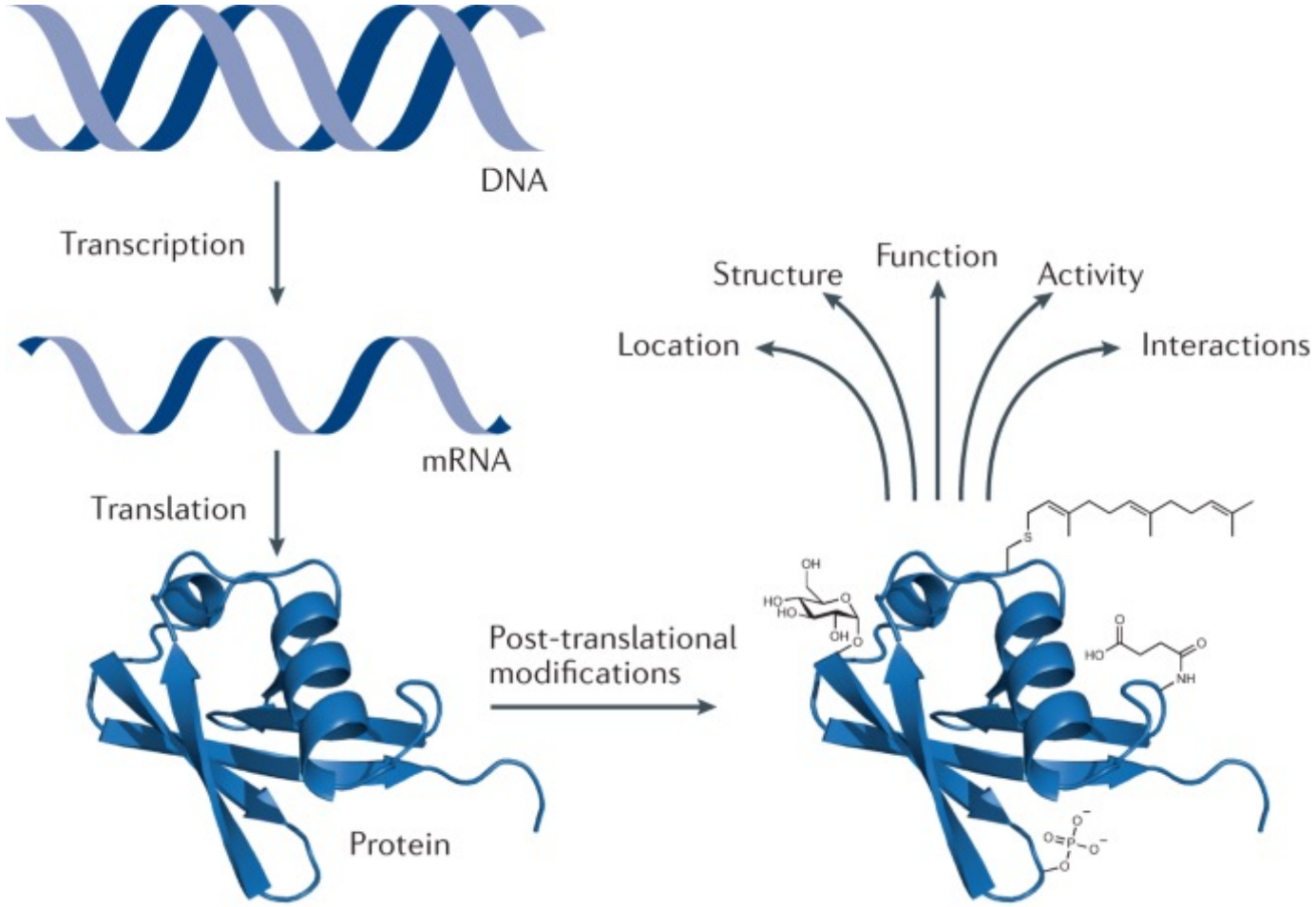
# LECTURE 5: PROTEIN POST-TRANSLATIONAL MODIFICATIONS

# Protein Post-translational Modifications (PTMs)




- PTM analysis is a discipline unique to proteomics.
  - Detect and/or quantify as many PTMs as possible from a complex mixture o proteins;
  - Find “all” PTMs in a single protein or small group of proteins.

Conibear, A.C. Deciphering protein post-translational modifications using chemical biology tools. *Nat Rev Chem* **4**, 674–695 (2020). https://doi.org/10.1038/s41570-020-00223-8

# Protein Post-translational Modifications (PTMs)

- What are they?
  Addition of groups or deletion of parts/amino acids to make functionally different proteins.

- Types of PTMs
  - Methylation
  - Acetylation
  - Glycosylation
  - Phosphorylation
  - Ubiquitination
  - etc. (how many??)

Currently about 1,530 documented modifications

**A Modification Database**

**UNIMOD** protein modifications for mass spectrometry

Help

Unimod  Logged as **Guest** Log out  Change password  Advanced search

Search for: Any field | Contains | Search | Show all

Details found: 1530
Page 1 of 77

Records Per Page:: 20

| | Accession # | PSI-MS Name | Interim name | Description | Monoisotopic mass | Average mass | Composition |
|---|---|---|---|---|---|---|---|
| View | 1287 | | Arg-loss | Loss of arginine due to transpeptidation | -156.101111 | -156.1857 | H(-12) C(-6) N(-4) O(-1) |
| View | 765 | | Met-loss | Removal of initiator methionine from protein N-terminus | -131.040485 | -131.1961 | H(-9) C(-5) N(-1) O(-1) S(-1) |
| View | 676 | | Trp->Gly | Trp->Gly substitution | -129.057849 | -129.1586 | H(-7) C(-9) N(-1) |
| View | 313 | Lys-loss | -K | Loss of Lysine | -128.094963 | -128.1723 | H(-12) C(-6) N(-2) O(-1) |
| View | 1224 | | Trp->Ala | Trp->Ala substitution | -115.042199 | -115.1320 | H(-5) C(-8) N(-1) |
| View | 1239 | | Tyr->Gly | Tyr->Gly substitution | -106.041865 | -106.1219 | H(-6) C(-7) O(-1) |
| View | 646 | | Arg->Gly | Arg->Gly substitution | -99.079647 | -99.1344 | H(-9) C(-4) N(-3) |
| View | 673 | | Trp->Ser | Trp->Ser substitution | -99.047285 | -99.1326 | H(-5) C(-8) N(-1) O |
| View | 400 | Tyr->Dha | DehydroalaY | Dehydroalanine (from Tyrosine) | -94.041865 | -94.1112 | H(-6) C(-6) O(-1) |
| View | 1237 | | Tyr->Ala | Tyr->Ala substitution | -92.026215 | -92.0954 | H(-4) C(-6) O(-1) |
| View | 1093 | | Phe->Gly | Phe->Gly substitution | -90.046950 | -90.1225 | H(-6) C(-7) |
| View | 766 | | Met-loss+Acetyl | Removal of initiator methionine from protein N-terminus, then acetylation of the new N-terminus | -89.029920 | -89.1594 | H(-7) C(-3) N(-1) S(-1) |
| View | 1232 | | Trp->Pro | Trp->Pro substitution | -89.026549 | -89.0947 | H(-3) C(-6) N(-1) |
| View | 1235 | | Trp->Val | Trp->Val substitution | -87.010899 | -87.0788 | H(-1) C(-6) N(-1) |
| View | 1189 | | Arg->Ala | Arg->Ala substitution | -85.063997 | -85.1078 | H(-7) C(-3) N(-3) |
| View | 1234 | | Trp->Thr | Trp->Thr substitution | -85.031634 | -85.1060 | H(-3) C(-7) N(-1) O |
| View | 674 | | Trp->Cys | Trp->Cys substitution | -83.070128 | -83.0670 | H(-5) C(-8) N(-1) S |
| View | 1117 | | His->Gly | His->Gly substitution | -80.037448 | -80.0880 | H(-4) C(-4) N(-2) |
| View | 679 | | Tyr->Ser | Tyr->Ser substitution | -76.031300 | -76.0960 | H(-4) C(-6) |
| View | 1090 | | Phe->Ala | Phe->Ala substitution | -76.031300 | -76.0960 | H(-4) C(-6) |

# Types of Protein Post-translational Modifications (PTMs)

| Modification | Description |
|---|---|
| Acetylation | *N*-terminal of some residues and side chain of lysine, +42 Da |
| Amidation | Generally at the *C*-terminal of a mature active peptide, -1 Da |
| Formylation | Generally of the *N*-terminal methionine, +28 Da |
| Glycosylation | *N*-linked (Asn), *O*-linked (Ser or Thr), and *C*-linked (Trp), more than +162 Da, up to kDa |
| Phosphorylation | Of serine, threonine, tyrosine, +80 Da |
| Sulphation | Of tyrosine, serine or threonine, +80 Da |
| Methylation | Of lysine and arginine, +14 Da |
| Ubiquitination | Of lysine, +114 Da |

For Δmasses, visit: https://abrf.org/delta-mass

# PTMs

| *Location* | *Modification* |
| --- | --- |
| Nucleus | acetylation, phosphorylation, methylation |
| Lysosome | mannose-6-phosphate labelled *N*-linked sugar |
| Mitochondria | *N*-formyl acylation |
| Golgi | *N*- and *O*-linked oligosaccharide, sulphation, palmitoylation |
| ER | *N*-linked oligosaccharide, GPI-anchor |
| Cytosol | acetylation, methylation, phosphorylation, |
| Ribosome | myristoylation |
| Plasma membrane | *N*- and *O*-glycosylation, GPI-anchor |
| Extraceullar fluid | *N*- and *O*-glycosylation, acetylation, phosphorylation |
| Extrallular matrix | *N*- and *O*-glycosylation, phosphorylation, hydroxylation |

# Why Are We Interested in Studying Protein Post-translational Modifications?

- Cells can rapidly respond to stimuli and perturbations
  - Protein expression is much too slow for quick adaption
- PTMs are crucial regulators
  - Important cellular mechanisms are tightly controlled
  - Activation, folding, stabilization, interaction, localization, and secretion
- Often, diseases (e.g., cancer) are due to aberrantly activated proteins

# Protein Methylation

- Multiple sites and to different degrees (mono-, di-, trimethyl)
  - Lys, Arg
  - Histones, Transcription factors, etc.

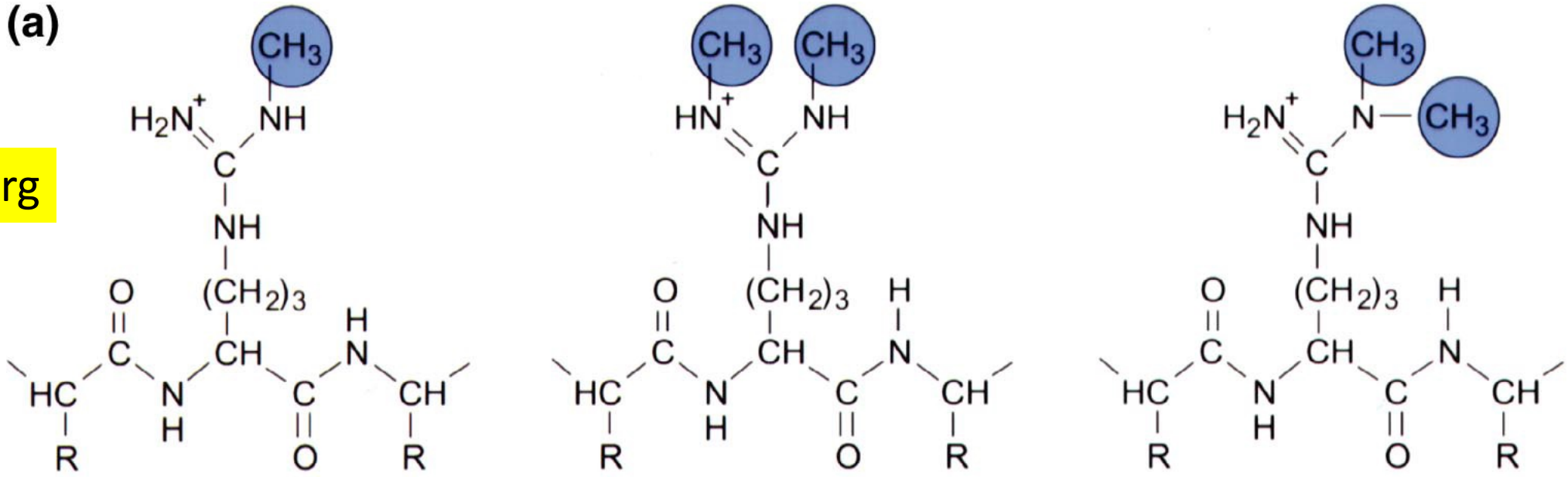
**Table I. Functions of protein-bound arginine and lysine methylation**

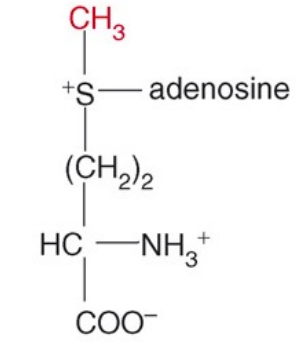
| Type of methylation | Postulated or proven function | Proteins involved |
|---|---|---|
| Protein-arginine | mRNA splicing | Motor neuron proteins |
| | Signal transduction | Interferon receptor |
| | Cellular proliferation | Transcription factor |
| | Chromatin remodeling | Histones |
| | Transcriptional coactivator | Nuclear receptor, p-53 |
| | Protein–protein interaction | Inter- and intra-molecules |
| | Translocation | hnRNP |
| | Myelogenesis | Myelin basic protein |
| Protein-lysine | Chromatin remodeling | Histones |
| | Increased protein stability *in vivo* | Cytochrome *c* |
| | Resistance to proteolytic digestion *in vitro* | Cytochrome *c* |
| | Biomineralization | Silaffins in marine diatom |
| | Modulation of NAD kinase | Calmodulin |

Paik, W. K., Paik, D. C., & Kim, S. (2007). *32*(3), 146–152. https://doi.org/10.1016/j.tibs.2007.01.006

(a)

