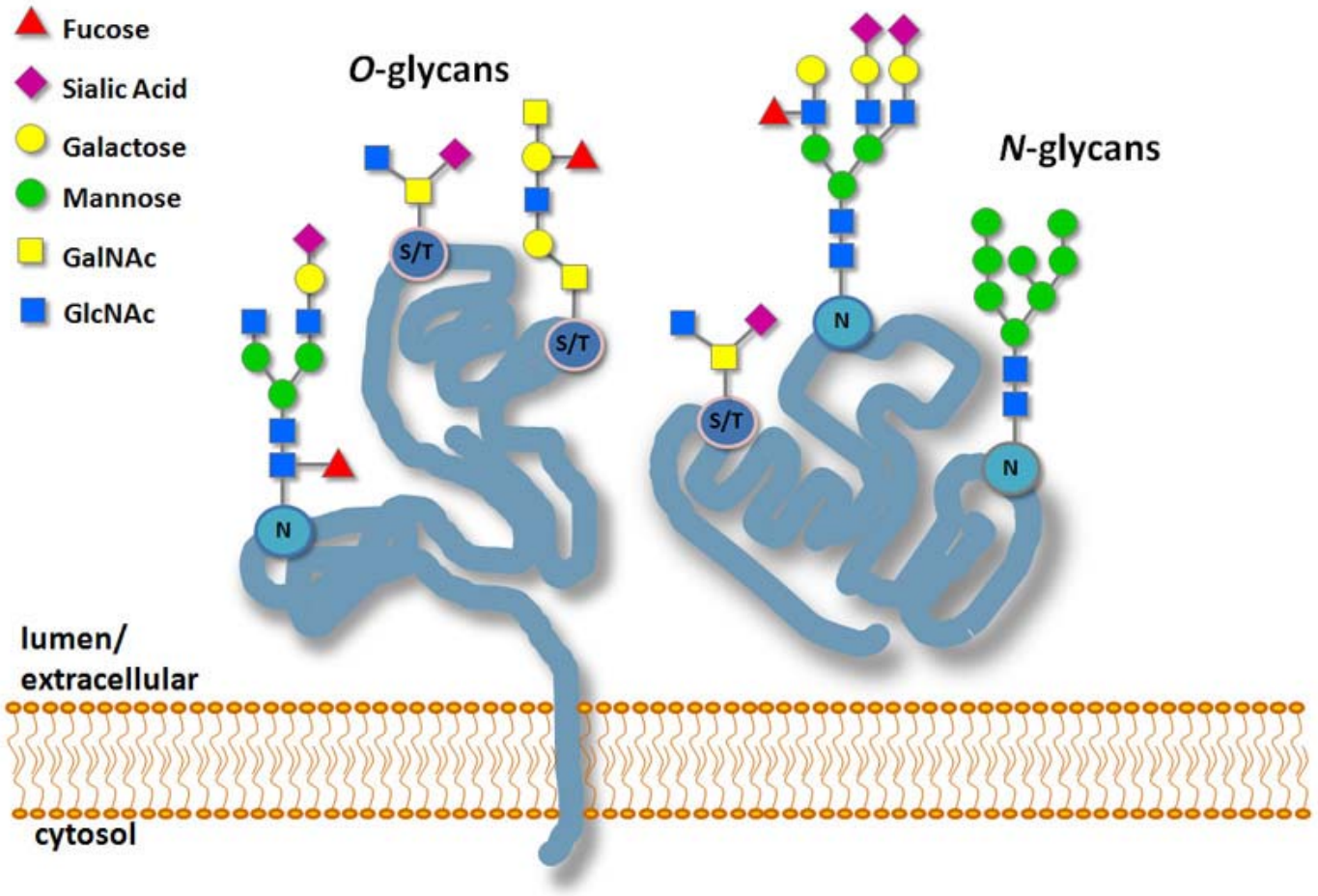
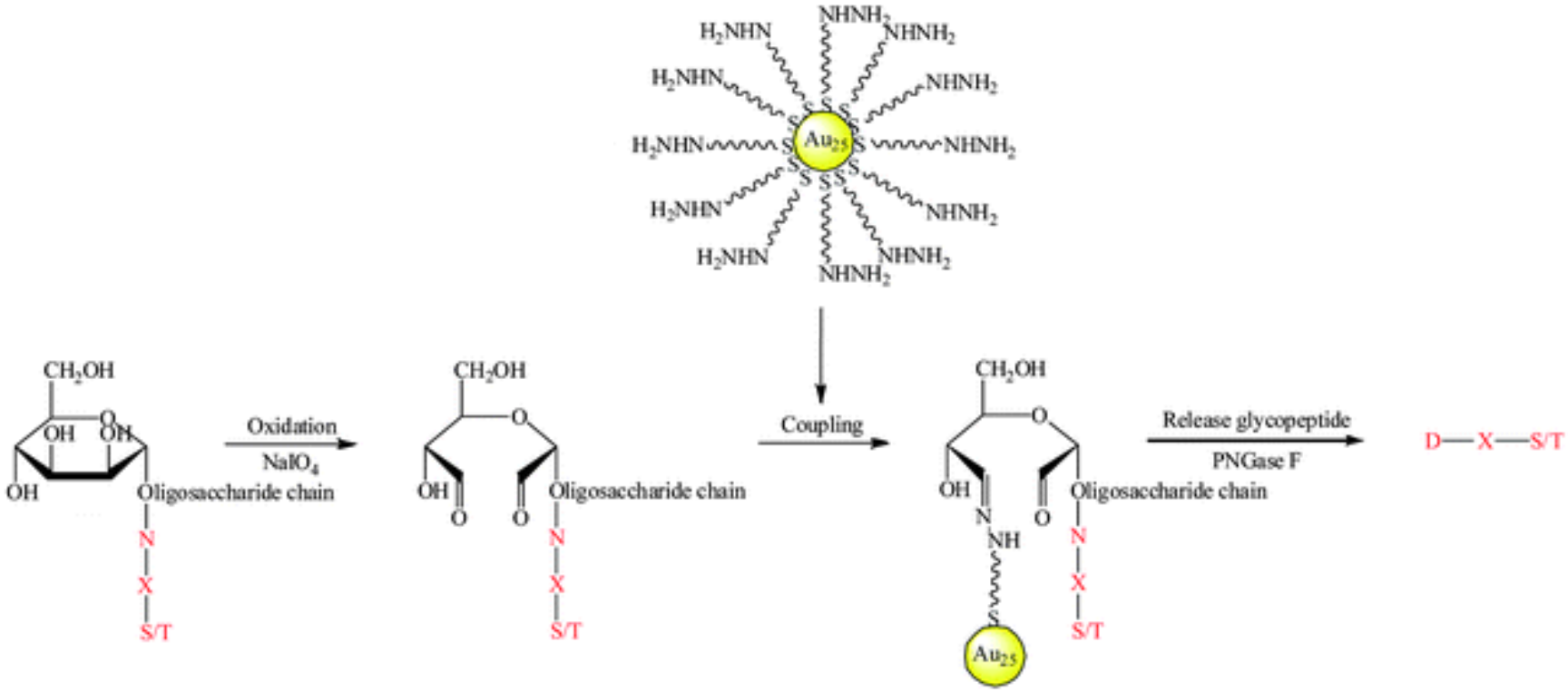
Proteomics of protein post-translational modifications

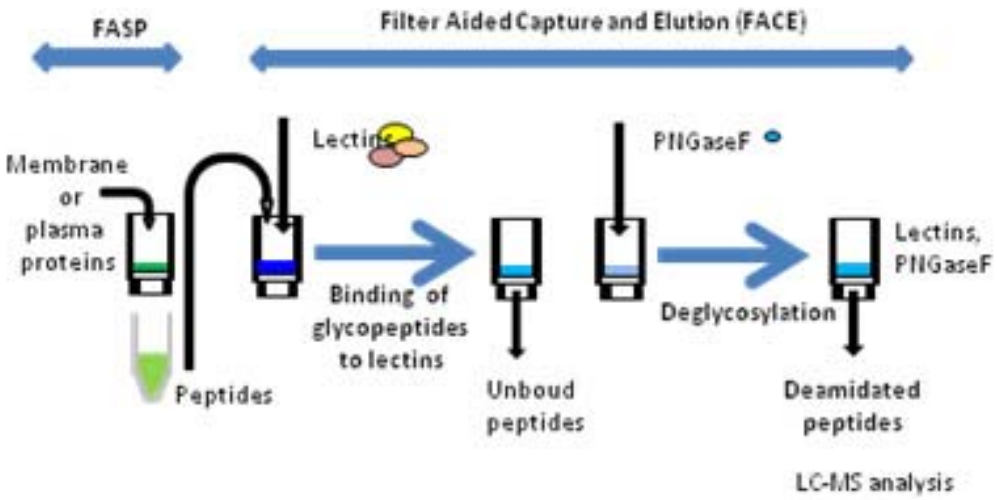
Glycosylation

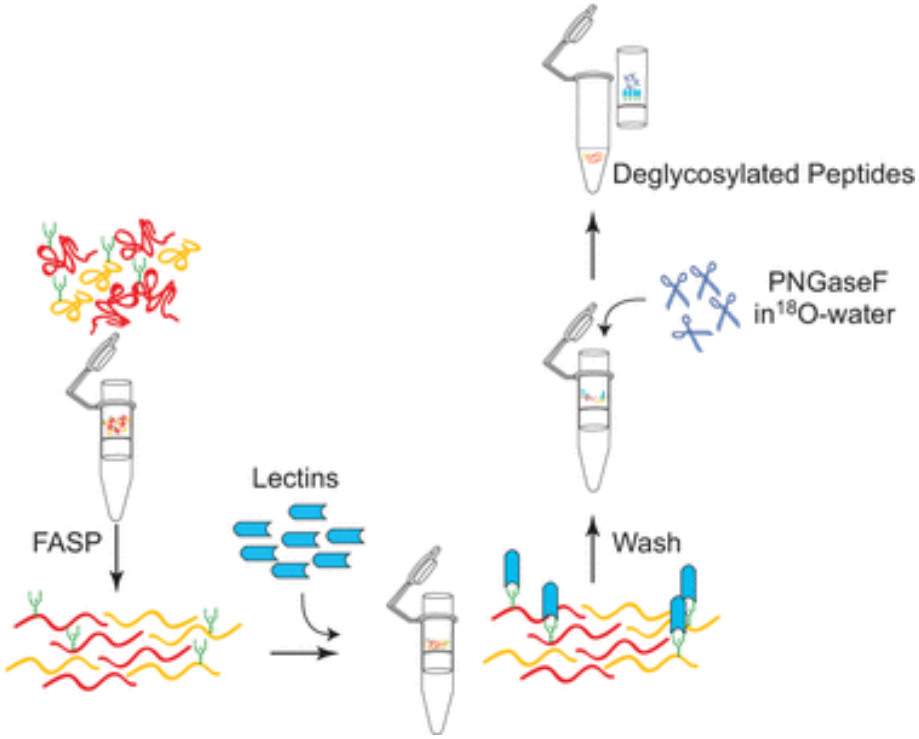


N-linked glycosylation : hydrazide chemistry



N-linked glycosylation : lectin-FASP (FACE)





Resource

Cell

Precision Mapping of an In Vivo N-Glycoproteome Reveals Rigid Topological and Sequence Constraints

Dorota F. Zielinska,[1,3] Florian Gnad,[1,2,3] Jacek R. Wiśniewski,[1,*] and Matthias Mann[1,*]

[1]Department of Proteomics and Signal Transduction, Max-Planck-Institute of Biochemistry, Am Klopferspitz 18, Martinsried D-82152, Germany

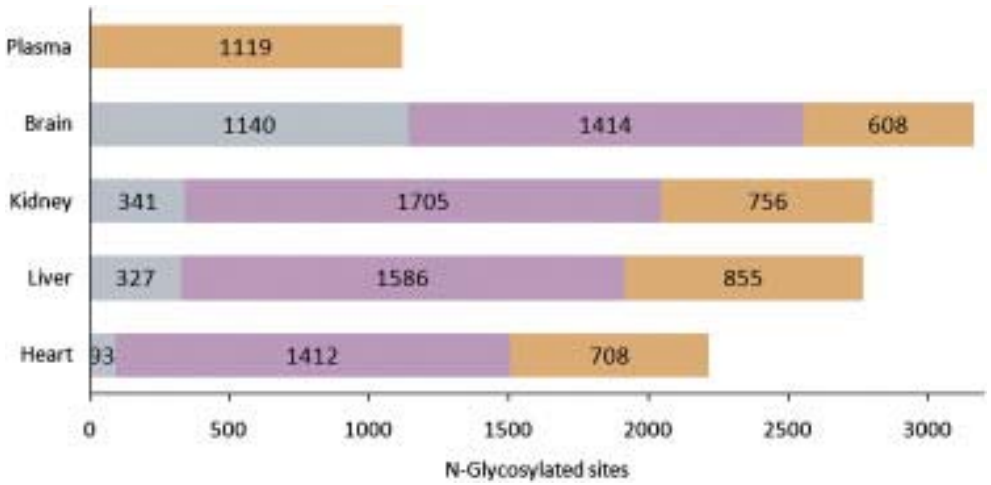
[2]Department of Systems Biology, Harvard Medical School, 200 Longwood Avenue, Boston, MA 02115, USA

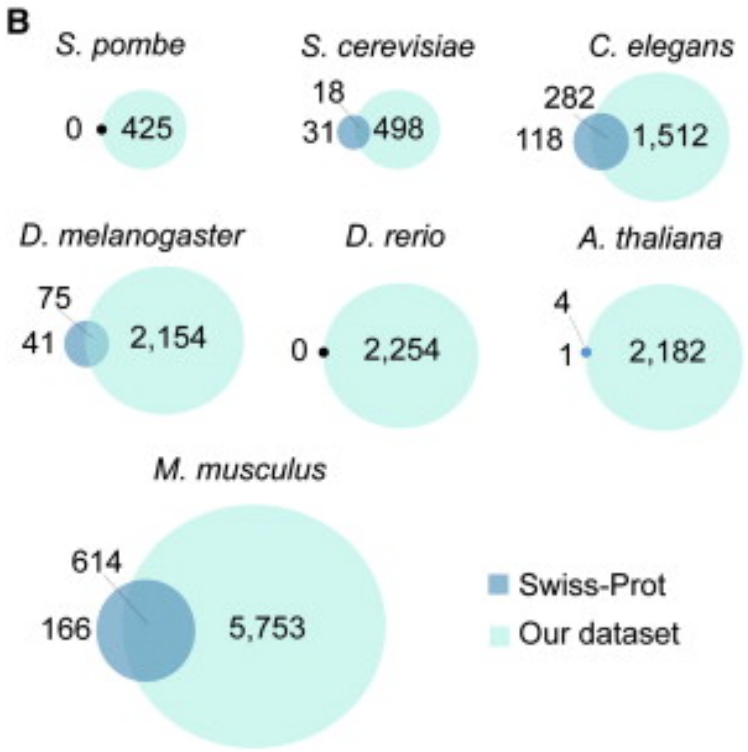
[3]These authors contributed equally to this work

*Correspondence: jwisniew@biochem.mpg.de (J.R.W.), mmann@biochem.mpg.de (M.M.)

