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A comprehensive database analysis of post-translational modifications with chemical and functional categories.
A throughout PTM study of 2022 databases.

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ABSTRACT:

One of the most important mechanisms that directly affect protein diversity and function is post-translational modification, which has grown exponentially and now encompasses a vast quantity of data. In order to better understand the nature of Post Translational modifications, in this study we elaborated those modifications and classified them into different perspectives

In order to base our investigations on high-confidence PTM occurrences, we confined our study to post-translational modifications that have been experimentally verified on humans, leaving out any annotated PTMs based solely on computation prediction. Utilizing chemical and functional details from external databases and literature, 170 distinct alterations from the neXtProt database were examined and identified. These modifications had a total occurrence of 187.220.

A careful examination of the data at hand led to the identification of 47 types that contained all 170 modifications. It was revealed that every amino acid underwent at least one type of modification, with cysteine serving as the target of the most diverse modifications—28 in all. Another finding was that most modifications function by converting the target amino acid, while small chemical groups are the most frequent functional groups.

The purpose of comprehensively classifying post-translational modifications is to provide an approach that may help understand the effects of these modifications on protein function. It's known that the physicochemical nature of these modifications exerts significant effects on function. However, there is a need to further deepen the current classifications

KEYWORDS: Post-translational modifications; amino acid modifications; reversible /irreversible modifications; Data analysis of modifications

1. INTRODUCTION

The diversity of the proteins occurs through two mechanisms: one is alternative splicing at the transcriptional level, and another is covalent modifications on proteins named post-translational modifications (PTMs). Beyond the diversity of proteins, large number of PTM types have been documented in the living organisms [1]. These modifications are prominent in many critically essential biological processes, including DNA repair and epigenetic, signalling cascades, apoptosis, intracellular locations, and protein - protein interaction etc [2]–[5]. On the other hand any malfunction in the formation or regulation of these modifications is frequently associated with diseases such as blood, cancer, and neurodegenerative disorders [6]–[8]. Therefore, PTMs are one of the primary objectives of present therapeutic and diagnostic studies [9]–[11]. Given the current level of assets in modern drug design, the mission is to reach novel compound with appealing chemical profiles that will facilitate for deeper investigation of the convergence of compounds and biological room. To develop therapeutics that function and are safe, it is essential to find small compounds with good ligand efficiency, high activity, and selectivity. Characterization of PTM

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patterns, on the other hand, is a sophisticated and time-consuming feature of biopharmaceutical development and production.

The essential components of PTMs vary broadly, from the addition or removal of a single atom to the addition of larger functional groups. These events occur reversibly as well as irreversibly [7], [12]. Reversible modifications are generally formed by attaching a modifying chemical group to an amino acid residue on the protein [13]. These PTM events vary widely, from the addition of small groups such as phosphorus (phosphorylation) and methyl (methylation) to proteins such as ubiquitin (ubiquitination). Conversely, changes that irrevocably impact the protein fate (proteolytic cleavage) or persistently change the protein's characteristics (deamidation, prenylation) are irreversible modifications[14]–[16]

In general, PTMs occur strongly or weakly on the side chains. Strong covalent binding occurs with amino acids C, M, S, T, Y, K, H, R, D, and E, whereas a weaker covalent modification takes place with N and Q [7]. These changes have a broad range of effects on the properties and behavior of proteins, such as influencing protein-protein interactions [17], modulation of intracellular signalling events [18] or regulating protein lifespan [19].

PTMs also display selectivity depending on the properties of amino acids. For example, phosphorylation of a protein typically occurs on S, T, and Y amino acids as they contain a free hydroxyl group to which phosphorus may be attached. On the other hand, methylation occurs primarily by the amino acids K and R [20], [21]. Moreover, PTMs are carried out on specific regions of the protein by highly specific modifying enzymes. For example, Type I protein arginine methyltransferases perform methylation specifically at the arginine glycine glycine (RGG) amino acid sequence on proteins [22].