Arg

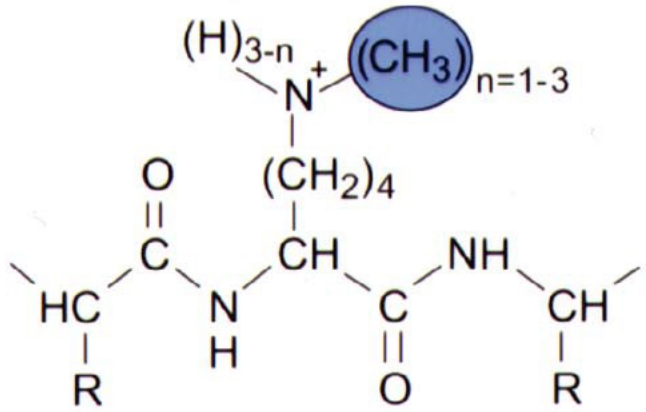





S-adenosyl-L-methionine (AdoMet)

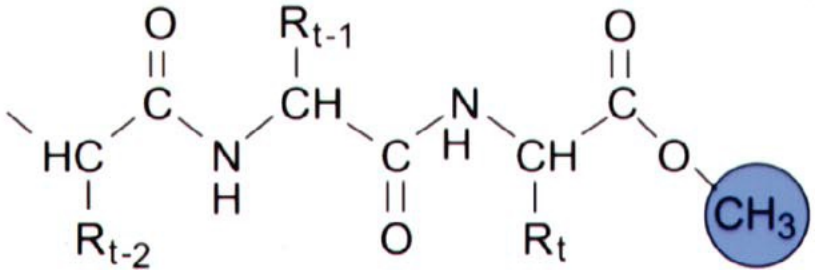
(b)

Lys




(c)

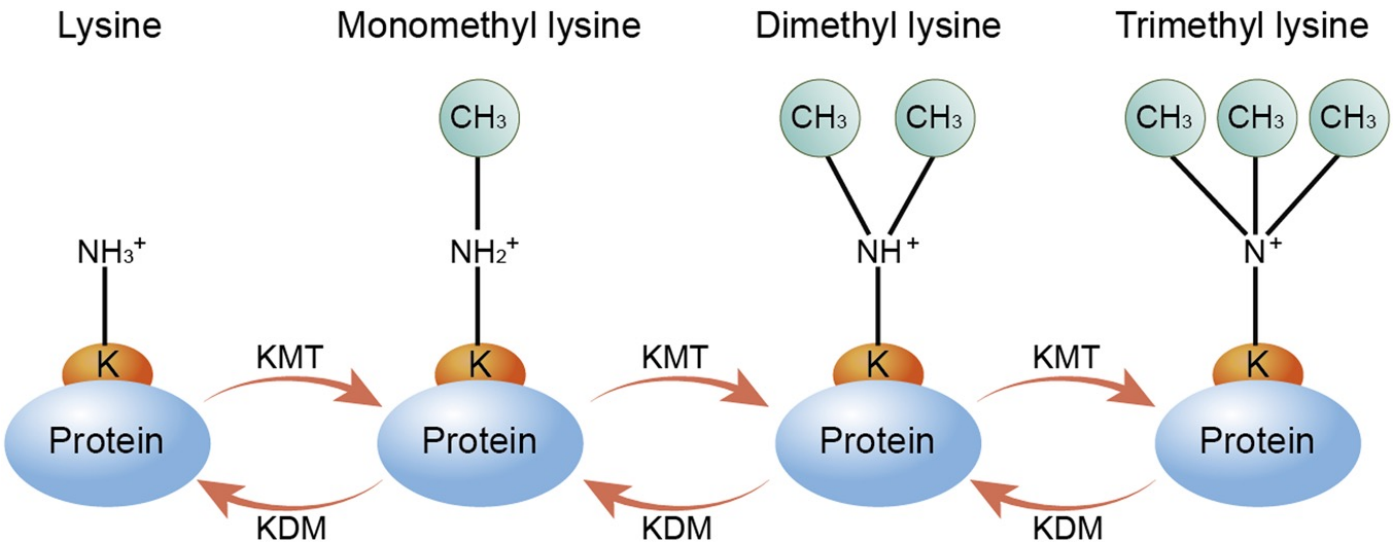
C-terminal carboxyl group




Aletta, J. M., Cimato, T. R., & Ettinger, M. J. (1998). *Trends in biochemical sciences*, *23*(3), 89–91.

# Lysine methylation of transcription factors in cancer

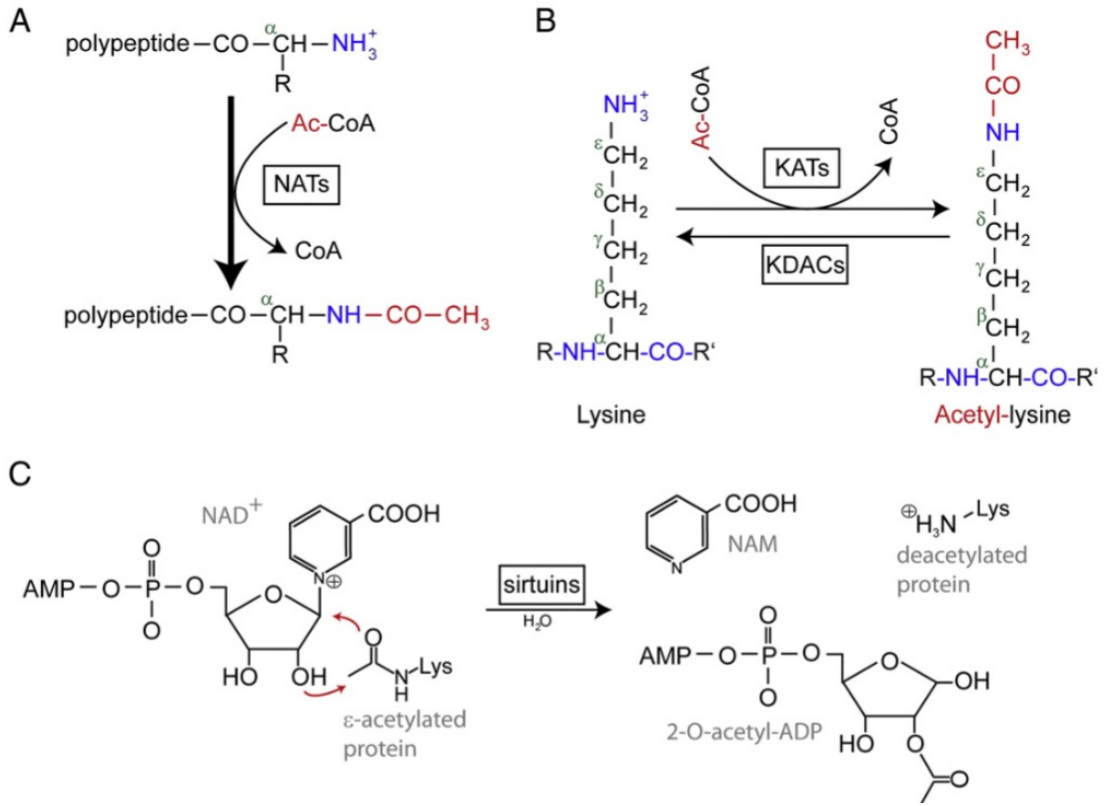
- Interfering with transcription factor lysine methylation can inhibit cancer cell proliferation, thereby reversing tumor progression.
- The inhibitors targeting lysine methyltransferases (KMTs) and lysine demethylases (KDMs) may be used as potential anticancer agents in the clinic.




Han, D., Huang, M., Wang, T., Li, Z., Chen, Y., Liu, C., Lei, Z., & Chu, X. (2019). *10*(4), 290. https://doi.org/10.1038/s41419-019-1524-2

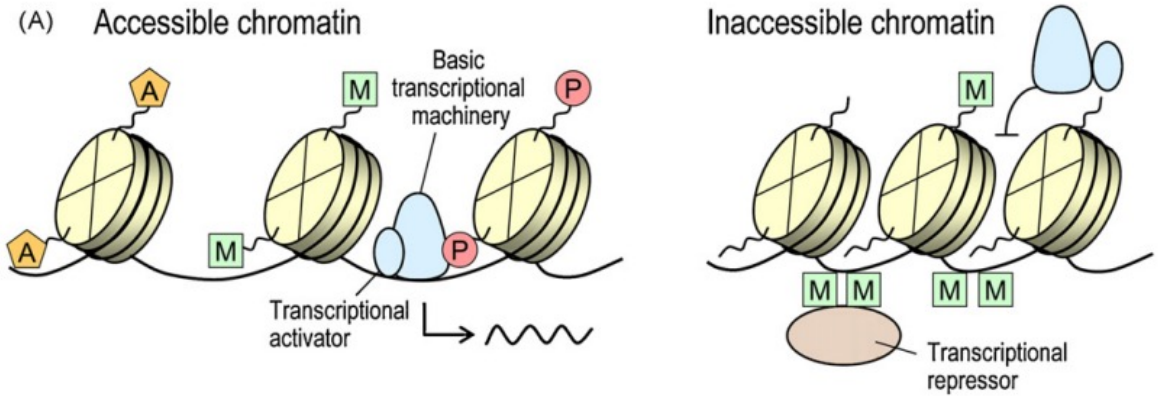
# Acetylation

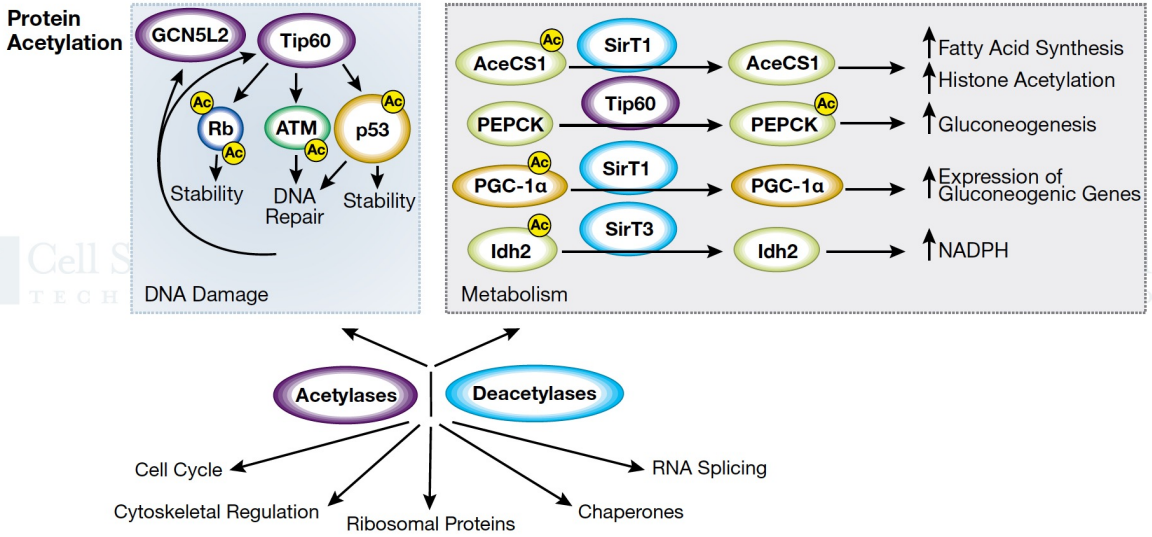
- Protein acetylation involves the process that acetyl group from acetyl coenzyme A (Ac-CoA) is transferred to a specific site on a polypeptide chain.




- In humans, a big majority (80-90%) of proteins are co-translationally acetylated at their N-termini by N-terminal acetyltransferases (NATs).
- Reversible acetylation on ε-amino group of a lysine residue is catalyzed by lysine acetyltransferases (KATs) and lysine deacetylases (KDACs) .

# Examples of Protein Acetylation



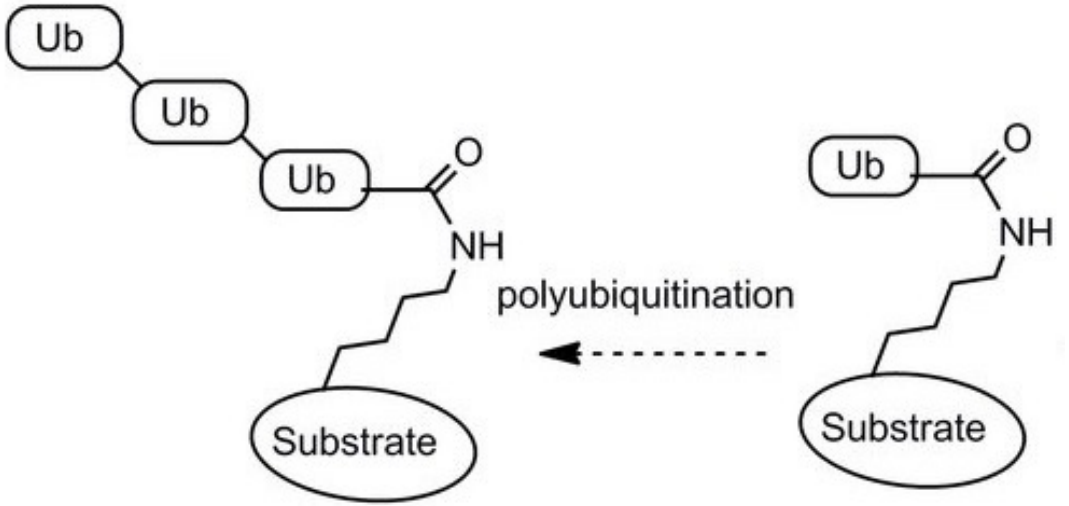




- Regulating chromatin structure and transcriptional activity
  - In histone acetylation, acetyl groups are attached to positively charged lysine in histone tails. Acetylation makes histones less positive, so less attraction to DNA. This generally loosens chromatin structure, promoting the initiation of transcription.
- Important role in immunity, circadian rhythmicity, and memory formation
- Favorable target in drug design for numerous disease conditions

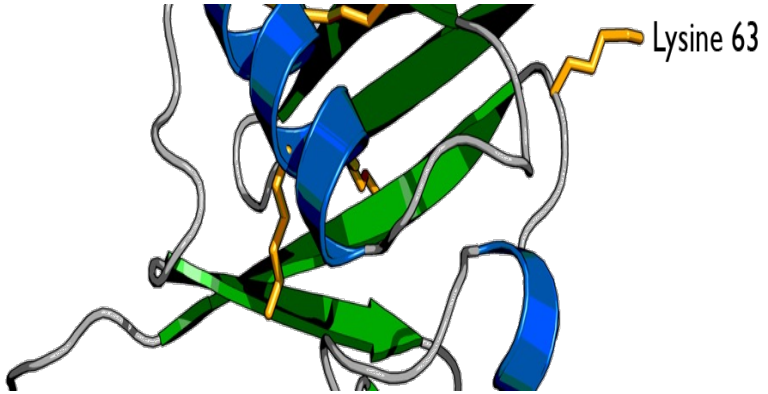
Johannes Graff, Isabelle M. Mansuy. Behavioural Brain Research 192 (2008) 70–87

# Ubiquitination

- Ubiquitination involves the attachment of the polypeptide **ubiquitin** to target proteins by a set of enzymes.
- Ubiquitin is a highly conserved 8 kD protein that tags proteins for destruction. Thereby denatured, damaged, or improperly translated proteins are removed from cells.