DOI 10.1016/j.cell.2010.04.012

N-linked glycosylation : lectin-FASP (FACE)

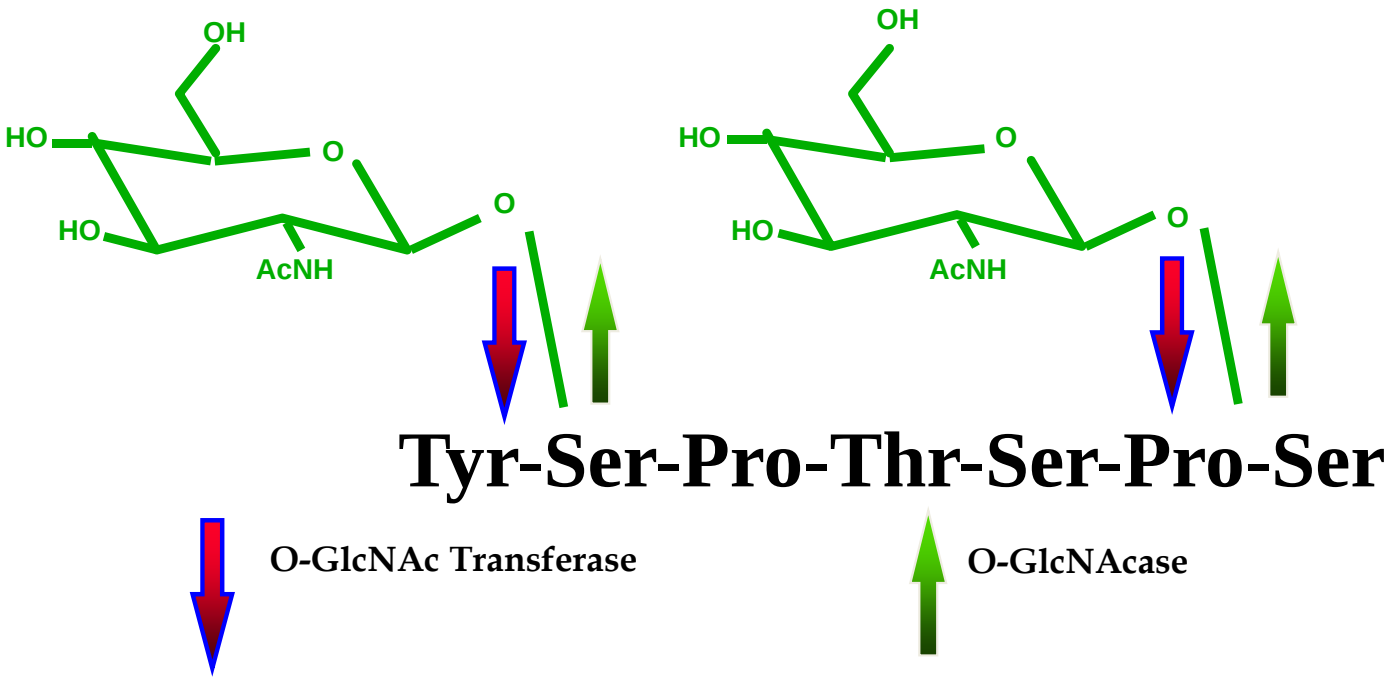




O-linked glycosylation: O-GlcNac

O- Linked N-Acetylglucosamine

A dynamic post-translational modification

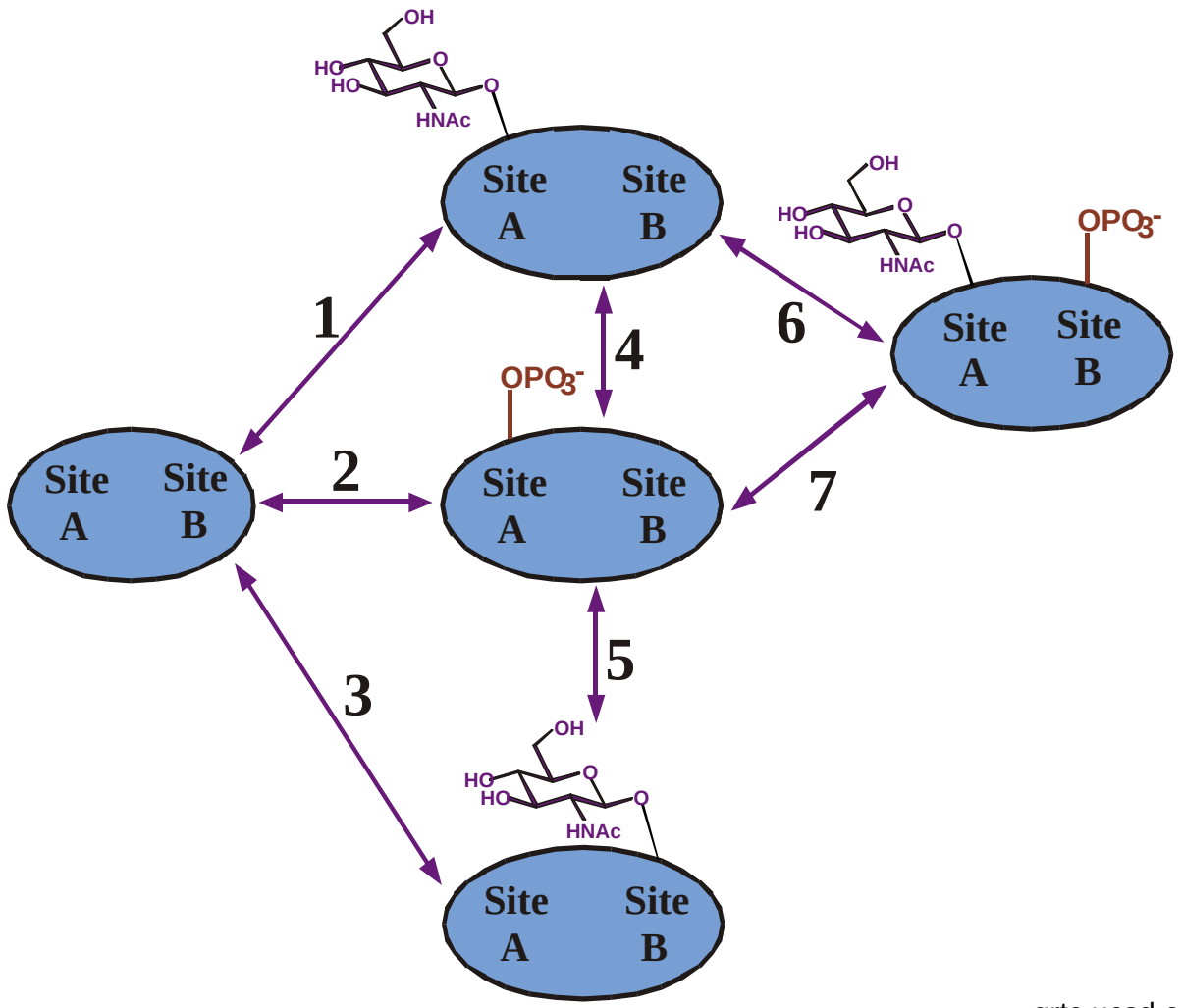


grtc.ucsd.edu/old_essentials/2004/lecture15

Key Features of O-GlcNAc :

- Localized to the cytoplasm and nucleus.
- Present in all higher eukaryotes studied.
- As abundant as phosphorylation;
  UDP-GlcNAc is Nearly as abundant as ATP.
- O-GlcNAc proteins are also Phosphoproteins
- O-GlcNAc and Phosphorylation are often reciprocal.
- Highly dynamic modification - a regulatory role.

Complex Interplay Between O-Phosphate & O-GlcNAc:



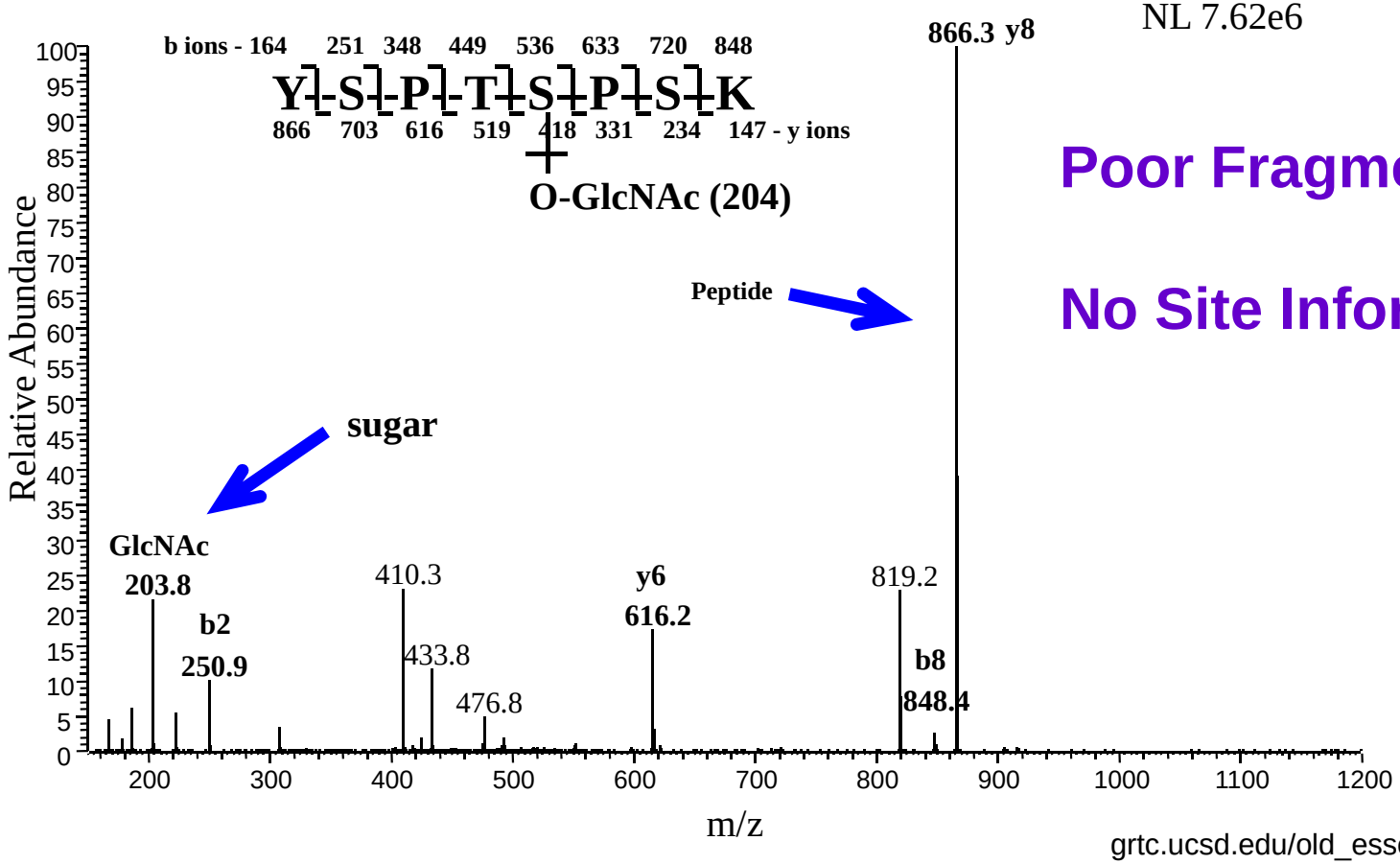
grtc.ucsd.edu/old_essentials/2004/lecture15

Identification of O-GlcNAc Modified Proteins by MS

Why Did O-GlcNAc Remain Undetected?

- Generally Not *Affect Gel Electrophoresis*
- Not Easily Labeled - No ^{32}P!
- Very Labile - both Chemically and Enzymatically
  - Falls Off in MS.
- Stoichiometry Similar to O-Phosphate.

