Spontaneous Post-translational Modification:

Crosstalk of Proteolysis, Autophagy, and Apoptosis in Aging

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Abbreviations

Alzheimer disease (AD). Parkinson disease (PD). Prion diseases (PrD).

Alpha-synuclein (aSyn),

Beta-amyloid (A-β).

Enzymatic and spontaneous post-translational modifications (PTMs ^{Enz} and PTMs ^{Sp}).

Non-equilibrium phase transitions (PhTs NE).

D-amino acids (D-AAs).

Deoxyribonucleic acid (DNA).

DNA methylation (DNAm).

DNA methyltransferases (DNAMTs).

Cataract (Ctr)

Long-lived proteins (LLPs).

Multiple sclerosis (MS).

Myelin basic protein (MBP).

Non-equilibrium phase transitions (PhTs ^{NE}).

Primarily on reproductive success (RS).

Prion proteins (PrP).

Pulmonary diseases (PulmD)/

Reproductive success (RS)

Ribonucleic acid (RNA).

Serine hydrolase (SerH)

Serine racemase (SerR)

Serine proteases (SerPr).

Short-lived proteins (SLPs).

Asparagine (Asn/N). Aspartate (Asp/D). Serine (Ser/S). Cysteine (Sys/C). Threonine (Thr/T). Tyrosine (Tyr/Y).

Cytosine-phosphate-guanosine (CpG).

Key Words: racemization, synaptic spine, cytoskeleton, post translational modification, apoptosis, spontaneous phase transitions, spontaneous racemization

There is no conflict of interest.

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Contents

Abstract

Biochirality is evident in the hierarchical relation of molecular and cellular physiology during organism development and aging. Molecular chirality influences the cellular physiology and higher levels of biological processes, such as perception, memory and cognition, through intermolecular interactions between DNA, proteins, and lipids. At the molecular level, an organism's aging is the accumulation of macro-molecules with the aberrant composition, chirality, folding, and function. Cellular aging is driven by the non-physiological phase transitions (PhTs) within membrane-bound and membrane-less compartments. Genomic instability and protein aging, as the interconnected root-causes of cell and organism aging, share two essential feature – spontaneous nature and accumulation over a lifetime. Consequently, we will analyze the interaction between the enzymatic (Enz) and spontaneous (Sp) post-translational modifications (PTMs ^{Enz} and PTMs ^{Sp}). Both forms of PTMs significantly contribute to the balance of L- and D-amino acids (L/D-AAs) in organisms, modulating the functions of nervous and immune systems. The most abundant form of PTM - enzymatic phosphorylation is bio-chemically associated with the spontaneous racemization (Rz ^{Sp}).

The crass talk of enzymatic phosphorylation and spontaneous racemization, as an essential determinant of protein aging and aggregation, associated with the aberrant autophagy, apoptosis, and cell signaling, is discussed in this review.

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Physical Ground of Biochirality

General

Biochirality reflected in the stereo-transformations of proteinogenic AAs is closely linked to the physics and thermodynamics of the spontaneous non-equilibrium phase transitions (PhTs $^{Sp/NE}$) [1, 2]. The prevalent translation of the L-AAs containing proteins and biosynthesis of L-phospholipids ^{I} is the thermodynamically unfavorable step. The out-of-equilibrium nonracemic steady state of a living organism can be maintained as long as the energy input exists. Such a dynamical asymmetric state of systems can "retain their asymmetry for times longer than their racemization time" [3]. It means that the thermodynamically uphill processes are necessary for the function of living systems. Any process directed to the equilibrium ~~racemic~~ state, such as racemization and aggregation of proteins, is thermodynamically favorable, i.e., irreversible process [4].

Protein Level

The primary determinants for peptide/protein function, stability, and susceptibility to enzymatic activity are the AA's composition, chirality, and sequence. All three factors are critical for protein degradation pathways, including proteolysis, fibrilization, and aggregation. Several studies suggest that resistance to proteolysis increases with increasing instability of the enzyme and the target protein's stability [5]. Proteinogenic amino acids (AAs) are not equivalent to known forms of enzymatic post-translational modifications (PTMs). First of all, it is relevant to phosphorylation and racemization. Our area of interest is the role of spontaneous PTMs (PTMs ^{Sp}), including racemization, in protein metabolism, cell and organism aging.

