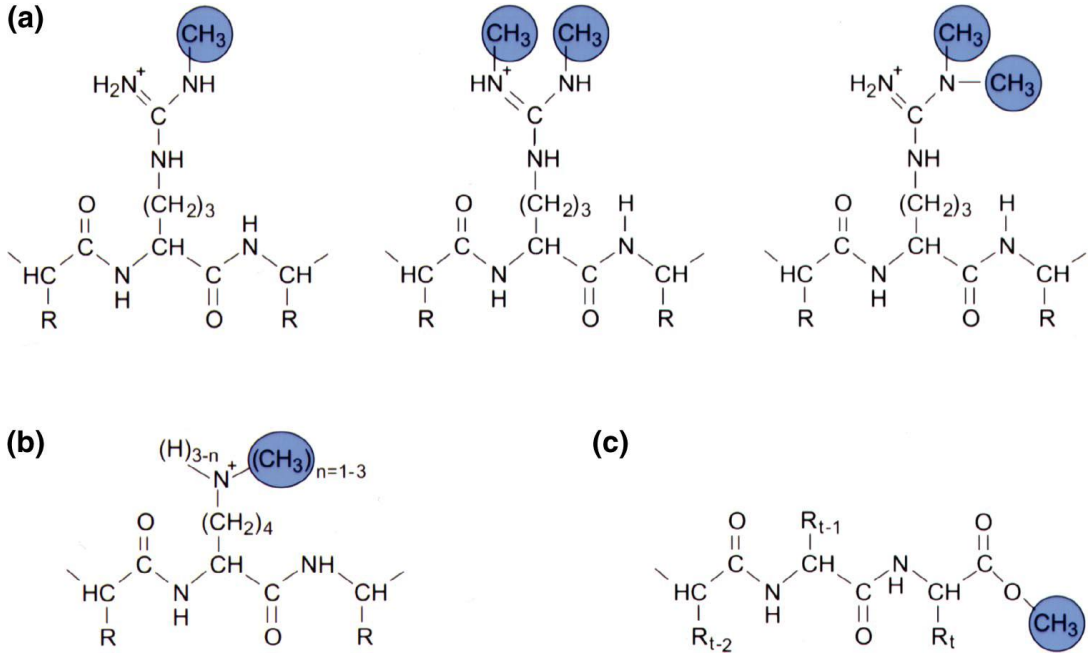
LECTURE 5-2: PROTEIN POST-TRANSLATIONAL MODIFICATIONS

Bio312

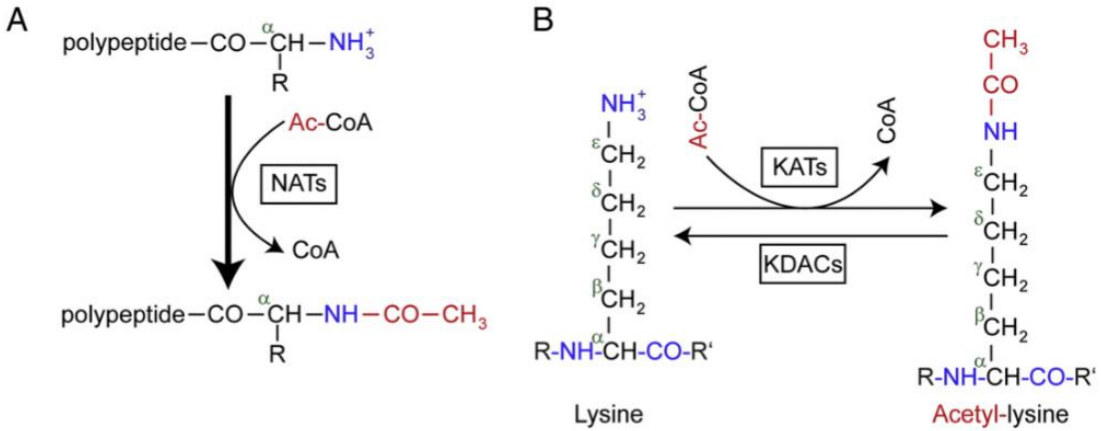
Instructor: Dr. Lanlan Han
E-mail: Lanlan.Han@xjtlu.edu.cn