Fragmentation of GlcNAc-CTD by CID-MS/MS
Parent ion 535 ([M+2H$^+$] + 1 GlcNAc)

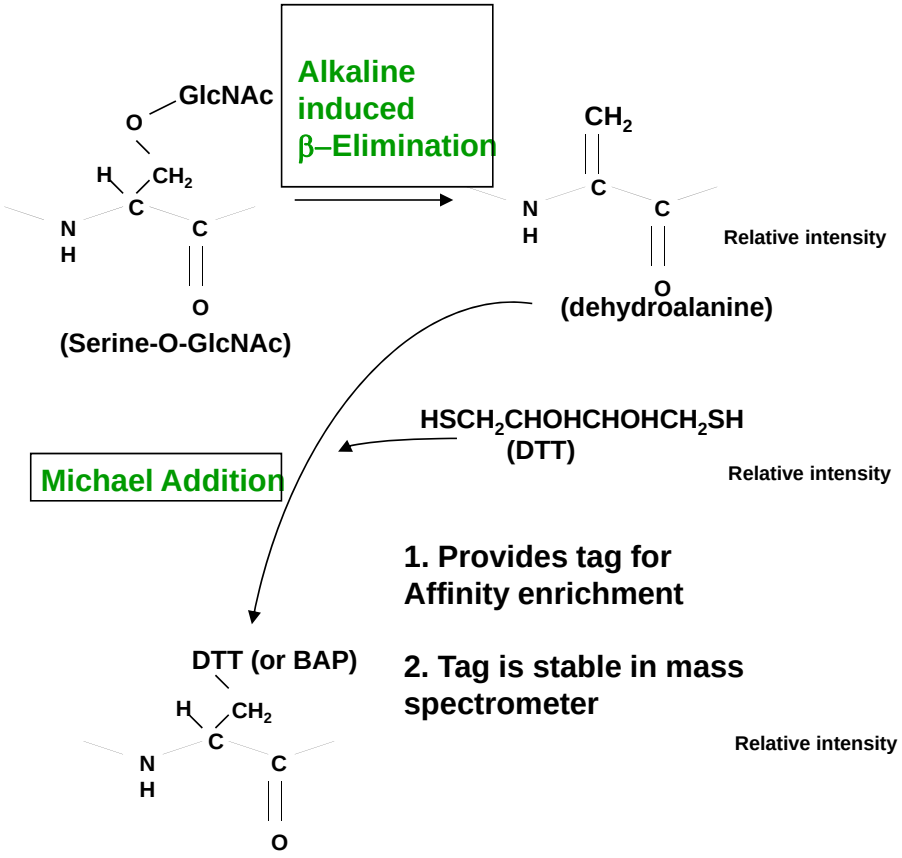


Poor Fragmentation

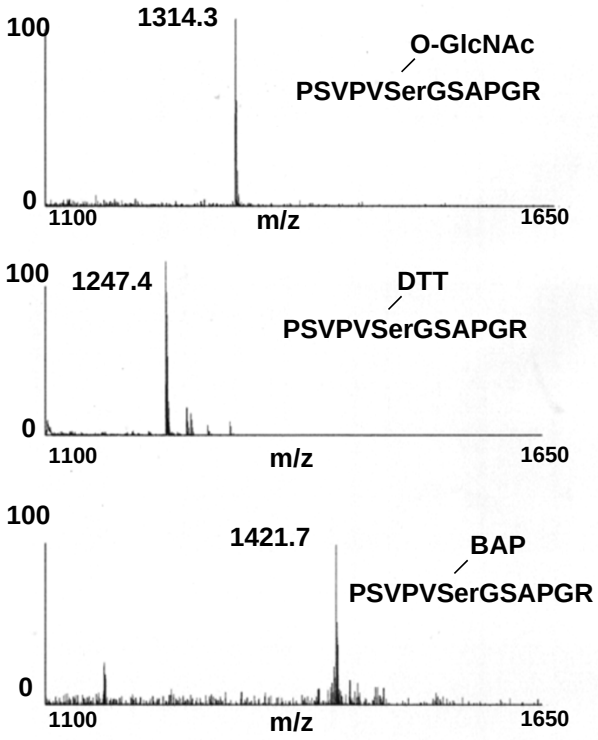
No Site Information.

grtc.ucsd.edu/old_essentials/2004/lecture15

BEMAD Strategy for O-GlcNAc/O-Phosphate site mapping



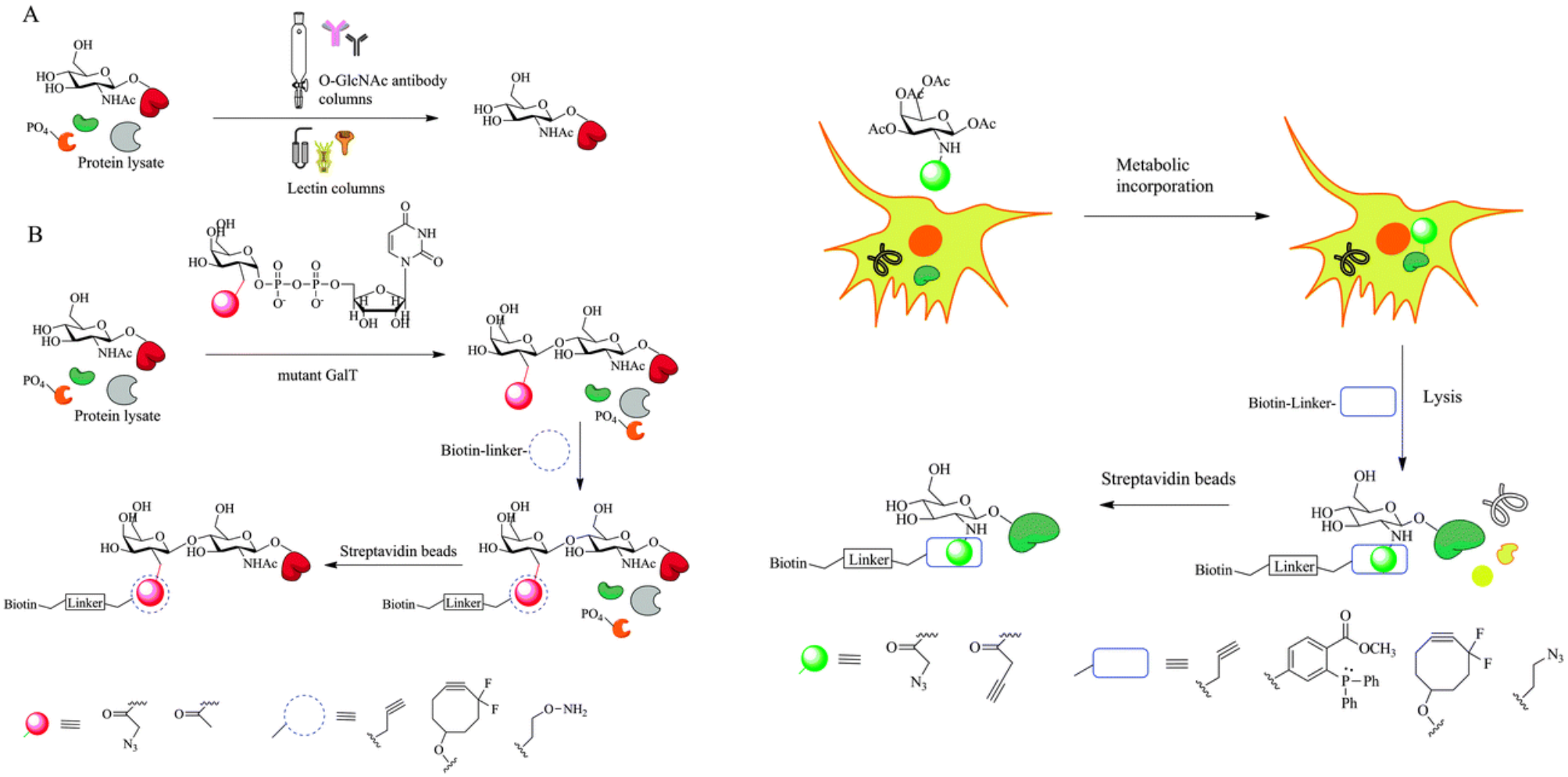
Replacement of O-GlcNAc with DTT Using β-elimination/michael addition

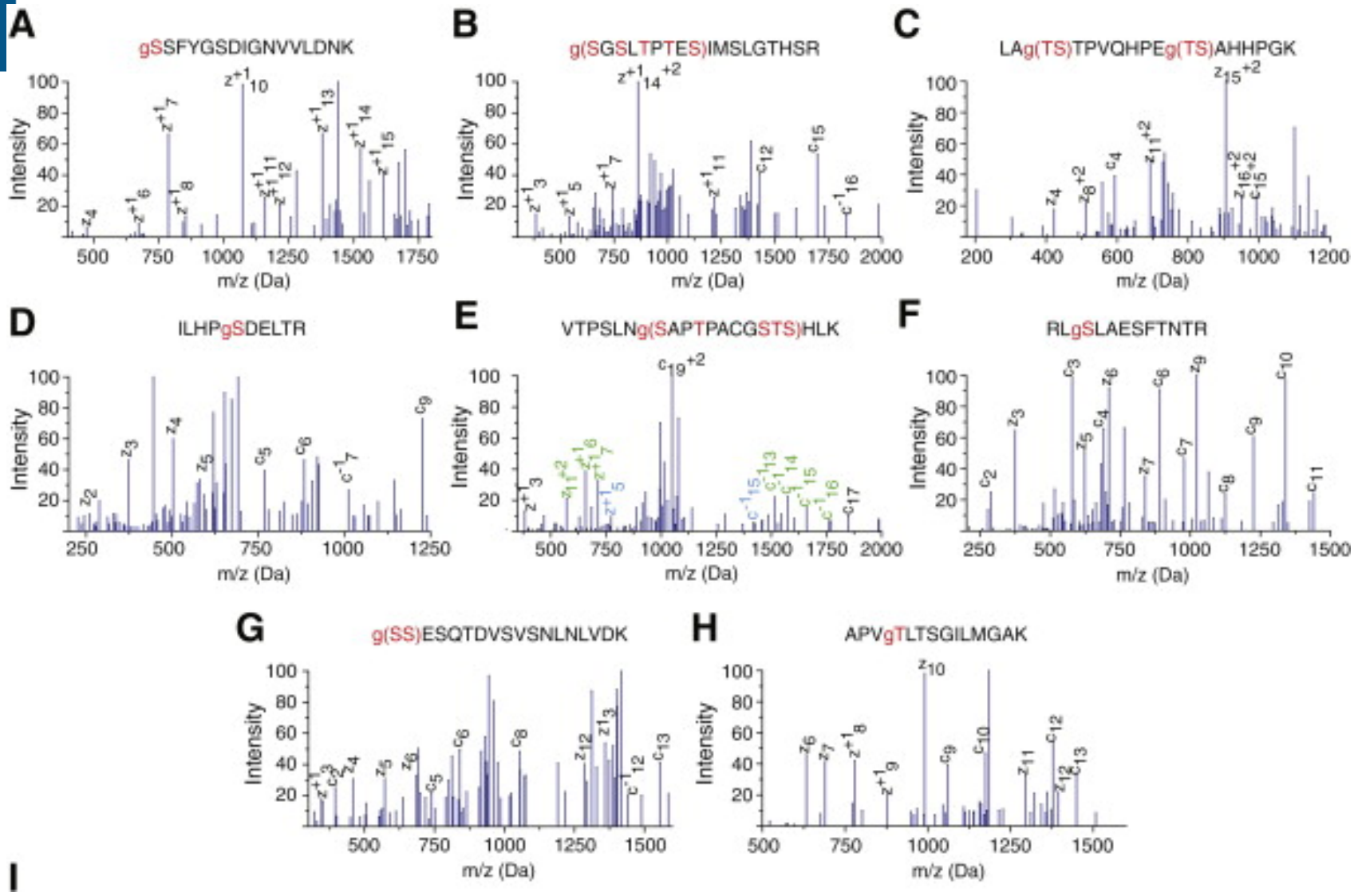


MALDI-TOF

grtc.ucsd.edu/old_essentials/2004/lecture15

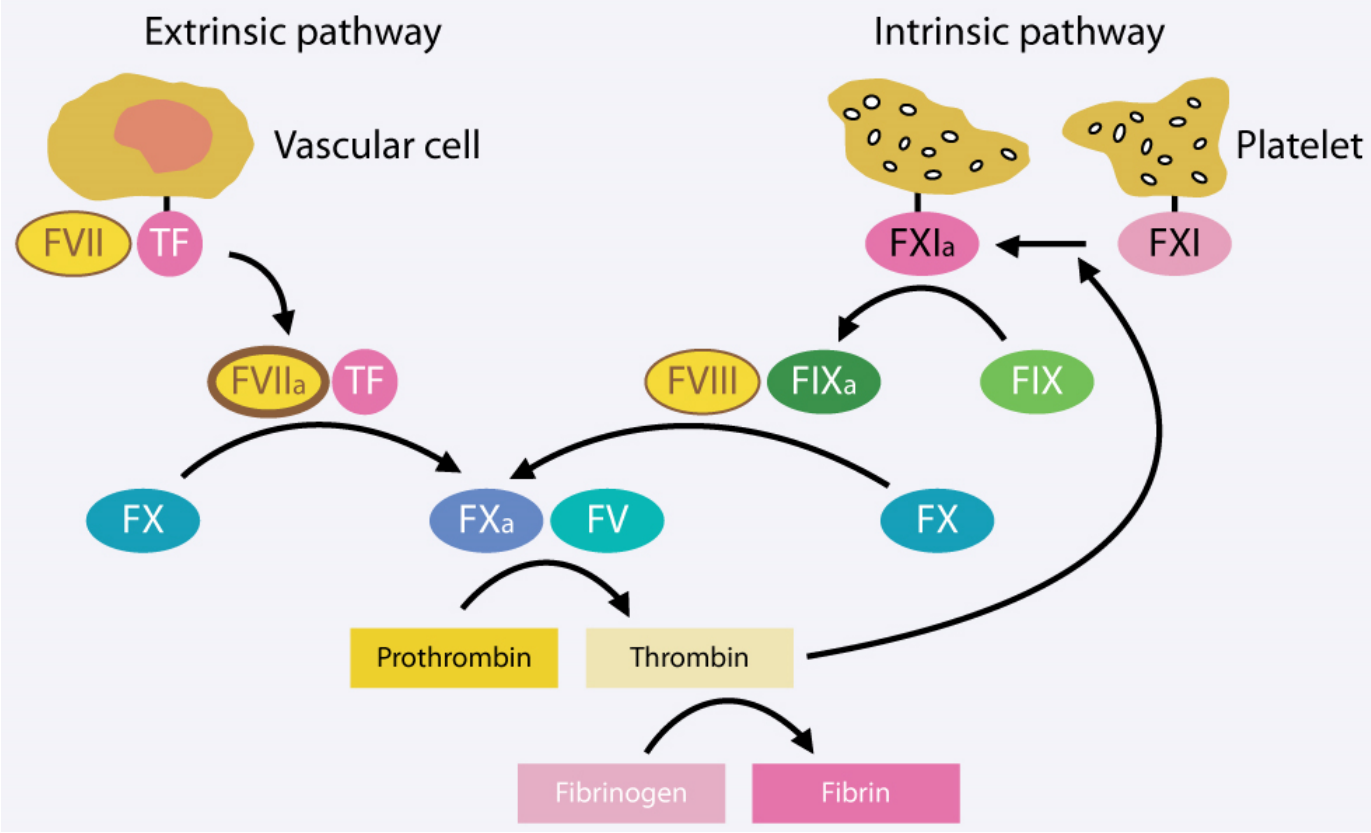
O-linked glycosylation: O-GlcNac




A
gSSFYGSDIGNVVLDNK
B
g(SGSLTPTES)IMSLGTHSR
C
LAg(TS)TPVQHPEg(TS)AHHPGK
D
ILHPgSDELTR
E
VTPSLNg(SAPTPACGSTS)HLK
F
RLgSLAESFTNTR
G
g(SS)ESQTDVSVSNLNLVDK
H
APVgTLTSGILMGAK
I
Intensity
m/z (Da)

