Chiral Interface of Amyloid Beta (Aβ): Relevance to Protein Aging, Aggregation and Neurodegeneration

Victor V. Dyakin [1],*, Thomas M. Wisniewski [2] and Abel Lajtha [1]

[1] Departmemts: Virtual Reality Perception Lab. (VV. Dyakin) and Center for Neurochemistry (A. Lajtha), The Nathan S. Kline Institute for Psychiatric Research (NKI), Orangeburg, NY 10962, USA; Abel.Lajtha@NKI.rfmh.org

[2] Departments of Neurology, Pathology and Psychiatry, Center for Cognitive Neurology, New York University School of Medicine, New York, NY 10016, USA; thomas.wisniewski@nyulangone.org

* Correspondence: Victor.dyakin@nki.rfmh.org

check for updates

Abstract: Biochirality is the subject of distinct branches of science, including biophysics, biochemistry, the stereochemistry of protein folding, neuroscience, brain functional laterality and bioinformatics. At the protein level, biochirality is closely associated with various post-translational modifications (PTMs) accompanied by the non-equilibrium phase transitions (PhTs [NE]). PTMs [NE] support the dynamic balance of the prevalent chirality of enzymes and their substrates. The stereoselective nature of most biochemical reactions is evident in the enzymatic (Enz) and spontaneous (Sp) PTMs (PTMs [Enz] and PTMs [Sp]) of proteins. Protein chirality, which embraces biophysics and biochemistry, is a subject of this review. In this broad field, we focus attention to the amyloid-beta (Aβ) peptide, known for its essential cellular functions and associations with neuropathology. The widely discussed amyloid cascade hypothesis (ACH) of Alzheimer's disease (AD) states that disease pathogenesis is initiated by the oligomerization and subsequent aggregation of the Aβ peptide into plaques. The racemization-induced aggregation of protein and RNA have been extensively studied in the search for the contribution of spontaneous stochastic stereo-specific mechanisms that are common for both kinds of biomolecules. The failure of numerous Aβ drug-targeting therapies requires the reconsolidation of the ACH with the concept of PTMs [Sp]. The progress in methods of chiral discrimination can help overcome previous limitations in the understanding of AD pathogenesis. The primary target of attention becomes the network of stereospecific PTMs that affect the aggregation of many pathogenic agents, including Aβ. Extensive recent experimental results describe the truncated, isomerized and racemized forms of Aβ and the interplay between enzymatic and PTMs [Sp]. Currently, accumulated data suggest that non-enzymatic PTMs [Sp] occur in parallel to an existing metabolic network of enzymatic pathways, meaning that the presence and activity of enzymes does not prevent non-enzymatic reactions from occurring. PTMs [Sp] impact the functions of many proteins and peptides, including Aβ. This is in logical agreement with the silently accepted racemization hypothesis of protein aggregation (RHPA). Therefore, the ACH of AD should be complemented by the concept of PTMs [Sp] and RHPA.

Keywords: biochirality; post-translational modifications; protein folding; protein aggregation; spontaneous chemical reactions; neurodegeneration; non-equilibrium phase transitions

1. Introduction

The amyloid cascade hypothesis (ACH) has played a crucial role in the understanding of the Alzheimer's disease (AD) etiology and pathogenesis. The deposition of β-amyloid (Aβ) and neurofibrillary tangles (NFTs) traditionally served as the essential neuropathological features of AD.

However, for many years, the attention to the stereochemistry of underlying spontaneous events was under-appreciated. Stereochemical errors in biomolecular structures, including proteins and peptides, have a dramatic impact on cell physiology [1]. The discovery of free D-aspartic acid (D-Asp) in rodents and man open a new window for understanding the mechanisms of protein synthesis and degradation [2]. Proteins, including glycoproteins, are the subjects of the reversible enzymatic (Enz) [3] and irreversible spontaneous (Sp) [4] post-translational modifications (PTM [Enz] and PTMs [Sp]). (see Figure 1). All physiological and pathological forms of proteins are the consequence of PTMs. We are focusing on the aberrant forms of PTM, such as racemization [Sp] and isomerization [Sp]. The relevance of the spontaneous modifications of amino acids (AAs) within peptides and long-lived proteins to protein aging, accumulation and pathologies is being increasingly recognized in the recent studies. Accordingly, the primarily biomarkers of aging and neurodegeneration are becoming the protein-cell-specific PTMs [Sp] of amino acids (AAs) [5–50].
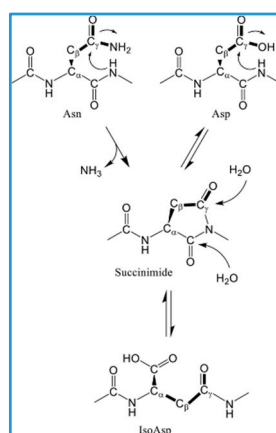