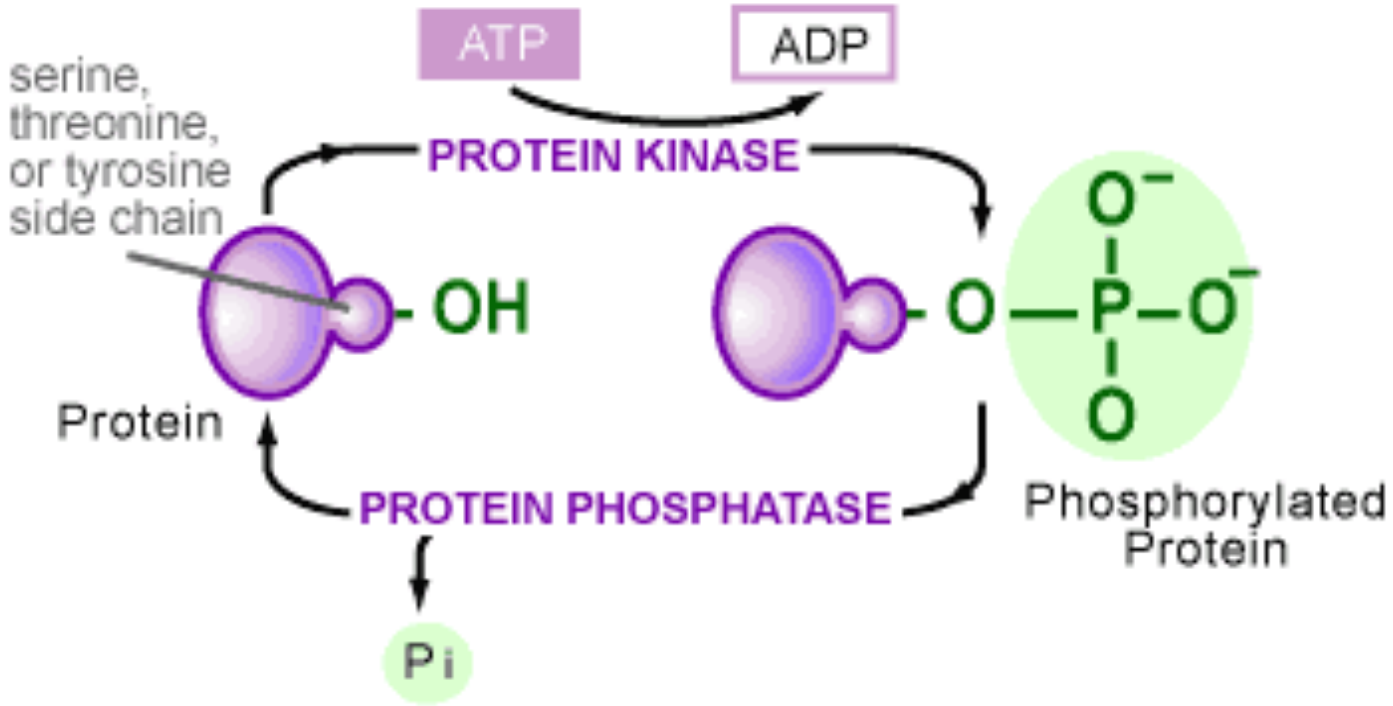



3 step ubiquitination




Ubiquitin

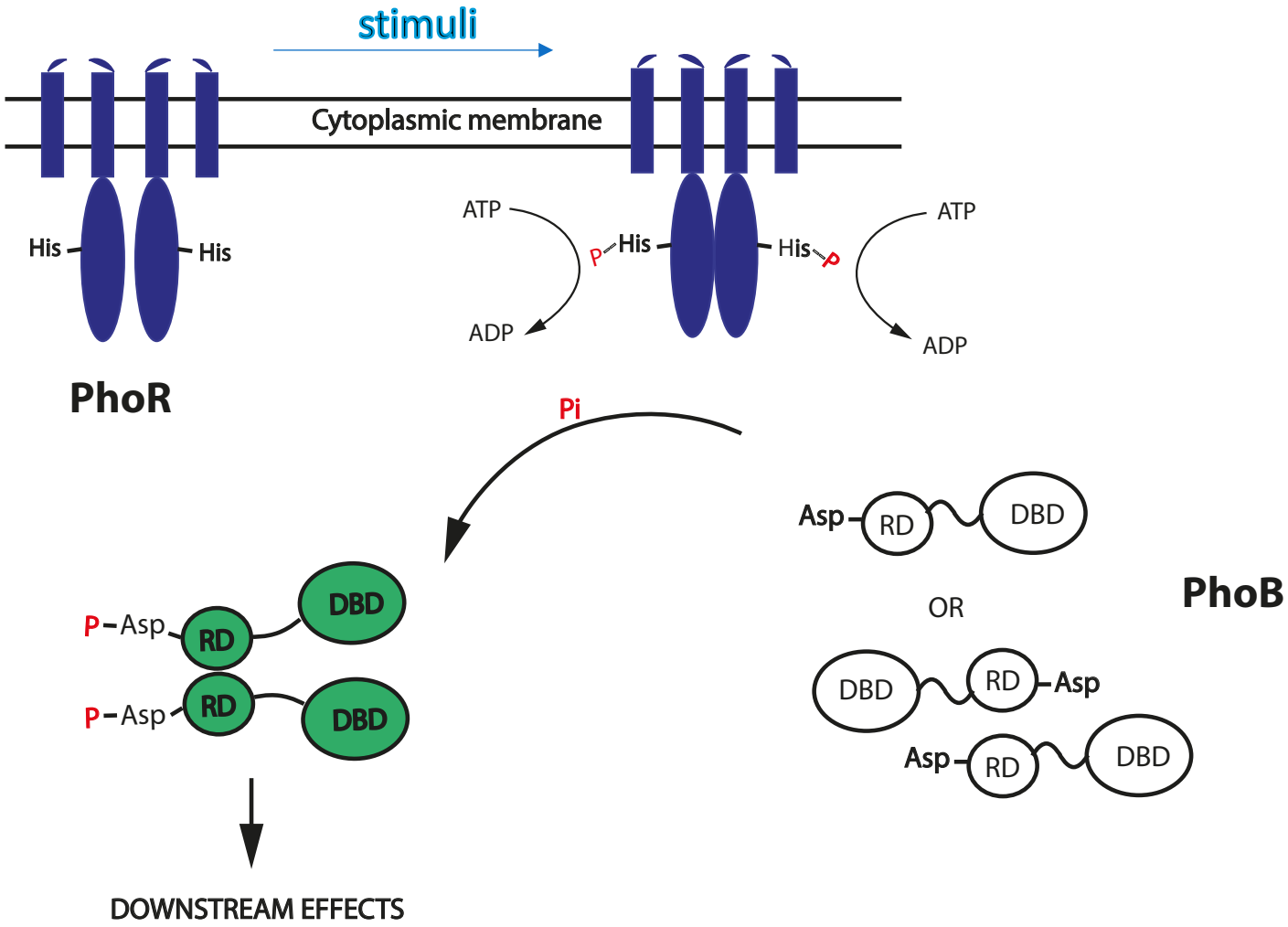
# Phosphorylation




- Protein phosphorylation is a reversible modification catalyzed by **kinases** and **phosphatases**.

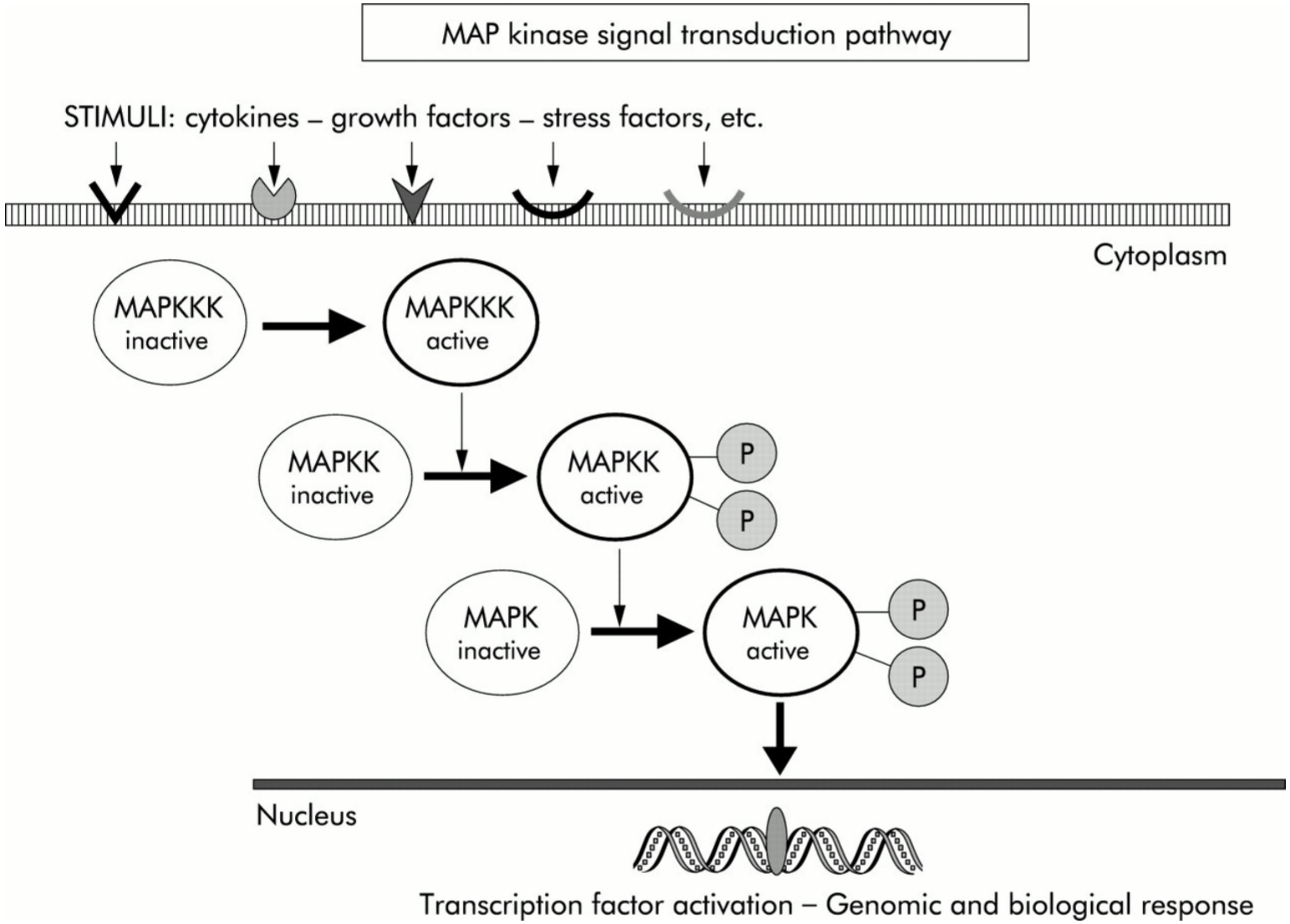
# Examples of Protein Phosphorylation

Two component system in prokaryotes



# Examples of Protein Phosphorylation

In eukaryotes



# Glycosylation

- Glycosylation, the attachment of sugar moieties to proteins.
- **The very important and complex form of PTMs**

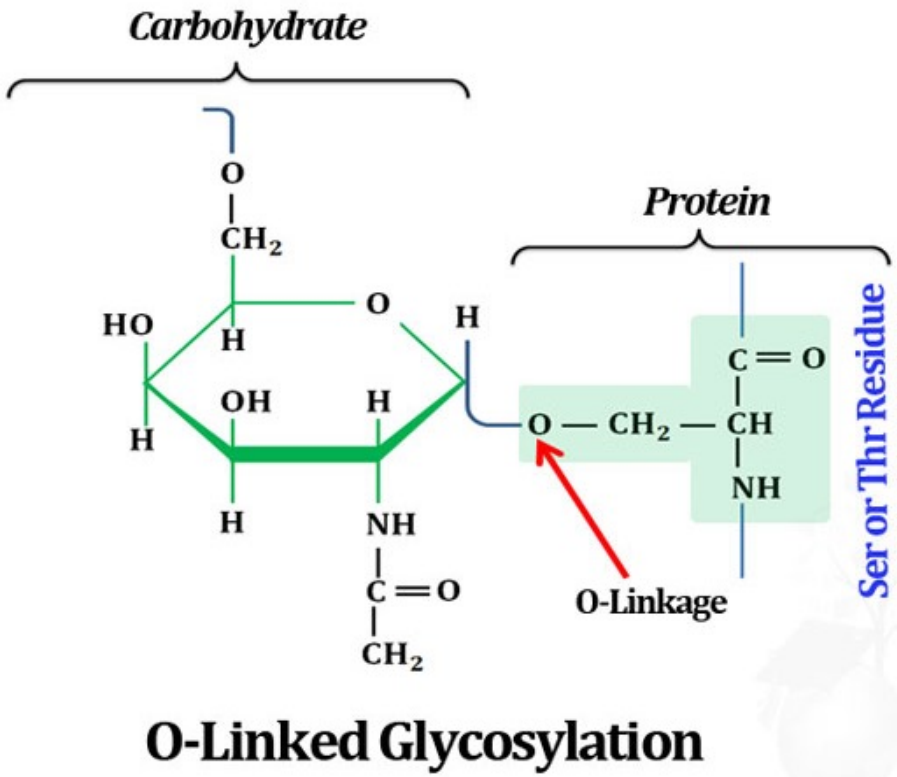
- Roles:
  - Folding (activity)
  - Localization
  - Stability (half life)
  - Interactions
  - Linked to diseases

