Post-Translation Modifications (PTM)

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On the agenda

• What are PTM, types and functional significance
• PTMs - characterization and prediction:
  ➢ Technical approach - crystallography, Mass Spectrometry, western
  ➢ Bioinformatics approach - database search, sequence based
    prediction methods (motif searches) and servers
Post-Translation Modifications

Post translation modifications are a series of chemical changes that occur in most translated proteins.

More than 350 post-translational modifications (PTMs) are known today in all protein types, affecting the physicochemical properties of proteins, and therefore its function.

The genetic code diagram showing the amino acid residues as target of post-translation modifications.
PTMs have Significant Biological Functions

➤ Increase diversity of gene products, especially under different cellular conditions.

➤ Extend the range of possible functions that can be exhibited by a protein by introducing new chemical groups: e.g., alter hydrophobicity of a protein (synthesis of membrane proteins).

➤ Activating or inactivating enzymes.

➤ Signal transduction mechanisms.

➤ Protein degradation.

➤ Blood coagulation.

➤ Immune system.

PTMs and Cancer - Development of new biomarkers and therapeutics

➤ Specific forms of post-translational modifications of histones (H3 and H4) can be used as tumor associated antigens for diagnosing prostate cancer.

➤ Study of the role of p53 post-translational modifications in carcinogenesis and cancer prevention is useful in the development of new strategies for treating and preventing cancer.

➤ Role of glycosylation in mediating the toxicity of hyperglycemia and in the control of the insulin gene expression.
Proteins that are membrane bound or are designed for excretion are synthesized by ribosomes in the endoplasmic reticulum (ER), and usually contain an N-terminus termed a signal sequence or signal peptide.

Signal sequence is a chain of 13-36 amino acids, predominantly hydrophobic.

The signal peptide is recognized by a multi-protein complex termed the signal recognition particle (SRP), which binds the protein and guides it to the ER and then to the Golgi.

The signal peptide is removed by signal peptidase following passage through the ER membrane.
### Examples of Signal Peptides

| Transport to the nucleus (NLS) | -Pro-Pro-Lys-Lys-Arg-Lys-Val-
| Transport to the endoplasmic reticulum | H2N-Met-Met-Ser-Phe-Val-Ser-Leu-Leu-Val-Gly-Ile-Leu-Phe-Trp-Ala-Thr-Glu-Ala-Glu-Gln-Leu-Thr-Lys-Cys-Glu-Val-Phe-Gln-
| Retention to the endoplasmic reticulum | -Lys-Asp-Glu-Leu-COOH
| Transport to the mitochondrial matrix | H2N-Met-Leu-Ser-Leu-Arg-Gln-Ser-Ile-Arg-Phe-Phe-Lys-Pro-Ala-Thr-Arg-Thr-Leu-Cys-Ser-Ser-Arg-Tyr-Leu-Leu-
| Transport to the peroxisome (PTS1) | -Ser-Lys-Leu-COOH
| Transport to the peroxisome (PTS2) | H2N----Arg-Leu-X5-His-Leu-

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http://proline.bic.nus.edu.sg/spdb/

http://www.signalpeptide.de/index.php
Proteolytic Cleavage

Most proteins undergo proteolytic cleavage following translation. The simplest form of this is the removal of the initiation methionine.

Examples: Pancreatic enzymes and enzymes involved in clotting are activated upon cleavage.

Insulin is secreted as pre-peptide from the pancreas. Following cleavage of the 24 amino acid signal peptide the protein folds into pro-insulin which is further cleaved yielding active insulin.
NLS and MTS

Nuclear localization signal (NLS) directs proteins into the nucleus. There is no cleavage of the peptide. All nuclear proteins are synthetized on free cytosolic ribosomes.

Mitochondrial targeting signal (MTS) directs proteins to the mitochondria. Mitochondrial proteins are made in cytosolic ribosomes.

Phosphorylation

Phosphorylation is one of the most common protein modifications that occurs in animal cells.

The enzymes that phosphorylate proteins are termed kinases and those that remove phosphates are termed phosphatases. Protein kinases catalyze reactions of the following type:

\[ \text{ATP + protein } \rightleftharpoons \text{ phosphoprotein + ADP} \]
Reversible protein phosphorylation regulates most aspects of cell life.

