- Protein *level* does not necessarily correlate with *function*

Rapid Commun. Mass Spec. **17**, 2380-2386.

Post-Translational Modifications

- Selected types
  - Phosphorylation
  - Acetylation
  - Nitration
  - Glycosylation
  - Myristoylation

- Active site directed protein regulation
  - Intrasteric
  - Complementary to allosteric

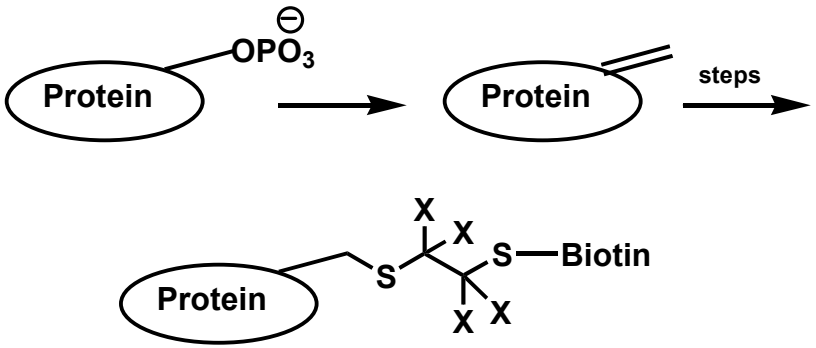
Nature **402**, 373-376.

Phosphoproteomics Strategies

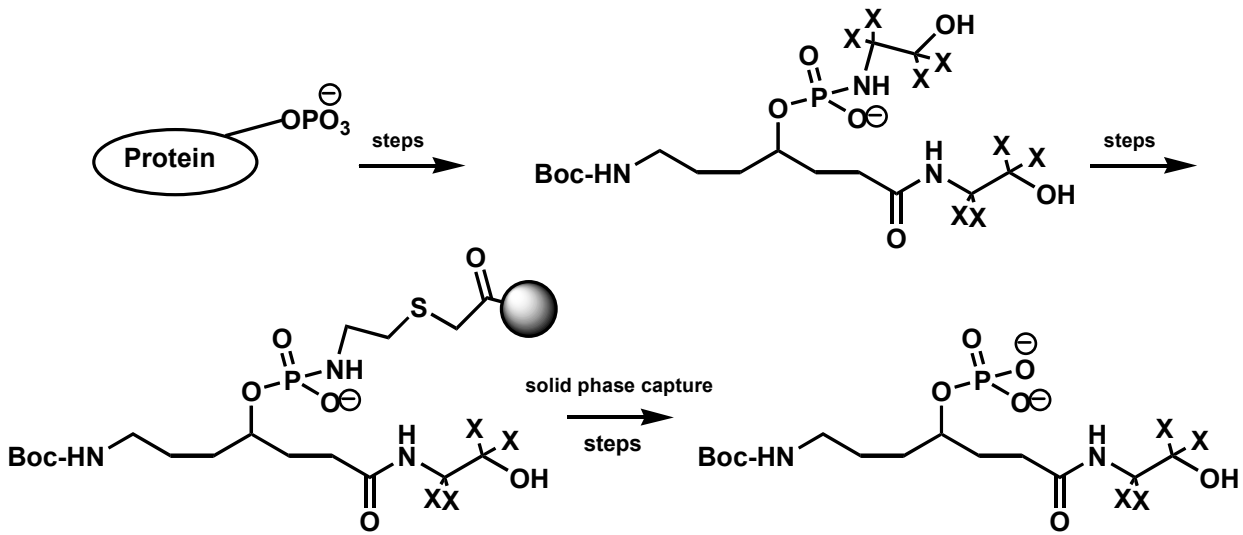
- Metabolic ^{32}P radiolabeling
- Phospho-specific antibodies
- Immobilized metal affinity column

Mol. Cell. Proteom. **1**, 781-790.

Chemical Phosphoproteomics



- β-elimination method
- Phosphoramidate method



Mol. Cell. Proteom. **1**, 781-790.