Proteolytic Processing

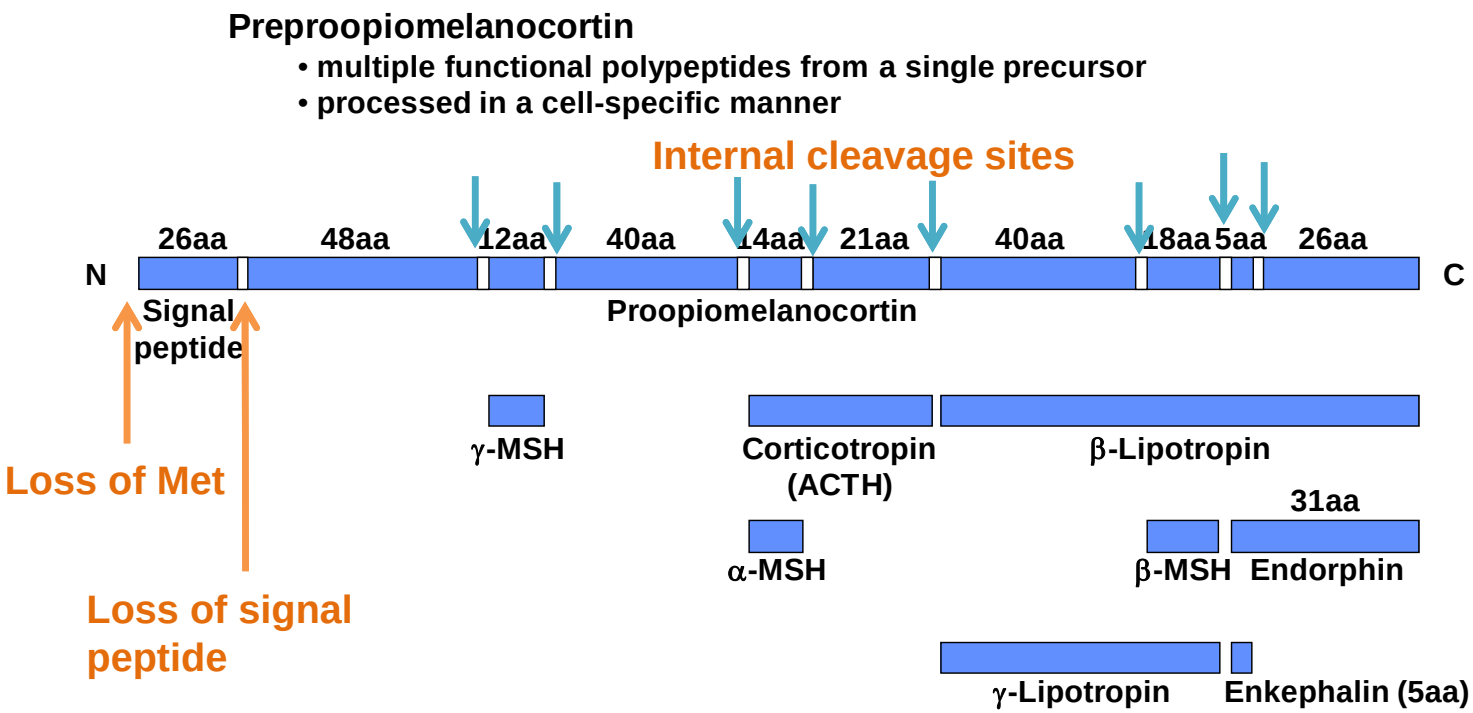
Example; coagulation cascade



Proteolytic Processing

- Protein functions in living organisms are naturally modulated by proteases through a process termed “proteolytic processing”
- New bioactive proteins are mostly generated through protease-substrate interactions, moreover the activities of proteases are also often modulated by other proteases.

Proteolytic Processing; example

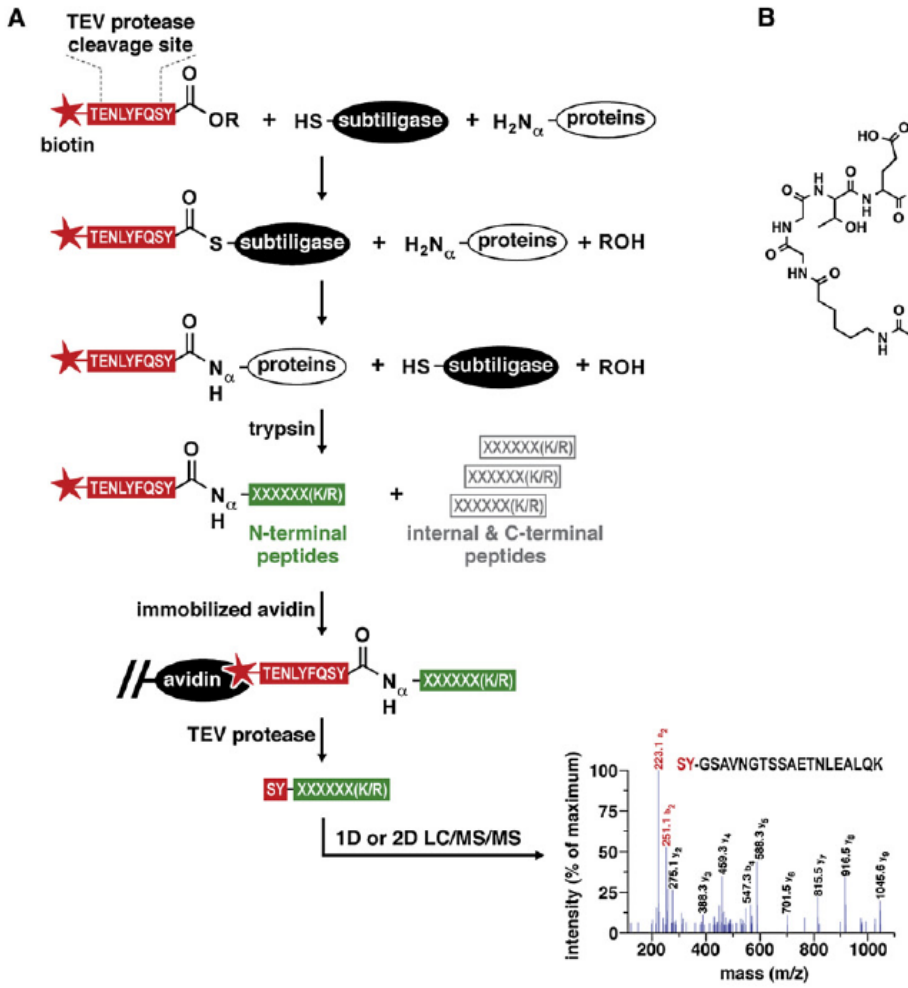


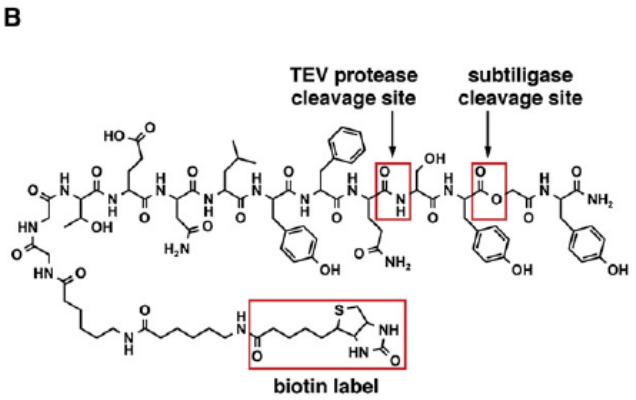
- ***Not predictable from genomics or transcriptomics***
- ***Greatly expands proteome complexity***
- ***Not covered by conventional proteomic approaches***

N-terminal proteomics

- Positive / Negative capture

1. Positive enrichment_*Biotin-Avidin System (Subtiligase/TEV)*

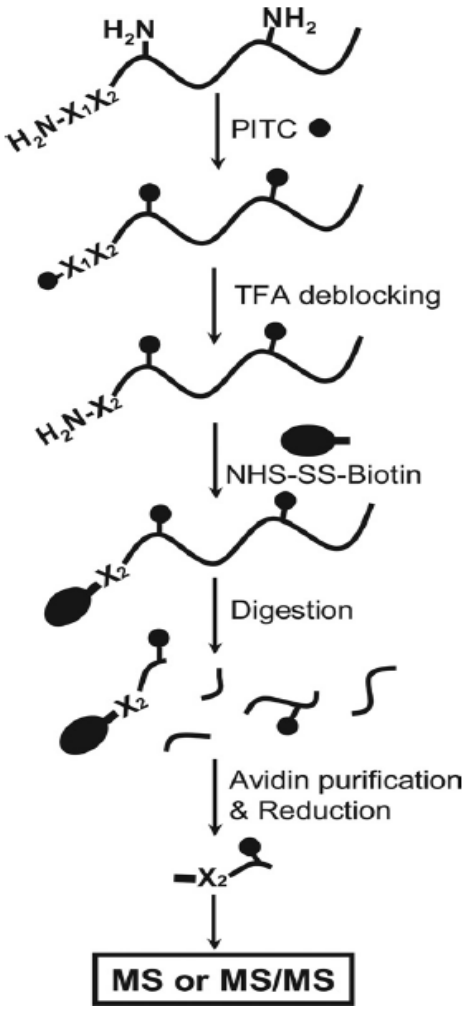


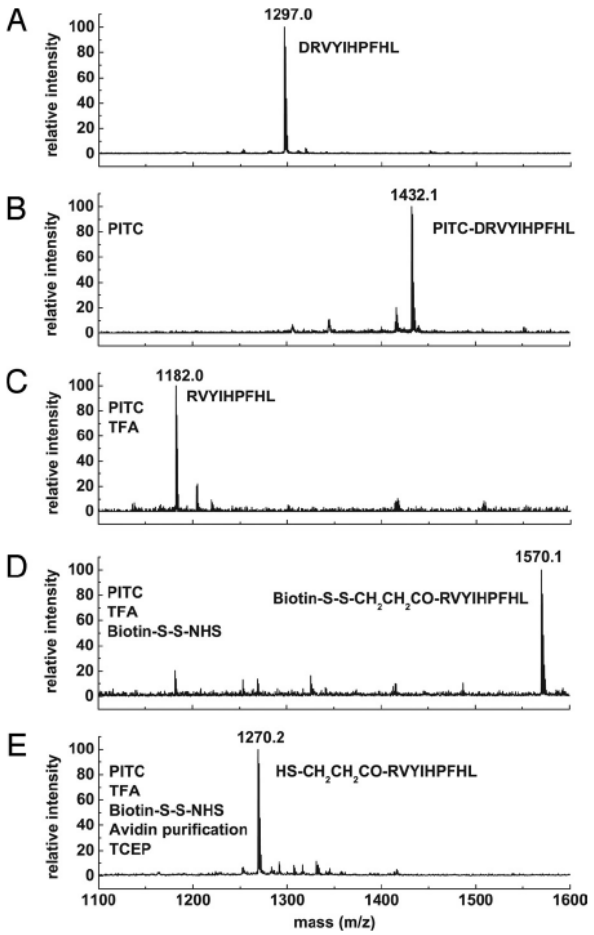


James A. Wells

Cell. **2008** Sep 5;134(5):866-76.