PTMs: Review

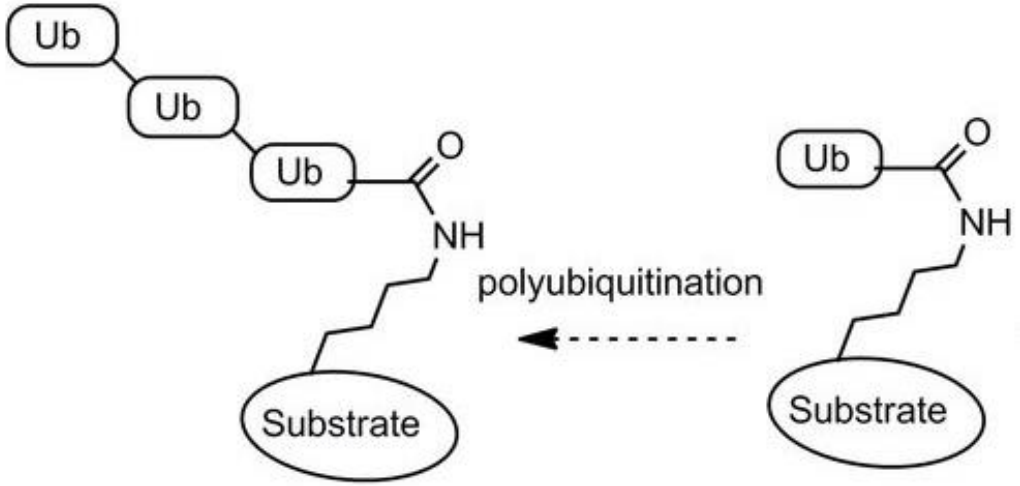
Methylation



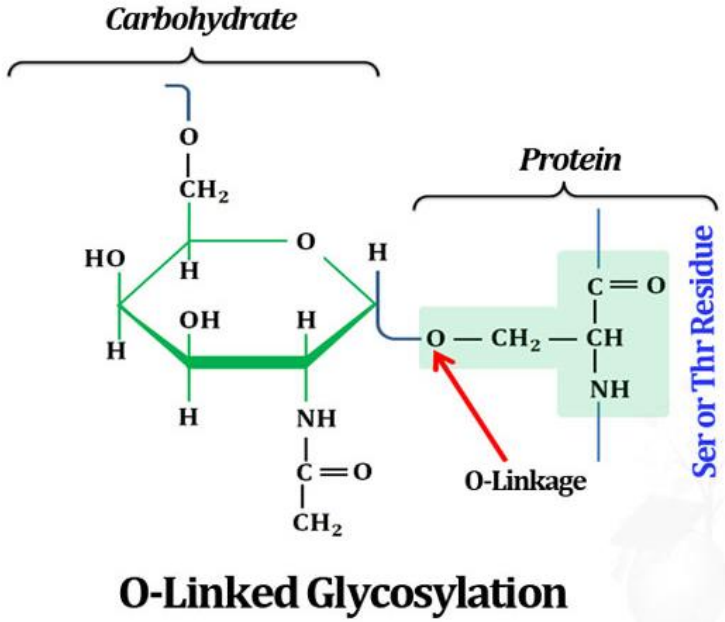
Acetylation

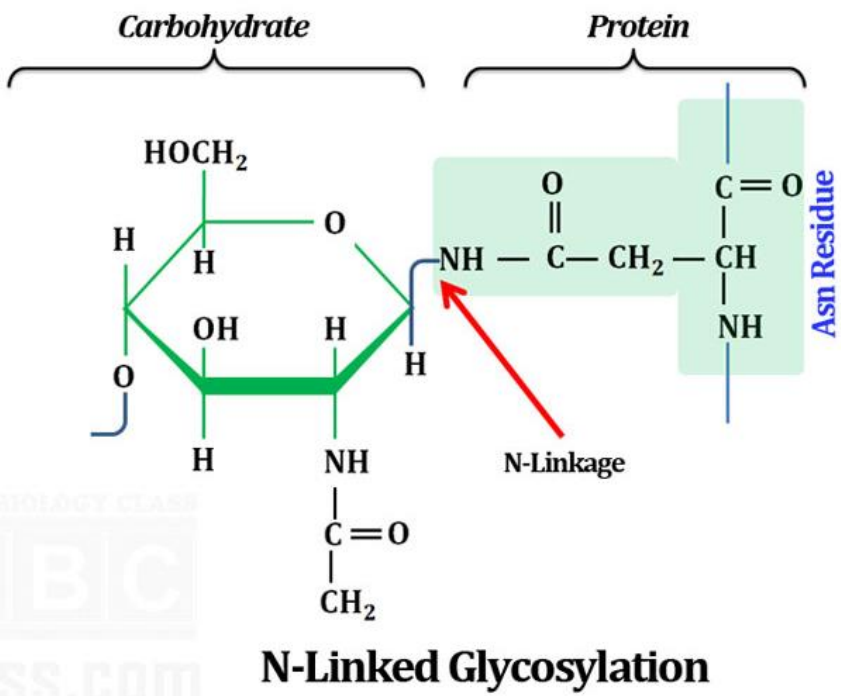


Ubiquitination

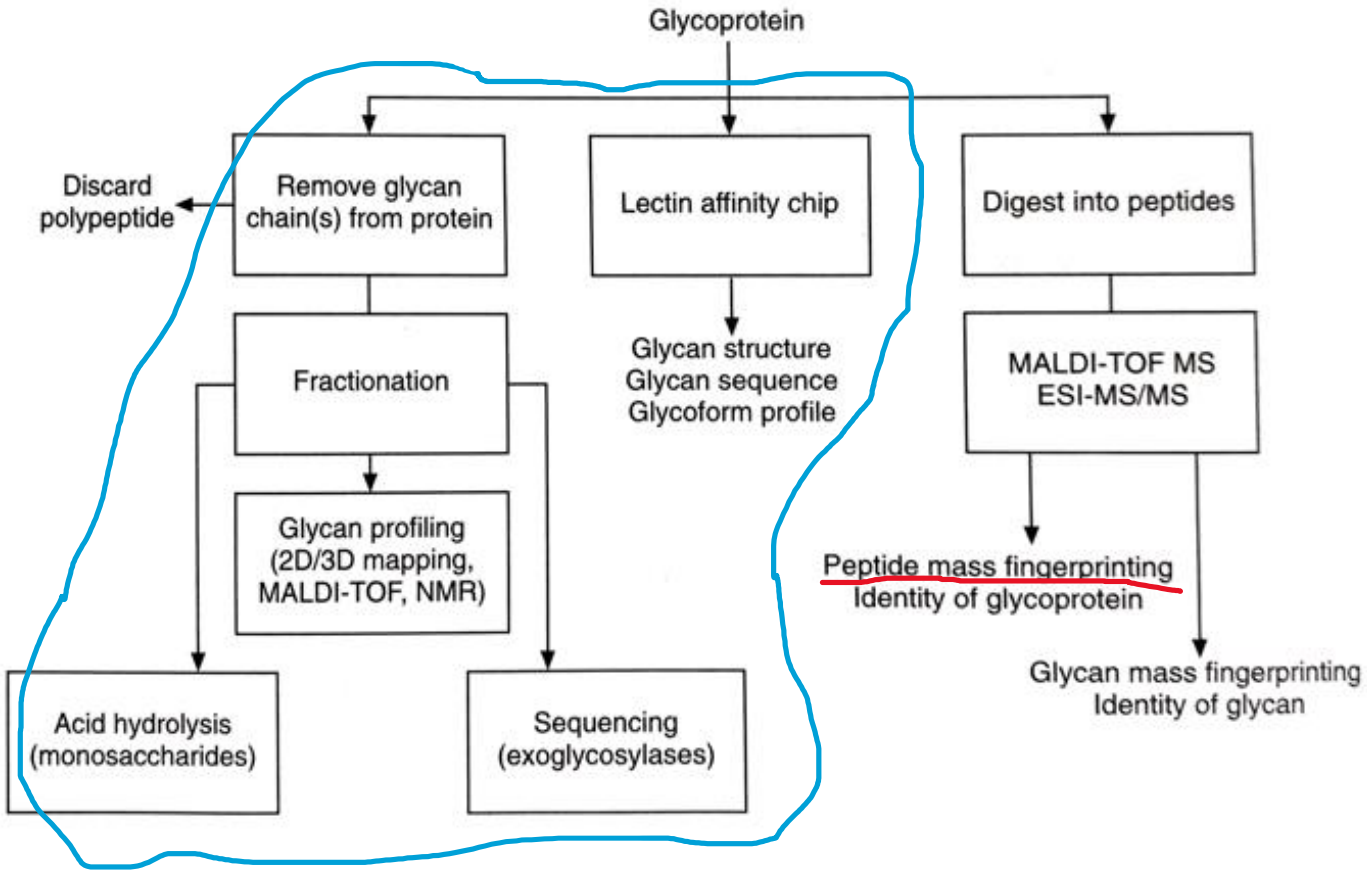


PTMs- **Glycosylation**: Review



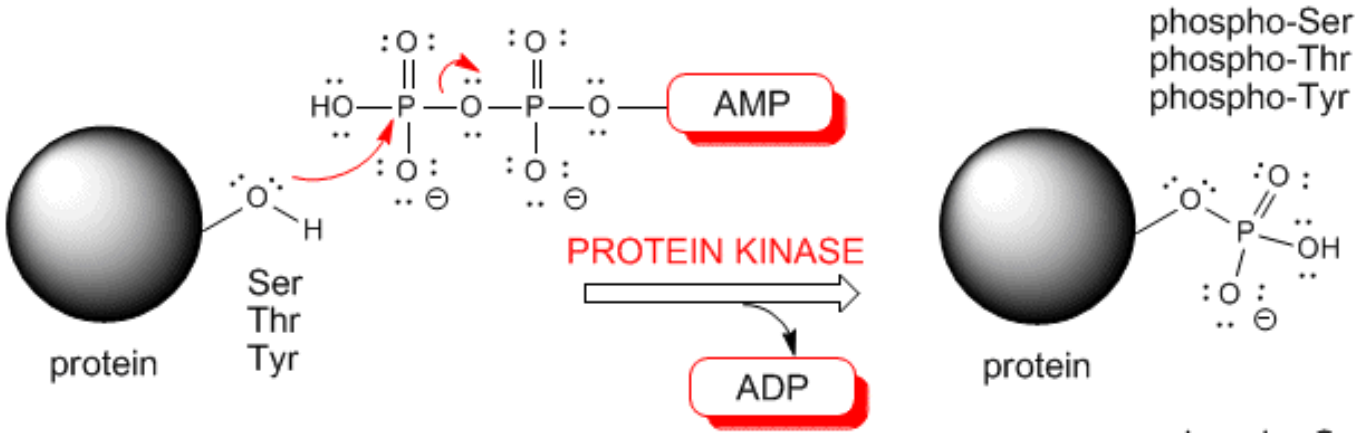


General Work-flow for the Full Analysis of Glycoproteins: Review

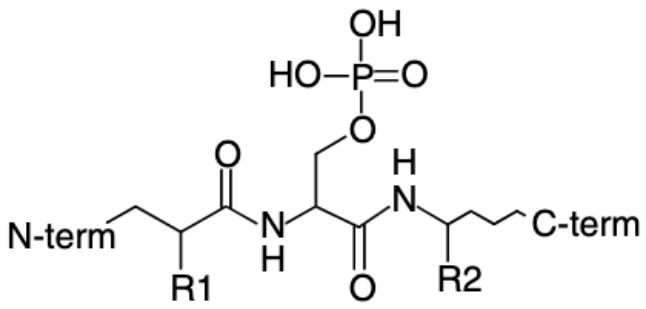


- The full analysis of glycoproteins must involve characterization of both the peptide and glycan.