Protein Microarrays

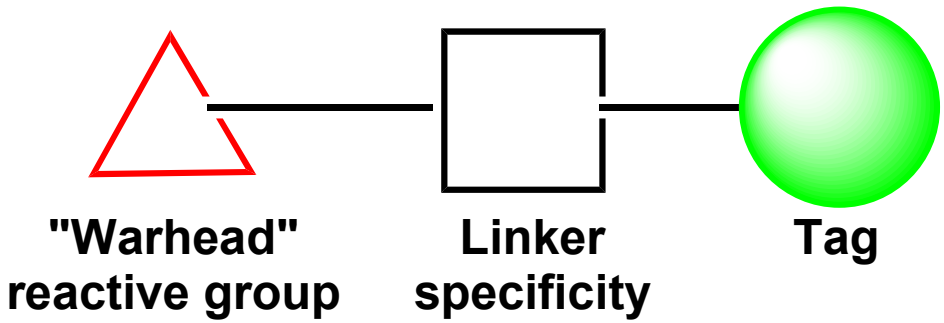
- Immobilized proteins on a solid support
  - The folded conformation of proteins is preserved.
- Examine interactions with tagged probes
  - Small molecules
  - Other proteins
- Develop molecular network profiles

Science **289**, 1760-1763.
Proteomics **3**, 2091-2100.

Activity Based Protein Profiling

- Abundance methods are indirect estimates of protein function.

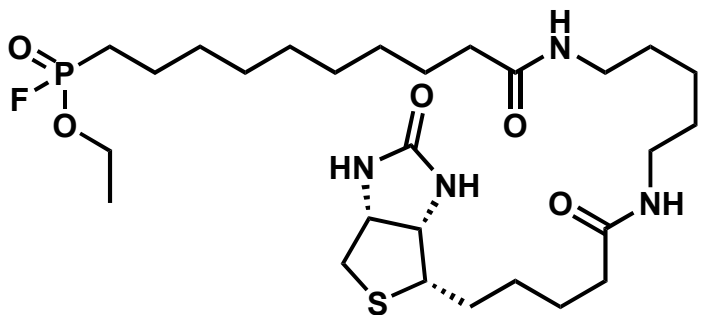
- ABP Probes report on the functional state of enzymes in complex proteomes.
  - React with specific class(es) of enzyme directly in complex proteomes
  - React in a manner correlating with catalytic activity
  - Possess a tag for rapid, sensitive detection and isolation of products



Curr. Opin. Chem. Biol. **4**, 663-668.
Mol. Cell Proteom. **1**, 781-790.

Activity-Based Protein Profiling

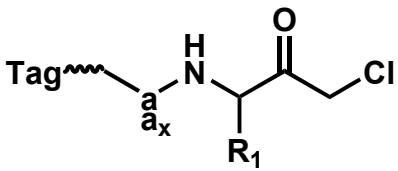
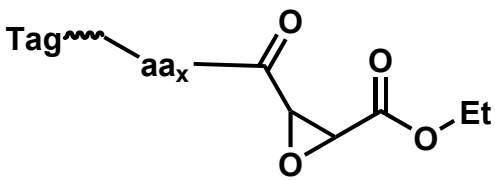
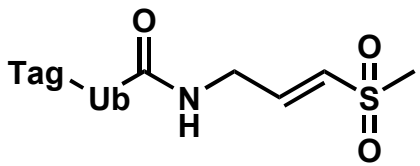
- Directed ABPP
  - Class specific irreversible inhibitors
- FP-Biotin
  - Readily labels *active* serine hydrolases
  - Labeled proteins ID'd by MALDI-MS/MS



Biochem. **40**, 4005
Mol. Cell Proteom. **1**, 781-790.

Activity-Based Protein Profiling

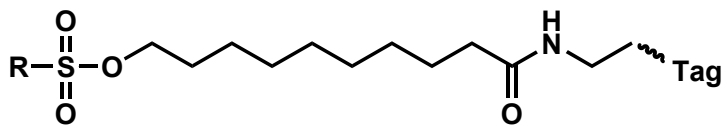
- Other classes of cognate affinity labels

	Serine protease
	Cysteine protease (papain)
	Cysteine protease (Ub hydrolase)

Curr. Opin. Chem. Biol. **7**, 296-303.