Figure 1. Spontaneous deamidation and isomerization of asparagine (Asn). Side-chain bonds of asparagine and aspartate are drawn as bold lines. Adopted from [4].

2. Racemization of the Aβ

With the recognition of the fact that many proteins (Aβ, TAU, prion protein Prion (PrP), Huntingtin and α-synuclein) are the substrate of the aggregation-prone PTMs [Sp] [51], we are focusing, primarily, on the racemization of the Aβ. The amyloid precursor protein (APP) is one of the most studied proteins concerning pathological misfolding. The products of APP processing by α-, β- and γ-secretases range from 16 to 49 AAs.

Most studied amyloid beta (Aβ) peptides includes Aβ (1–16), Aβ (1–19), Aβ (20–34), Aβ (20–33), Aβ (20–40), Aβ (23–34), Aβ (34–40), Aβ (35–40), Aβ (1–35), Aβ (1–40) and Aβ (1–42) [5,52] are characterized by differential chain of PTMs and susceptibility to PTMs [Sp]. PTMs of Aβ (1–42), the primary constituent of Aβ plaques in the AD brain, are extensively studied. Misfolding and aggregation of Aβ peptides is the convincing example of a link between the perturbations of the molecular chirality, deteriorated enzyme-substrate recognition, abnormal cell signaling (including neurotransmission) and cognitive dysfunction [3]. The spontaneous aggregation of Aβ peptides into amyloid plaques and in the walls of the cerebral vasculature is the unresolved issue of Alzheimer's disease (AD)-amyloid conundrum [6]. It is a common assumption that PTMs [Sp] can significantly alter the structure of the original polypeptide chain. The AAs that most frequently undergo racemization [Sp] and isomerization [Sp] in human proteins are aspartate (Asp), asparagine (Asn), glutamate (Glu), glutamine (Glu), serine (Ser), alanine (Ala) and proline [7]. For Aβ peptides, racemization-prone are found two non-essential AAs: serine (Ser) and aspartate (Asp) (Aβ-42 contains two Ser and three Asp residues (see Table 1)). For Asp, the mechanism acceleration of racemization [Sp] (about 10^5) is associated with the specific succinimide intermediates [8–10]. Both D-Ser and D-Asp play a crucial role in N-methyl-d-aspartate (NMDA) receptor-mediated neurotransmission. D-Ser26-Aβ1–40 possesses

a strong tendency to form fibrils [11]. AD patients have increased brain D-Ser levels [12]. This fact agrees with the activated spontaneous racemization (Rs^{Sp}) of Ser residue in Aβ, with an elevated level of D-Ser in amyloid plaques, impairment of the NMDA neurotransmission, memory loss and cognitive dysfunction. Racemization and isomerization of Asp are the most common types of non-enzymatic covalent modification that leads to an accumulation of aging proteins in numerous human tissues [13]. Asp-1, Asp-7 and Asp-23 of Aβ are crucial in the control of Aβ aging and aggregation [5].

Table 1. The frequency (f) of the AAs appearance in Aβ (1–42).