A key event in cell regulation is reversible protein phosphorylation. A protein kinase adds a phosphate group from ATP to the protein, its shape and function is altered. A protein phosphatase removes the phosphate and the protein reverts to its original state.

"for their discoveries concerning reversible protein phosphorylation as a biological regulatory mechanism"

Phosphorylation

Revealed a novel mechanism for enzyme control through reversible protein phosphorylation (discovery of the 50s').

How is the cell affected by protein phosphorylation

1. Receptor phosphorylation
2. Phosphorylation of cytoskeletal proteins
3. Phosphorylation of ribosomes
4. Phosphorylation of gene regulatory proteins
5. Increased hormone release
6. Muscle contraction or relaxation
7. Altered cell metabolism

Phosphorylation Sites - on Serine, Threonine, Tyrosine

- Serine/threonine kinases


The ratio of phosphorylation of the three different amino acids is approximately 1000/100/1 for serine/threonine/tyrosine. Although the level of tyrosine phosphorylation is minor, the importance of phosphorylation of this amino acid is profound. As an example, the activity of numerous growth factor receptors is controlled by tyrosine phosphorylation.

Phosphorylation site database:
http://cbm.bio.uniroma2.it/phospho3d

NetPhos 2.0 Server:
http://www.cbs.dtu.dk/services/NetPhos/

Sulfate modification of proteins occurs at tyrosine residues such as in fibrinogen and in some secreted proteins (e.g. gastrin). The universal sulfate donor is 3'-phosphoadenosyl-5'-phosphosulphate (PAPS).

Sulfate is added permanently - it is necessary for the biological activity and not used as a regulatory modification like that of tyrosine phosphorylation.

Sulfation

http://www.expasy.ch/tools/sulfinator/
Glycosylation

Glycosylation is the addition of saccharide to a protein or a lipid molecule.

Glycosylation can occur:

N-Linked Glycosylation (the usual glycoproteins in mammals) - through the amide group of asparagine.
Consensus sequence: Asn-X-Ser/Thr
X is any amino acid except proline

O-Linked Glycosylation - is to the hydroxyl of serine, threonine or hydroxylysine.

Clinical Significances of Glycoproteins

Most proteins that are secreted, or bound to the plasma membrane, are modified by carbohydrate attachment.

Intracellular proteins are less frequently modified by carbohydrate attachment. Several transcription factors and RNA polymerase II have been shown to be modified by O-GlcNAc linkage.

Glycoproteins on cell surfaces are important for communication between cells, for maintaining cell structure and for self-recognition by the immune system (for example: blood groups).
Enzyme Defects in Degradation of Asn-GlcNAc Type Glycoproteins

<table>
<thead>
<tr>
<th>Disease</th>
<th>Enzyme Deficiency</th>
<th>Symptoms/Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartylglycosaminuria</td>
<td>aspartylglycosaminidase</td>
<td>progressive mental retardation, delayed speech and motor development, coarse facial features</td>
</tr>
<tr>
<td>b-Mannosidosis</td>
<td>b-Mannosidase</td>
<td>primarily neurological defects, speech impairment</td>
</tr>
<tr>
<td>a-Mannosidosis</td>
<td>a-Mannosidase</td>
<td>mental retardation, dystonia, hepatosplenomegaly, hearing loss, delayed speech</td>
</tr>
<tr>
<td>GM, Gangliosidosis</td>
<td>b-Galactosidase</td>
<td>also identified as a glycosphingolipid storage disease</td>
</tr>
<tr>
<td>GM, Gangliosidosis (Sandhoff-Jatzkewitz disease)</td>
<td>b-N-acetylhexosaminidases A and B</td>
<td>also identified as a glycosphingolipid storage disease</td>
</tr>
<tr>
<td>Sialidosis (also identified as Mucolipidosis I)</td>
<td>Neuraminidase (sialidase)</td>
<td>myoclonus, congenital asciites, hepatosplenomegaly, coarse facial features, delayed mental and motor development</td>
</tr>
<tr>
<td>Fucosidosis</td>
<td>a-Fucosidase</td>
<td>progressive motor and mental deterioration, growth retardation, coarse facial features, recurrent sinus and pulmonary infections</td>
</tr>
</tbody>
</table>

http://www.modares.ac.ir/relearning/minades/Structural/BiologyIntro_20081118/Pages/post-translational_modification.htm

Acylation

Acylation is the process of adding an acyl group to a compound. In most cases the initiator methionine is hydrolyzed and an acetyl group is added to the new N-terminal amino acid. Acylation modification allows association of the modified protein with membranes.