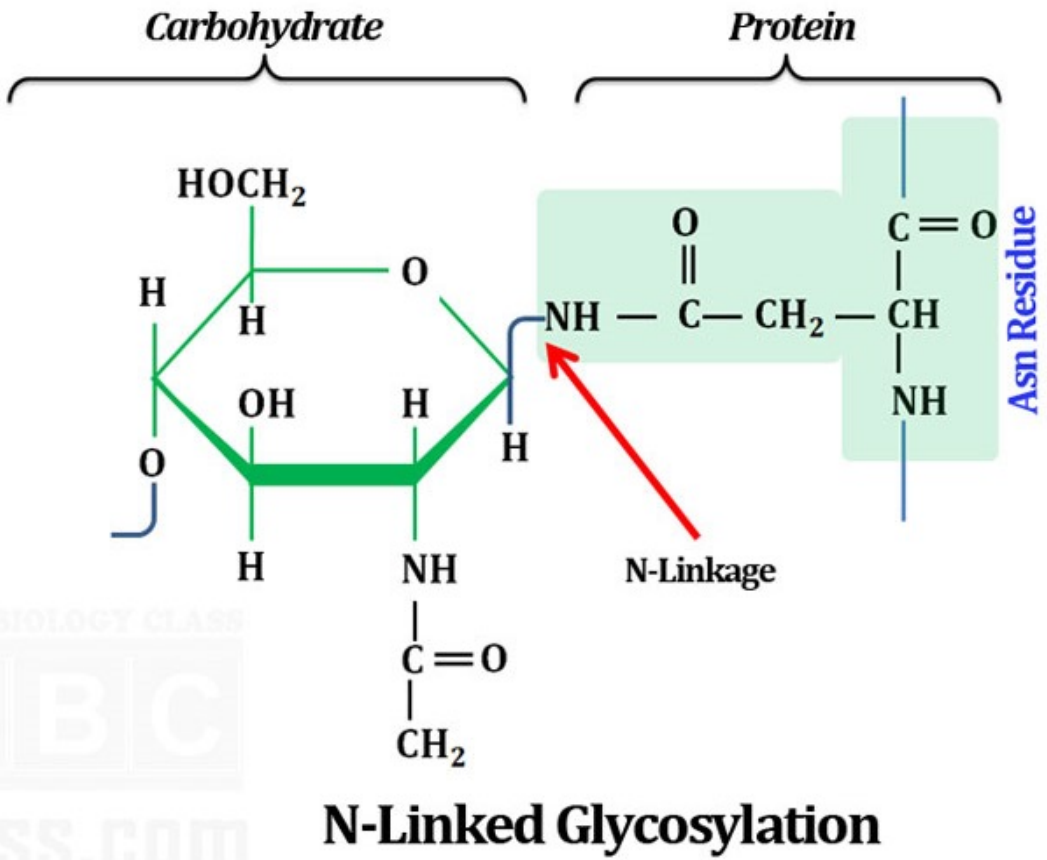
| Diseases | Glycoprotein | Alteration |
|---|---|---|
| Hepatic cancer | $\alpha$-fetoprotein | Different N-glycan structures |
| Immune disorder | CD43 | Different O-glycan structures |
| Rheumatoid arthritis | IgG | N-glycans, reduction in terminal galactosylation |
| Choriocarcinoma | hCG | N- and O-linked glycans, hyperbranching |
| Alcohol abuse | Transferrin | N-glycans, desialylation |

# Types of Glycosylation

- Based on the nature of the sugar-peptide bond and the oligosaccharide attached, glycosylation includes:
  - N-linked: Glycan binds to amino group of Asn (Asn-X-Ser/Thr/Cys) in the ER
  - O-linked: Monosaccharides bind to OH group of Ser/Thr in the Golgi stack
  - Glypiation: Glycan core links a phospholipid and a protein
  - C-linked: Mannose binds to the indole ring of Trp
  - Phosphoglycosylation: Glycan binds to Ser via phosphodiester bond

# Chemistry of Glycosylation



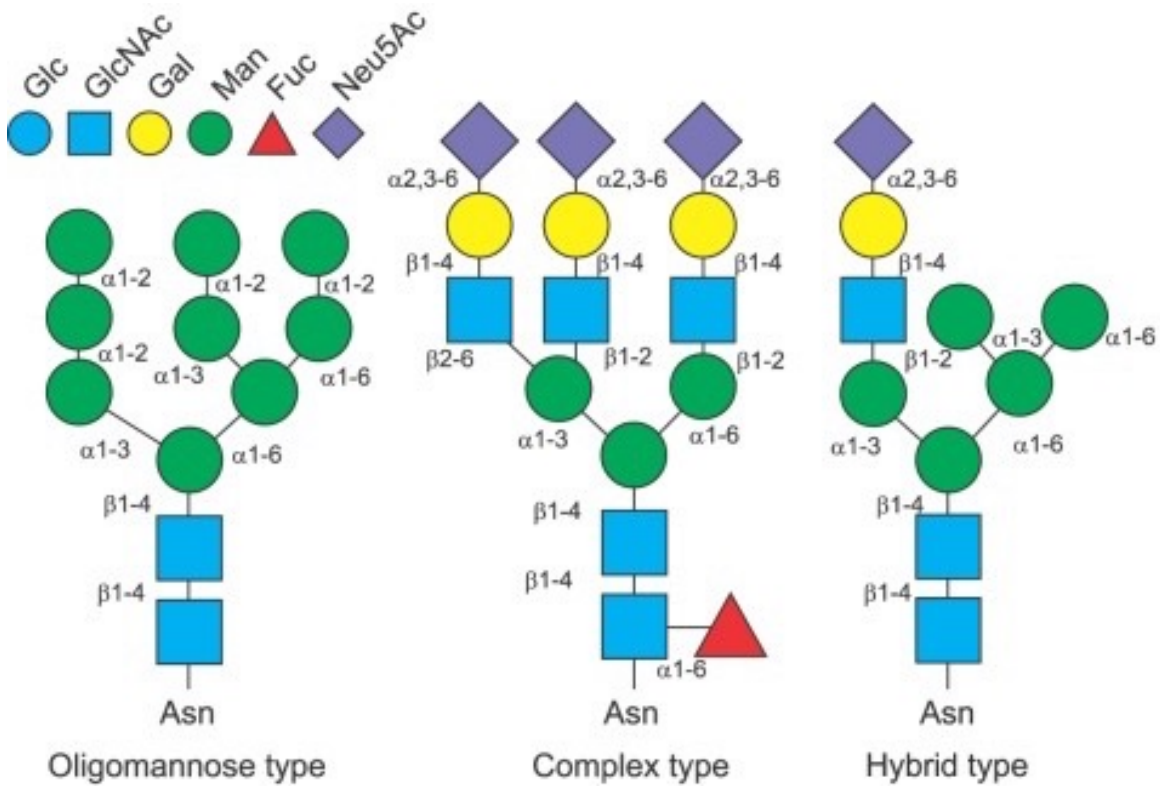



# Sugar Abbreviations

| | |
|---|---|
| Glucose | Glc |
| Galactose | Gal |
| Mannose | Man |
| Rhamnose | Rha |
| Fucose | Fuc |
| Glucosamine | GlcN |
| Galactosamine | GalN |
| Mannosamine | ManN |
| N-acetylglucosamine | GlcNAc |
| N-acetylgalactosamine | GalNAc |
| N-acetylmannosamine | ManNAc |
| Glucuronic acid | GlcA |
| Galacturonic acid | GalA |
| Mannuronic acid | ManA |
| N-acetylneuraminic acid | NeuNAc |
| 3-deoxy-D-manno-2-octulosonic acid | Kdo |

# *N*-Linked Glycans

- *N*-linked glycans are covalently attached to Asn residues within a consensus sequence (Asn-Xaa-Ser/Thr/Cys), enabling prediction of the modification sites by protein sequence analysis
- All *N*-linked glycans share a common pentasaccharide core (GlcNAc2Man3) recognized by **lectins** and ***N*-glycanase enzymes (PNGase F)**
- These reagents have been used to visualize proteins bearing *N*-linked glycans from cell or tissue lysates and to enrich them for mass spectrometry analysis



# *O*-Linked Glycans

- Comparable tools are lacking for the study of proteins bearing *O*-linked glycans.

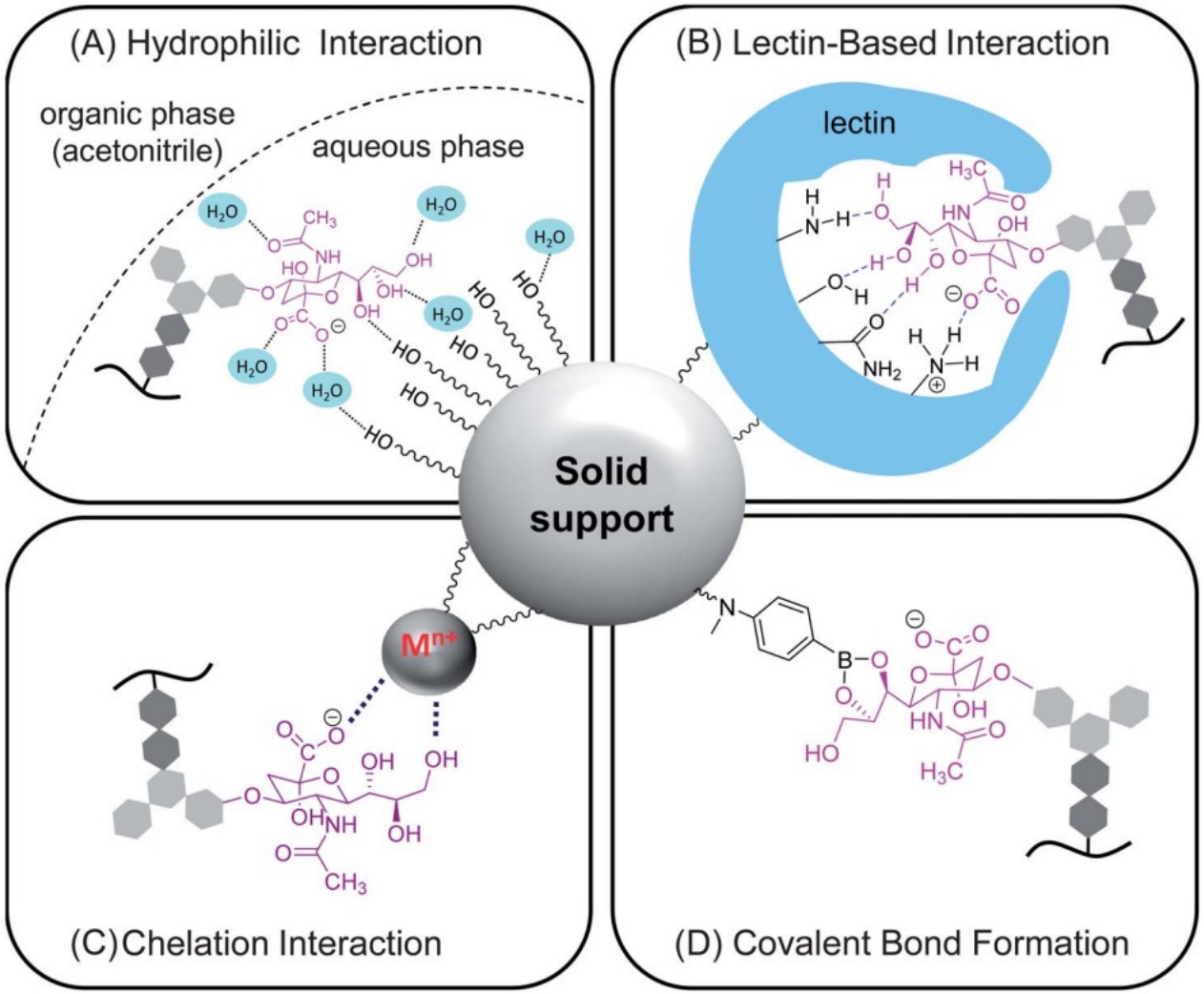
- Mucin-type, the most prevalent *O*-linked glycosylation is characterized by an *N*-acetylgalactosamine (GalNAc) residue -linked to the hydroxyl group of Ser or Thr. GalNAc residue is installed by a family of 24 *N*-acetyl-galactosaminyltransferases, then further elaborated by a series of glycosyltransferases to generate higher-order *O*-linked structures.

- Because of the complex biosynthetic origin, *O*-linked glycans are not installed at a defined consensus motif and their presence cannot be accurately predicted based on the protein's primary sequence.