Studies on PTMs are carried out on two main experimental methods [23] First, there are traditional methods like Western blot and mutagenesis techniques; second, there are modern methods like mass spectrometry and large-scale proteomic studies that provide large amounts of information. Advancements in mass spectrometry-based detection methods, radioactive label method, chromatin immunoprecipitation (ChIP), and liquid chromatography [21], [24] and large-scale genome sequencing [5] have generated enormous quantities of annotation data of PTMs and Proteins. In addition to aforementioned approaches which are very laborious and expensive methods, Tools for bioinformatics based on artificial intelligence can quickly and cost-effectively predict PTM and binding sites [25]

Numerous databases, software, and applications have been developed in an effort advance our understanding of the diverse PTMs found in different species and to aid in the analysis of sophisticated PTM data easier. Due to the complexity and diversity of PTMs, These databases are dedicated to single focus, like Phosphositeplus (https://www.phosphosite.org), which emphasizes on phosphorylation, and Ubiprot (http://ubiprot.org.ru), that provides information on ubiquitination. Additionaly, there are specific databases that link PTMs to illnesses such as PTMD (http://ptmd.biocuckoo.org) and dbPTM (http://dbptm.mbc.nctu.edu.tw/)

Consequently, The most recent subject in biochemistry and molecular biology is the continuously increasing variety of proteins and PTMs. In particular, understanding the pathophysiology of diseases and developing novel types of pharmaceuticals require knowledge of these diversities, which experimental validation and association with biological processes and functions give.

It is crucial to categorise those PTMs according to their physico-chemical and conformational alterations in order to better understand their function, impacts and possible drug design. In this study, the experimentally verified PTM's were classified according their type of reaction, type of functional group and type of modification. An overview of the most common PTM's and the number of different PTM's per amino acid is also given.

2. RESULTS

The first finding was the fact, that all amino acids have at least one experimentally verified PTM to their name. Cystine with 28 different PTM's is the most diverse amino acid. Figure 1 gives the amino acids with their

number of different PTM's. One can see, that amino acids with strong covalent bindings tend to be more diverse than weaker ones.

Another important statistic is the frequency of the PTM Types themselves. Figure 2 shows the most common 15 PTM Types in log10 notation. As expected and in accordance with broad literature, the prevalent PTM type is phosphorylation with 114.687 occurrences. The huge gap is observable, with sulfation on rank 15 with only 157 occurrences in total. All the other PTM's summed up occur 795 times.

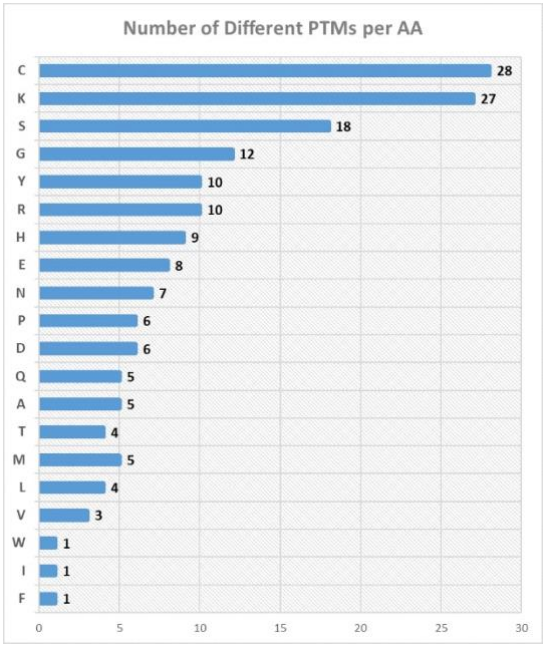

Figure 1: Number of PTM's per amino acid (AA); Alanine: A, Arginine: R, Asparagine: N, Aspartate: D, Aspartate: B, Cysteine: C, Glutamate: E, Glutamine: Q, Glutamate: Z, Glycine: G, Histidine: H, Isoleucine: I, Leucine: L, Lysine: K, Methionine: M, Phenylalanine: F, Proline: P, Serine: S, Threonine: T, Tryptophan: W, Tyrosine: Y, Valine: V