Combinatorial/ Nondirected ABPP Strategy

- Mild electrophile: sulfonate ester
- Labels enzymes from various mechanistic classes
  - Aldehyde dehydrogenases
  - Glutathione S-transferases
  - phosphofructokinase



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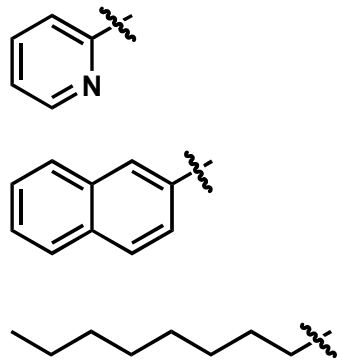
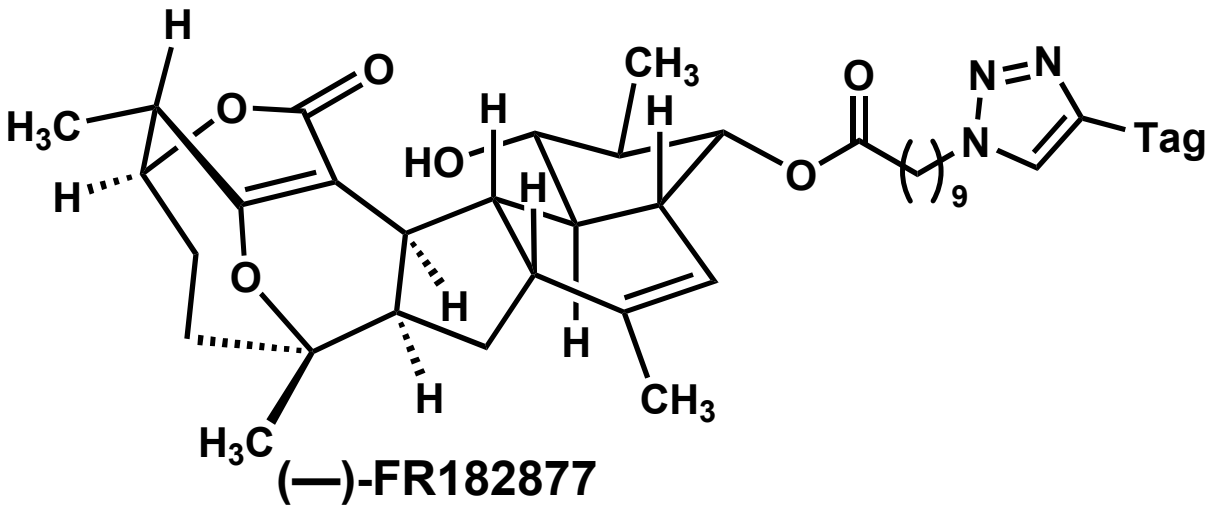


Chem. Biol. **8**, 81-95.

ABPP Applications

- The activity of a novel serine hydrolase correlates with ovarian cancer cell invasiveness.
- FR182877 is a potent and selective inhibitor of carboxylesterase-1



(—)-FR182877

PNAS **99**, 10335-10340
ACIEE **42**, 5480-5484.