- For glycan analysis,
  - Stepwise degradations with specific reagents (e.g., *O*- or *N*- glycosidase) that reveal bond position and stereochemistry
  - Mixture separated by chromatography
  - Overall composition and analysis by GC, Mass Spec and NMR

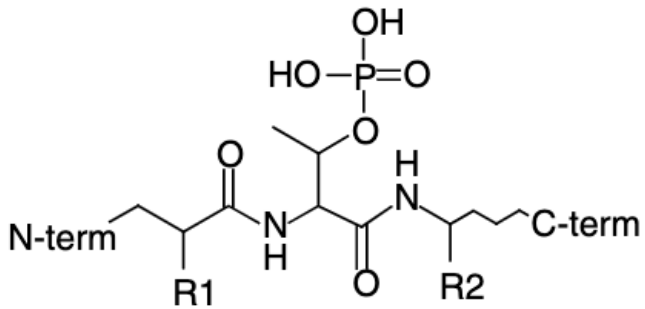
PTMs- **Phosphorylation**: Review



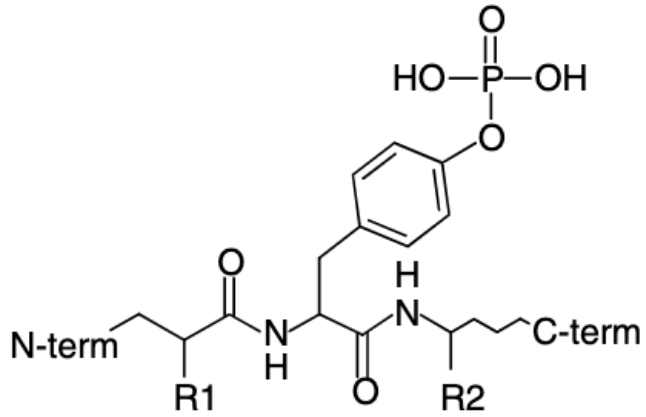
Note: In eukaryotes, phospho groups are predominately attached the S, T and Y residues (other phosphorylated amino acids exists (H,L or R), but are very rare)