I. Phosphatidyl-D-serine (PSer-D) accounts for 0.9 % of the total PS in the rat cerebral cortex [6].*

Lifetime of Organism: Cell, Proteins. and Lipids Aging

Biochirality is evident in the hierarchical relation of molecular and cellular physiology during organism development and aging [7, 8]. The accumulation of age-related changes occurs preferentially in long-lived postmitotic cells, including neurons, retinal pigment epithelium (RPE), cardiac myocytes, and skeletal muscle fibers [9]. Cellular aging is driven by the aberrant phase transitions (PhTs) within membrane-bound and membrane-less compartments [10]. At the molecular level, an organism's aging is the accumulates of macro-molecules with aberrant composition, chirality, and folding observed in long-lived proteins (LLP) [11] and phospholipids [6]. [II] Cell membrane aging occur through time-dependent changes in membrane-bound proteins and lipids. Among the thousands of known lipids in each brain cell, the specific families found to be altered throughout aging and AD's progress of Alzheimer's Disease (AD). The role of sphingolipid and phospholipid metabolism in aging, lifespan regulation, and age-related disease were frequently reviewed [12, 13]. AD-related changes were linked [14] mostly to phospholipid composition. However, the contribution of aberrant phospholipids and lipid chirality to cell aging has been ignored (mostly due to the technical challenges). Time-dependent alterations at the molecular and cellular levels are controlled by both inherited and non-inherited factors. The natural selection of protein's and phospholipid's functions is accompanied by the cell type-specific events (including autophagy, proteolysis, cell cycle, and apoptosis) is based primarily on reproductive success (RS) and not so much on post-reproductive survival (although the latter can affect the RS of kins).

In most animals, females live longer than males, and there are dramatic differences in lifespan between species. A genomic predictor of lifespan in vertebrates is deoxyribonucleic acids methylation (DNAm) DNAm, linked to the density of cytosine-phosphate-guanosine (CpG) sites, may predict maximum lifespan in vertebrates [16]. DNA methylation, catalyzed by the DNA methyltransferases (DNAMTs),

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II. Aging of lens indicated by the decrease in the transparency is associated with the aging of many different molecular constituents, the main of which are proteins and phospholipid [15].

is a crucial player in epigenetic silencing of transcription [17]. Altered DNA methylation might also be a post-translational modification that can affect transcription of key genes involved in the response to epigenic cues [18]. Female's median lifespan is, on average, 18.6% longer than that of conspecific males, whereas, in humans, the female advantage is, on the average, 7.8% [19].

Spontaneous Events in Development and Aging

Epigenetic modifications are heritable and non-heritable changes in gene expression not encoded by the DNA sequence. The factors representing the epigenetic landscape of aging include DNA methylation, the histone code, noncoding RNA, nucleosome positioning, and PTM of proteins. In addition to sex- and species-specific genetic programs, aging of an organism is affected by many additional factors. Most studied of them involve aberrant signaling [18, 20] and accumulation of dysfunctional macro-molecules including DNA [21], lipids [13, 15, 22], and proteins [2]. The common causal factors for all three kind of macromolecules are spontaneous, age-related events at the molecular and cellular levels [23, 24]. Spontaneous events are observed through all hierarchical levels of biological processes, including

- DNA mutation [25],
- protein folding [26, 27, 28],
- aggregation of Aβ peptides [29],
- insertion of proteins into membranes [30],
- G-protein-coupled signaling [31, 32],
- symmetry breaking during cell division [33],
- fluctuation of the resting membrane potential [34],
- T cell proliferation [35],
- intracellular Ca^{2+} oscillations [36],
- activity smooth muscle [37],
- spontaneous eye movement [38],

- attenuation of sensory perception [39],

- electrical brain activity [40],

- default-mode networks (DMN) activity [40],

- fluctuations in attention [41, 42],

- involuntary autobiographical memories [43, 44],

- spontaneous cognitive processes [44, 45, 46, 47; 48], and

-spontaneous behavior [47, 49].