# Glycoproteomics

- **Glycoproteomics** analyzes the totality of **glycoproteins** (e.g., molecules consisting of a protein part and a sugar part [**glycan**]).
- Glycosylation of proteins mainly takes place in the endoplasmic reticulum (ER) and the Golgi
- Enzymes, such as glycosyltransferases and glycosidases, attach or remove the sugar groups to/from the proteins
- These enzymes mainly target S, T and N
- It impacts on charge, conformation and protein stability
- Glycosylation is the most complex of all PTMs
  - Glycans (polysaccharides) represent a huge variety in their composition

# Enrichment of Glycopeptides




- Due to low abundance glycoproteins/glycopeptides usually need to be enriched
- **Lectin affinity chromatography**
  - Lectins are sugar-binding proteins
  - Lectins play a role in many cellular processes, for example in virus attachment to host cells, where the lectin's affinity to glycosylated membrane proteins is used by the virus
  - Many lectins are available that have very specific ligands

Chen-Chun Chen *et al*. Analyst, 2014, 139, 688–704

# Lectins- carbohydrate-binding proteins

Lectins read sugar code and mediate many biological processes :

- Cell-cell recognition
- Signaling
- Adhesion
- Intracellular targeting of newly synthesized proteins

Commonly used lectins for enrichment of glycoproteins or glycopeptides

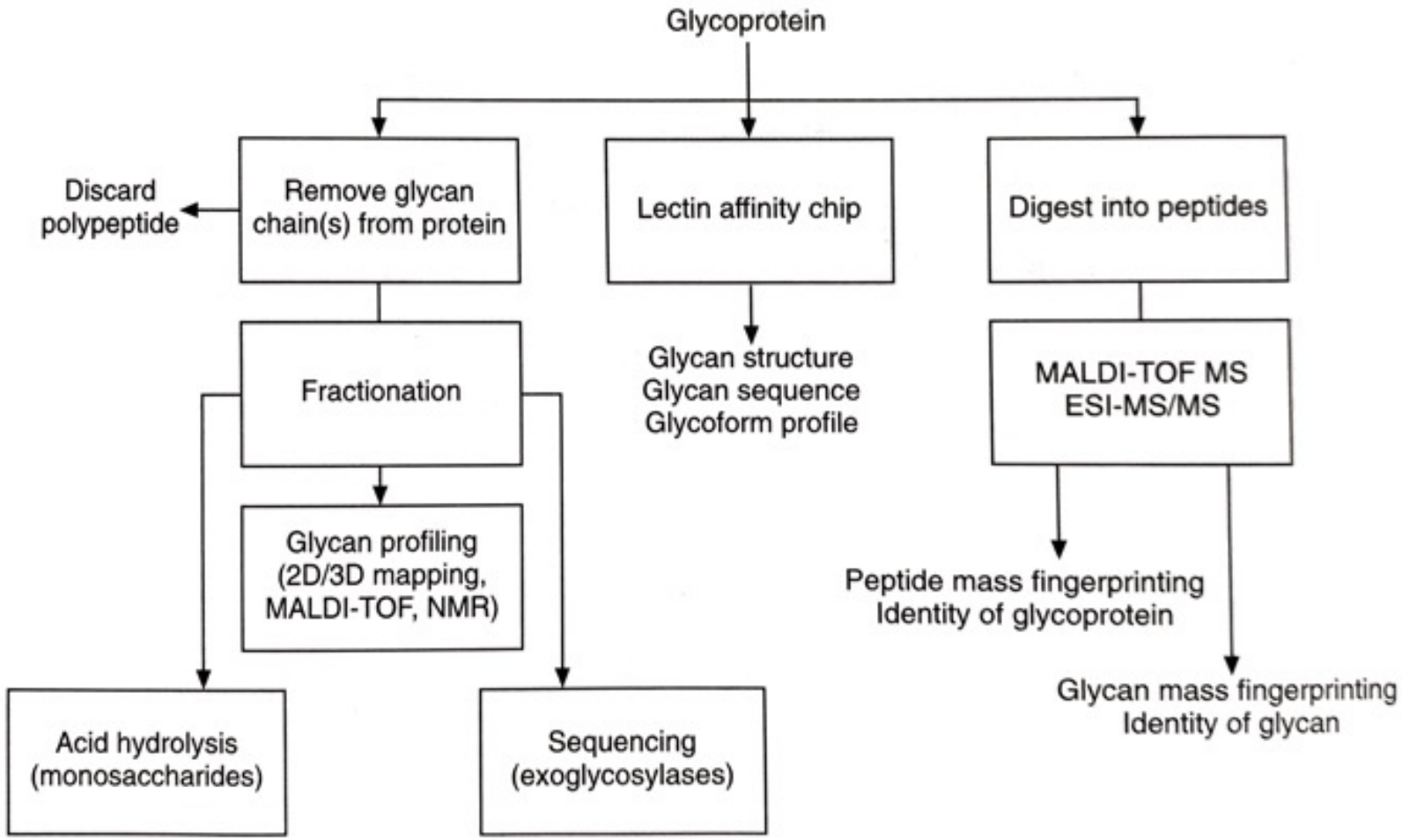
| Lectin | Specific Affinity |
|---|---|
| *Aleuria aurantia* lectin (AAL) | Fucα1-6 GlcNAc, Fucα1-3(Galβ1-4)GlcNAc |
| Concanavalin A (Con A) | High-Mannose, Manα1-6(Manα1-3)Man |
| Jacalin | Galβ1-3GalNAc, GalNAc |
| *Phaseolus vulgaris* leucoagglutinin (PHA-L) | Tri/tetra-antennary complex-type *N*-glycan |
| *Ricinus communis* agglutinin-I (RCA-I) | Galβ1-4GlcNAcβ1-R |
| *Sambucus nigra* lectin (SNA, EBL) | Siaα2-6Gal/GalNAc |
| *Ulex europaeus* agglutinin-I (UEA-I) | Fucα1-2Galβ1-4GlcNAc |
| Wheat Germ Agglutinin (WGA) | Chitin oligomers, Sia |

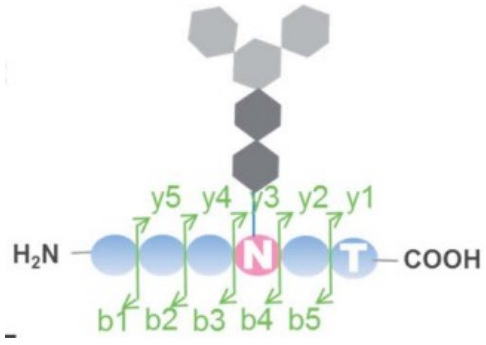
Chen-Chun Chen *et al*. Analyst, 2014, 139, 688–704

# Mass Spec Analysis of Glycopeptides

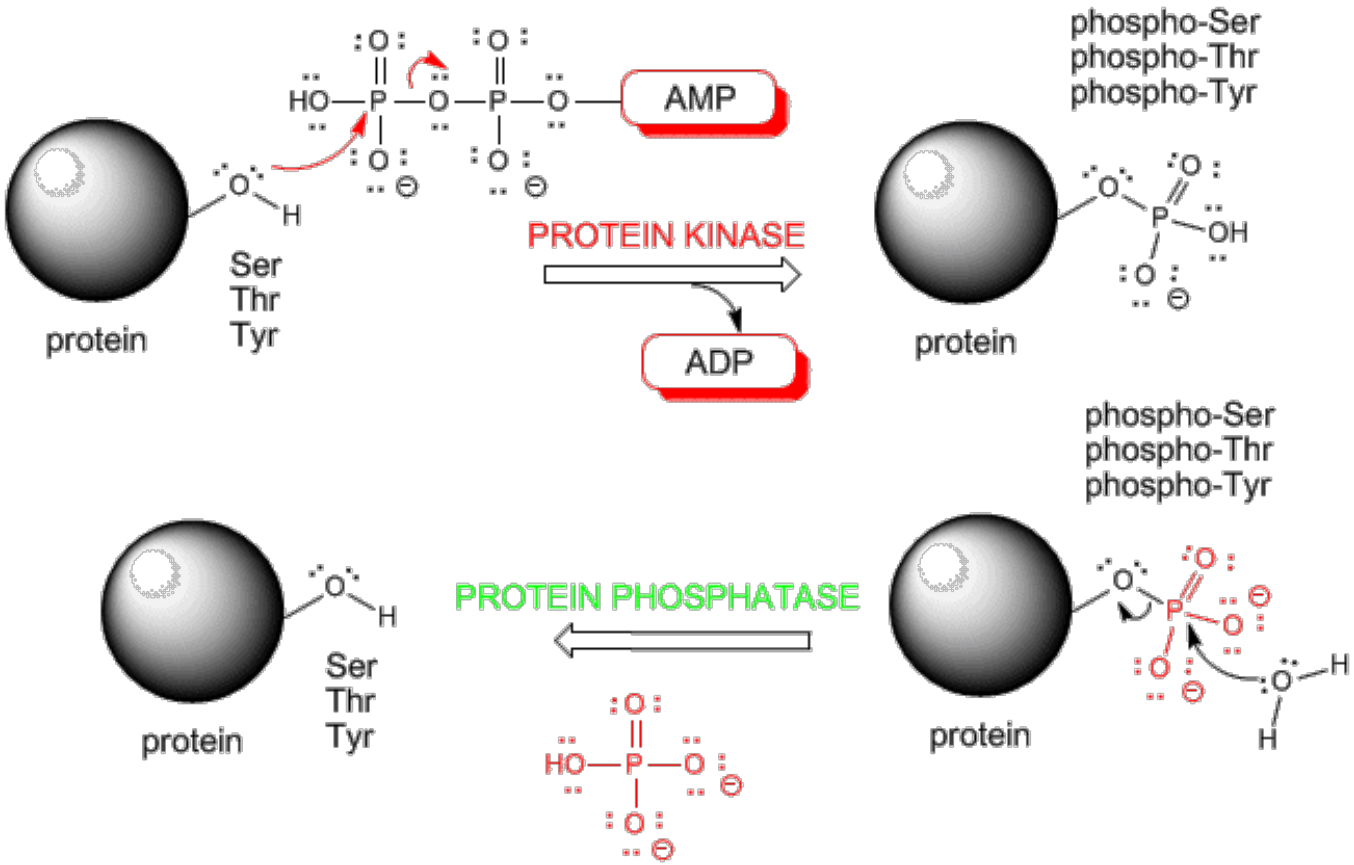
- The hydrophilic nature of glycans limits the surface activity and the ionization efficiency
  - Natural and basic glycoconjugates can be protonated
  - Acidic glycoconjugates can only be deprotonated (negative ESI mode!)
- Often, derivatization is used to increase hydrophobicity and volatility (and thereby ionization efficiency)
- As for unmodified peptides, MS/MS can be performed to sequence the glycan as well as the underlying peptides
- MS/MS of glycopeptides is more complicated than that of unmodified peptides, since the chemical properties of the peptide and glycan are not similar
  - Heterogeneity in glycan size and charge
- Using CID, the collision energy is highly important to the content of the MS/MS spectrum

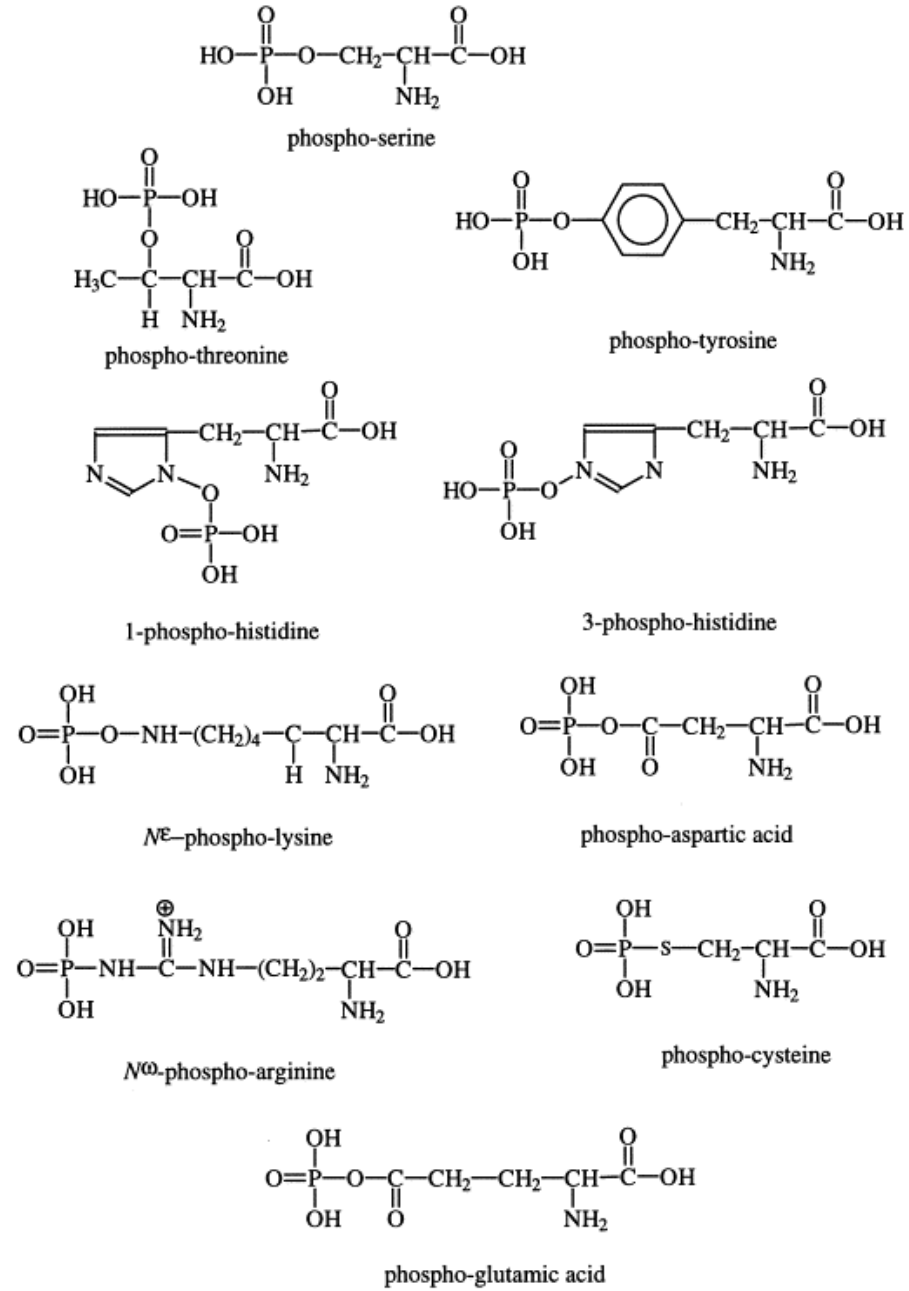
# General Work-flow For The Full Analysis Of Glycoproteins