Opportunities for Chemistry in the Post-Genome Era

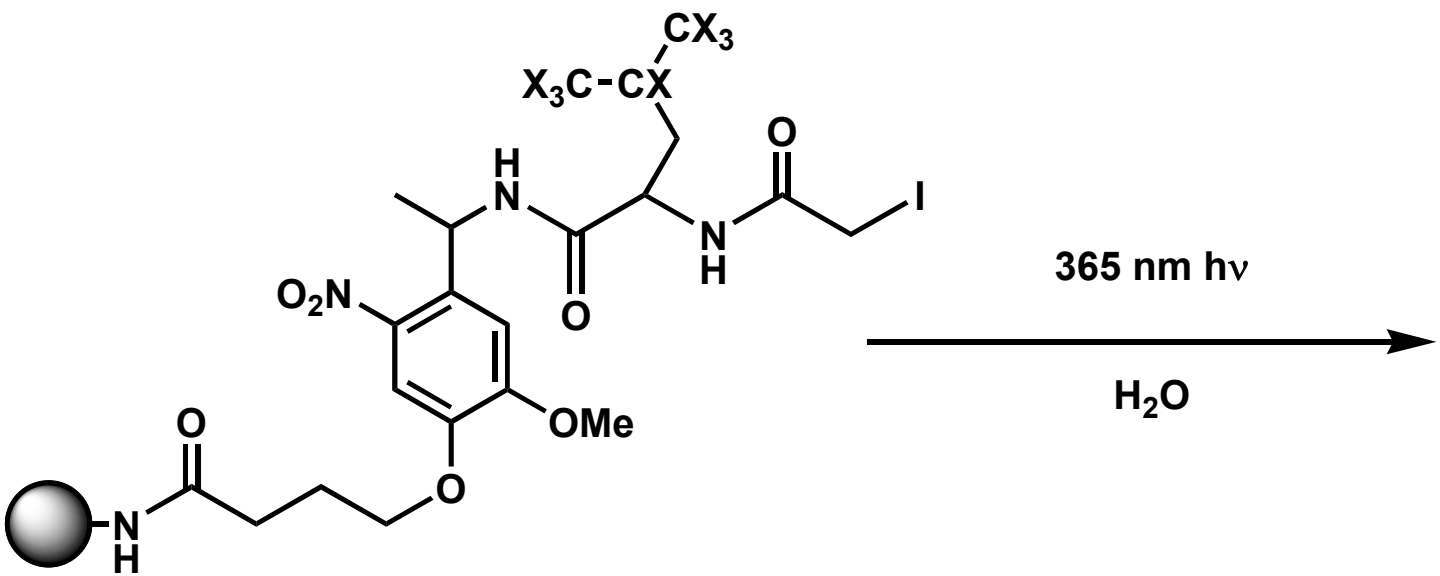
"The field of synthetic organic chemistry is uniquely suited to furnish proteomics initiatives with new concepts and tools that are complementary and, in many cases, superior to those in use by today's researchers."

Chemical methods will make significant contributions to research efforts aimed at recording variations in protein activity.

Curr. Opin. Chem. Biol. **4**, 663-668.

Problem 1

Provide a mechanism for and the products of the transformation below.



Nature Biotech. **19**, 512-515.
J. Chem. Technol. Biotechnol. **74**, 835-851.

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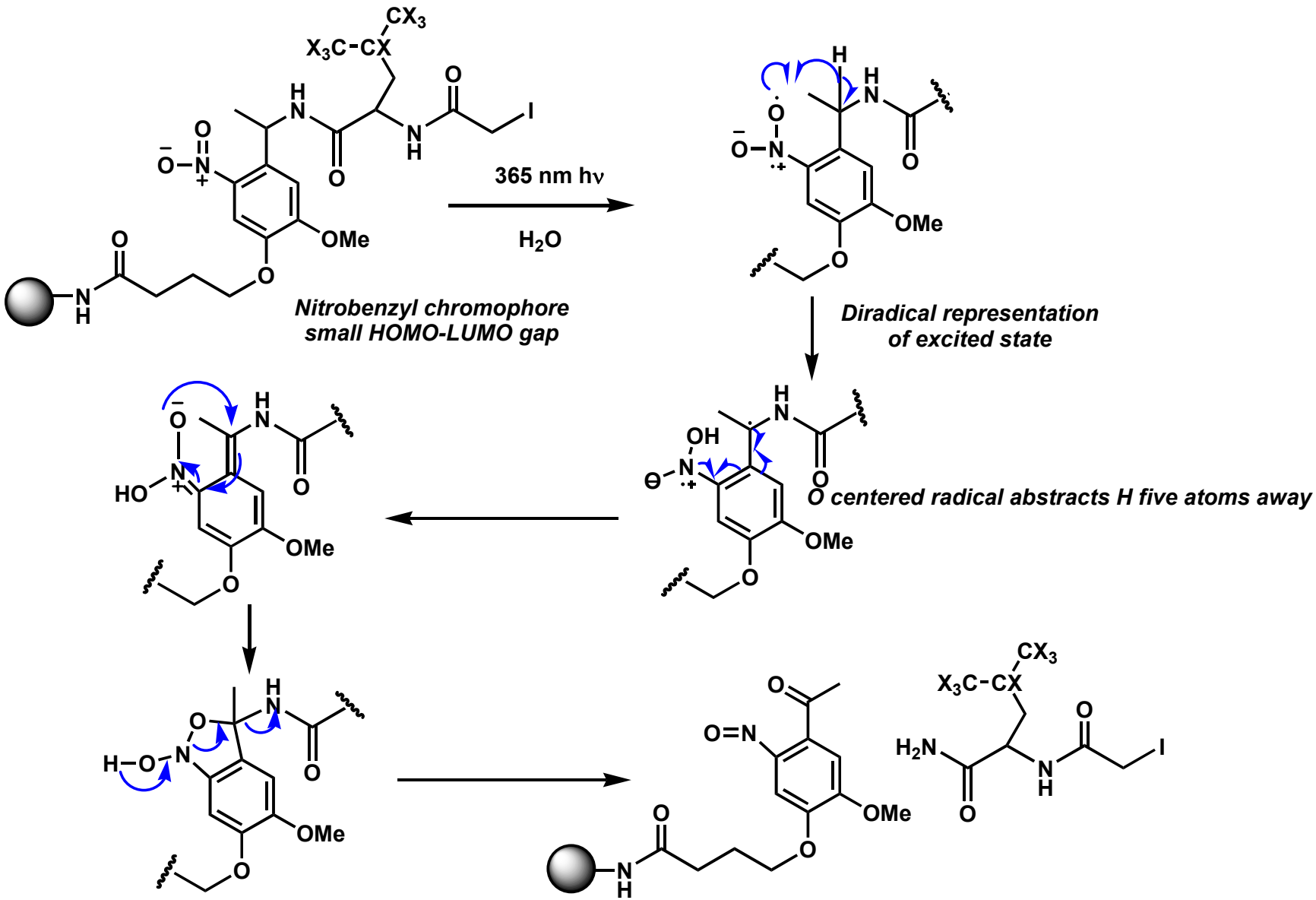