The inter-connection between them received increasing attention in association with the PTMs ^{Sp} of proteins [28] and spontaneous non-equilibrium phase transitions PhTs ^{SpNe} [2].Holding in mind the inherent integrity of all complex of biological events, we will be focused on the spontaneous events on the molecular level. More specifically, on the spontaneous PTMs ^{Sp} of proteins.

Spontaneous and Enzymatic PTMs

At the molecular level spontaneous event are observed in the biochemical reactions. Spontaneous (Sp) biochemical reactions are the major contributor to the non-physiological (non -enzymatic) PTMs such as glycation [50] and spontaneous racemization [29]. Racemization, as manifestation of molecular biochirality, is a consistent target of attention. Spontaneous racemization is well studied at DNA and protein levels, but much less so in lipids. DNA biochirality is attributed to the predominant existence of keto-isomers. [III] Two other forms (known as a smoking gun for DNA mutation mechanism) are aberrant, serving as the molecular triggers of the DNA repair pathway [51]. Epigenetic modifications are essential for PTMs of proteins, cell proliferation, and organism development. However, all three processes are influenced by the spontaneous age-dependent DNA mutation [52] and aberrant protein folding [53, 54]. Aging, as a slow, time-dependent decline of a set of multiple biological functions, can be discriminated

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III. Each of the bases in DNA can appear in one of three geometric forms (keto-, imino-, and enol-), called tautomers, which are isomers. The keto form of DNA is predominant (or natural).

into well-defined categories, including the accumulation of genomic damage (leading to chromosomal instability), reactive oxygen species-induced damage to mitochondrial functions, reduced energy production, stem cell depletion, accumulation of damaged proteins, and alterations of the epigenome. In each human cell, the frequency of spontaneous events within DNA molecule comprises nearly 600 mutations per hour [25]. A similar number is attributed to spontaneous modification of proteins. Genomic instability and protein aging are considered as interconnected root causes of organism aging, which share essential features – a spontaneous nature and accumulation over a lifetime. As an example of such an interaction, we can refer to the fact that AAs sequences of many DNA-associated proteins, including human enzyme DNA polymerase, (1-527) contains evolutionarily conserved residues, several of which (including Ser), are racemization prone [55, 56]. Consequently, DNA polymerase is known as a highly mutable enzyme [56]. Now we will narrow our attention to the aging of an organism caused by the accumulation of aberrant proteins and the corresponding dysfunction of cellular organelles. The biochirality at the protein level is traditionally considered through the prisms of two sciences: biophysics and biochemistry. The former is related to the non-equilibrium phase transitions (PhTrs ^{NE}), the latter is related to the interaction between the mechanisms of enzymatic (Enz) and spontaneous (Sp) PTMs (PTMs ^{Enz} and PTMs $^{Sp)}$. Biomolecular condensates containing proteins, lipids, and RNAs undergo spontaneous (Sp) non-equilibrium (NE) phase transitions (PhTs) {NE-PhTs ^{Sp}} accompanied by spatial separation [29, 57, 58]. Within biomolecular condensates, PhTrs ^{NE} are closely linked to the abnormal forms of PTMs [2] and dysfunction of membrane-less organelles (MLOs) [10]. The mechanisms of interplay between enzymatic (i.e., physiological) versus spontaneous (i.e., non-physiological) processes in biomolecular condensates remain unclear. This uncertainty is associated with the lack of systematic attention to the spontaneous post-translational modifications PTMs ^{Sp} at the protein levels.

Protein Aging.

The sources of aberrant proteins, beyond erroneous synthesis by cytoplasmic and mitochondrial ribosomes, include damage induced by reactive oxygen and nitrogen species, glycation and cross-linking

by glucose, age-related changes in the immune system, excessive phosphorylation, and spontaneous events such as deamidation, isomerization, and racemization of AAs. It has been recognized that two major PTMs influencing protein aging are (a) excessive phosphorylation and (b) spontaneous racemization. Notable, that for the running of both mechanisms the small subset of proteinogenic AAs was evolutionary selected. Although D-AAs are well-known landmarks of many neurodegenerative diseases, the role of Rz ^{Sp} is not consistently addressed. We will analyze the interaction between the mechanisms of enzymatic (Enz) and spontaneous (Sp) PTMs (PTMs ^{Enz} and PTMs ^{Sp}). Both forms of PTMs provide a significant contribution to the level, of D-amino acids (D-AAs) in organism.