phosphoserine



phosphothreonine

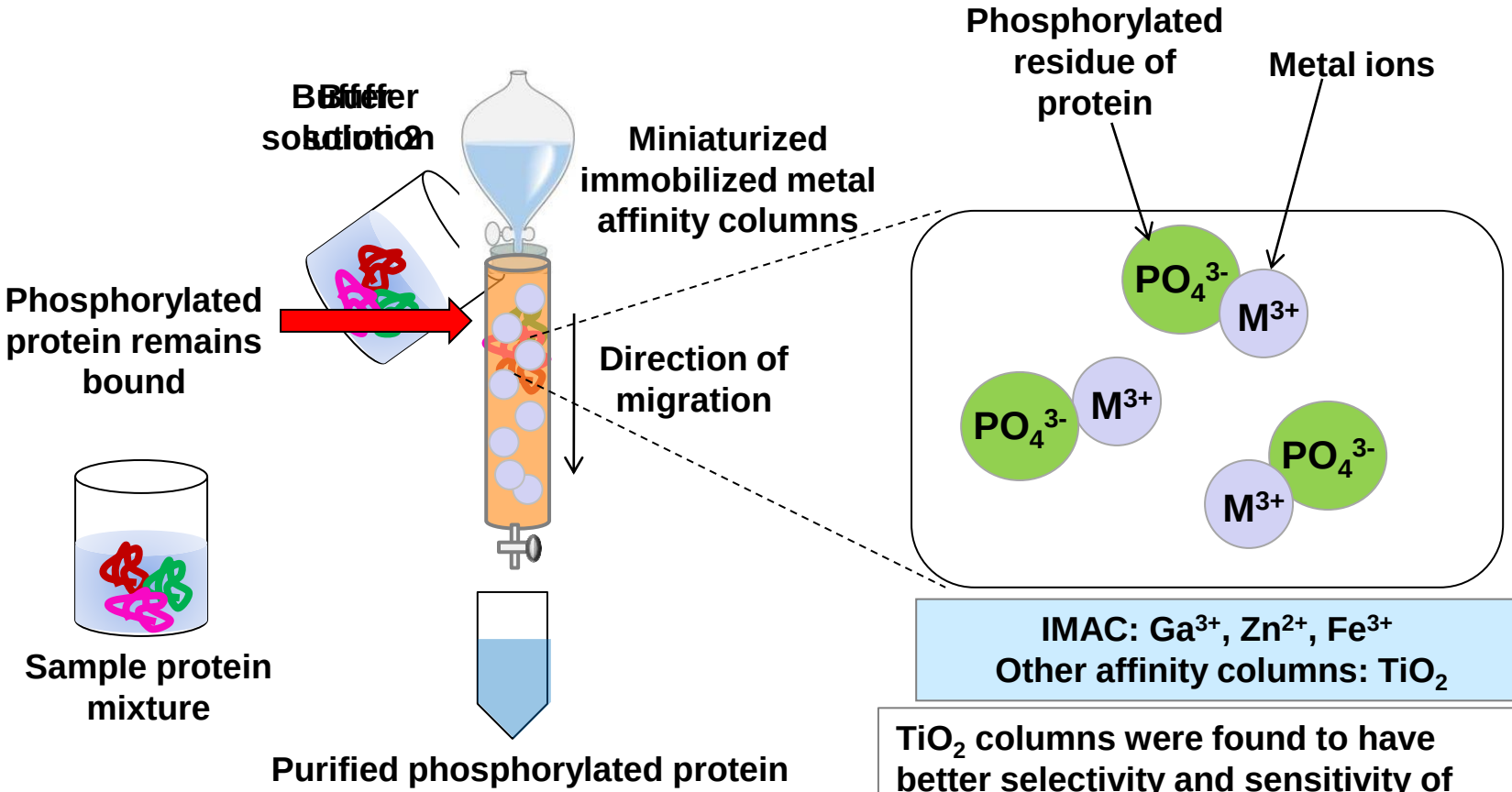


phosphotyrosine

Enrichment of Phosphoproteins: Review

1. Antibodies (anti-phospho-Tyr)
   - limited in throughput and hard to automate
2. IMAC
   - Interactions between negatively charged phosphate groups and positively charged trivalent metal ions or TiO_2
   - Relatively low selectivity
3. SCX
   - Difference in the solution charge state of phosphorylated and non-phosphorylated peptides
   - Phophopeptides come out **earlier** than non-phosphorylated peptides
   - multiply phosphorylated peptides will be in the flow-through fraction
4. Chemical modification
   - β–elimination reaction→addition of Biotin→bind to immobilized streptavidin
     - Only for phospho-Ser and phospho-Thr, it doesn't work with phospho-Tyr
     - O-glycosylated Ser/Thr can also be derivatized
   - Carbodiimide condensation reaction→Cystamine added-->binds to Iodoacetylated beads

LC-MS/MS based approach – Liquid chromatography



IMAC: Ga^{3+}, Zn^{2+}, Fe^{3+}
Other affinity columns: TiO_2

TiO_2 columns were found to have better selectivity and sensitivity of detection for phosphorylated peptide binding when compared to trivalent metal based IMAC.

MS for Phosphopeptides Detection: **Review**

1. MALDI-TOF MS combining with alkaline phosphatase treatment

- Intact peptides: match against the theoretical peptides of known proteins.
- Phosphopeptides: compare the mass spectra for mass shift of 80 x N Da (N is the number of phosphoryl groups)

- But it does not identify the phosphorylated residues directly.

2. Electron-based dissociation methods (ECD/ETD)

- It leaves PTMs intact during fragmentation
- c and z ions

3. Neutral loss ion scanning

- QqQ MS (scanning mode in Q1 and Q3)
- Detects neutral loss of 98 Da and 80 Da between precursor and fragment ions.

4. **Precursor/Reporter Ion Scanning**

- Q-q-TOF/Ion trap(q: Higher collision energies)
- Negative ion mode to detect the presence of phosphopeptides (97, 79, and 63 Da).
- Positive ion mode to determine the tandem mass spectra of identified phosphopeptides.

- Less sensitive QqQ can be used to detect diagnostic fragment ions. (Q1: scanning; Q3: fixed)
  - 216 Da in positive mode for p-Tyr peptides.