2. Positive Enrichment_*Biotin-Avidin System (PITC)*

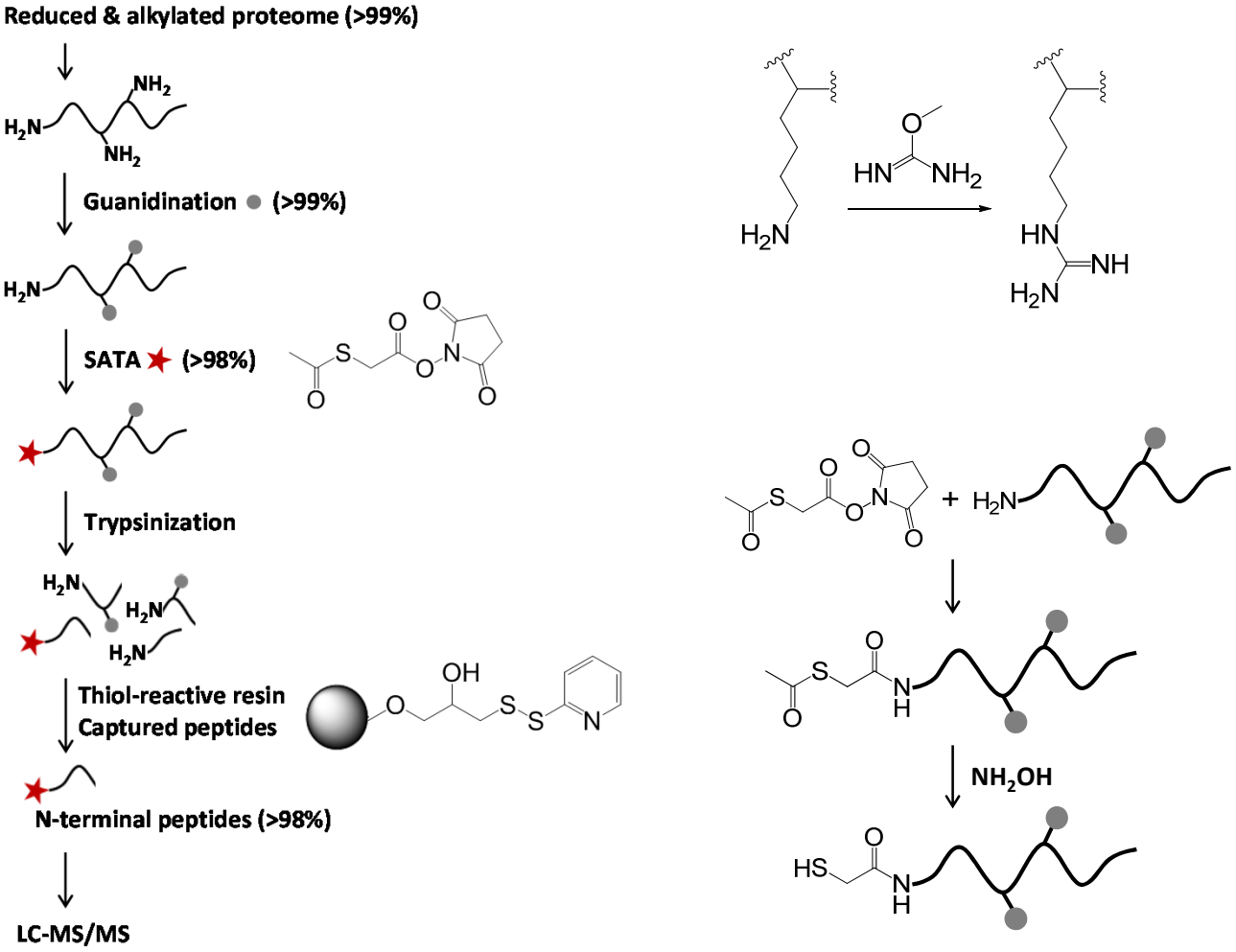




Samie R. Jaffrey

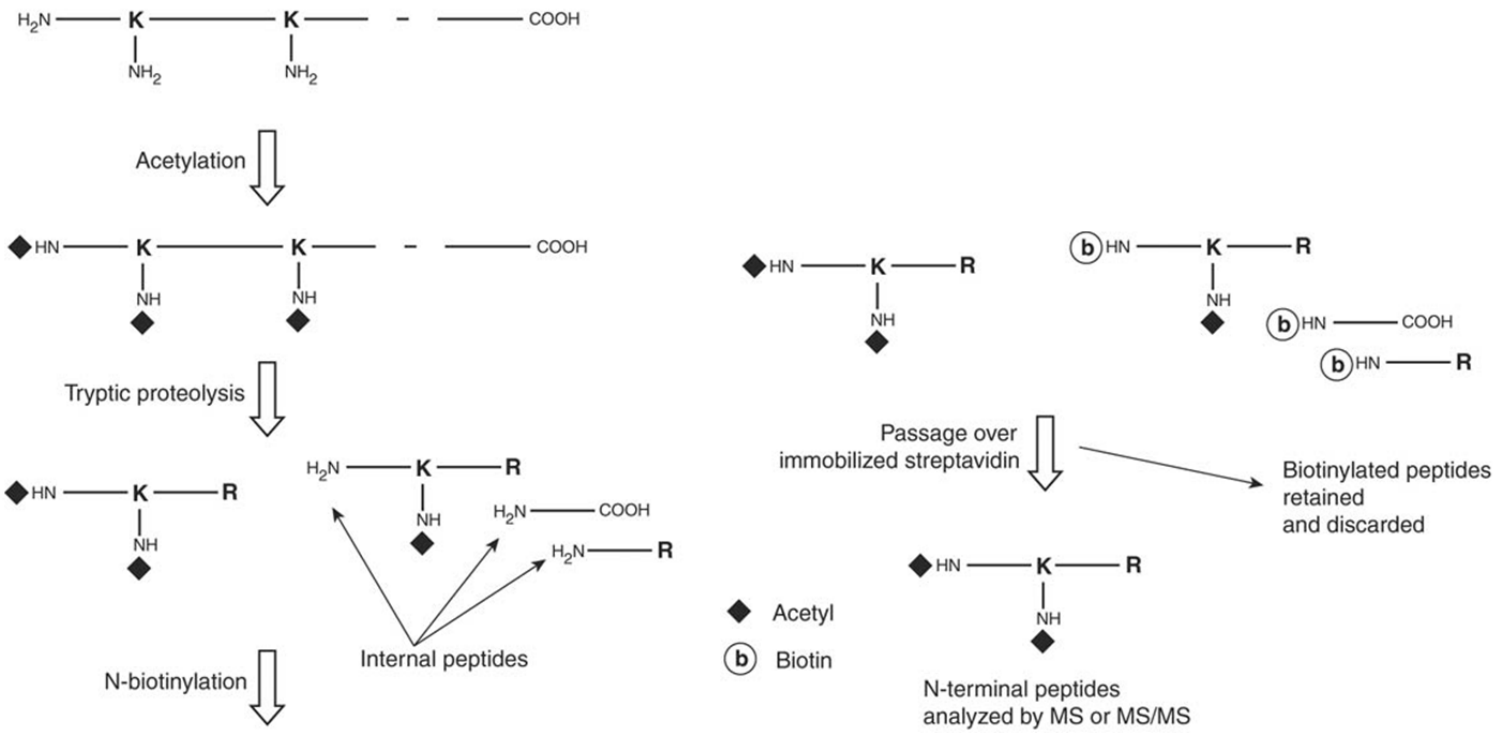
Proc Natl Acad Sci U S A. **2009** Nov 17;106(46):19310-5

3. Positive enrichment_resin assisted capture



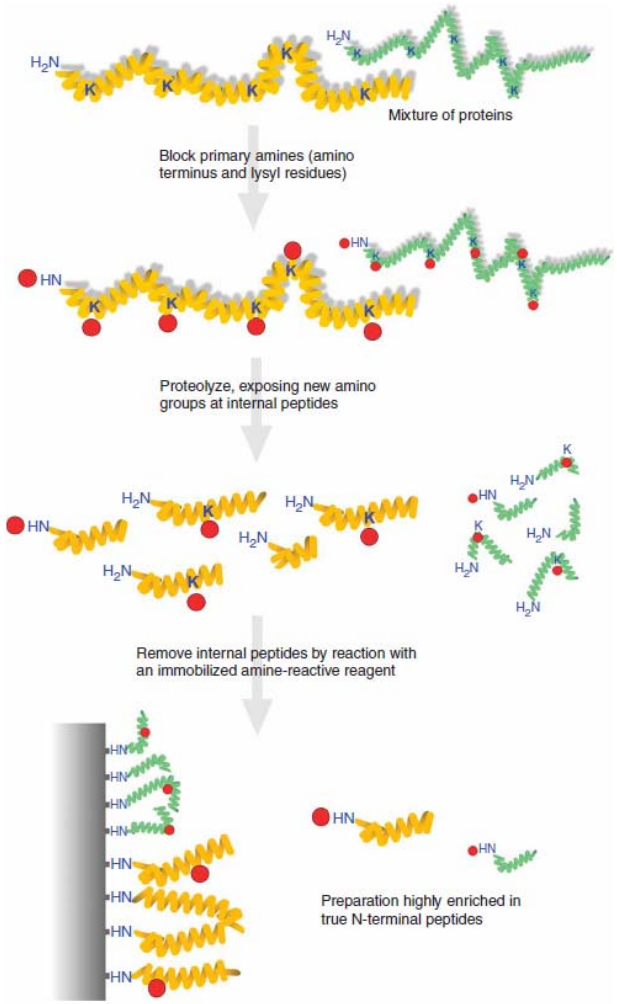
1. Acetylation/biotin-avidin

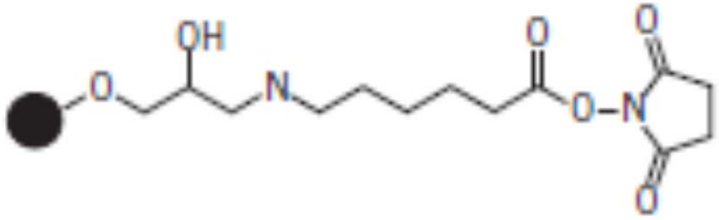
Nat Methods. 2005 Dec;2(12):955-7 **Lucy McDonald**



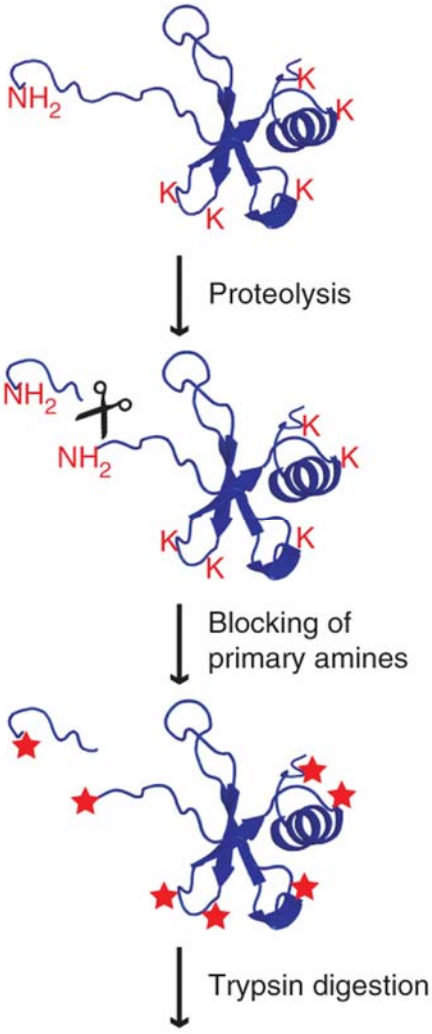
2. Acetylation/NHS-activated Sepharose

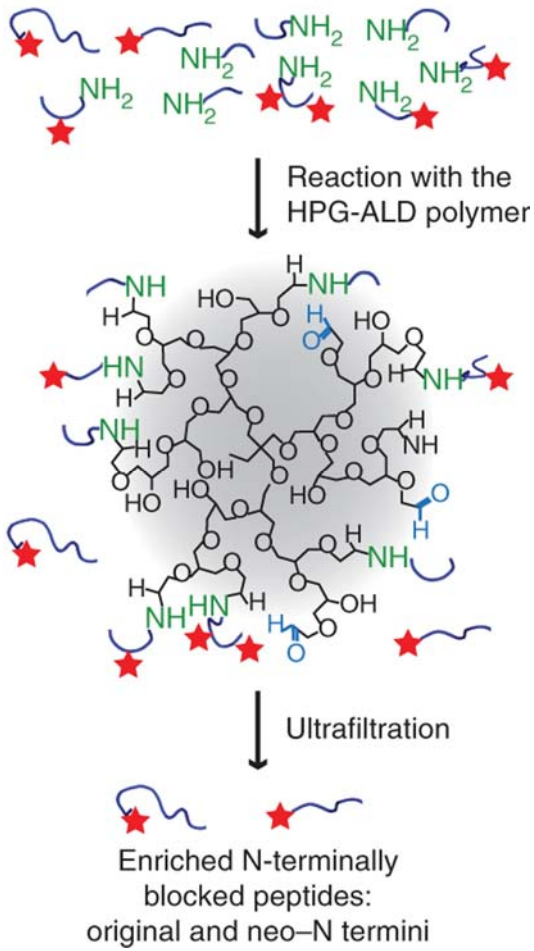
Nat Protoc. 2006;1(4):1790-8 **Lucy McDonald**





3. TALIS : Dimethylation/Aldehyde-dendrimeric polymer

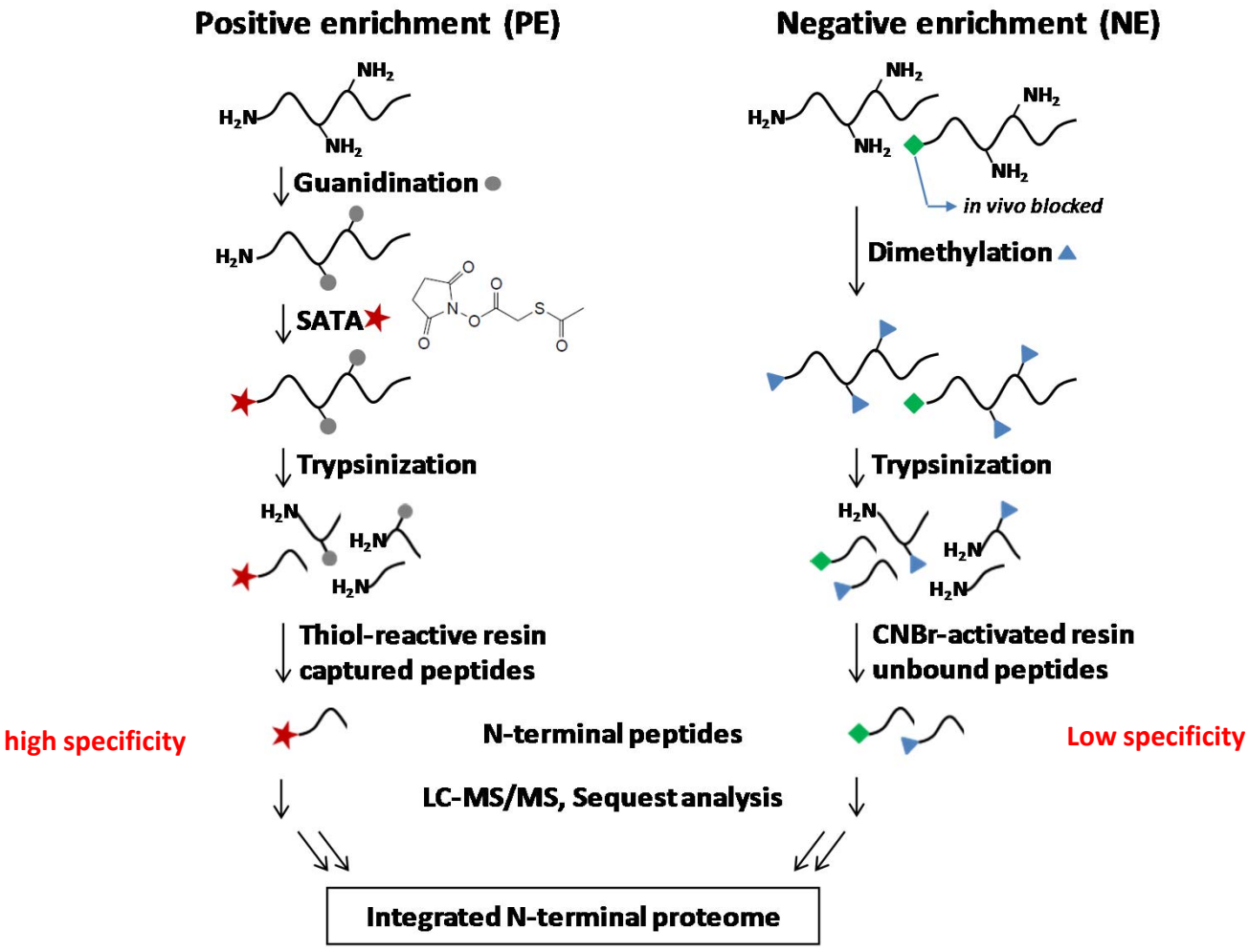




Christopher M Overall

Nat Biotechnol. 2010 Mar;28(3):281-8.