# PhosphoProteomics



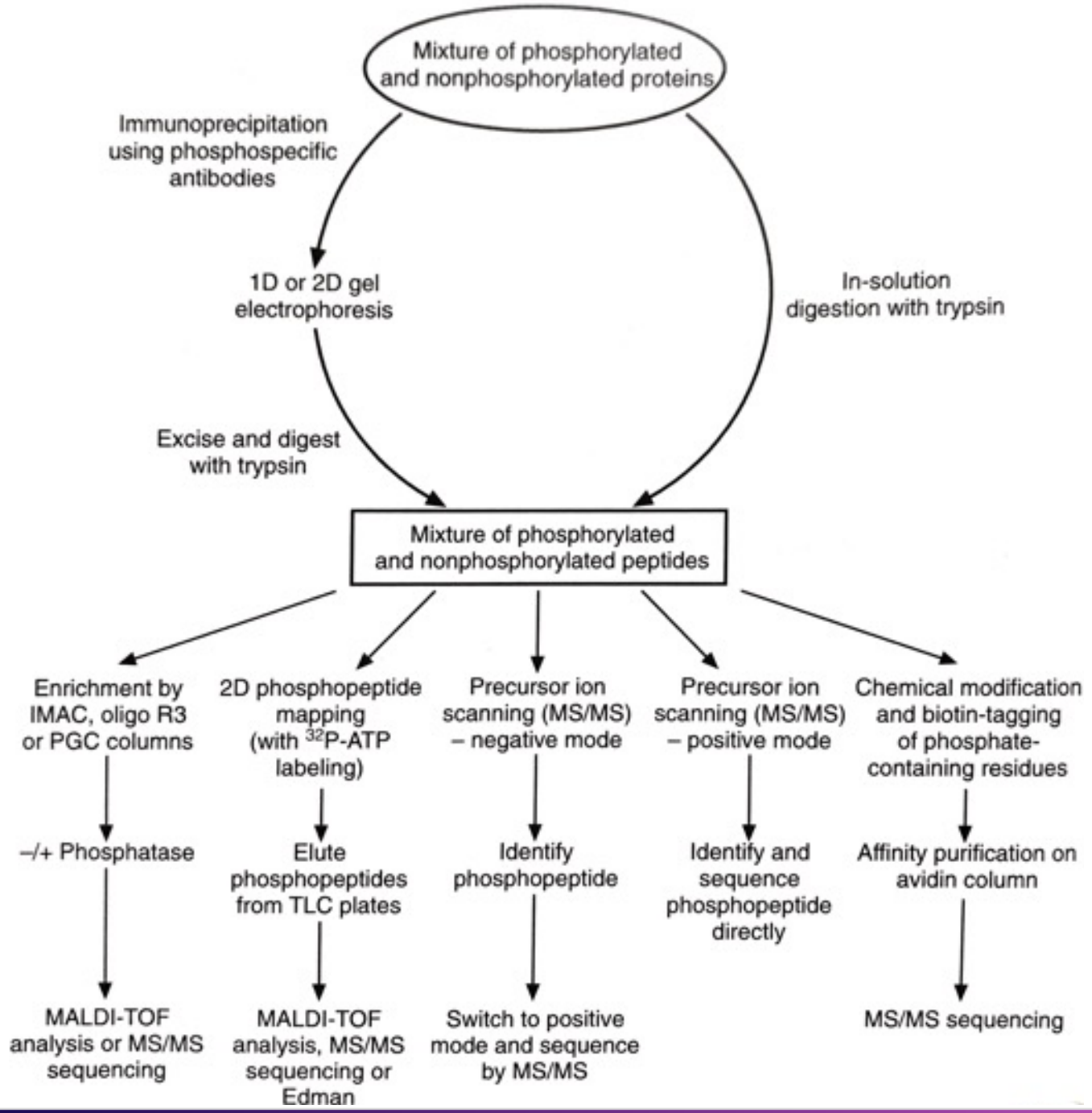



# PhosphoProteomics

- Phosphorylation lies at the heart of many many biological processes, including signal transduction, gene expression, and the regulation of cell division.
- Most phosphoproteins have more than one phosphorylation site and exist as a mixture of alternative phosphoforms.
- The phosphoproteome is not only complex but also extremely dynamic.
  - A catalog of all phosphoproteins
  - Distribution of phosphorylation site
  - Abundance of alternative phosphoforms under different conditions

# Different Techniques for Phosphoproteins Analysis

- Because low abundance of many phosphoproteins, enrichment is usually necessary.



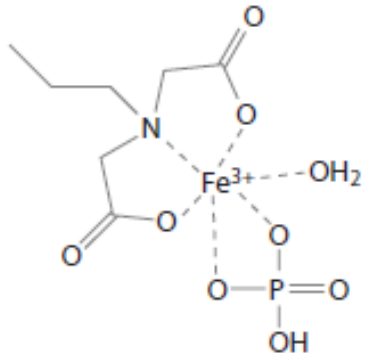
# Enrichment of Phosphoproteins: Antibodies

- Immunopurification with immobilized anti-phospho-Tyr antibodies
- Antibody-based method is well established for Tyr, but not for other residues (Ser & Thr)
  - Antiphosphoserine or Antiphosphothreonine antibodies don’t have satisfactory immunoprecipitation result
- The antibodies don’t bond phosphopeptide strongly, so antibodies are usually employed before digestion
- It is limited in throughput and hard to automate

# Enrichment of Phosphoproteins: $IMAC_1$

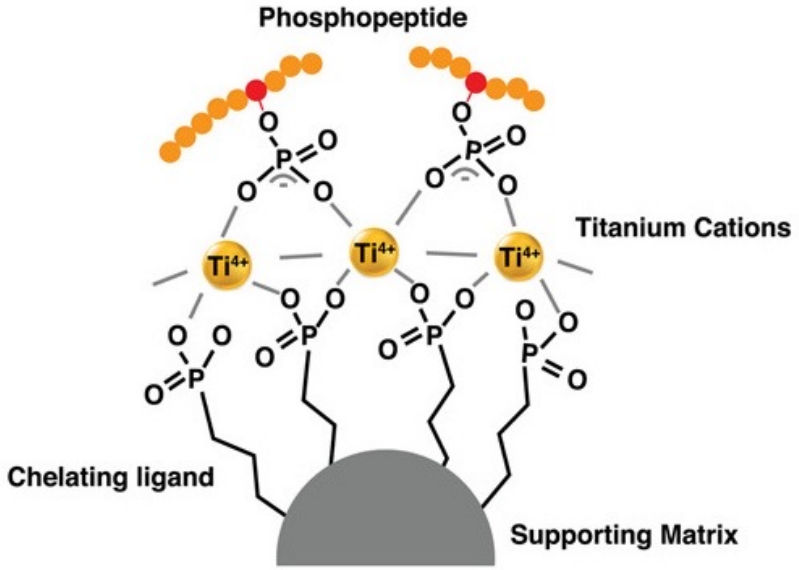
- Immobilized Metal Affinity Chromatography
  - Phosphates have high affinity to trivalent metal ions
  - Metal ions are immobilized on columns
  - A variety of metals has been used, including $Fe^{3+}$, $Ga^{3+}$, $Al^{3+}$ and $Zr^{3+}$

- Problems with IMAC
  - If the pH during the loading is very basic, then non-phosphopeptides bind as well
  - Strongly acidic peptides (rich in E and D) are also affine to the metal complexes



# Enrichment of Phosphoproteins: $IMAC_2$

- High chemical stability
- Unique amphoteric ion-exchange properties
- Organic phosphates are effectively adsorbed to $TiO_2$ in acidic conditions and desorbed in alkaline conditions
  - Selective enrichment for phosphopeptides
- Dihydroxy benzoic acid (DHB) as a competitive binder in $TiO_2$ enrichment to avoid unspecific binding of D and E

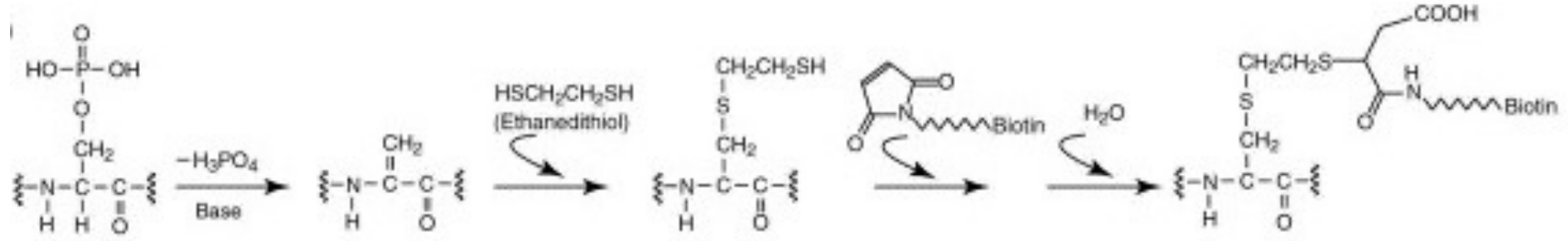


# Enrichment of Phosphoproteins: SCX

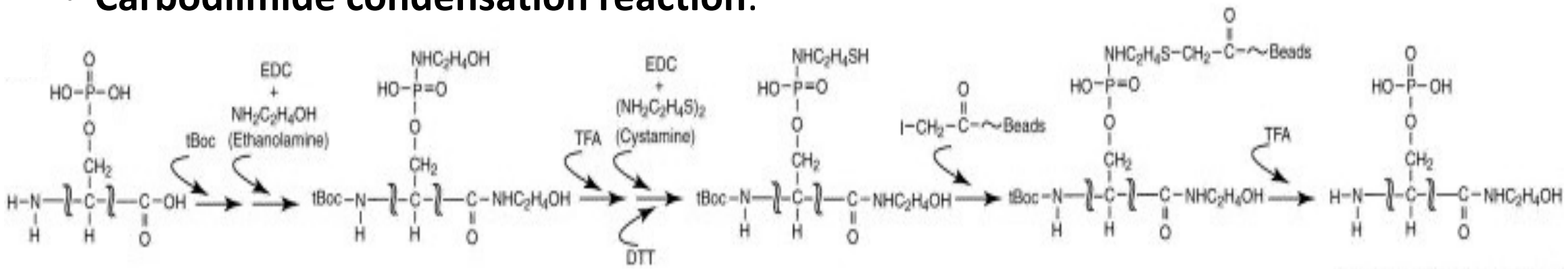
- Strong Cation Exchange (SCX) strategy is based on the difference in the solution **charge state** of phosphorylated and non-phosphorylated peptides
  - At pH 2.7 a (typical) tryptic peptide has charge z = +2 (N-terminal amine group + C-terminal K or R)
  - If this peptide is phosphorylated -> z = +1, since the phosphate group is negatively charged
  - Using a linear salt gradient the phosphopeptides can be enriched in early SCX fraction.
  - Note that multiply phosphorylated peptides will be in the flow-through

# Enrichment of Phosphoproteins: Chemical Modification

- For larger amounts of starting samples, affinity purification with chemical modifications of phosphates can be used.
  - **$\beta$–elimination reaction**:




  - **Carbodiimide condensation reaction**:

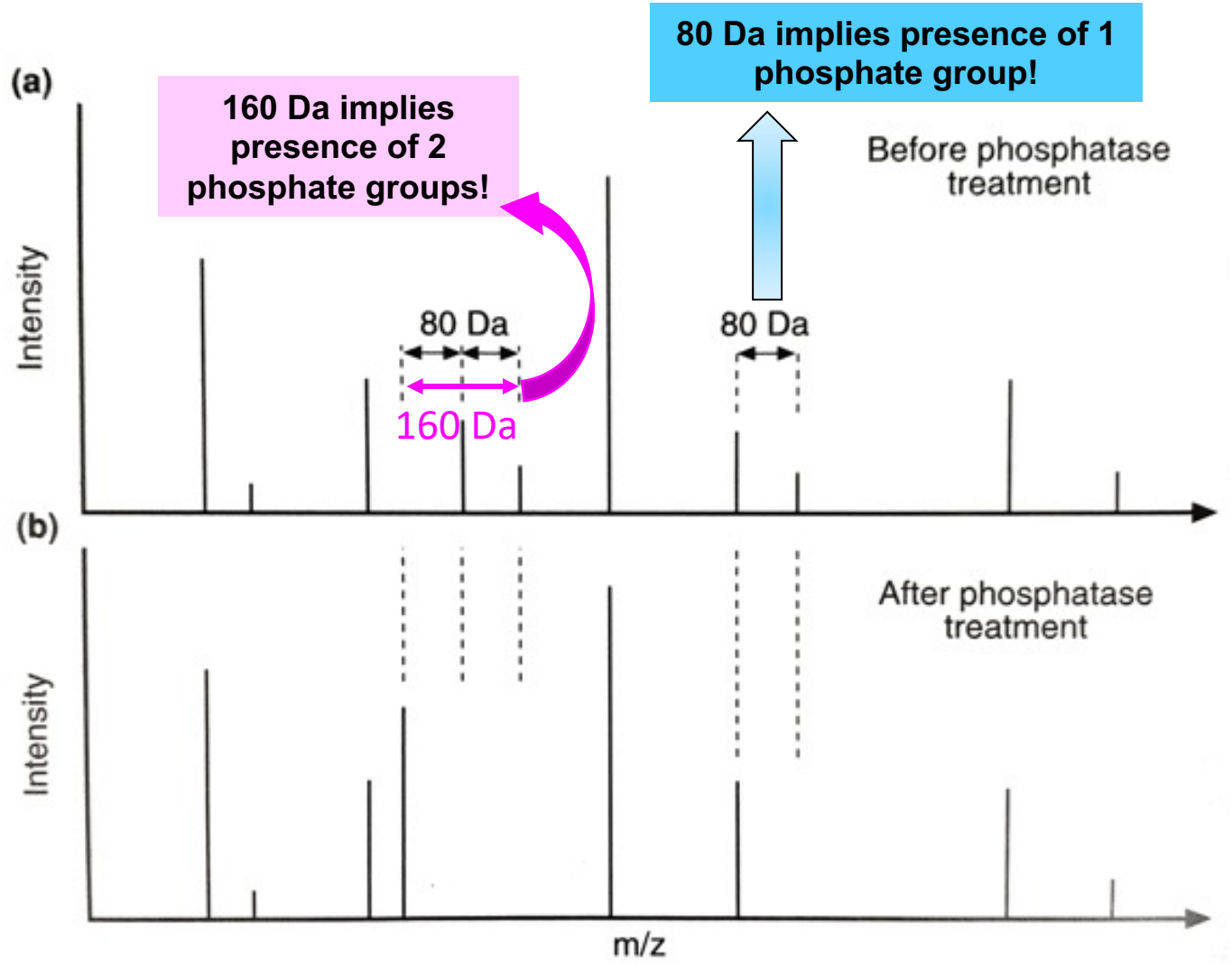


# MS for Phosphopeptides: **MALDI-TOF MS**

- **MALDI-TOF MS** is most often used to analyze intact peptides, and correlative database searching (peptide mass fingerprinting) allows the derived masses to be matched against the theoretical peptides of known proteins.