Methylation

Post-translational methylation occurs at lysine residues in some proteins such as calmodulin and cytochrome c.
Large Modifications: Ubiquitin

Ubiquitin is a small protein occurring in all eukaryotes, of 76 conserved (human and yeast ubiquitin share 96% sequence identity) amino acids.

Polyubiquitylation can target proteins for proteasome-mediated degradation and thereby has an important function in the regulation of protein abundance and turnover in cells.

Human ubiquitin sequence:

MOIVEKTLTGKITLEVEPSD这不是一个完整的句子。它可能需要一个缺失的单词或标点符号。

Function of Ubiquitin Modifications

Ubiquitination function on cellular processes:
- Antigen processing
- Apoptosis
- Biogenesis of organelles
- Cell cycle and division
- DNA transcription and repair
- Differentiation and development
- Immune response and inflammation
- Neural and muscular degeneration
- Morphogenesis of neural networks
- Modulation of cell surface receptors, ion channels and the secretory pathway
- Response to stress and extracellular modulators
- Ribosome biogenesis
- Viral infection

PTMs can be Characterized or Predicted

**Experimental methods**
- Crystallography
- Mass Spectrometry
- Western

**Bioinformatics methods**

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**Experimental Methods**

**PTM Detection by Crystalization/NMR**

X-ray diffraction/NMR is the method of choice to determine structural information from biological macromolecules to atomic resolution (macromolecular crystallography, crystallography at very high resolution).

**PTM Detection by Western-blot**

Method relies on prior knowledge of the type and position of specific modifications, and availability of specific antibodies.
What is Mass Spectrometer?

![Diagram of Mass Spectrometer Analysis](image)

- Amino acid residue modified with PTM
- Amino acid residue
- N-terminal amino acid residue
- C-terminal amino acid residue

Table 1. Mass Values for Some Posttranslational Modifications and Frequently Observed Bifurcation Losses and Diagnostic Ion Signals

<table>
<thead>
<tr>
<th>PTM</th>
<th>$\Delta m$ (Da)</th>
<th>$\Delta m$ (MS/MS)</th>
<th>MS/MS Diagnostic ion (m/z)</th>
<th>References (examples)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphotyrosine</td>
<td>79.066</td>
<td>79.066</td>
<td>-79.066 (M+H+Na)</td>
<td>18, 17</td>
</tr>
<tr>
<td>Phosphoserine</td>
<td>79.066</td>
<td>97.077</td>
<td>-79.059 (M+H+Na)</td>
<td>17</td>
</tr>
<tr>
<td>Phosphothreonine</td>
<td>26.011</td>
<td>143.116</td>
<td>143.109 (M+H+Na)</td>
<td>15, 15</td>
</tr>
<tr>
<td>Dimethylglycine</td>
<td>14.085</td>
<td>See ref.</td>
<td>144.103 (M+H+Na)</td>
<td>19, 19</td>
</tr>
<tr>
<td>Methylamine</td>
<td>14.085</td>
<td>144.103</td>
<td>144.103 (M+H+Na)</td>
<td>19, 19</td>
</tr>
<tr>
<td>Dimethylarginine</td>
<td>28.021</td>
<td>183.558</td>
<td>183.558 (M+H+Na)</td>
<td>67, 67</td>
</tr>
<tr>
<td>Trimethyllysine</td>
<td>48.347</td>
<td>242.079</td>
<td>242.079 (M+H+Na)</td>
<td>65, 65</td>
</tr>
<tr>
<td>N-methylglycine</td>
<td>162.053 (m/z)</td>
<td>450.053</td>
<td>450.053 (M+H+Na)</td>
<td>19</td>
</tr>
<tr>
<td>263.079 (m/z)</td>
<td>204.497</td>
<td>255.503</td>
<td>255.503 (M+H+Na)</td>
<td>19</td>
</tr>
<tr>
<td>351.105</td>
<td>301.105</td>
<td>301.105</td>
<td>301.105 (M+H+Na)</td>
<td>19</td>
</tr>
<tr>
<td>(m/z)</td>
<td>355.140</td>
<td>355.140</td>
<td>355.140 (M+H+Na)</td>
<td>19</td>
</tr>
<tr>
<td>Citrulline</td>
<td>44.365</td>
<td></td>
<td></td>
<td>19</td>
</tr>
<tr>
<td>Pyridoxal</td>
<td>298.230</td>
<td>272.217</td>
<td></td>
<td>23</td>
</tr>
<tr>
<td>Acetylation</td>
<td>55.000</td>
<td>See ref.</td>
<td></td>
<td>23</td>
</tr>
<tr>
<td>Ubiquitin</td>
<td>162 (acetyl3)</td>
<td>See ref.</td>
<td></td>
<td>70</td>
</tr>
<tr>
<td>Methionine oxidation</td>
<td>15.925</td>
<td>63.998</td>
<td></td>
<td>71</td>
</tr>
<tr>
<td>Trypsin inactivation</td>
<td>338.965</td>
<td>313.965</td>
<td></td>
<td>72</td>
</tr>
</tbody>
</table>