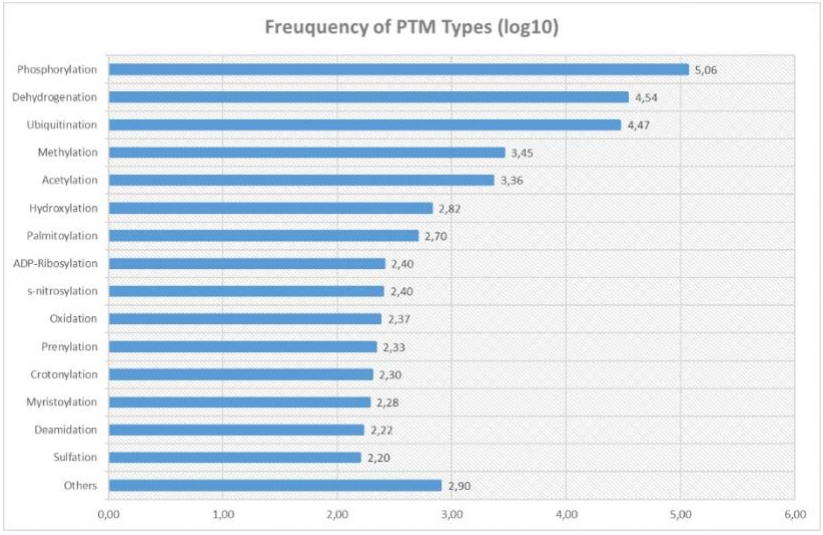

Figure 2: Frequency of PTM Types

Most PTM's prefer the C or N Terminal of the proteins. Only 18,94% of PTM's happen on other parts of the protein. On the other hand, over 97% of all PTM's are reversible. Therefore, a more interesting distribution is to look at the reaction type in combination with the reaction terminal. Figure 3 shows that irreversible PTMs only happen on CT or NT, with a slight preference for NT.

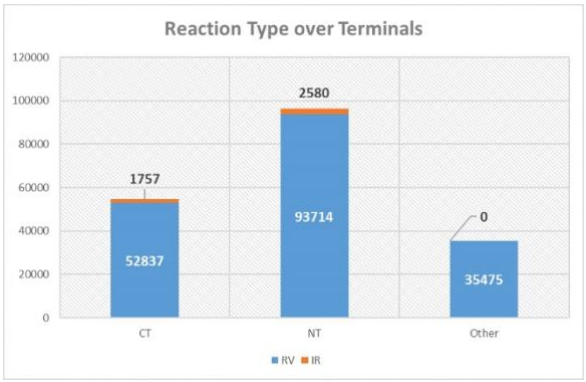

Figure 3: Distribution of Reaction Type (RV: Reversible, IR: Irreversible) over Terminals (CT: C -Terminal, NT: N-Terminal, Others)

In the next step, the type of modifications and the type of functional groups were investigated. Figure 4 shows, that most PTM's work by converting an amino acid (CA), closely followed by disulfide bonds (DS) and cross-linking (CL). Figure 5 shows that small chemical groups (SC) are the most common functional group, followed by lipidation (LP) and nucleic acids (NA). One can also see, that a huge part of the PTM's remain unclassified, highlighting an important area for future research.

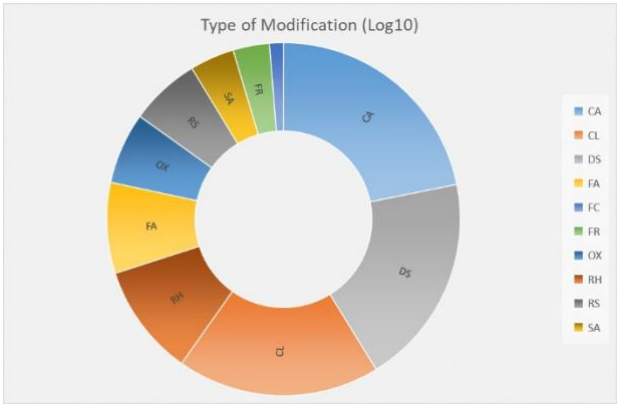

Figure 4: Distribution of PTM's according to their type of modification CA: Convert amino acid, CL: Cross-Link amino acid, DS: DiSulfide Bond, FA: Forming an adduct with one ADP-ribose, FC: Forming an adduct between cysteine residue, FE: Forming an adduct with epicocconone, FR: Forming an adduct with a riboflavin compound, OX: Oxygenation, RH: Replacing H, RS: Replacing a residue sulfanyl group, SA: Substituting amino acid, X: Unclassified

