

POST-TRANSLATIONAL MODIFICATION

Folding, Chemical Modification, and Regulated Degradation of Proteins After Synthesis

1. Introduction

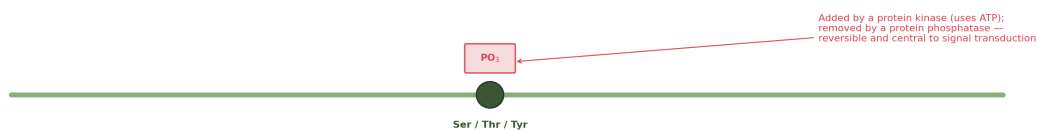
A newly synthesised polypeptide emerging from the ribosome is rarely functional on its own.

Post-translational modification (PTM) refers to the covalent chemical changes made to a protein after translation — folding, proteolytic processing, and the enzymatic addition or removal of chemical groups — that determine a protein's final structure, localisation, activity, interactions, and lifespan.

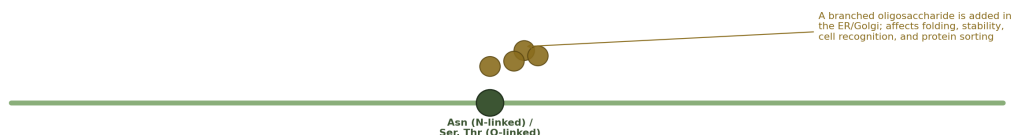
- PTMs vastly expand functional diversity beyond what is encoded by the genome: a single gene can give rise to a protein with many different modified forms, each with distinct activity, localisation, or stability.
- Because PTMs are added and removed by dedicated enzymes, most are **reversible and dynamically regulated**, making them central to cell signalling, the cell cycle, and rapid responses to changing conditions.
- Broad categories include: **folding and proteolytic processing** (chaperones, signal peptide/propeptide cleavage), **small chemical group addition** (phosphorylation, acetylation, methylation), **complex group addition** (glycosylation, lipidation), and **protein-protein conjugation** (ubiquitination, SUMOylation).

Major Types of Post-Translational Modification (PTM)

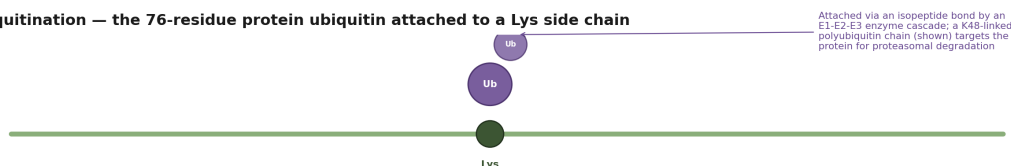
1. Phosphorylation — a phosphate group added to Ser, Thr, or Tyr side chains



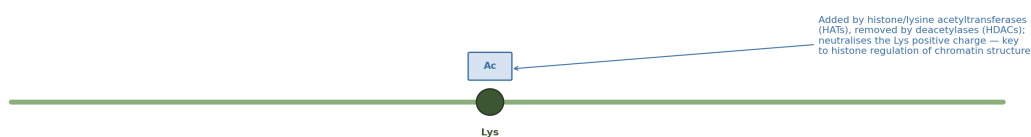
2. Glycosylation — sugar chains attached to Asn (N-linked) or Ser/Thr (O-linked)



3. Ubiquitination — the 76-residue protein ubiquitin attached to a Lys side chain



4. Acetylation — an acetyl group added to a Lys side chain (or the N-terminus)



5. Lipidation — a lipid group attached to anchor the protein to a membrane

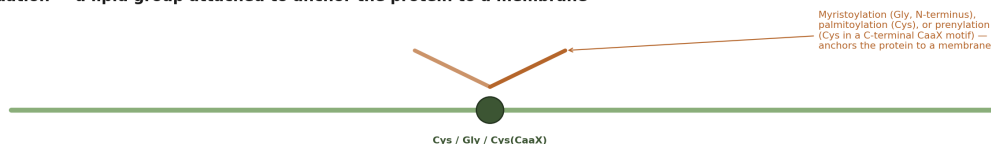


Fig. 1 — Five major classes of post-translational modification, each acting on a characteristic amino acid side chain.

2. Folding and Proteolytic Processing

(a) Chaperone-Assisted Folding

- Molecular **chaperones** (e.g. the Hsp70 and Hsp60/chaperonin families) bind exposed hydrophobic surfaces of nascent or stress-denatured polypeptides, preventing aggregation and assisting correct folding, often in an ATP-dependent cycle.