Enzymatic Phosphorylation

Protein phosphorylation is a fundamental biological process controlling transcription and translation of proteins, regulating of the cell cycle, neuronal signaling, energy metabolism, lifespan of an organism, as well as the learning and memory function. The transfer of phosphate groups between an ATP molecule and various substrate proteins is a primary mechanism of protein kinases and protein phosphatases [59]. Twenty-two proteinogenic AAs are not equivalent in many aspects, including their nutritional values and susceptibility to PTMs ^{Enz}. First of all, it is relevant to the enzyme driven phosphorylation and racemization. The first phosphoprotein (casein) was discovered in 1883, while corresponding enzyme responsible was identified more than 100 years later [60]. Protein kinase and phosphatase allow the tight spatial and temporal regulation of cell signal transduction. The molecular networks of mammalian protein phosphorylation have been extensively investigated due to its abundance and relevance to biological signaling and its associations with many human disorders [61]. The phosphorylation of the canonical hydroxyl-containing AAs the serine (Ser), threonine (Thr), and tyrosine (Tyr) (known as the phosphorylation-prone AAs) are the primary sites of phosphorylation-mediated signaling in non-plant eukaryotes (see Fig. I and Tabl. I} [62, 63, 64, 65]. The stereochemical identity of Ser, Thr, and Tyr (including the presence of an hydroxyl group) was causal for the evolutionary selection serving the major biological perception-related signaling pathway [66].

The illustrative example of such selection is the phosphor-sites of proteins from human eye lenses [67]. Due to the involvement of many sequence-dependent and site-specific biochemical factors [IV], the list of primary phosphorylation-prone AAs can be different from well know the Ser, Thr, and Tyr triplet. Indeed, experimental studies reveal many exceptions, such as phosphorylation of histidine and aspartic acid (Asp) residues in two-component signal transduction systems [68, 69, 70, 71], phosphorylation of the SerSerAsp triplet in dentin proteins [70], and Ser/Asp phosphorylation in amyloid-b (A- β) peptide [2].

Spontaneous Racemization

Spontaneous racemization impacts specific sub-set of proteinogenic AAs and just a few from many known proteins (see Table I.). The spontaneous processes, altering the structure and function of proteins in vivo, are characterized by a relatively short lifetime than the neuronal cells lifetime. The study of model synthetic peptides at the "natural conditions" (T = 37 "C, pH = 7.4) show that the halftime of aspartyl (Asp) racemization halftime is about 19.5 h. More essential that, in this particular peptide(L-Val-L-Tyr-L-Pro-L-Asn-L-Gly-L-Ala), the racemization time of any specific AAs can be easily modified et last in 10th fold in both directions, just by modification of neighboring (to Asp) residues [72, 73]. The facts mentioned above support the notion that the relatively short but repetitive increments of protein's lifetime will favor the accumulative age-dependent impacts of spontaneous PTMs. Even this brief excurses in the field of spontaneous racemization's thermodynamics is convincing to understand the causal role of spontaneous PTMs in the developing dis-functional proteins, protein aggregates, aberrant autophagy, triggering apoptosis, and neurodegeneration. Unfortunately, the role of spontaneous \PTMs is frequently and systematically overlooked [74]. The contribution of spontaneous racemization (Rz^{Sp}) to protein aging and corresponding constraints to

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IV. For the trans-membrane proteins, the degree of topological rearrangement upon phosphorylation are lipid charge– and lipid environment–dependent [71].

proteolysis was recently reviewed in relation to the pathophysiology of amyloid β (A-β) peptides [29]. Considering the biochirality at the protein level, we should take to account the multiple sources of D-AAs in the organism. These include (i) the endogenous (internal) microbial flora, (ii) ingestion/digestion of food, (iii) environmental microorganisms, internal (iv) enzymatic, and (v) spontaneous racemization of L-AAs incorporated into polypeptides during aging [29, 75, 76, 77]. It is widely recognized that, Thr, and Tyr residues are the most racemization prone. However, due to the involvement of many biochemical factors, such generalized recognition can be misleading. Indeed, according to other sources, the list of racemization-prone AAs (in order of decreasing racemization rate) begins from residues Ser, Asp, and Lys = His [78, 79, 80]. It is notable that in different evaluation protocols, Ser retain the leading position.