Poll Question

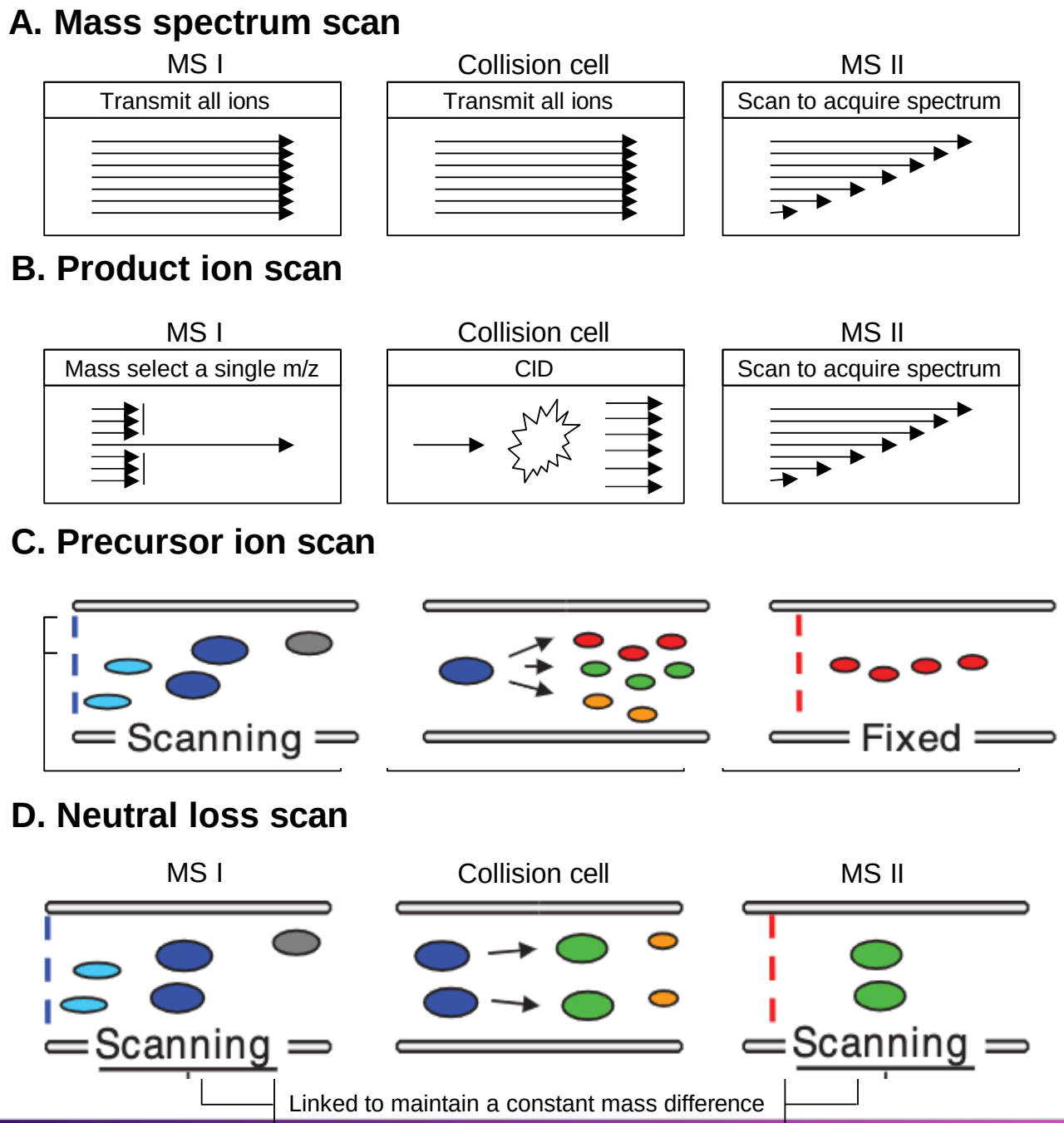
- To detect the presence of phosphotyrosine residues in peptides, which is the most suitable tandem MS assay below:

A. Mass spectrum scan

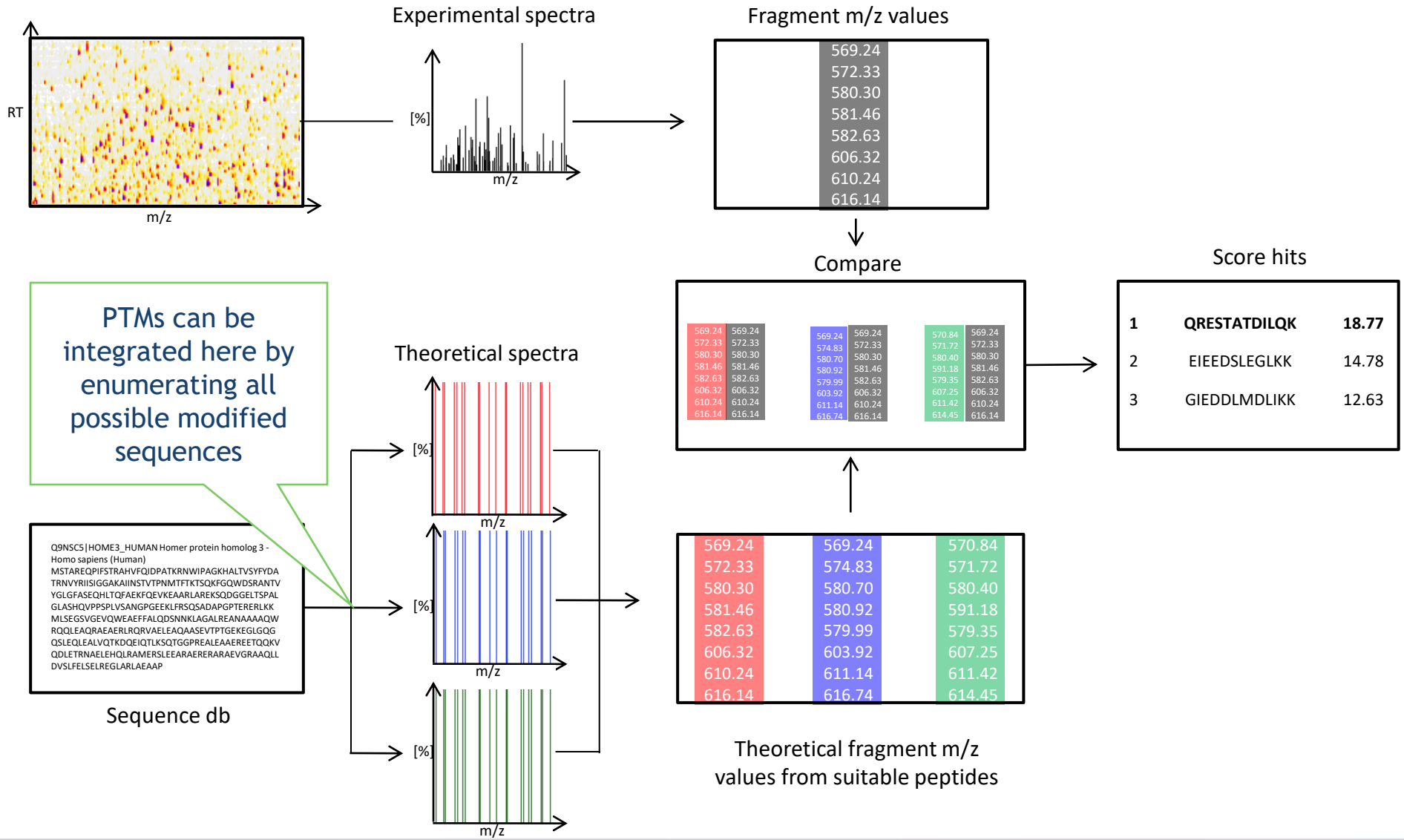
B. Product ion scan

C. Precursor ion scan

D. Neutral loss scan



Database Search for Large Scale PTMs



PTMs Increase Search Space

- PTMs are considered variable modifications for database search: they can be present at a certain amino acid, but do not have to be
- Each PTM position thus increases the search space: the original sequence and the modified sequence need to be generated
- Search space is further increased by nearby PTMs – multiple PTMs within one (tryptic) peptide led to an exponential number of sequence

D I G S E S T E K

D I G S*E S T E K

D I G S E S*T E K

D I G S E S T*E K

D I G S*E S*T E K

D I G S*E S T*E K

D I G S E S*T*E K

D I G S*E S*T*E K

Example:
This peptide contains three potential phosphorylation sites (marked by asterisks). We thus obtain $2^3 = 8$ potential peptide sequences.