- In the endoplasmic reticulum, **disulfide bonds** between cysteine residues are formed and rearranged by protein disulfide isomerase (PDI), stabilising the tertiary structure of secreted and membrane proteins.

(b) Proteolytic Processing

- **Signal peptide cleavage** — an N-terminal signal sequence that directs a protein into the ER is removed by signal peptidase once the protein has been translocated.
 - **Zymogen activation** — many enzymes and hormones are synthesised as inactive precursors (zymogens/proproteins) that require specific proteolytic cleavage to become active — for example, trypsinogen to trypsin, or proinsulin to insulin (which also removes the connecting C-peptide).
 - **Protein splicing** — some proteins contain an internal segment called an **intein** that catalyses its own excision, ligating the flanking **extein** sequences together — a rare, self-contained form of post-translational processing.
-

3. Phosphorylation

Phosphorylation is the most common regulatory PTM, estimated to affect roughly a third of all human proteins at any given time.

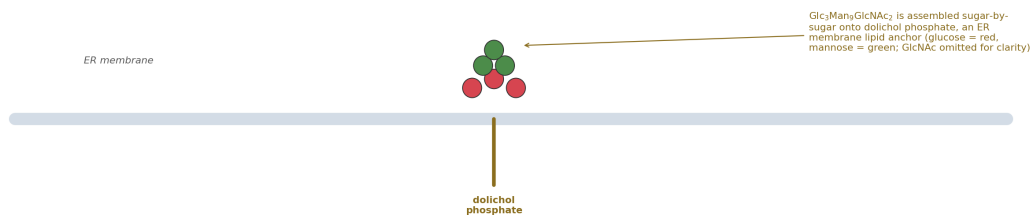
- **Protein kinases** transfer the γ -phosphate of ATP onto the hydroxyl group of serine, threonine, or tyrosine side chains; **protein phosphatases** remove it — the two activities together create a reversible molecular switch.
 - Phosphorylation changes a protein's charge and shape, which can activate or inhibit enzymatic activity, create or destroy a binding site for other proteins, or trigger relocalisation or degradation.
 - Phosphorylation cascades (e.g. MAP kinase pathways) are a core mechanism of intracellular signal transduction, allowing a single extracellular signal to be amplified and diversified inside the cell.
-

4. Glycosylation

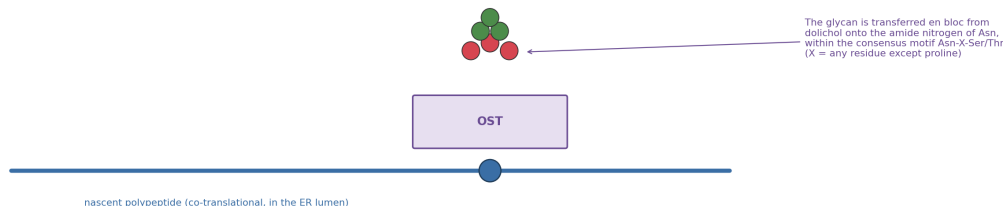
Glycosylation attaches carbohydrate chains (glycans) to a protein, and is the most structurally complex common PTM. It occurs mainly as proteins pass through the secretory pathway (ER and Golgi).

N-Linked Glycosylation: from the ER to the Golgi

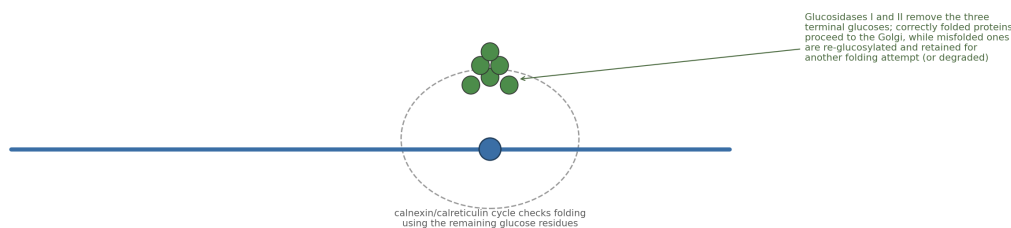
1. A 14-sugar precursor is built on a lipid carrier (dolichol phosphate) in the ER membrane