PTM, posttranslational modifications; MS, mass spectrometry; MS/MS, tandem mass spectrometry; m/z, mass-to-charge ratio.
MS detection of PTM

Proteins are typically digested by trypsin and/or endoproteinase Lys C to generate peptides in the mass range 0.5–4 kDa that are suitable for MS analysis (a & b).

Comparative MS analysis of proteolytic peptides that were derived from a purified and modified (phosphorylated) protein.

The mass difference between the peptides at the mass-to-charge ratio (m/z) 1411.6 (part c) and m/z 1331.6 (part d) is 80 Da, and this difference corresponds to the addition of an HPO₃⁻ moiety to the peptide.

Most functionally interesting post-translational modifications (PTMs) are present at low levels in cells and tissues. Mass Spectrometry (MS) and Bioinformatics

Most functionally interesting post-translational modifications (PTMs) are present at low levels in cells and tissues.
Bioinformatics Methods: Integrative Computational Approaches

- Database search of known PTMs.
- Computational sequence based prediction methods (motif searches and conservation).

Take into account:
1. **Context (motif):**
   Example: PKC phosphorylation site: [ST]-x-[RK]
   NetPhorest: database of motifs of phosphorylation signaling
   http://netphorest.info/index.php
   KinBase: http://kinase.com/kinbase/

2. **Conservation:**
   Is the motif found in human, conserved in related organisms (for instance, in chimp)?
**Consensus-Pattern-Profile/Motif**

**multiple alignment**

<table>
<thead>
<tr>
<th>Consensus</th>
<th>Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTTG</td>
<td>[AC]-A-[GC]-T-[TC]-[GC]</td>
</tr>
</tbody>
</table>

Sensitivity: consensus < pattern < profile

**Motif Patterns - syntax**

A sequence motif = a certain sequence that is widespread and conjectured to have biological significance.

- The standard IUPAC one-letter codes.
- 'x': any amino acid.
- '[': residues allowed at the position.
- '{'}: residues forbidden at the position.
- '(' : repetition of a pattern element are indicated in parenthesis. X(n) or X(n,m) to indicate the number or range of repetition.
- '-' : separates each pattern element.
- '‹' : indicated a N-terminal restriction of the pattern.
- '›' : indicated a C-terminal restriction of the pattern.
- '.': the period ends the pattern.
Searching Prosite

http://www.expasy.org/prosite/

Query UniProt for PTM

http://www.expasy.org/uniprot/P00533 => PTM databases
GlycoSuiteDBP00533 => https://glycosuite.proteomesystems.com/glycosuite/glycodb
PhosphoSiteP00533 => http://www.phosphosite.org/proteinAction.do?id=592&showAllSites=true

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PTM Databases - examples

- **General PTM Databases**
  - dbPTM
  - PTM-Switchboard - PTM of TFs
    - [http://cagr.pcbi.upenn.edu/PTMswitchboard/](http://cagr.pcbi.upenn.edu/PTMswitchboard/)