Nature Biotech. **19**, 512-515.
J. Chem. Technol. Biotechnol. **74**, 835-851.

Problem 2

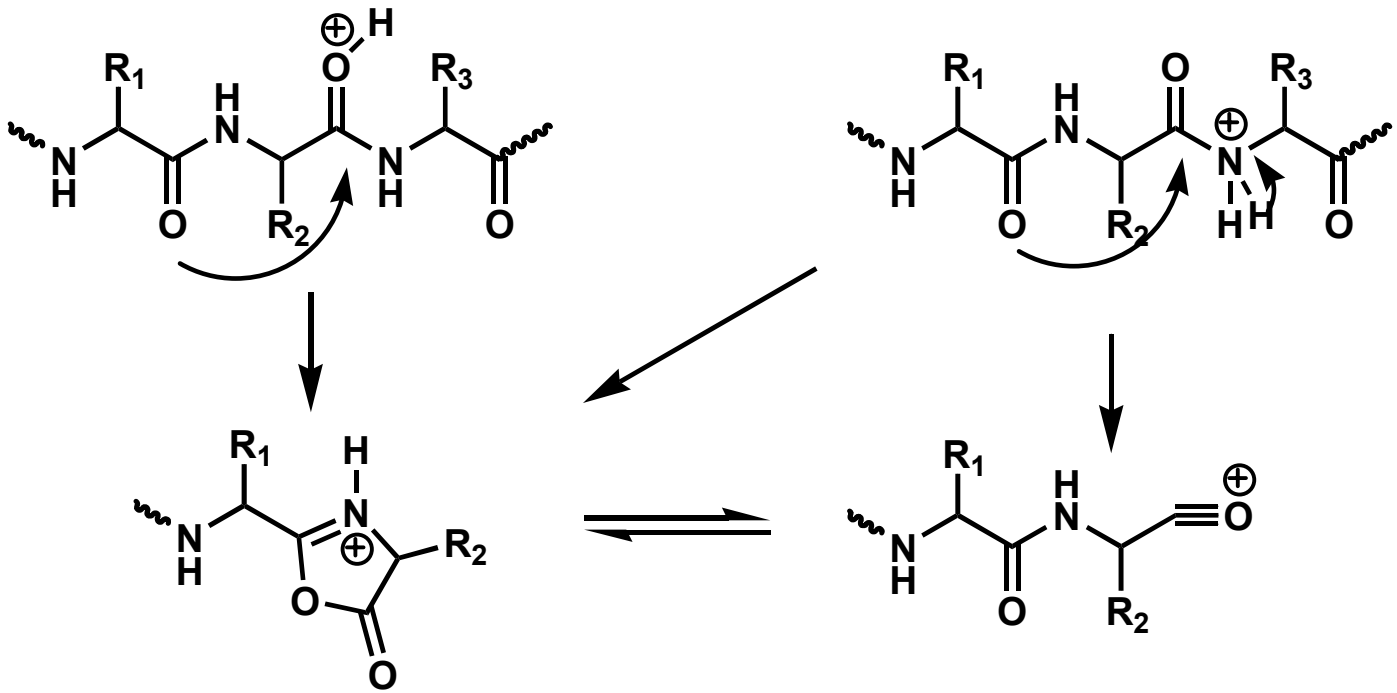
Cleavage at or near histidine is enhanced for peptides in MS. Can you propose a fragmentation mechanism?