Sample Preparation

Several Modification can be induced during the sample preparation

- Carbamidomethylation (Cys + 57 Da): protection of reduced sulfide groups with iodoacetamide.
- Oxidation (Met +16 Da): Exposure to air
- Pyro-Glu (N-terminal Glu/Gln – 18/17 Da): spontaneously
- Deamidation ([Asn – Gly] +1 Da): spontaneously
- Sodium adducts (Asp, Glu + 22 Da) from salt
- Carbamylation (N-terminus and Lys + 43 Da): from metabolites of urea

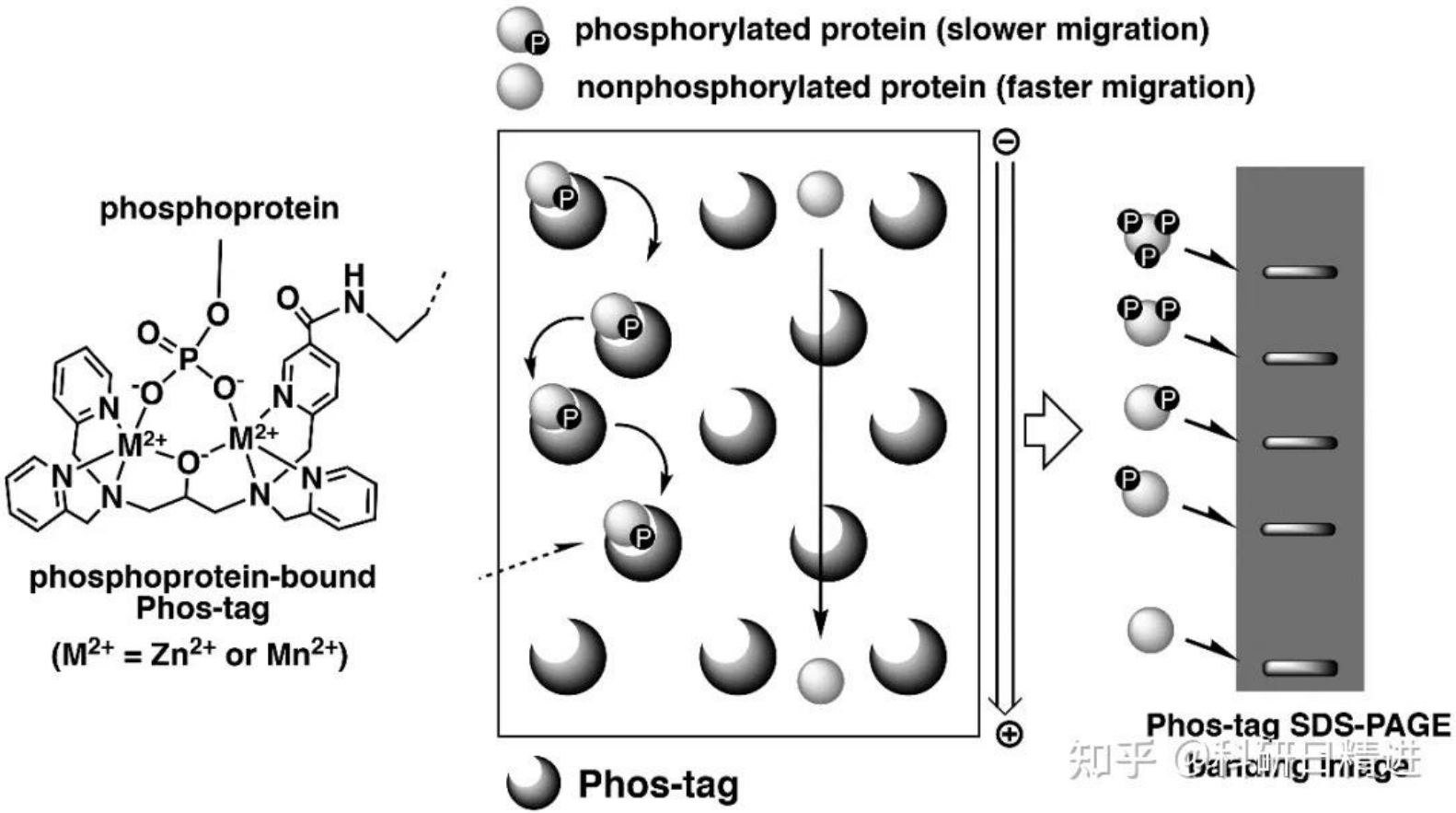
- Note: the modification masses here are nominal masses

Advantage of High Mass Accuracy

- Modifications often have similar masses
- Accurate precursor measurement improves discrimination of these possibilities