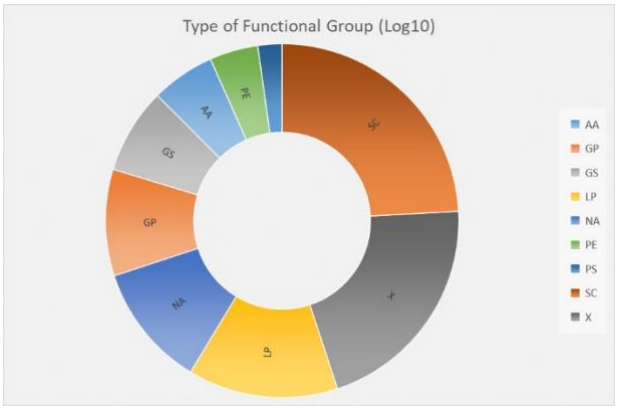

Figure 5: Distribution of PTM's according to their type of functional group; AA: amino acid, GP: Glycoprotein, GS: Glycoslation, LP: Lipidation, NA: Nucleic Acid, PE: Peptid, PS: Polysaccharides, SC: Small Chemical Group, X: Unclassified

3. DISCUSSION

PTM is the collective term for the alterations to the primer structure that arise during the maturation of proteins. These alterations occur either once a group is removed from the protein's primary structure or when the monomers that form this structure form covalent bridges with other groups both inside and outside the structure. As a result, the physicochemical characteristics and sizes of proteins change substantially, which in turn alters, enhances, or inhibits specific functions of proteins or guides the protein to a new subcellular location.

Given the rising availability of data on PTMs, the influence of PTMs on the structure and functionality of proteins has come to the forefront as a research topic. However, the functional significance of many PTMs has yet to be defined. The purpose of this study was to update the data on PTMs from different database and resources to generate helpful data on the nature of these modifications from many perspectives that may potentiate our understanding

Most literature about PTMs concentrate naturally on the more common types and it is easy to find very detailed information about those. The less common PTM's however are not always documented appropriately and to our knowledge there is no complete list considering the aspects investigated in this paper. So the given complete list of all experimentally verified PTM's in human proteins should be an important information source. There are still some unknown or uncategorized aspects, which display the areas for future work.

In our study, we revealed that all genetically encoded amino acids, without exception, undergo the PTM process (Table 1). The heterogeneity of PTMs is guided by the physicochemical characteristics of the side chains of amino acids [20]. For instance, serine (S), an amino acid with a hydroxymethyl side group, is classified as a polar amino acid, making it very attractive to many PTMs. S receives 18 distinct types of PTM, as seen in Table 1. However, it should be noted that an amino acid's propensity to take up PTM could be influenced by causes other than how frequently it appears in proteins. Tryptophan, for instance, which is the least encoded amino acid in proteins, appears to have the least PTM (table 1).

First, we categorised the PTMs according to the ontologically given names of these molecules, resulting in 47 PTM types comprising 170 PTMs. These are the well-known modifications akin to phosphorylation, methylation, and ubiquitination, etc. In this study, we summerized those PTMs as the most common 15 PTMs (Figure 2)

One of the immediately noticeable results in our study was the non-appearance of some PTMs, which are known to be the most common PTMs in numerous publications [29]–[31]. For instance, whilst sumoylation is one of the most common modifications, we have been unable to spot any sumoylation or a related modification in our study's validated human database. We checked the situation in the UNIMOD database (www.unimod.org) in this context. UNIMOD is a comprehensive and consistent protein modification database designed for mass spectrometry [32]. It has been noted that the sumoylation modifications with UNIMOD Accession Nr: 846, 877, 876, 932, 933, 960, 961, 1293, 1405, 1406 and 1799 have not been validated. As a result, such modifications were inevitably excluded from this study. An intriguing situation that suggests that the ends of the proteins may affect the reaction type of PTMs (reversible) is encountered in lipidation modifications as a functional group. Myristoylation and palmitoylation (N terminal modification) are reversible PTMs, while prenylation (C terminal modification) is irreversible [33].

For a modification to ensue, access to that site should be provided by the protein. This criterion become more probable to be fulfilled in nonglobular parts of the sequence [34]. These PTMs are considered to as canonical PTMs because they are notably found at the ends of proteins, such as the N- and C-terminals. As seen in Table 3, only 35475 of the 186363 PTMs fall outside this definition. Moreover, PTMs occur in reversible and irreversible manners. When we jointly analyse which type of reaction occurs at which terminal, as shown in Table 3 all PTMs outside both ends are reversible, and the irreversible PTM rate is 2.3% of all PTMs. This type of modifications occurs during proteolysis and redox signaling [35].