J. Mass Spec. **35**, 1399-1406.

Problem 2 Hint

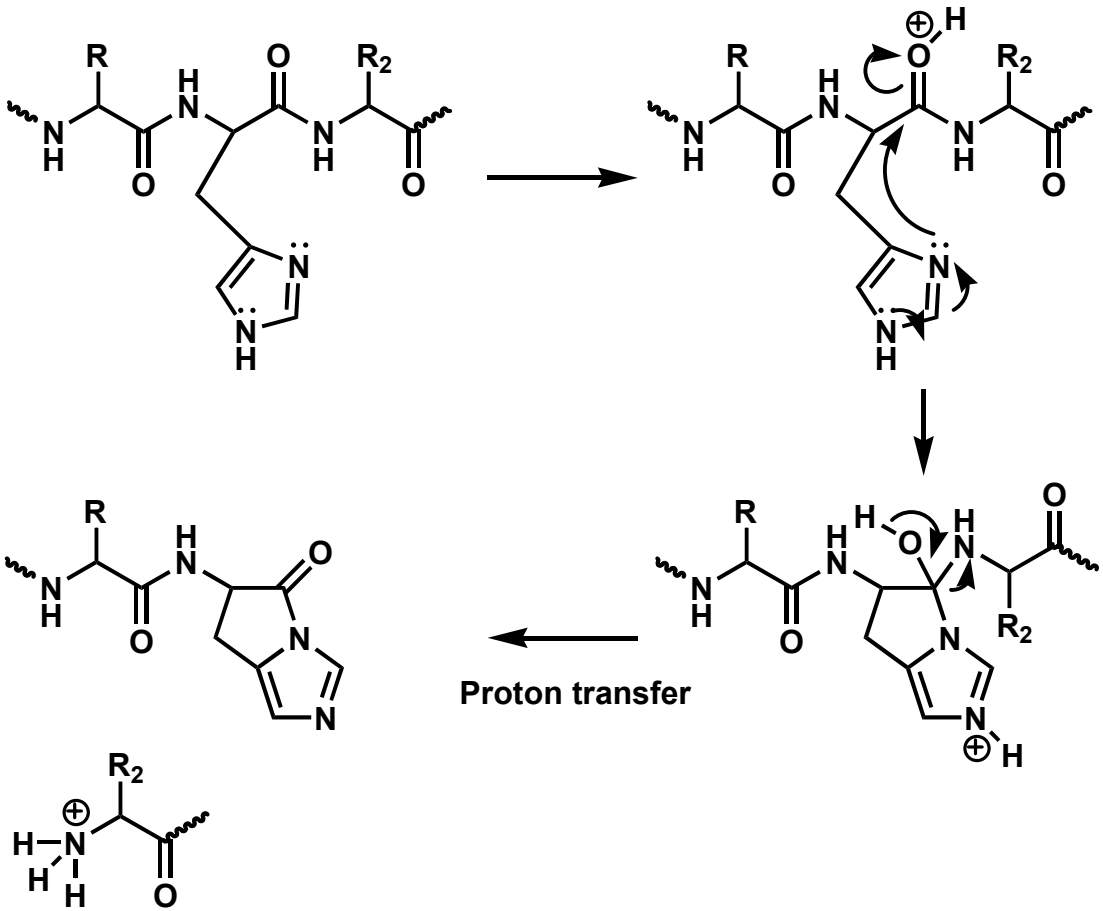
Cleavage at or near histidine is enhanced for peptides in MS. Can you propose a fragmentation mechanism?

Below: "Normal" MS peptide fragmentation



J. Mass Spec. **35**, 1399-1406.

Problem 2



J. Mass Spec. **35**, 1399-1406.