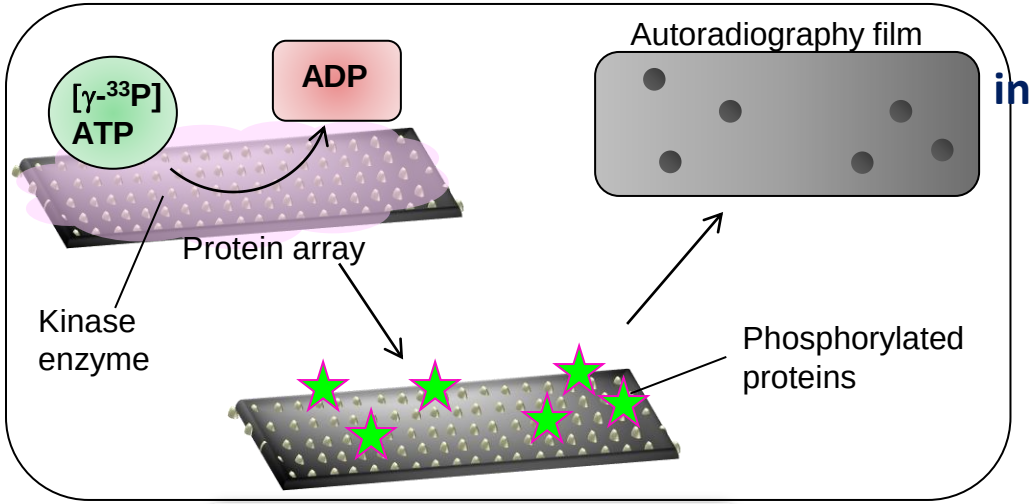
	Accession #	PSI-MS Name	Interim name	Description	Monoisotopic mass	Average mass	Composition
View	1	Acetyl	Acetyl	Acetylation	42.010565	42.0367	H(2) C(2) O
View	1197		Ser->Glu	Ser->Glu substitution	42.010565	42.0367	H(2) C(2) O
View	52	Guanidinyl	Guanidination	Guanidination	42.021798	42.0400	H(2) C N(2)
View	440	Amidino	amidino	amidino	42.021798	42.0400	H(2) C N(2)
View	37	Trimethyl	tri-Methylation	tri-Methylation	42.046950	42.0797	H(6) C(3)
View	575		Gly->Val	Gly->Val substitution	42.046950	42.0797	H(6) C(3)
View	1047		Ala->Xle	Ala->Leu/Ile substitution	42.046950	42.0797	H(6) C(3)
View	1305		Propyl	Propyl	42.046950	42.0797	H(6) C(3)
View	1163		Asn->Arg	Asn->Arg substitution	42.058184	42.0830	H(6) C(2) N(2) O(-1)