Cell Aging

Cell aging is a divided into two types: intrinsic and extrinsic aging. The intrinsic determinant of cell aging includes aberrant PTMs of proteins. The aging of skin was shown to be associated with the phosphorylation of Ser, Thr, and Tyr residues in dermal cell proteins and activation of apoptotic pathways [81]. This is commonly recognized that pathological protein aging and aggregation is linked to the failure of major proteolytic pathways involved in protein turn-over and cell cycle [82]. It is in agreement with the known fact that spontaneous racemization of AAs within protein diversifies protein toxicity, resulting in unusually stable structures [83]. This stability is associated with the altered relative stereochemistry of adjacent chiral centers within a macromolecule. The practical significance of toxicity rests with the effects of the physical properties of the polymer. The regulation of proteasomal activity by PTMs is a well-established phenomenon [84]. The most-frequently occurring PTMs are reversible enzymatic phosphorylation which interact with many other forms of PTMs, including racemization and proteolysis [84]. Correspondently the role of phosphorylation / racemization interlay in protein turn-over and proteolysis has been the most studied [85]. Notably, a globally lifetime increase of phosphorylation

was observed for many proteins and phosphor-sites [86]. The illustrative example is the phosphorylation of Ser-129 in alpha-synuclein (αSyn), leading to a decrease in protein turnover and, ultimately, to the to cell death [87, 88, 89]. The interaction of phosphorylation with the proteosomes is also a hotspot of many current studies. The interaction of phosphorylation and racemization is known to be the mechanism responsible for the age-related aggregation of many long-lived proteins (LLPs), including alpha-crystalline [90]. However, the contribution of spontaneous racemization, to protein aging, the collapse of proteolytic mechanisms, and protein aggregation, has not been systematically reviewed and summarized [2]. The interplay of non-enzymatic and enzymatic reactions is necessary to the evolution of metabolic pathways and the function of the metabolic network in the modern organism (see review [91]. Among others, the complex of PTMs increases the diversity of proteolytic pathways controlling fundamental physiological and pathological processes including protein turnover [92]. The imbalance between PTMs ^{Enz} and PTM ^{Sp}, in favor of spontaneous reactions, is associated with many metabolic disorders related to the protein and cell aging [93]. It is essential to emphasize that protein aging impacts both the enzymes and their corresponding substrates. Several lines of evidence support the view that enzyme activity is altered as a function of the enzyme/substrate proteins lifetime and the age of the organism [94, 95, 96, 97]. Time-dependent accumulation of the product of spontaneous PTMs, including D-amino acids (D-AAs) containing peptide and proteins, maybe a key event in age-related pathology. Spontaneous biological processes, including spontaneous autophagy and spontaneous apoptotic cell death, are currently under discussion [97, 98].

Apoptosis

The toxic effects of D-AAs and their ability to trigger apoptosis is well known. Current advancements in the chiral discrimination methods allow for a better understanding of the underlying mechanisms [99]. Different types of proteins have different turnover rates, which are determined by the specificity of the pathways of involved PTMs. The hydrophobic and more structured proteins tend to live longer. The majority of brain proteins have a lifetime of less than 10 days. The proteins associated

with cellular organelles have a lifetime less than one day. Many synaptic proteins, including proteins of dendritic spines and actin cytoskeleton proteins, have a lifetime shorter than a second. Short lifetime (which are difficult to measure in vivo) allow fast cellular response to extracellular signaling events [100 101]. Axonal neurofilaments (NFs) have a relatively long (~55 days) lifetime [102]. The lifetime of NFs within dendritic spines is unknown. The presence of long-lived proteins (LLPs) (including neurofilaments) in the synapse was demonstrated in several studies [82, 103]. It was hypothesized that synaptic LLPs are necessary for the long-term memory function [82]. In synaptic spines, the information processing (i.e., synaptic plasticity) is regulated by local protein synthesis and tuning of protein turn-over by synaptic proteasomal systems [104, 105]. One of the dynamic members influencing synaptic plasticity is the actin cytoskeleton [106]. Dysregulation of protein turn over by racemization will impact normal neuronal function. The distinct role of proteolytic clearance in short-lived proteins (SLPs) and LLPs in age-related diseases has attracted extensive attention [107]. The predominant pathway for clearance of SLPs is enzymatic proteolytic degradation which protects them against spontaneous forms of PTMs. Notably synaptic actin cytoskeleton protein does not undergo spontaneous racemization despite possessing multiple racemization-prone Ser residues [108]. LLPs (with half-lives significantly longer than the lifetime of the cell) are cleared through the process of cell division and proliferation. Cells transition from dividing (short-lived) to non-dividing (long-lived) states, initiates a natural tendency to accumulate LLPs beyond their physiological limit [109]. [V]