- **PTM Databases for Specific Proteins**
  - Histone sequence database:
  - Plasma Proteome Database
    - [http://www.plasmaproteomedatabase.org/](http://www.plasmaproteomedatabase.org/)

- **Example databases for Specific PTMs**
  - Phospho.ELM - [http://phospho.elm.eu.org/](http://phospho.elm.eu.org/)
  - GlycoSuiteDB, SweetDB - [https://glycosuite.proteomesystems.com/glycosuite/glycodb](https://glycosuite.proteomesystems.com/glycosuite/glycodb)
    - [http://www.glycosciences.de/sweetdb/](http://www.glycosciences.de/sweetdb/)

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Example:

```
http://dbptm.mbc.nctu.edu.tw/search_result.php?search_type=db_id&swiss_id=EGFR_HUMAN
```

```
http://dbptm.mbc.nctu.edu.tw/tutorial.php
```

```
http://dbptm.mbc.nctu.edu.tw/download.php
```
Browse dbPTM

An Information Repository of Protein Post-translational Modification

http://dbptm.mbc.nctu.edu.tw/browse.php

Four external biological databases related to protein post-translational modification information, Swiss-Prot, Phospho.EM, O-GLYCBASE, and UBProt, are integrated into the proposed resource, as shown in statistics page. Furthermore, the experimentally validated sites in each type of PTM were categorized by the modified amino acid, and the number of experimentally validated sites is provided in the summary table, as shown in below. Users can investigate into the substrate peptide specificity of each categorized PTM.

DBPTM 1.0

Latest News:
Jan. 29, 2009:  The structural information, protein dephosphorylation, will be introduced on dbPTM in Feb. 2009.

http://dbptm.mbc.nctu.edu.tw/browse.php

http://www.cbs.dtu.dk/researchgroups/PTM.php

Protein post-translational modification (PTM)

Group leader: Thomas Nielsen
Group members: Tue Anh, Eivind Hlavac, Rune Brorson, Sorensen, Hlavac, Larsen, Pihlin, Thomas Nielsen, Paterson, Ben Paterson

CBS has a long history of predicting various types of protein PTMs (post-translational modifications). Among the first publicly available CBS prediction servers was Predict, which predicts secondary protein structures.

It should be noted that while the "P" in PTM stands for "protein," some of these modifications actually occur in non-proteins. They are important because they are often considered important in the function of the protein.

http://www.cbs.dtu.dk/researchgroups/PTM.php

http://dbptm.mbc.nctu.edu.tw/browse.php
ExPASy Proteomics tools


- ChromP - Prediction of chloroplast transit peptides
- LipP - Prediction of lipoproteins and signal peptides in Gram negative bacteria
- MTP/PROT - Prediction of mitochondrial targeting sequences
- PATS - Prediction of aperoxiporin targeting sequences
- PNAS - Prediction of mitochohndrial transit peptides in Plasmodium falciparum
- PredTar - Prediction of mitochondrial and plastid targeting sequences
- PredOTLS- Predicts O-linked carbohydrates containing proteins
- SignalP - Prediction of signal peptide cleavage sites
- DcytOGlyc - Prediction of O-GlcNAc O-glycosylation sites in Dictyostelium
- NetOGlyc - Prediction of O-GlcNAc (mucin type) glycosylation sites in mammalian proteins
- NetGlyc - O-glycyrlation sites in Lysozyme in mammalian proteins
- NetOGlyc - Prediction of O-glycosylation sites in human proteins
- OGGT - Prediction of O-GalNAc (mucin type) glycosylation sites in eukaryotic (non-protein) proteins
- YinCYc - O-beta-GalNAc attachment sites in eukaryotic protein sequences
- TIGOR predictor - GPI Modification Site Prediction
- CDS - Prediction of GPI anchor and cleavage sites (Ulimor site)
- ProtTREX - Prediction of the human Kohonen Soft Organizing Map
- MyoMyosite - Prediction of N-terminal myosin by neural networks
- NMT - Prediction of N-terminal N-myosinylations
- CSS-Palm - Palmitoylation site prediction with CSS
- PappS - Prelysin Prediction Suite
- NetAcet - Prediction of N-acetyltransferase A (NalA) substrates (in yeast and mammalian proteins)
- NetPhos - Prediction of Ser, Thr and Tyr phosphorylation sites in eukaryotic proteins
- NetPhosYeast - Serine and threonine phosphorylation sites in yeast proteins
- GPS - Prediction of kinase specific phosphorylation sites for 482 human protein kinases in hierarchy
- Sulfotran - Prediction of tyrosine sulfation sites
- Sulfotrane - Prediction of tyrosine sulfation sites
- mRNA2Chrom - Prediction of chromatin localization sites
- SUMOchop - Prediction of sumoylation sites
- TermPred - Prediction of N-terminal modification (version 3)
- NetPSORtRNA - Prediction of protease cleavage sites in prorotosomal proteins
- NetChroma - Chromosome 3C-like protease cleavage sites in proteins
- PseP - Arginine and lysine protease cleavage sites in eukaryotic protein sequences