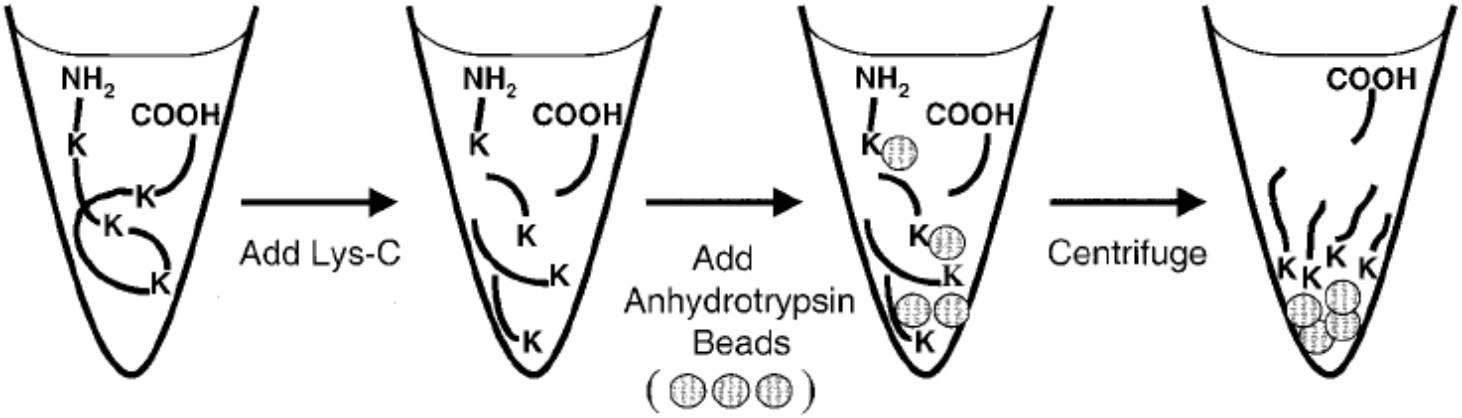
Methodology overview



C-terminal proteomics

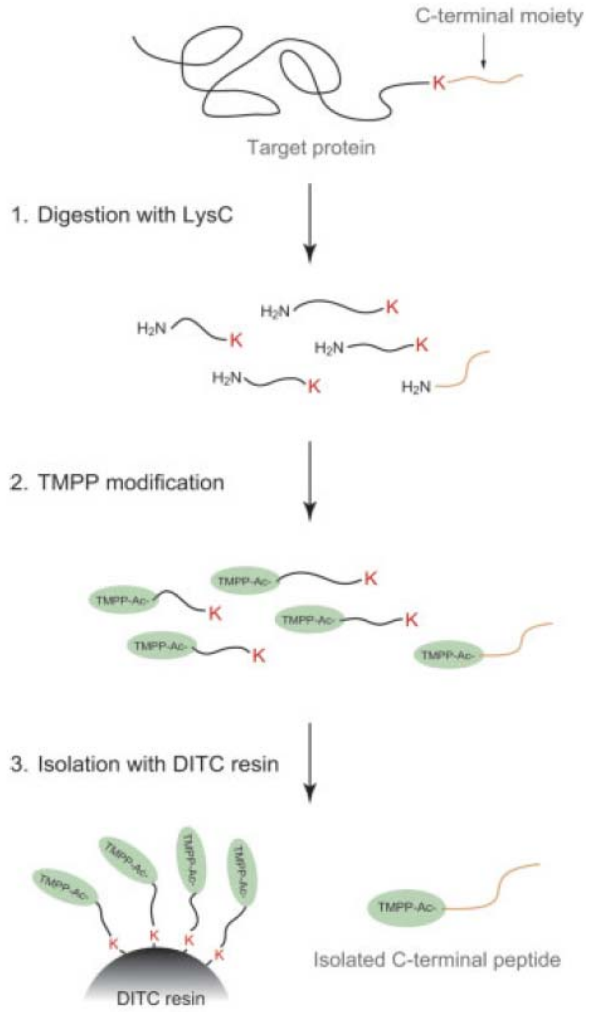
1. Anhydrotrypsin Bead

Anal Chem. 2000 Jul 15;72(14):3374-8. **Chait BT**



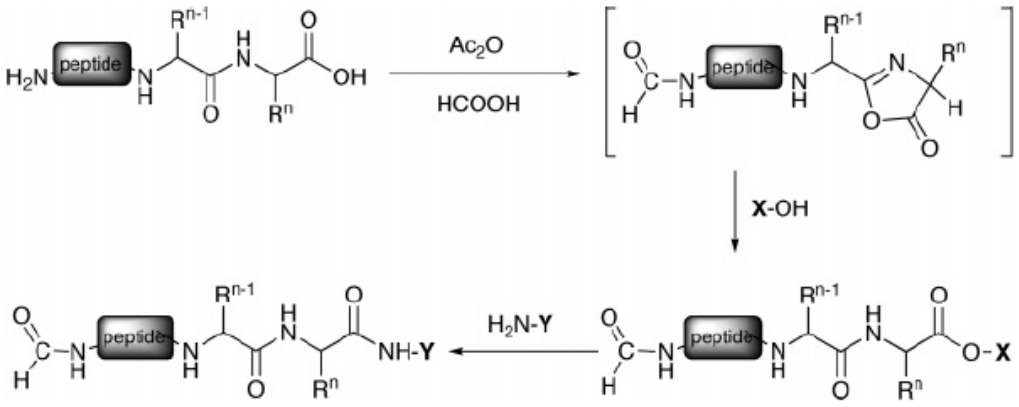
2. LysC/TMPP labeling DITC resin

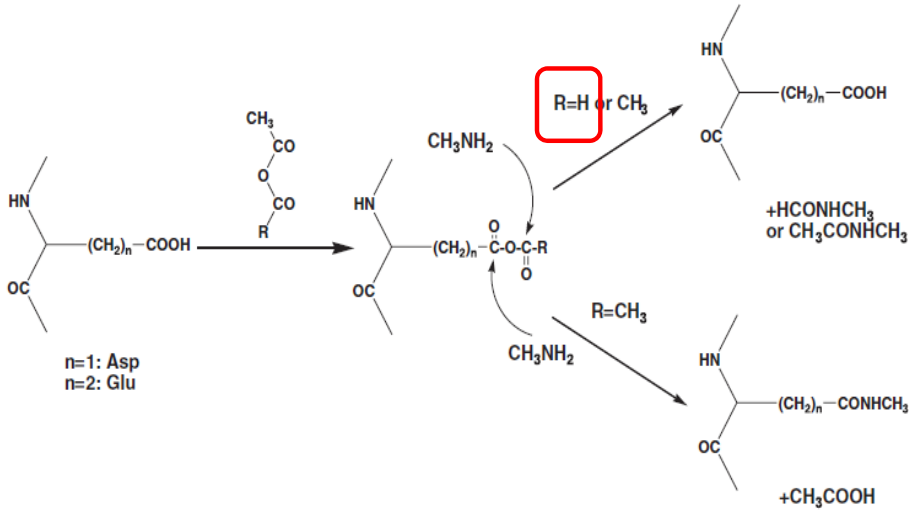
Proteomics. 2008 Apr;8(8):1539-50 **Kuyama, Hiroki**