The effect of cell aging linked to that of protein aging is disruptive to cells. It believed that the overload of the cytoplasm by LLPs leads to the activation of proteostatic lysosomal and autophagic mechanisms.

Autophagy

Protein aggregation accompanied by aberrant autophagy is a well-known landmark of

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V. Age dependent rate of collagen turnover in human is influenced by the racemization of aspartic acid (Asp) [114].

neurodegenerative diseases including Alzheimer's disease (AD) [110, 111, 112, 113]. However, the role of spontaneous racemization in these studies has not addressed. The failure of these processes then triggers proteinaceous AD-related aggregation and apoptotic cell death [82]. However, the comprehensive~~ly~~ analysis of the role of spontaneous PTMs in the cellular accumulation of LLPs has not been carried out [115]. Although the pathological accumulation of LLPs is linked to spontaneous racemization [25, 82, 116]. It took a long time to found that D-AAs containing peptide initiate the apoptosis in in the lung cell [117]. Enzyme Ser racemase (SR) is a critical modulator of NMDAR dependent shift between apoptosis-necrosis pathways [118]. However, the crosstalk among proteolysis, autophagy, and apoptosis in neuronal cells, under the condition of D-AAs overload has not been broadly studied. Recently, the prior suggestion that primary proteolytic pathways, including autophagy could be a major process in neurodegenerative pathologies including Parkinson disease [119], Huntington disease [120], and AD [121] has been confirmed [122]. These finding suggest a role for phosphorylation-racemization interplay in mammalian proteolytic pathways. Based on catalytic mechanisms and structure, mammalian proteases are classified into five classes which include 221 serine proteases (SerPr), 192 metallo-proteases, 160 cysteine proteases, 29 threonine proteases, and 24- aspartic proteases (numbers provided for human SerPr [123, 124]. SerPr play a primary role in diverse physiological functions and different pathological processes. Most studied processes are: an airway function [124], gastric [125; 126], and neurodegenerative [127, 128, 129, 130] diseases. SerPr enzymes [VI], including trypsin and neurosin [VII`] its substrates, and inhibitors contain multiple racemization-prone Ser residue. Giving these facts it is logical to expect a devastating impact of spontaneous racemization

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VI. SerPr activity contains a highly conserved sequence motif - the catalytic triad of three AAs serine (Ser/S), aspartate (Asp/D, and histidine (His/H) [124].

VII. The neurosin, a novel type of trypsin-like serine protease, is preferentially expressed in the human brain [133].

on the molecular, cellular, and cognitive functioning. Therefore, the dysfunction of proteolytic enzymes, induced by the spontaneous racemization, may contribute significantly to neurodegeneration. Indeed, more than 30 different proteins are known to form long-lived deposits in humans [131], including the beta-amyloid (A-β) and prion proteins (PrP) aggregations [132, 133]. In support of the significance of the PTMs is the fact that has been identified as molecular mechanism resulting in lysosomal failure that occurs in AD [82].

Afterword.