- If the identity of the protein is known or can be deduced, phosphopeptides can be identified simply by examining the mass spectrum for masses shift of 79.983 ($HPO_3$ = 80 Da) compared to predicted masses.

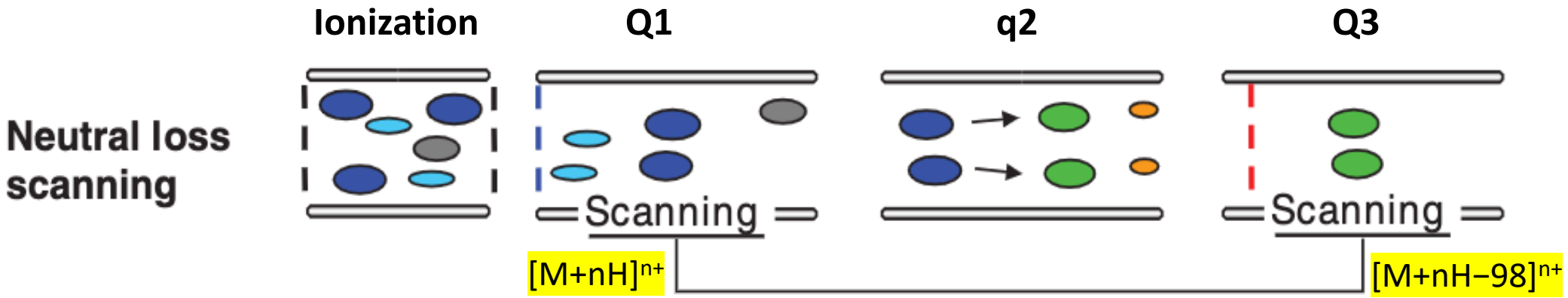
# Phosphopeptide identification by MALDI-TOF MS combining with alkaline phosphatase treatment




- This method does not identify the phosphorylated residues directly.
- If there is only one possible phosphorylation site or if a consensus kinase target site is present, then the site can be identified accurately.

# MS for Phosphopeptides: **Neutral-Loss-Dependent $MS^n$**

- Fragmentation of phosphopeptides by CID is often dominated by neutral losses
  - pS: predominantly $H_2PO_4$ (-98 Da)
  - pT: $H_2PO_4$ (-98 Da) and $HPO_3$ (-80 Da)
  - pY: $HPO_3$ (-80 Da) or ($HPO_3$ + $H_2O$) (-98 Da)
- **Neutral loss ion scanning** on a triple quadrupole mass spectrometer can be used to detect the loss of the phosphate group from phosphopeptides.
  - Q1 is used to scan the whole mass range
  - q2 is used as a collision cell
  - Q3 is set to scan at $[M+nH-98]^{n+}$ to detect phosphopeptides that lose the phosphate group.




P. J. Boersema, S. Mohammed and A. J. R. Heck. J. Mass. Spectrom.2009,44, 861–878

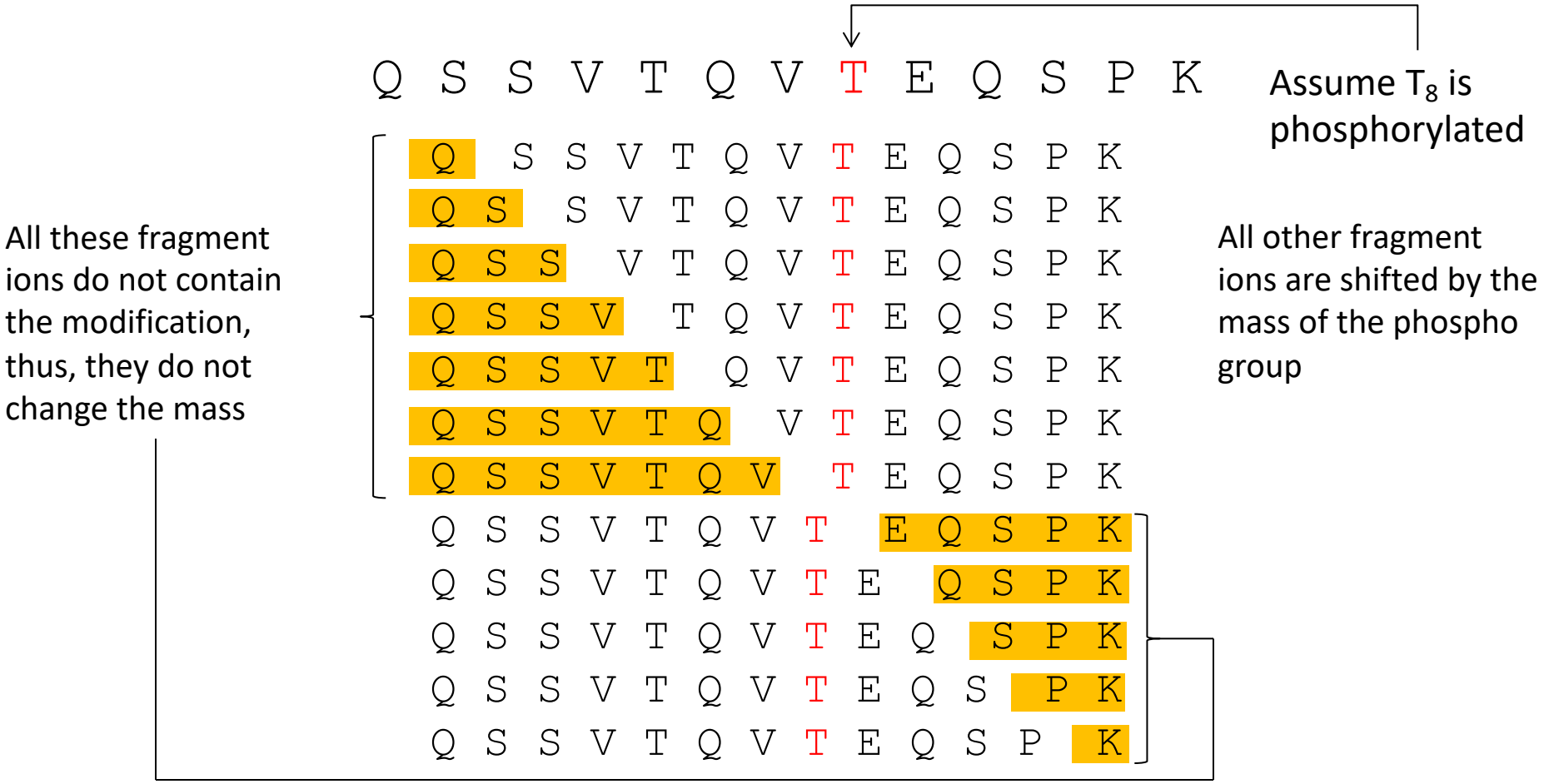
Domon B, Aebersold R. *Science*. 2006 Apr 14;312(5771):212-7. doi: 10.1126/science.1124619.

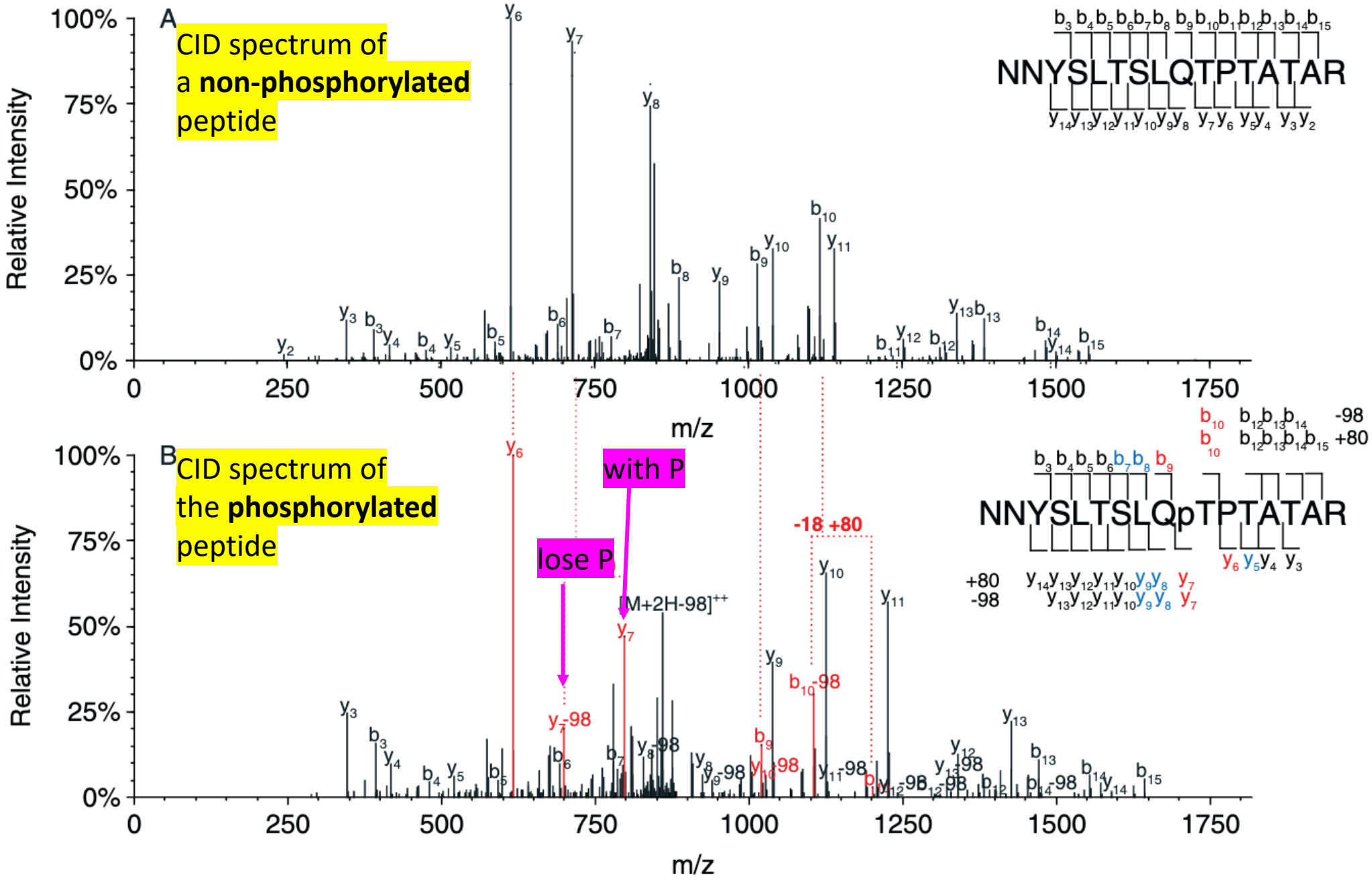
# Phosphorylated Example

- QSSVTQVTEQSPK is phosphorylated at one position
  - precursor weight of unphosphorylated version + weight of phospho group
- Which fragment ions masses are changed in the $MS^2$ spectrum?

# Phosphorylated Example

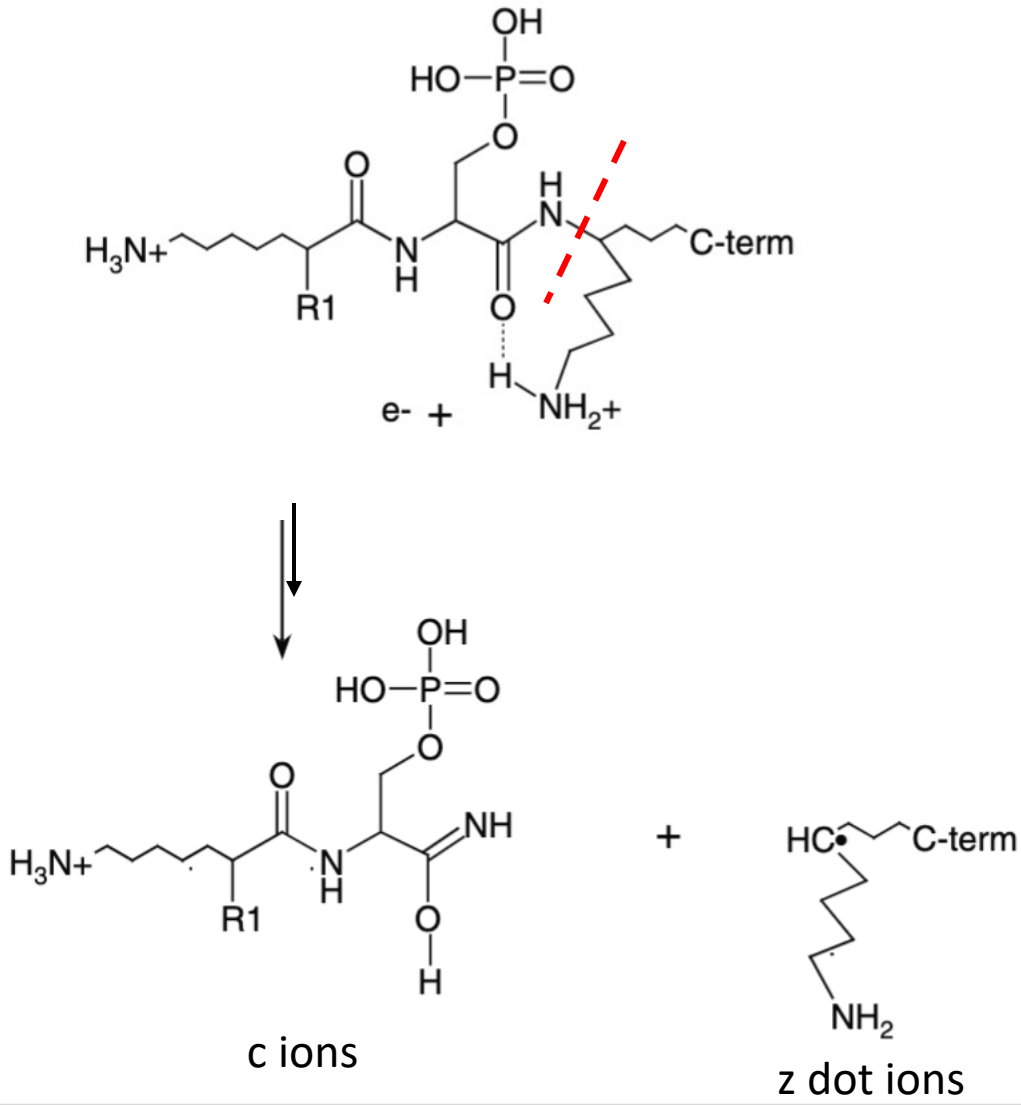
- QSSVTQVTEQSPK is phosphorylated at one position
  - precursor weight of unphosphorylated version + weight of phospho group
- Which fragment ions masses are changed in the $MS^2$ spectrum?