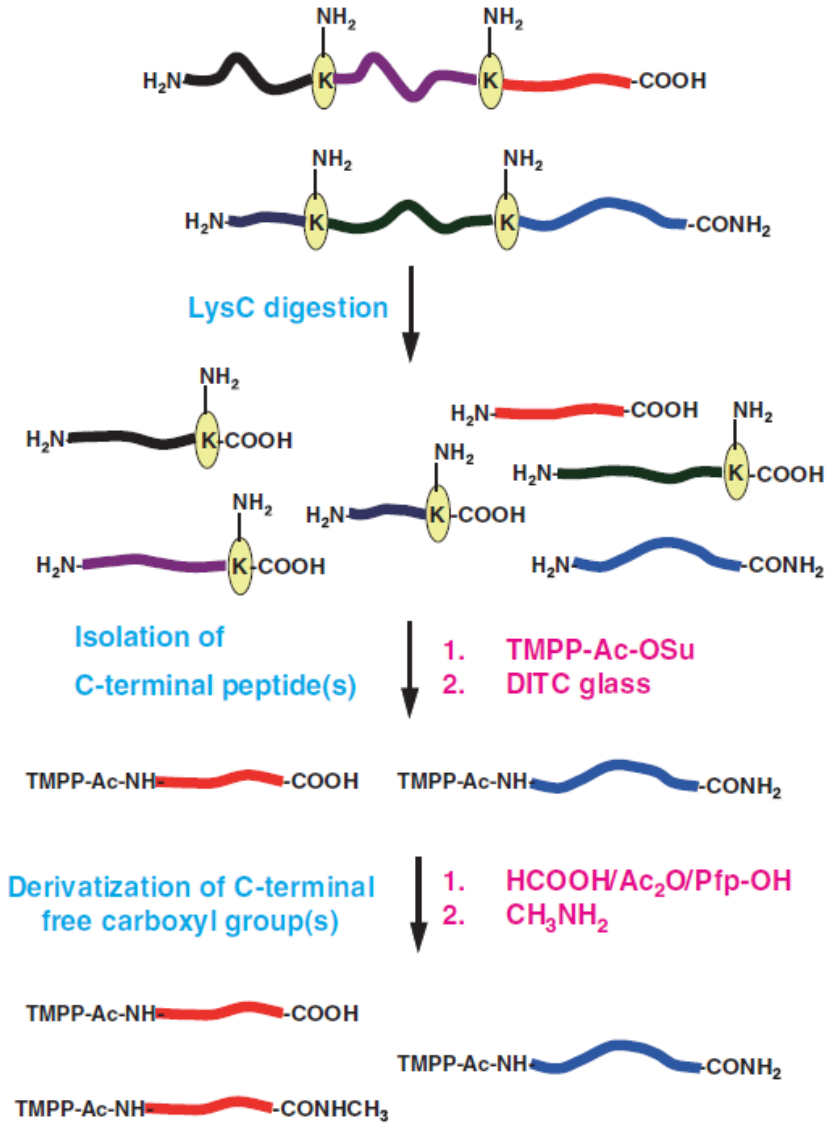


3. Oxazolone chemistry

Proteomics. 2009 Aug;9(16):4063-70 **Kuyama, Hiroki**

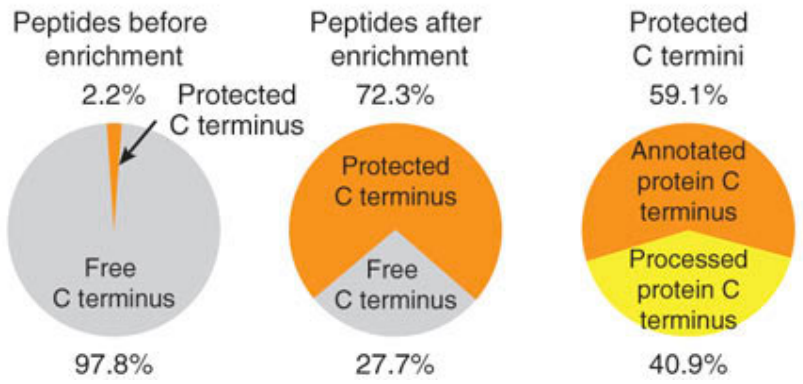




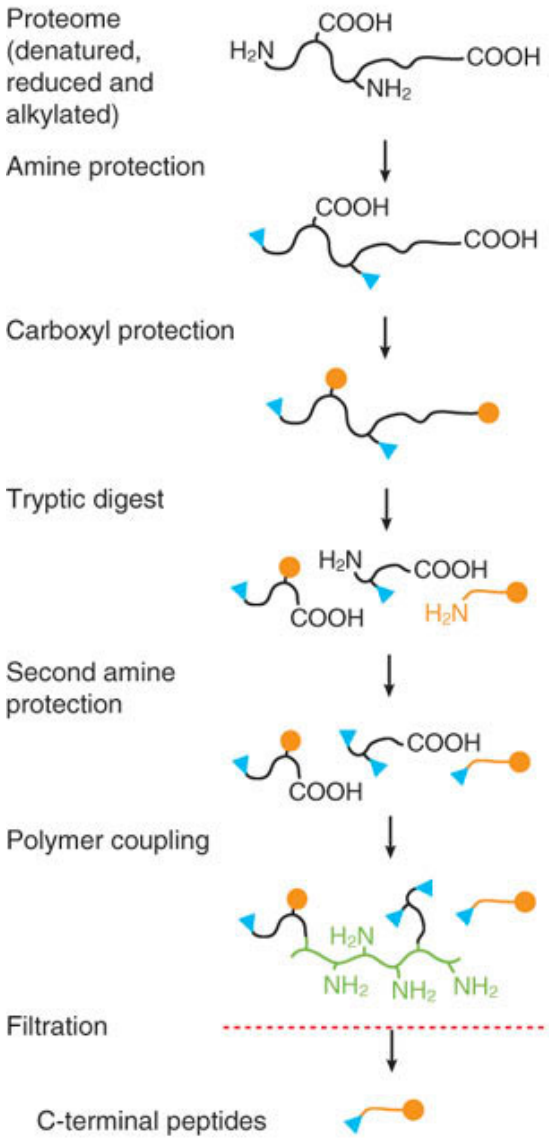


4. CTAILS: EDC coupling/polymer bead

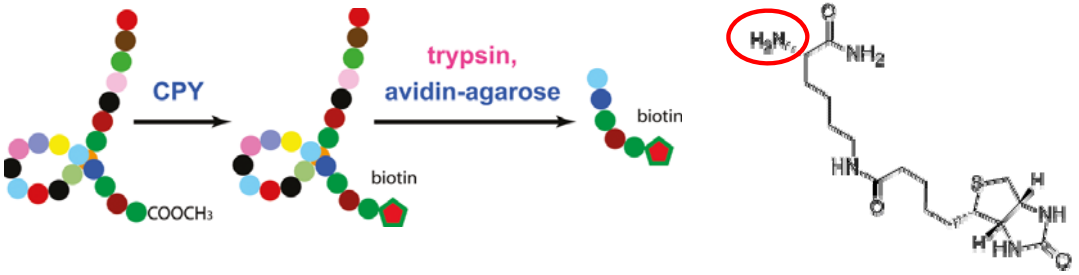
Nat Methods. 2010 Jul;7(7):508-11 **Christopher M Overall**

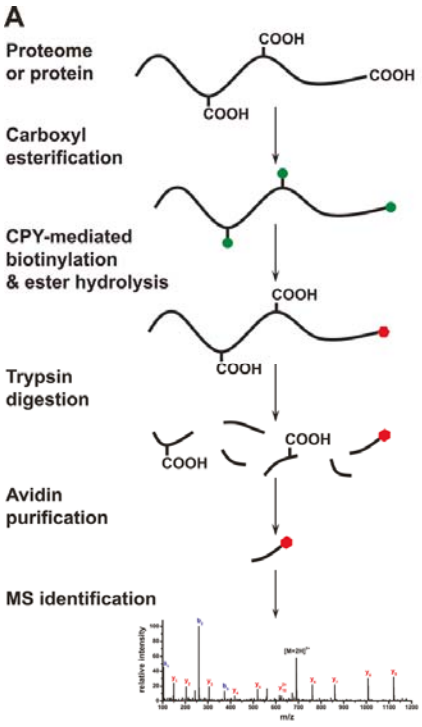


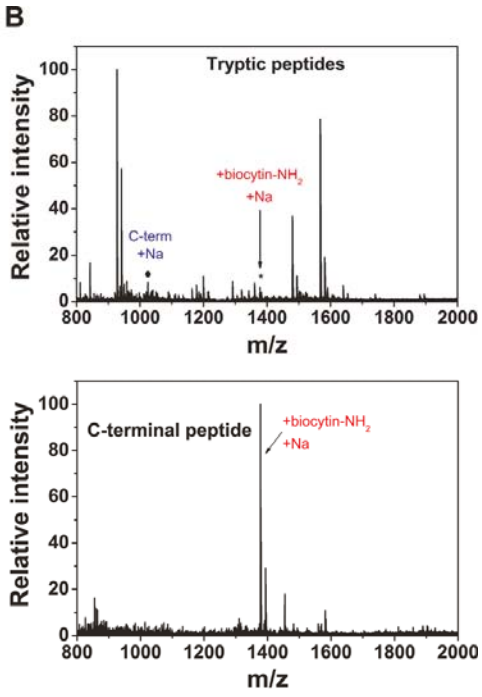
1. Time consuming : ~72 h for chemical labeling.
2. Relatively low labeling efficiency of EDC coupling for COOH
3. 460 pep/196 pro in 2 LC-MS/MS