The intriguing and challenging field of protein folding has recently undergone significant evolution within bio-physical and biochemical sciences. Different proteins are involved in unique systems and pathways of spontaneous PTMs. Until recently the racemization was not considered as an essential form of PTMs [152]. The advances in the thermodynamics of protein folding represents a platform for the uniformly characterization of broad phenomena [134, 135]. Theoretical conclusions indicate that statistical fluctuations are an unavoidable source of asymmetry in a single chiral object (A) and in the system of chiral objects (B). However, the racemization thermodynamics of A and B are different. The collection of weakly interacting chiral objects is prone to racemize towards an equilibrium state. In contrast, the collection of strongly interacting chiral objects can retain an asymmetry of non-equilibrium state [136]. These theoretical considerations are applicable to the comparative description of racemization of free and protein-bound AAs in the terms of non-equilibrium phase transitions (PhTs ^{NE}) of protein folding [2 ,137]. The chain of possible folding PhTs (i.e., the chain of spatial condensation), beginning from the initial unfolded state, include the transition from the statistical coiled states to compact globular states [138, 139]. The main goal is a solution of the protein folding problem in principle [135]. However, it is evident, that this overarching (generalized) achievement must be complemented by a systematic protein fold DATA-BASE, which is only now being developed.

Crosstalk between reversible and irreversible PTMs, linked to numerous developmental disorders and human diseases, is a well-studied effect in eukaryotic biology [154].

The typical example is PTMs of histone protein involving the interaction between phosphorylation, acetylation, ubiquitination, O-GlcNacylation, and methylation [155, 156]. Given the commonality of the most targeted residues (Se, Tyr, and Thr), significant connections are expected between enzymatic phosphorylation and spontaneous racemization, which could be either positively or negatively directed processes [157].

Ser is a substrate in numerous metabolic pathways, including protein, nucleotide, and lipid synthesis [158]. These facts suggest that the critical role of Ser racemization could not be restricted to the protein PTMs. Not in the less degree it is related to the membrane lipid synthesis, transport, and localization and [159]. Ser phosphorylation in lipids metabolism is well studied, while Ser residue's racemization is waiting for attention. However, lipid metabolism is beyond the topic of our current review.

Figure legend.

Fig. 1. Phospho-amino acids. The hydroxyl group of L-Ser, L-Tyr, and L-Thr can be easily phosphorylated. Adopted from [Chen & Cole. 2015].

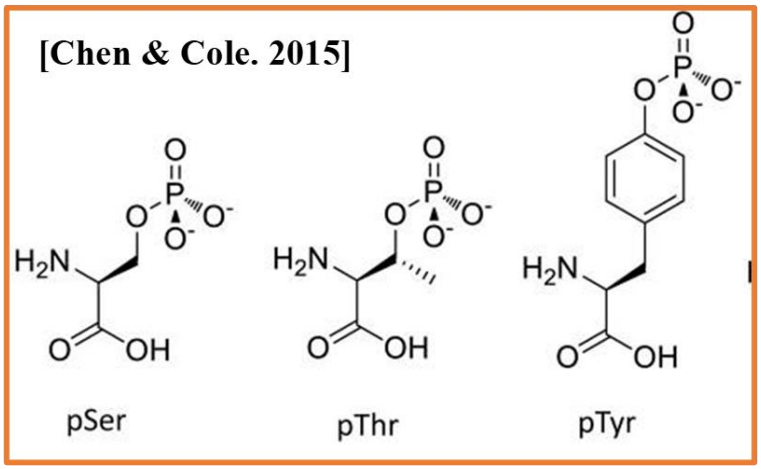

Table I. Phosphorylation-prone AAs (Ser, Thr, and Tyr).

Database PDB	Number of proteins	Ser/S (a)	Thr/T (b)	Tyr/Y (c)	Total
[Karabulut & Frishman. 2016]	5444	14661/348754	2832/215735	424/100083	17917/664572
	a -Number of Ser phosphorylated sites/non-phosphorylated sites				
	b -Number of Thr phosphorylated sites/non-phosphorylated sites				
	a -Number of Tyr phosphorylated sites/non-phosphorylated sites				

Table II. Racemization-prone AAs in peptides and proteins associated with the disease conditions.

Protein or Peptide	Amino Acids	References	Diseases
αA-Cryst.	Asp, Ser,Thr	[80, 90, 140, 141]	Ctr
A-β	Asp, Ser	[142, 143, 144]	AD
α–Syn	Pro	[145]	PD
Collagen	Asp	[146]	PulmD
MBP	Asp, Ser	[143, 146, 147]	MS
PrP	Asn, Asp	[148]	PrD
TAU	Asp	[149, 150]	AD

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