Please choose from the following PSORT programs for localization prediction:

- PSORTb v.2.0 (Gardy et al., 2004) (v.1.0: Gardy et al., 2003) for bacterial sequences
- Wolf PSORT (Horton et al., 2006) recently updated version of PSORT II for the prediction of eukaryotic sequences
- PSORT II (Nakai and Horton, 1997) for eukaryotic sequences
- PSORT (Nakai and Kanehisa, 1991) for plant sequences
- iPSORT (Bannai et al., 2002) for classification of eukaryotic N-terminal sorting signals

http://www.psort.org/index.html
ExPASy Proteomics tools

- NePRES: Leucine-rich nuclear export signals (NES) in eukaryotic proteins
- PSCAT: Prediction of protein subcellular localization
- SecretomeP: Non-classical and leaderless secretion of proteins
- TargetP: Prediction of subcellular location
- TaffP: Twin-arginine signal peptides
- DAM: Prediction of transmembrane regions in prokaryotes using the Domain Alignment Sulfate method (Stockholm University)
- HMHTOP: Prediction of transmembrane helices and topology of proteins (Hungarian Academy of Sciences)
- PredictProtein: Prediction of transmembrane helix location and topology (Columbia University)
- SOSUI: Prediction of transmembrane regions (Nagoya University, Japan)
- TMHMM: Transmembrane helices in proteins (CBS, Denmark)
- TMpred: Prediction of transmembrane regions and protein orientation (EMBnet-Ch)
- TopPred: Topology prediction of membrane proteins (France)

http://www.expasy.ch/tools/

Conclusion: PTMs are site-specific and affect protein function

| Table 1. Some common and important post-translational modifications |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| PTM type | Mass/Da | Stability | Function and notes |
| Phosphorylation (Ser, Thr) | +80 | +/++ | Reversible, activation/inactivation of enzyme activity, regulation of molecular interactions, signaling |
| Acetylation | +42 | +++ | Protein stability, protection of N-terminus, regulation of protein-DNA interactions (histones) |
| Methylation | +14 | +++ | Regulation of gene expression |
| Acylation, fatty acid modification | +304 | +++ | Cellular localization and targeting, membrane trafficking, regulation of protein-protein interactions |
| Glycosylation, N-linked (144 Da) | +800 | +/++ | glycoprotein, cell-cell recognition/signalling, O-GlcNAc, cell adhesion, regulatory functions |
| GPI anchor | +1,000 | ++ | Glycosylphosphatidylinositol (GPI) anchor, membrane trafficking of enzymes and receptors, highly to outer leaflet of plasma membrane |
| Hydroxylation | +15 | +++ | Protein stability and protein-ligand interactions |
| Sulfonation (O-SO3) | +80 | + | Modulation of protein-protein and receptor-ligand interactions |
| Cysteine bond formation | -2 | ++ | Intramolecular interactions, protein stability |
| Deamidation | +1 | +++ | Possible regulator of protein-ligand and protein-protein interactions, also a common chemical artifact |
| Pyrrolidonyl acid | -17 | +++ | Protein stability, blocked N-terminus |
| Ubiquitination | +1,000 | +/++ | Deactivation signal, removal of protein, ubiquitin-protein site is modified with the Glu-Glu dipeptide |
| Nitrosation of threonine | +85 | +/++ | Oxidative damage during inflammation |

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