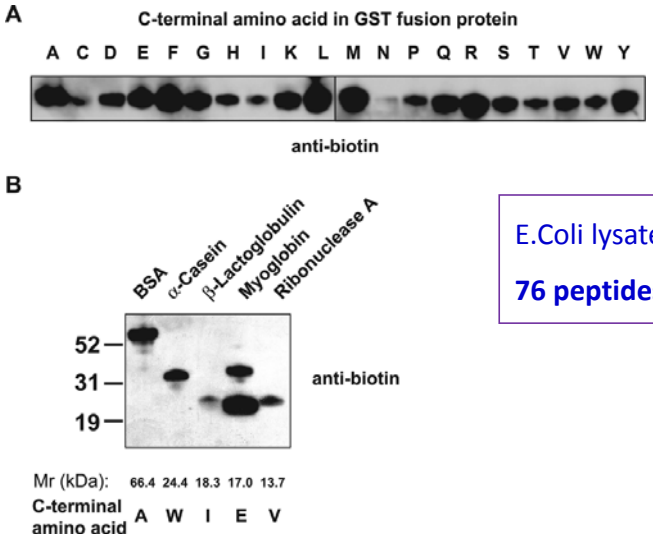


5. Chemoenzymatic labeling of protein C-termini







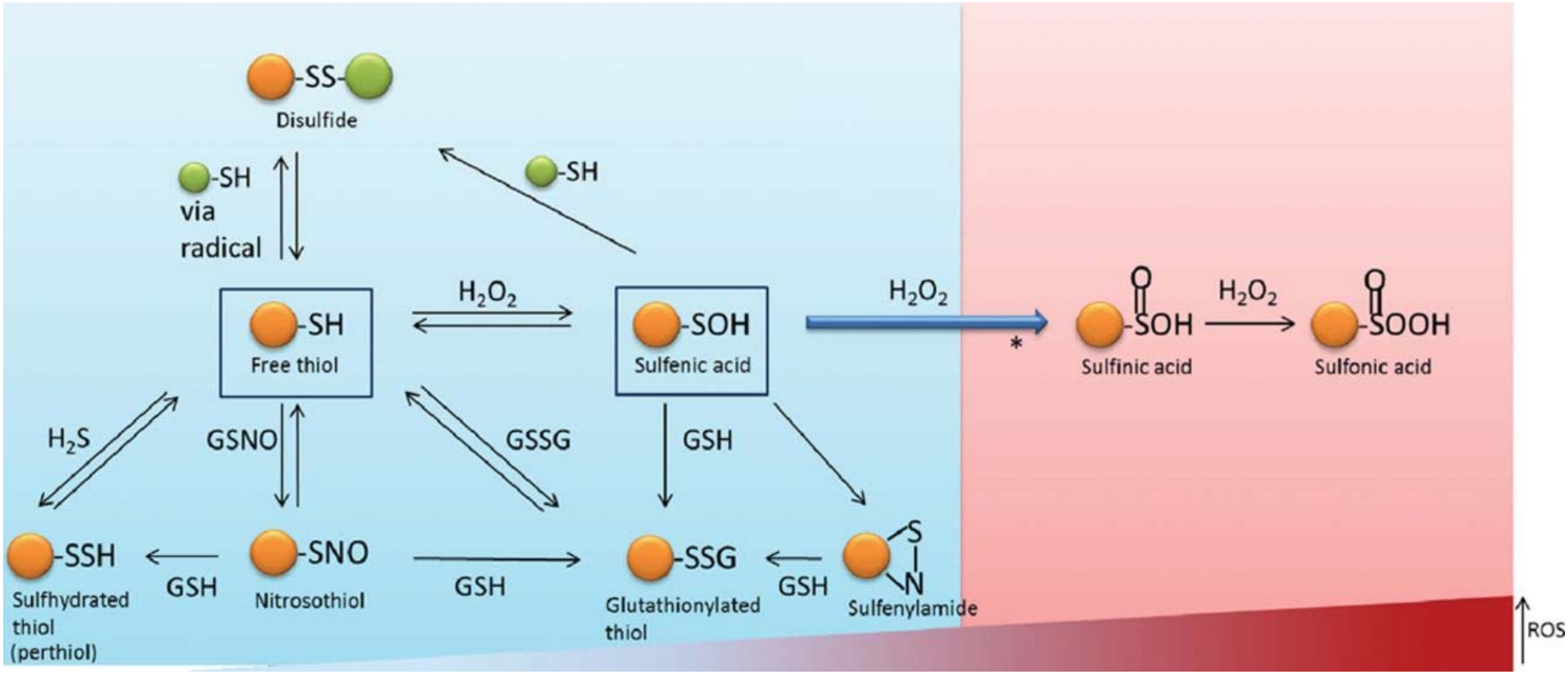


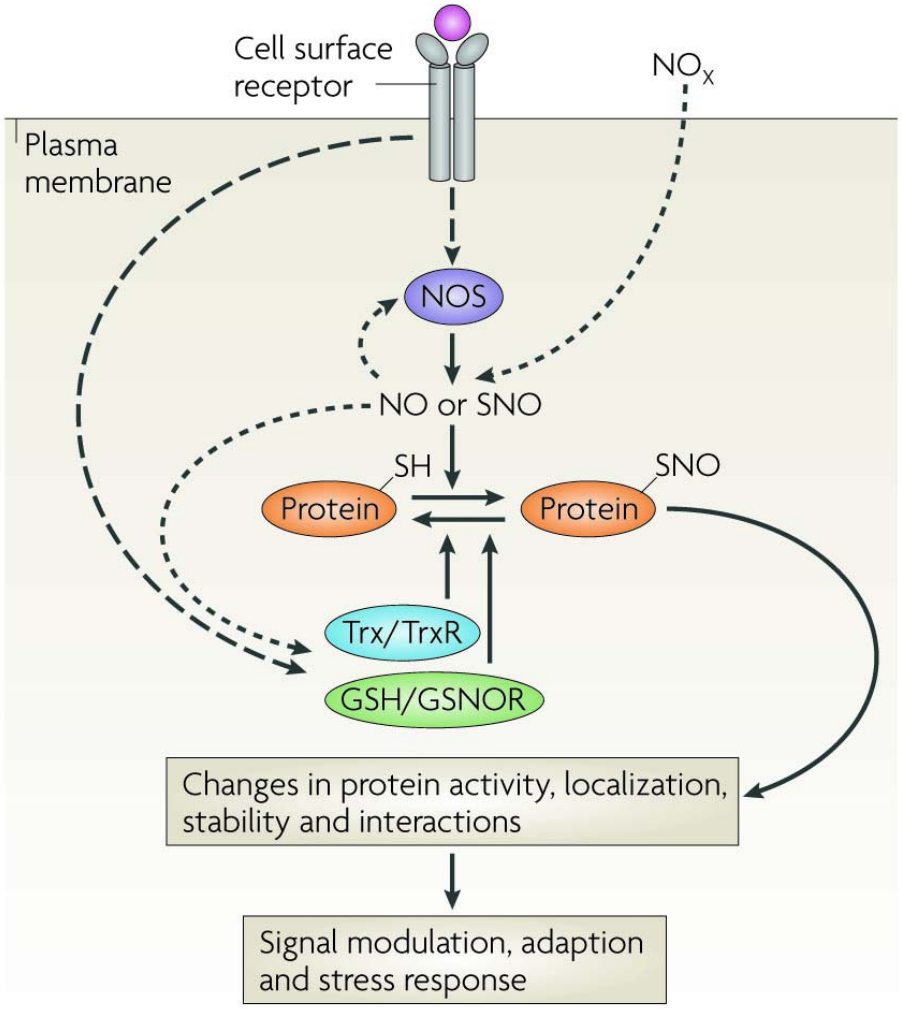
E.Coli lysate

76 peptides/76 proteins

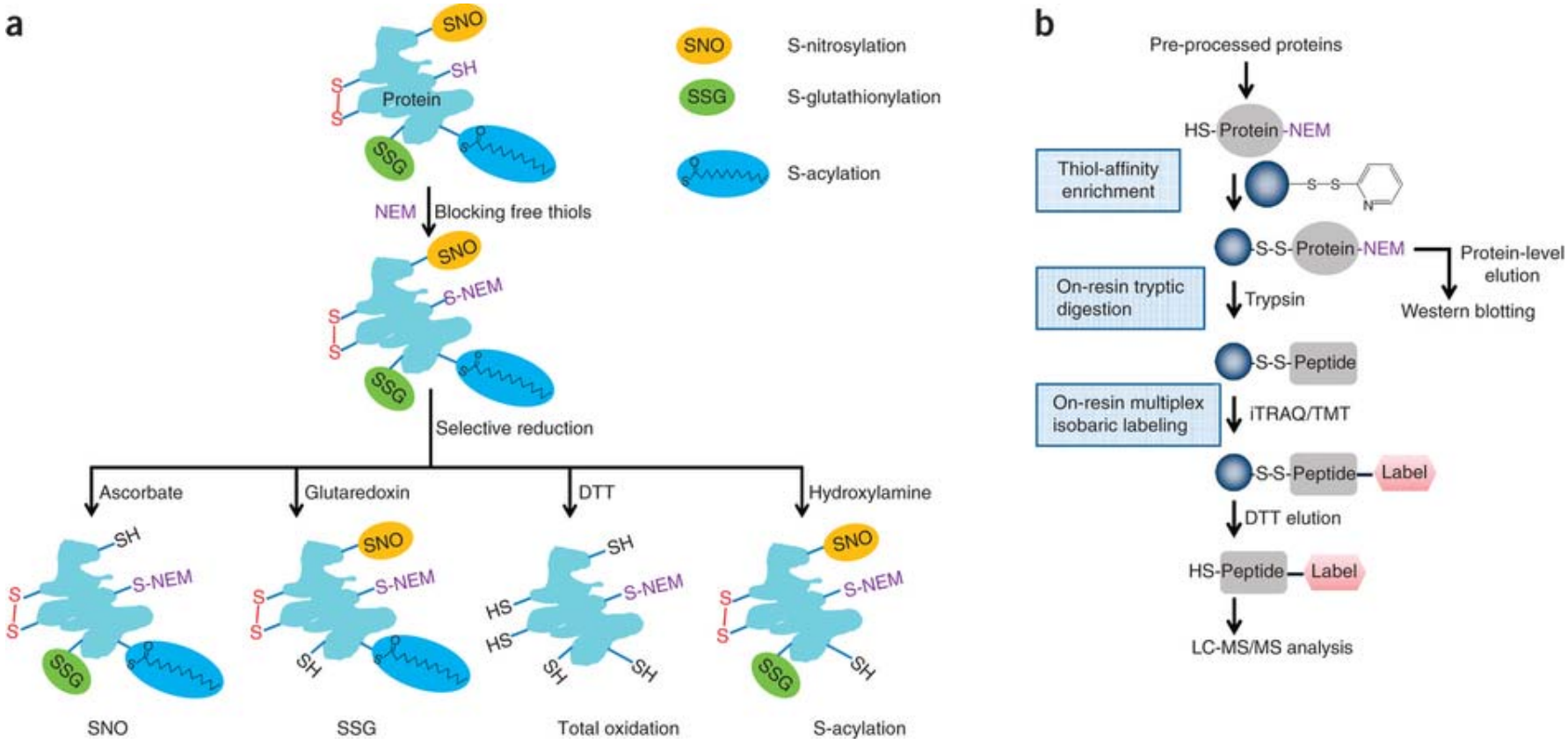
ACS Chem. Biol. **2011** Samie R. Jaffrey

Redox modifications


-SS-
Disulfide
-SH
via
radical
-SH
-SH
Free thiol
H_2O_2
-SOH
Sulfenic acid
H_2O_2
*
-SOH
Sulfinic acid
H_2O_2
-SOOH
Sulfonic acid
H_2S
GSNO
GSSG
GSH
-SSH
Sulfhydrated
thiol
(perthiol)
GSH
-SNO
Nitrosothiol
GSH
-SSG
Glutathionylated
thiol
GSH
S
N
Sulfenylamide
ROS

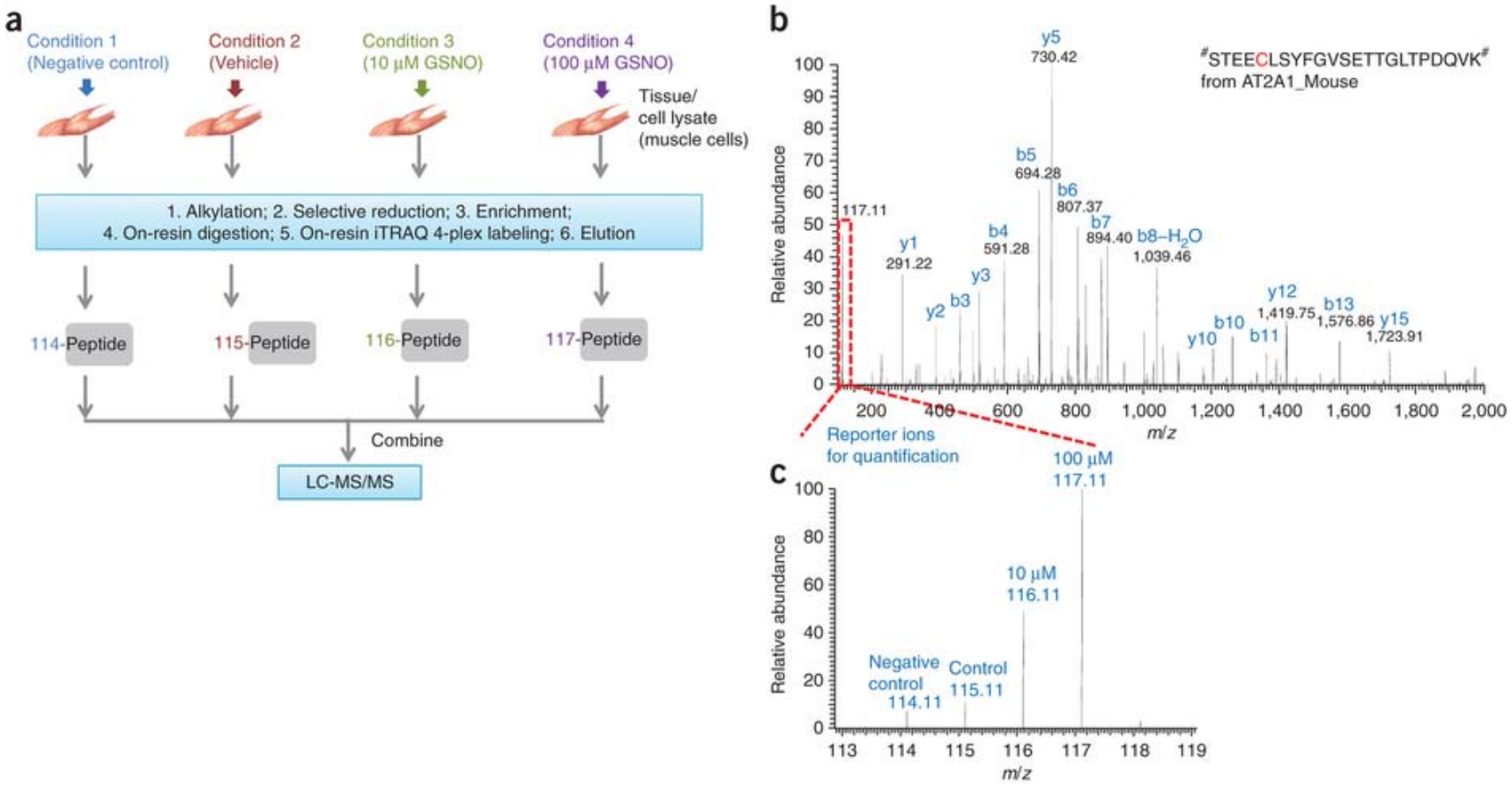

Cell surface receptor
NO_X
Plasma membrane
NOS
NO or SNO
SH
SNO
Protein
Protein
Trx/TrxR
GSH/GSNOR
Changes in protein activity, localization, stability and interactions
Signal modulation, adaption and stress response

Resin-assisted enrichment of cysteine-modifications

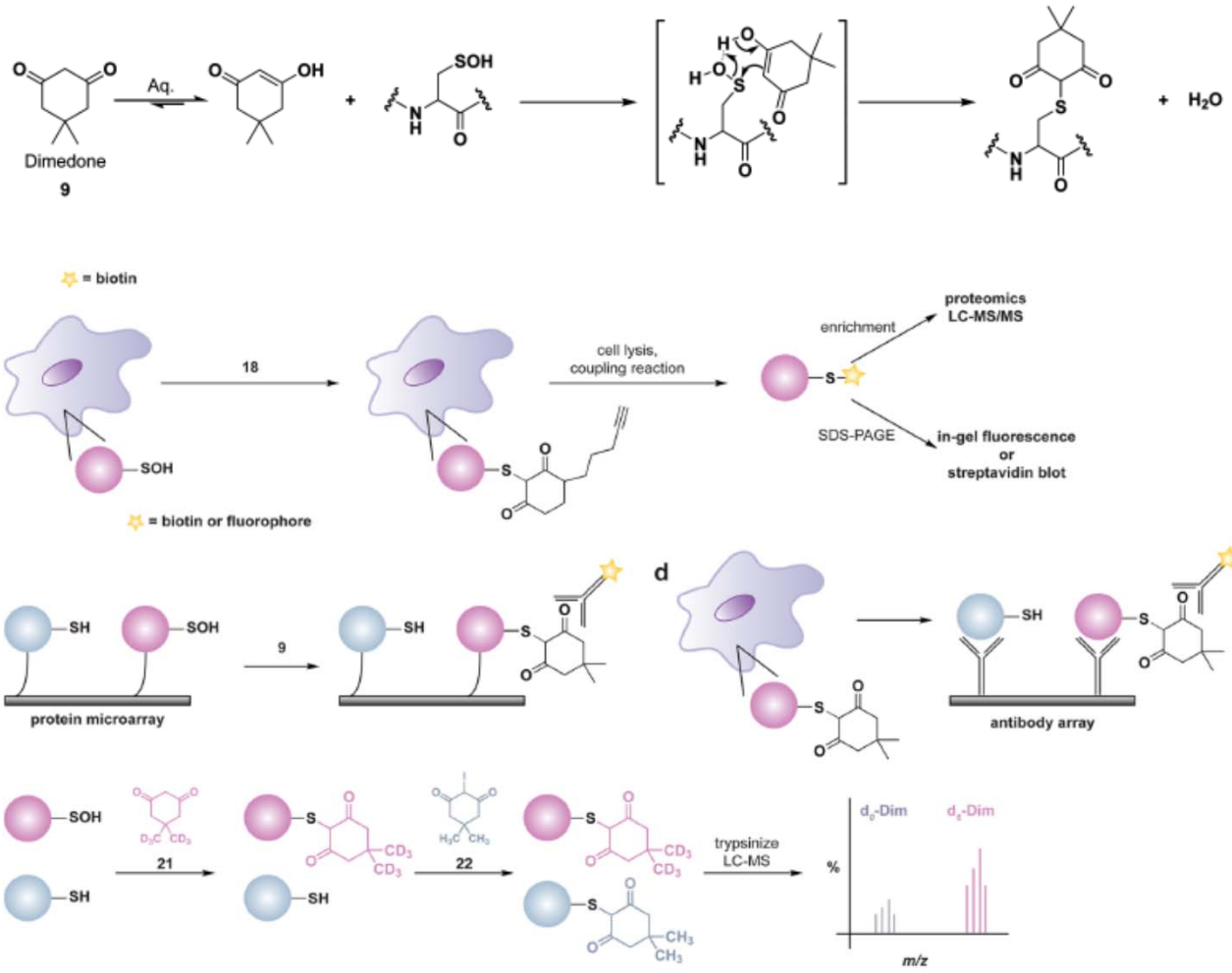


Qian et al ***Nature Protocols*** 2014 Jan;9(1):64-75

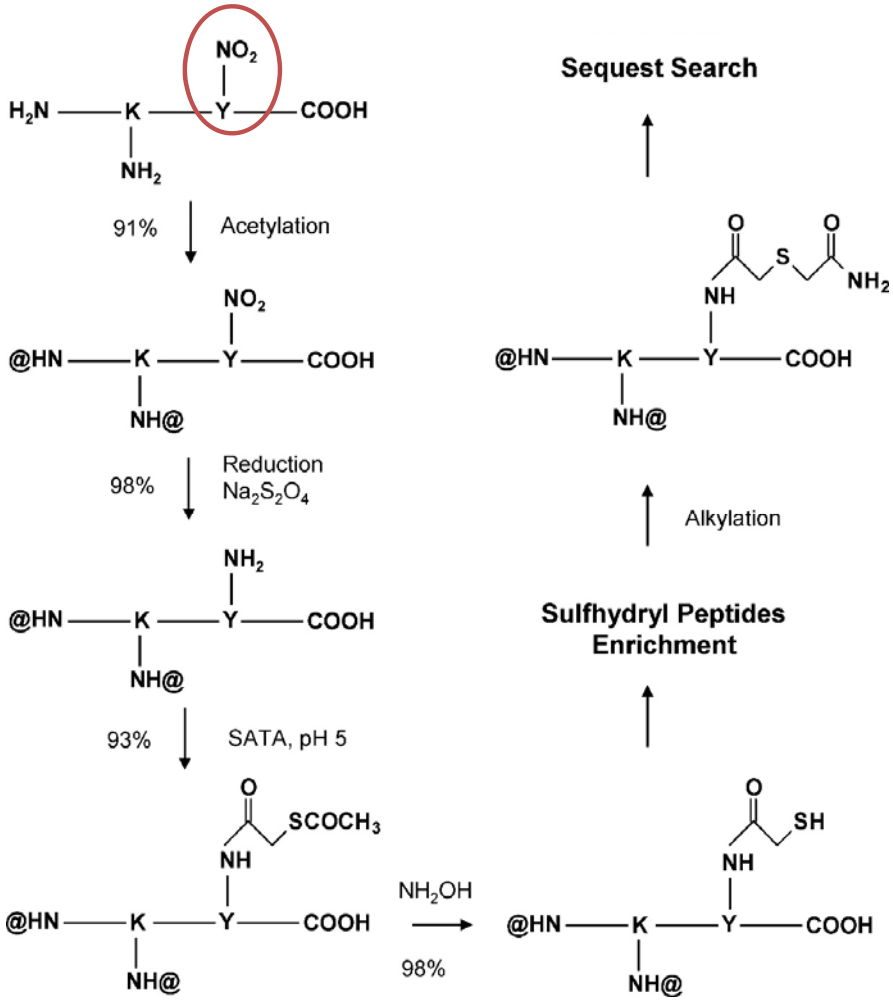
Resin-assisted enrichment of cysteine-modifications



Protein S-sulfenylation



Resin-assisted enrichment of nitrotyrosine



J Proteome Res. 2007 Jun;6(6):2257-68

Post-Translational Modifications (PTMs)

1. Phosphorylation
2. Ubiquitination
3. Lysine PTMs (acetylation, methylation, …)
4. Arginine methylation
5. Glycosylation (N-linked & O-linked)
6. Proteolytic processing (N-/C-terminomics)
7. Cysteine redox PTMs …
8. Tyrosine nitration