2. Oligosaccharyltransferase (OST) transfers the whole glycan onto an Asn of the nascent chain



3. ER trimming and quality control — glucosidases trim the glycan; misfolded proteins are retained



4. Golgi remodelling — mannoses are trimmed and new sugars added, building diverse complex glycans

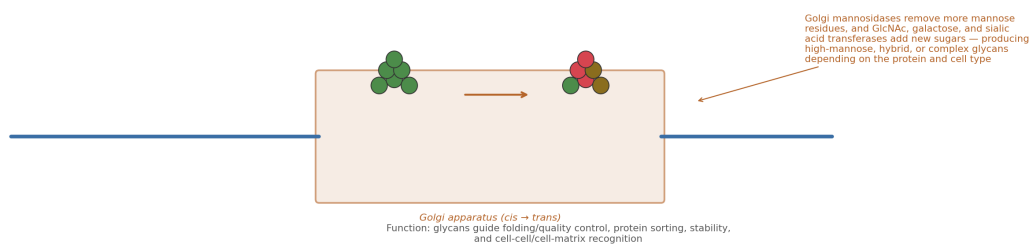


Fig. 2 — N-linked glycosylation begins with a pre-assembled 14-sugar precursor transferred en bloc in the ER, followed by extensive trimming and remodelling in the Golgi.

- **N-linked glycosylation** attaches a glycan to the amide nitrogen of an asparagine within the consensus sequence Asn-X-Ser/Thr; the entire pre-assembled 14-sugar precursor (built on the lipid dolichol phosphate) is transferred at once by oligosaccharyltransferase (OST), co-translationally, in the ER.
- The glycan is then trimmed and used by **calnexin/calreticulin** as a folding quality-control signal, before further trimming and elaboration in the Golgi produces the final high-mannose, hybrid, or complex glycan structure.

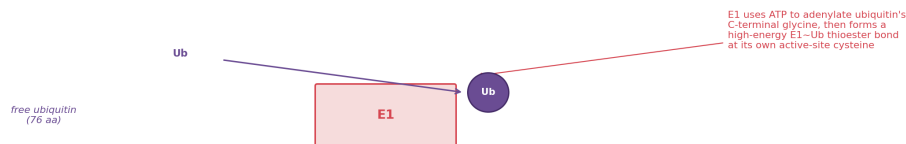
- **O-linked glycosylation** attaches sugars (commonly starting with GalNAc) directly to the hydroxyl of serine or threonine, is added entirely in the Golgi (no lipid-linked precursor), and is common on secreted mucins and many membrane proteins.
- Glycosylation influences protein folding and stability, protects against proteolysis, and mediates cell-cell recognition and adhesion — changes in glycosylation patterns are also a hallmark of many cancers.

5. Ubiquitination and the Proteasome

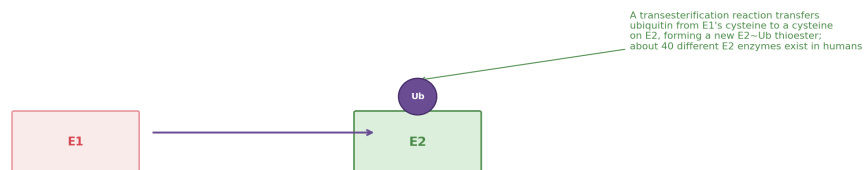
Ubiquitination attaches the small, highly conserved 76-residue protein **ubiquitin** to a substrate, most often as a signal for regulated destruction by the proteasome.

The Ubiquitin-Proteasome Pathway

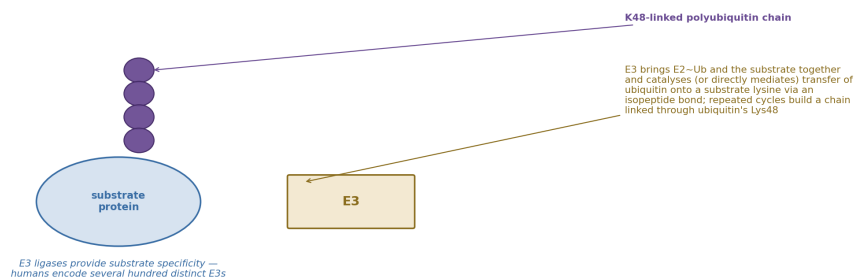
1. Activation — E1 (ubiquitin-activating enzyme) activates ubiquitin using ATP