Gel-based Technique for Phosphoproteins Detection



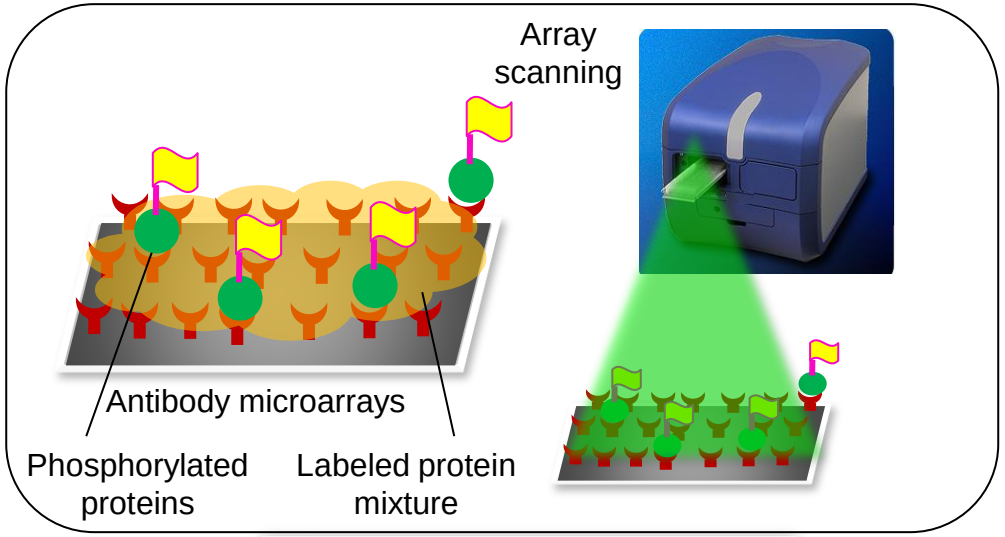
Western blot detection

Microarray-based detection techniques for Phosphorylation



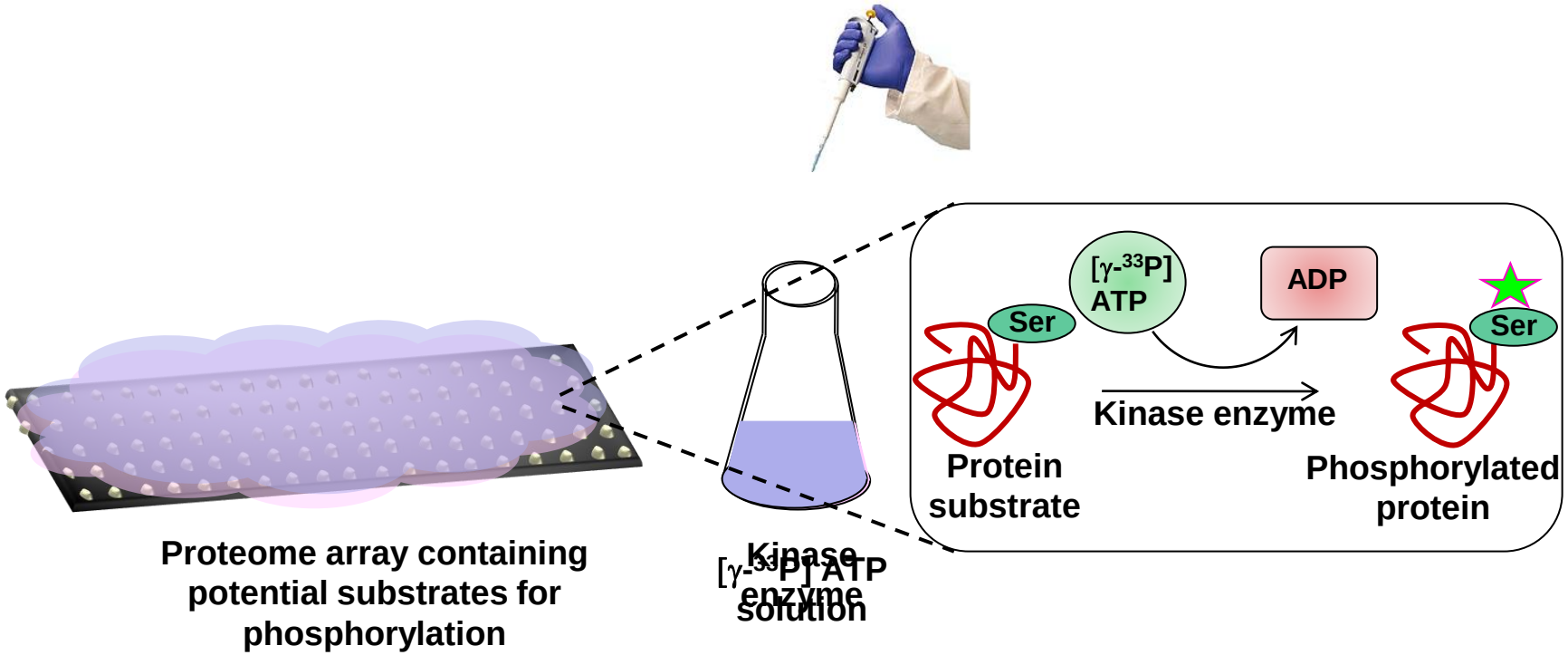
in vitro detection with known kinases

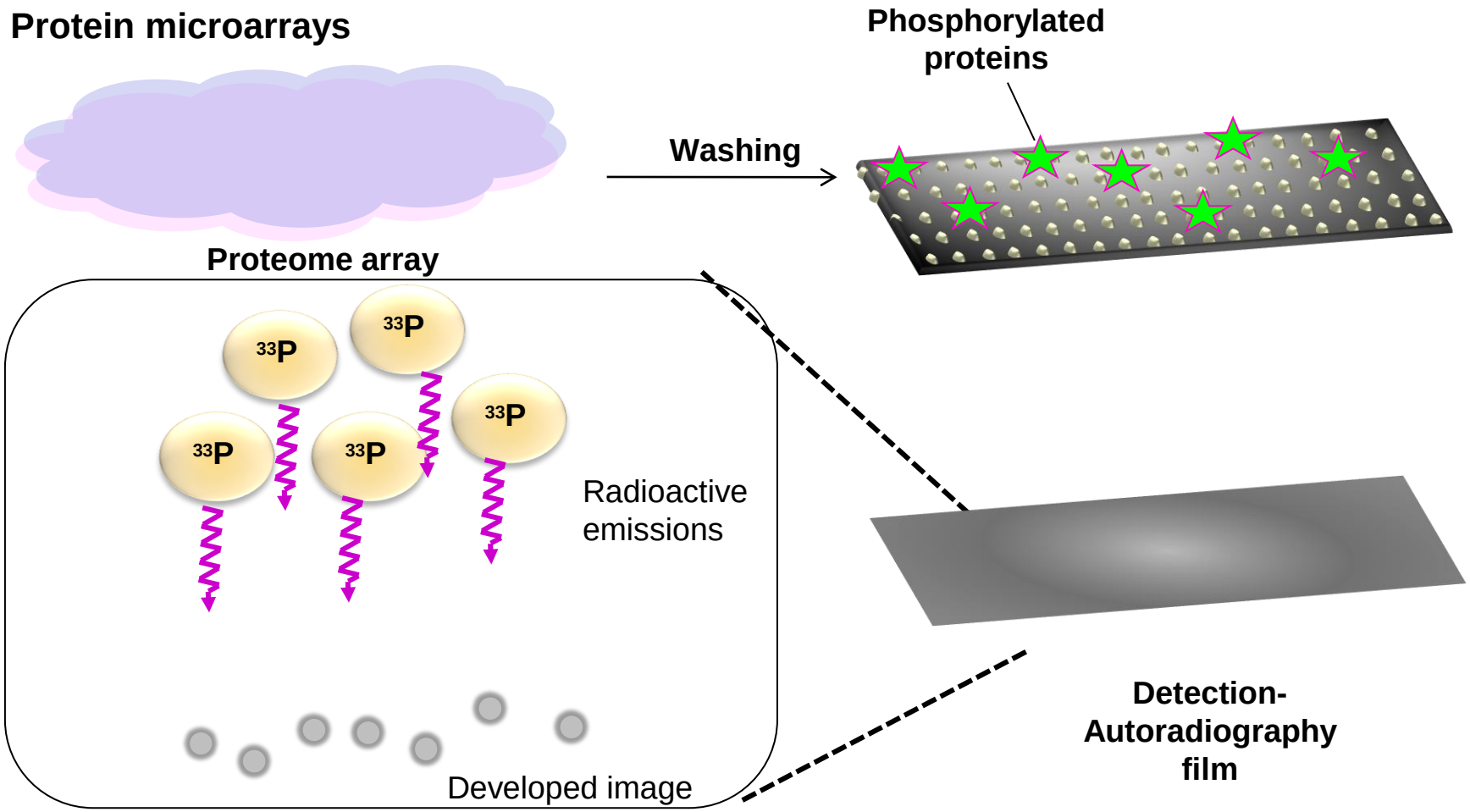
1. Protein microarrays



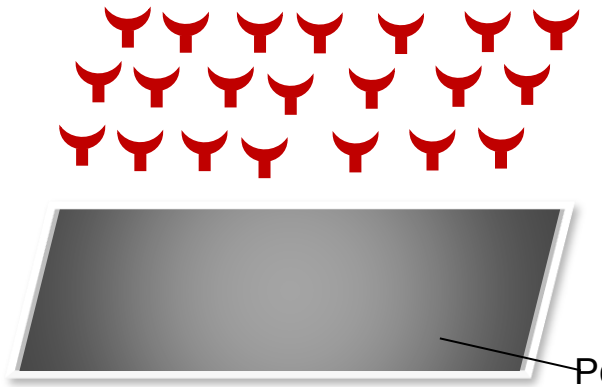
2. Antibody microarrays

Protein microarrays



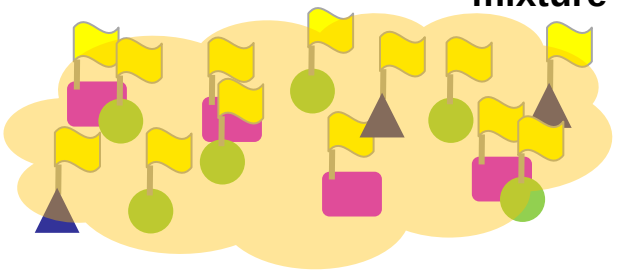

Protein microarrays
Phosphorylated proteins
Washing
Proteome array
33P
33P
33P
33P
33P
Radioactive emissions
Developed image
Detection-Autoradiography film

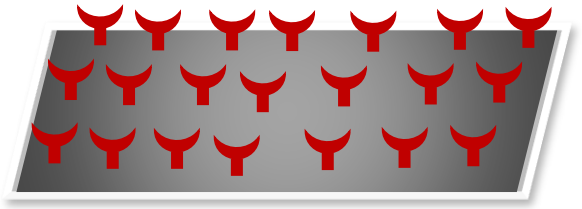
Antibody microarrays



Anti-phospho serine, threonine and tyrosine antibodies immobilized on array

Labeled protein mixture added





Specific binding of phosphorylated proteins

Antibody microarrays