Second, when we classify alterations based on their chemical properties and the chemical interactions they create on the structure (Figure 4), we determine that additions or deletions of groups modify the structure of

the amino acids in the protein's primary sequence. Figure 4 shows, that most PTM's work by converting an amino acid (CA), closely followed by DiSulfide Bonds (DS) and Cross-Linking (CL). Twenty amino acids are encoded in protein translation. According to Table 4, most of the PTMs convert amino acids in the primary sequence into different amino acids that are not coded by the genome. So, through these modifications, the limits of genetic code are exceeded. Incidentally, figure 5 shows that small chemical groups (SC) are the most common functionally classified group which is also overlap with the converted amino acid structure.

In the literature one can find different approaches for classifying PTM's [34]–[36] . Few research [36]–[39] that integrate PTM data from various sources to characterise correlations between PTMs are known to exist, to the best of our knowledge. In this work, we tried to unite these approaches in the light of our analysis of current data gathered from several up-to-date databases. Thus we were able to shed some light on the effects of PTM's on the protein functions.

4. CONCLUSION

Post-translational modifications are a major contributor to protein diversity. We need to better comprehend the PTMs, which give the functionality of the proteins engaged in all biological processes and the cell's building blocks based on their functionalities. However, our expertise in PTMs' functions is currently very limited.
In this work, we examined a wide range of data by categorising the chemical properties of PTMs ontologically. Our meta-analysis and computer-assisted data mining studies on this topic are still ongoing
It will be more straightforward to develop second-generation pharmaceuticals with a PTM profile designed to maximise therapeutic usefulness if these structural-functional linkages are well understood.

5. MATERIALS AND METHODS

In this work, the use of the database neXtProt [26] as the main source of data was chosen because it combines the data about human proteins available in the most common protein databases, including the PTM information and differentiating between experimentally verified results and others.

In the data release from 2021-02-15, there are 20.379 proteins and 42.368 isoforms with a total of 191.837 PTM's, covered by a total of 528.233 publications. To avoid multiple countings, isoforms were disregarded in this study. Also only experimentally verified proteins were included, thus leading to a final total of 18.371 proteins and 187.220 PTMs.

neXtProT doesn't classify PTM's by their nature, rather gives the raw name of the modification. For example the most common modification Phosphorylation happens mainly on three aminoacids, namely S, T and Y. The name of the PTM's are respectively "O-phospho-L-serine", "O-phospho-L-threonine" and "O4'-phospho-L-tyrosine". While it is easy to group those 3 PTM's to a PTM Type called Phosphorylation, this isn't straightforward for all the 170 PTM's. Furthermore, there is no structured information about their chemical or reaction properties.

Utilizing sources like UniProt [27] and DisProt [28], the type of reaction was annotated. While most PTM's are Reversible (R), some are Irreversible (IR), and a few couldn't be categorized (X=Unclassified). Another important aspect is the reaction terminal, with the classifications CT (C-Terminal), NT (N-Terminal) and others. A detailed study of the chemical properties of the PTM's and their functioning resulted in the detailed classifications given in Table 1.

Table 1: Classifications for Type of Modification and Type of Functional Group

Type of Modification		Type of Functional Group	
Abr.	**Name**	**Abr.**	**Name**
CA	Convert amino acid	AA	amino acid
CL	Cross-Link amino acid	GP	Glycoprotein
DS	DiSulfide Bond	GS	Glycoslation
FA	Forming an adduct with one ADP-ribose	LP	Lipidation
FC	Forming an adduct between cysteine residue	NA	Nucleic Acid
FE	Forming an adduct with epicocconone	PE	Peptid
FR	Forming an adduct with a riboflavin compound	PS	Polysaccharides
OX	Oxygenation	SC	Small Chemical Group
RH	Replacing H	X	Unclassified
RS	Replacing a residue sulfanyl group		
SA	Substituting amino acid		
X	Unclassified		

With this data available in relational tables, univariate statistical analyses were performed to calculate frequencies regarding the above introduced classifications. The most important ones are given in the result section. An emphasis was given on statistical information found in older publications so that with this newer and broader dataset, some important updates were found. This updated information along with some novel findings will be guiding researchers to more focused future work.

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Conflict of interest statement: The authors declare there are no competing interests.

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