2. Conjugation — ubiquitin is passed from E1 to an E2 (ubiquitin-conjugating enzyme)



3. Ligation — E3 (ubiquitin ligase) transfers ubiquitin onto a substrate lysine



4. Degradation — the 26S proteasome recognises, unfolds, and destroys the tagged protein

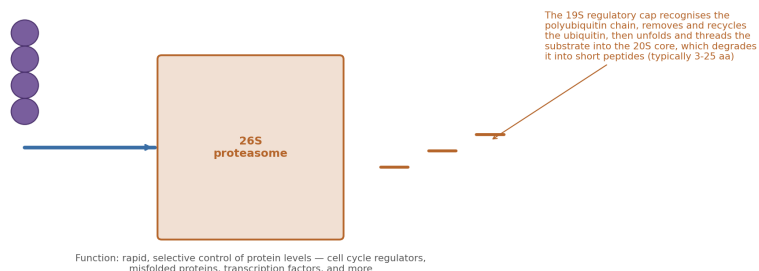


Fig. 3 — The E1-E2-E3 enzyme cascade builds a polyubiquitin chain on a substrate protein, which the 26S proteasome then recognises and degrades.

- **E1 (activating enzyme)** uses ATP to activate ubiquitin's C-terminal glycine and forms a high-energy E1~ubiquitin thioester bond; humans have only a couple of E1 enzymes.
- **E2 (conjugating enzyme)** receives the activated ubiquitin from E1 via a second thioester bond; roughly 40 different E2s exist.
- **E3 (ligase)** provides substrate specificity, bringing the E2~ubiquitin conjugate and a specific substrate together and mediating transfer of ubiquitin onto a substrate lysine via an isopeptide bond; humans encode several hundred distinct E3 ligases.
- Repeated rounds build a **polyubiquitin chain**; a chain linked through ubiquitin's **lysine 48 (K48)** is the canonical signal for degradation by the **26S proteasome**, whose 19S cap recognises the chain, recycles the ubiquitin, and unfolds and threads the substrate into the barrel-shaped 20S core for proteolysis into short peptides.
- Other linkage types have non-degradative roles — for example, **K63-linked chains** and **monoubiquitination** regulate processes such as DNA damage signalling, endocytosis, and protein trafficking rather than marking a protein for destruction.

6. Other Common Modifications

Modification	Residue(s)	Representative role
Acetylation	Lysine (or N-terminus)	Neutralises Lys's positive charge; central to histone regulation of chromatin (histone acetyltransferases/deacetylases)
Methylation	Lysine, arginine	Alters protein-protein interactions; histone methylation marks activate or repress transcription depending on the site
SUMOylation	Lysine	Attachment of the ubiquitin-like protein SUMO; regulates nuclear transport, transcription, and DNA repair
Myristoylation / palmitoylation	Glycine (N-terminus) / cysteine	Adds a fatty-acid anchor that tethers the protein to a membrane
Prenylation	Cysteine (C-terminal CaaX motif)	Adds a farnesyl or geranylgeranyl lipid group; membrane anchoring, especially for small GTPases (e.g. Ras)
Hydroxylation	Proline, lysine	Required for stable collagen triple-helix formation; catalysed by prolyl/lysyl hydroxylases (vitamin C-dependent)

7. Summary: Enzymes and Reversibility

PTM	Adding enzyme	Removing enzyme	Reversible?
Phosphorylation	Protein kinase	Protein phosphatase	Yes

PTM	Adding enzyme	Removing enzyme	Reversible?
Acetylation	Acetyltransferase (HAT)	Deacetylase (HDAC)	Yes
Methylation	Methyltransferase	Demethylase	Yes
Ubiquitination	E1-E2-E3 cascade	Deubiquitinase (DUB)	Yes
Glycosylation	Glycosyltransferases (OST, Golgi enzymes)	Glycosidases (limited)	Largely no, once mature
Proteolytic cleavage	Specific proteases	None (irreversible)	No

8. Quick Revision Summary

Process	Key point
Folding/processing	Chaperones assist folding; signal peptides and propeptides are cleaved; zymogens are proteolytically activated
Phosphorylation	Kinases add, phosphatases remove phosphate on Ser/Thr/Tyr; core signal transduction switch
Glycosylation	N-linked: 14-sugar precursor transferred en bloc in the ER, then trimmed/remodelled in the Golgi; O-linked: added directly in the Golgi
Ubiquitination	E1-E2-E3 cascade builds a K48-linked polyubiquitin chain, targeting the substrate to the 26S proteasome for degradation
Acetylation/methylation	Modify Lys/Arg; especially important in histone-based regulation of chromatin and transcription
Lipidation	Myristoylation, palmitoylation, and prenylation anchor proteins to membranes
Reversibility	Most small-group PTMs (phosphorylation, acetylation, methylation, ubiquitination) are reversible; proteolytic cleavage is not

Compiled study note — molecular biology / biochemistry reference. Figures are original schematic diagrams prepared for this note and are not to scale.