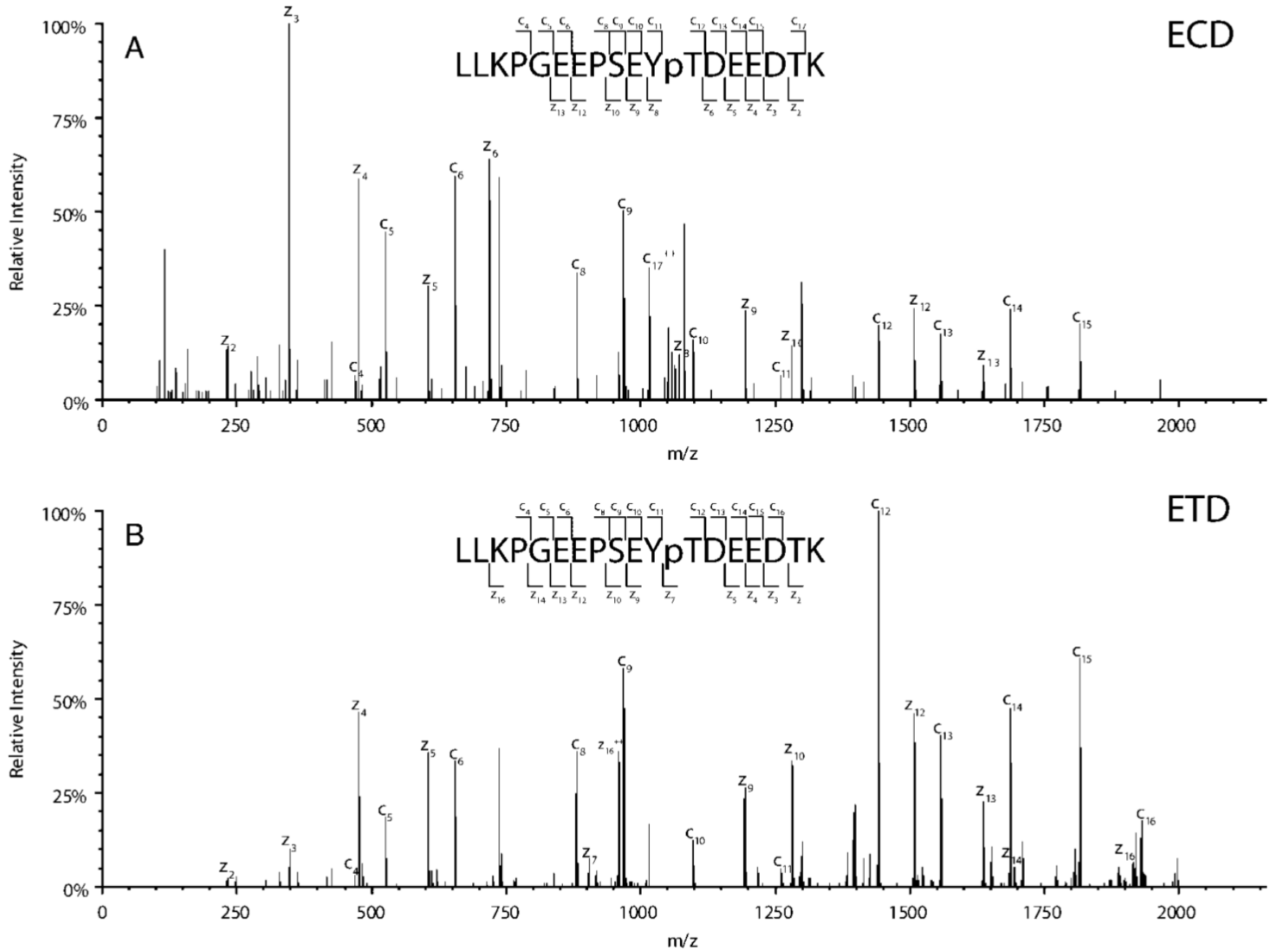



A
CID spectrum of
a **non-phosphorylated**
peptide
Relative Intensity
100%
75%
50%
25%
0%
0
250
500
750
1000
1250
1500
1750
m/z
NNYSLTSLQTPTATAR
B
CID spectrum of
the **phosphorylated**
peptide
with P
lose P
-18 +80
[M+2H-98]++
NNYSLTSLQpTPTATAR
+80
-98

# MS for Phosphopeptides: **Electron-Driven Dissociation Methods**

- As discussed above, fragmentation of phosphopeptides by CID is often dominated by neutral losses which can seriously hamper identification and phosphosite determination.
- **Electron-based dissociation methods (ECD/ETD)** have been introduced that leave posttranslational modifications such as phosphorylation and glycosylation intact during fragmentation.

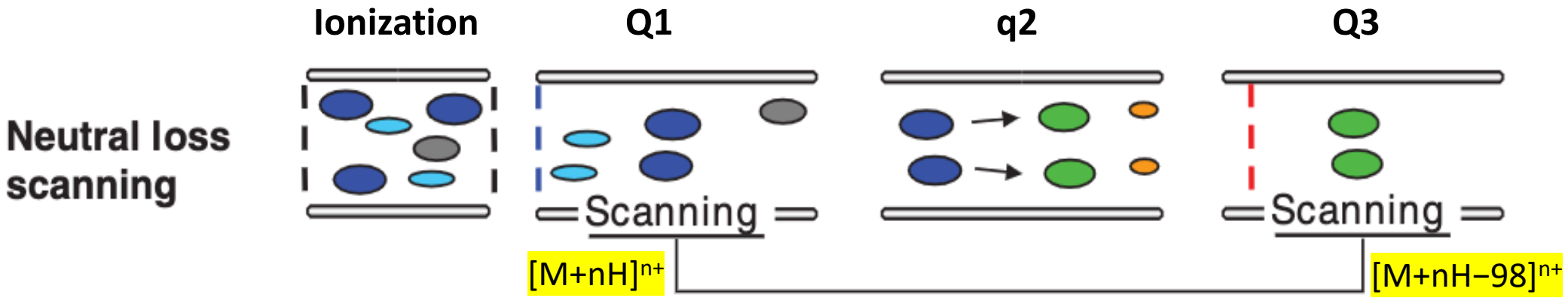





No neutral loss

# MS for Phosphopeptides: **Neutral-Loss-Dependent $MS^n$**

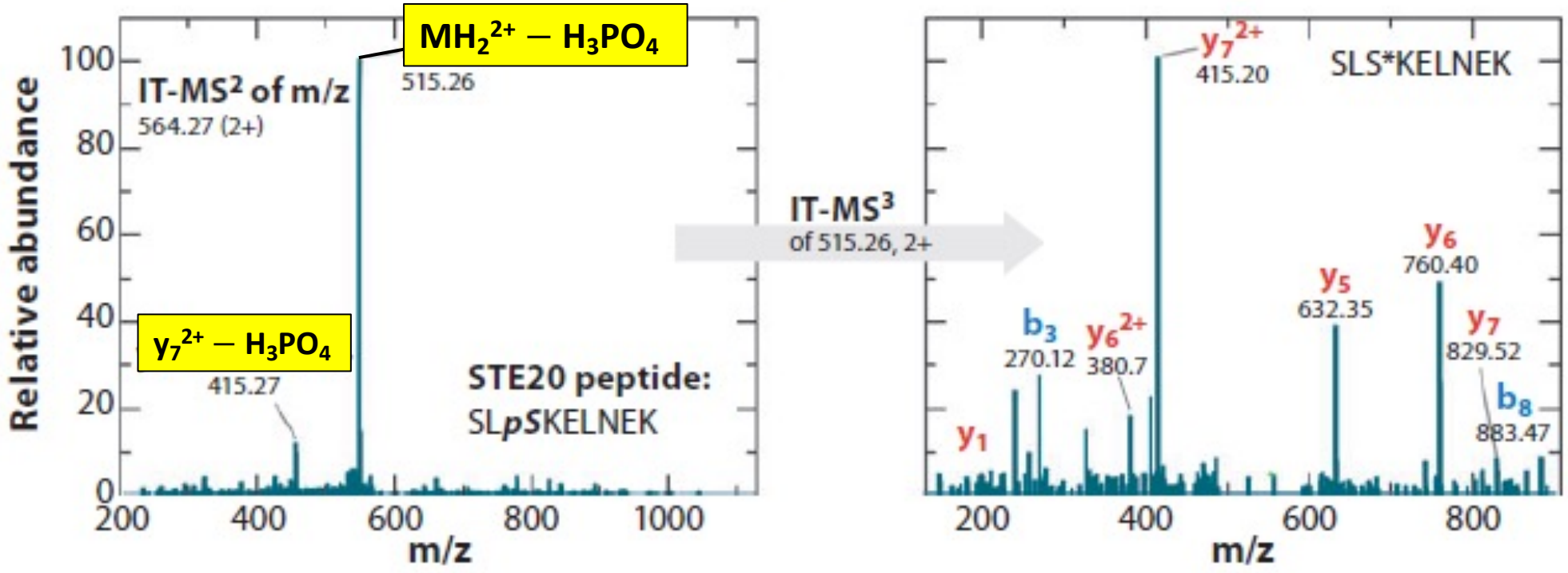
- Fragmentation of phosphopeptides by CID is often dominated by neutral losses
  - pS: predominantly $H_2PO_4$ (-98 Da)
  - pT: $H_2PO_4$ (-98 Da) and $HPO_3$ (-80 Da)
  - pY: $HPO_3$ (-80 Da) or ($HPO_3$ + $H_2O$) (-98 Da)
- **Neutral loss ion scanning** on a triple quadrupole mass spectrometer can be used to detect the loss of the phosphate group from phosphopeptides.
  - Q1 is used to scan the whole mass range
  - q2 is used as a collision cell
  - Q3 is set to scan at $[M+nH-98]^{n+}$ to detect phosphopeptides that lose the phosphate group.




P. J. Boersema, S. Mohammed and A. J. R. Heck. J. Mass. Spectrom.2009,44, 861–878

Domon B, Aebersold R. *Science*. 2006 Apr 14;312(5771):212-7. doi: 10.1126/science.1124619.

- When spectra are noisy or when backbone fragments have been suppressed by phosphate groups, an additional MS event ($MS^3$) can be triggered at m/z [m/z precursor – neutral loss ($H_3PO_4$ = 98 Da)/z]

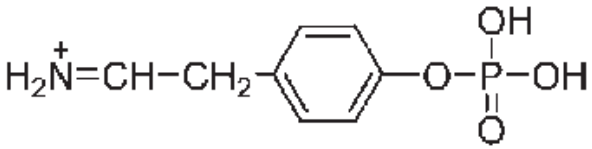


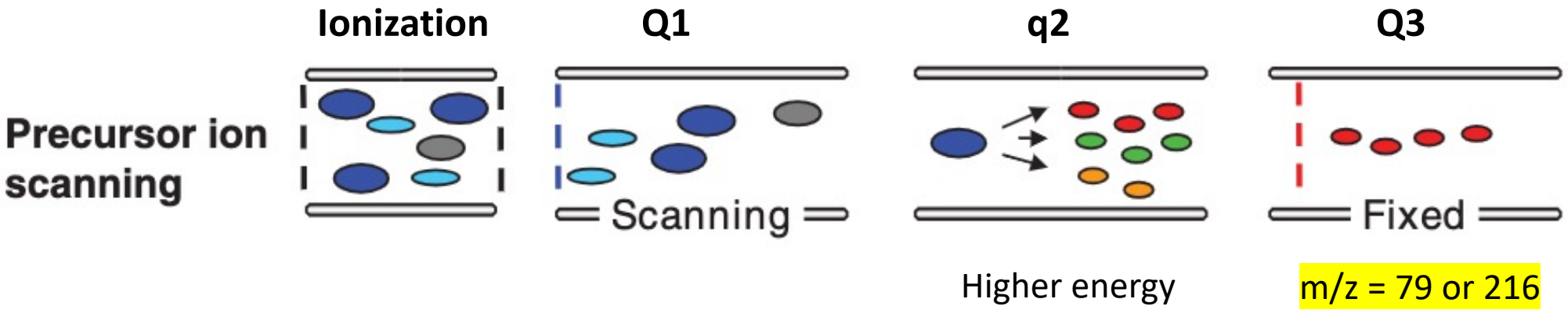
# MS for Phosphopeptides: **Precursor/Reporter Ion Scanning**

- Phosphopeptides preferentially yield diagnostic, phosphate-specific fragment ions, which indicates the presence of phosphopeptides.
  - $H_2PO_4^-$ (97 Da)
  - $PO_3^-$ (79 Da)
  - $PO_2^-$ (63 Da)
- Since the phosphate group (above) provides a negative charge, phosphate-specific fragment ions are usually obtained in **negative ion mode.**
- **Higher collision energies** are used to generate these phosphate fragments
- The Precursor/Reporter Ion Scanning is usually carried out in triple quadrupole or more sensitive hybrid Q-q-TOF/Ion trap.
- The mass spectrometer can then be switched to positive ion mode to perform CID on only the peptide precursors that showed the loss that was scanned for.

# MS for Phosphopeptides: **Precursor/Reporter Ion Scanning**

- Triple quadrupole (QqQ) instruments can be used to detect diagnostic fragment ions (e.g., at m/z of 79 for $PO_3^-$) using precursor ion scanning in negative mode
  - For the analysis of Tyr phosphorylation, the reporter ion scanning method is used to detect the **p-Tyr immonium ion** (cleavage at both sides of p-Tyr) at **m/z 216.043** at positive mode. (very specific for p-Tyr!)




Domon B, Aebersold R. *Science*. 2006 Apr 14;312(5771):212-7. doi: 10.1126/science.1124619.