The Frequency (f) of the AAs Appearance in A-beta (1–42)				
f	Amino Acids			
6	Gly	Val		
4	Ala			
3	**Asp**	Glu	Phe	His	Ile
2	Lys	Leu	**Ser**		
1	Met	Asn	Arg	Gln	Tyr

Residues Asp-1 and Asp-7 of Aβ in amyloid plaques are a mixture of L-, D-, L-iso- and D-iso-aspartate [14]. D-Asp-7 enhances the aggregation process by shifting the equilibrium of Aβ from the soluble to the insoluble form [15]. Therefore, the set of PTMs ^{Sp}, including racemization ^{Sp} and isomerization ^{Sp}, is an efficient modifier of Aβ metabolism. In 2011, Kumar promoted the hypothesis that enzymatic phosphorylation of Aβ triggers the formation of toxic aggregates [16], which has been confirmed by later studies [3]. In 1994, Szendrei discovered that spontaneous isomerization of Asp affects the conformations of synthetic peptides [17]. However, the role of the PTMs ^{Sp} in Aβ aggregation and neurotoxicity remains in the shadow. Consequently, many structural details of misfolded Aβ have remained elusive for a long time [7,18]. This short review provides a summary of information regarding events of PTMs ^{Sp} in Aβ. The heterogeneity of Aβ proteolytic forms in AD brain is represented by at least 26 unique peptides, characterized by various N- and C-terminal truncations. The N- and C-terminal truncated fragments (in contrast to canonical Aβ) are allowing to distinguish between the soluble and insoluble aggregates. The N-terminal truncations are predominating in the insoluble material and C- terminal truncations segregating in the soluble aggregates [19,20]. Aβ peptides exhibit a high sensitivity of the secondary structure and fibril morphologies to the chirality of ligands [21] and enzymes of PTMs. Only for a small part of Aβ isoforms exist information regarding the pathways of PTMs ^{Sp}.

Currently, available data for Aβ42 peptides are summarized in Tables 1–3. The data in Tables 2 and 3 demonstrate two essential facts: first, the coincidence of phosphorylation and spontaneous racemization/isomerization (enzymatic phosphorylation can be accompanied by the enzyme-driven or spontaneous racemization) events at the Ser-8, Ser-26 (Ser-26 residue is located within the turn region of Aβ), and Asp23 residues, second, currently available information covering the PTMs ^{Sp} of Aβ is limited only to 4 from the 16 types of AAs, which means that much remains to be explored. Most recent attempts to overcome previous limitations of the ACH are concentrated on many additional essential aspects of metabolism, contributing to progress in understanding [22–42]. However, most them, do not pay enough attention to the stereochemistry of the PTM in general and the impact of spontaneous racemization (^{Sp}) on Aβ assembly, aggregation and functions. At the same time, the progress in methods of chiral discrimination has produced new, stereochemistry-oriented, experimental results regarding aberrant PTMs ^{Sp}. Growing evidence suggests that proteins undergo several unusual, previously unknown PTMs associates with the interplay between physiological protein modification, spontaneous aging-associated molecular processes [7,10,11,13], stress conditions [23,24], accidental co-localization of the enzyme and substrate or PTMs ^{Sp}. For Aβ, Asp and Ser are known as the most racemization prone residues. For the illustration purpose, we provide several of many existing molecular pathways where the racemization ^{Sp} of Ser can be critical.

Table 2. Coincidence of enzymatic and spontaneous PTMs at Ser and Asp residues of Aβ in the neurodegenerative amyloid aggregates.

Peptide	Disease	Residue	PTMs		
			Rcm.	Ism.	Ph.
			Spontaneous		Enzymic
A-β (40–42)	AD	**Ser-8**	[24, 35]		[3, 40, 41, 42]
		Ser-26	[13, 35, 36]		[43. 44]
		Asp-23	[35, 37, 38]		[40]
A-β (20–34)		**Asp-23**		[39]	

Post-translational modification (PTMs): Spontaneous (Sp) and Enzymic (Enz), Racemization (Rcm). Isomerization (Ism). Phosphorylation (Ph).

Table 3. Coincidence of phosphorylation and racemization at Ser and Asp residues of Aβ (1–42).

		A-Beta (1–42)																																									
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	
		Asp	Ala	Glu	Phe	Arg	His	Asp	**Ser**	Gly	Tyr	Glu	Val	His	His	Gln	Lys	Leu	Val	Phe	Phe	Ala	Glu	**Asp**	Val	Gly	**Ser**	Asn	Lys	Gly	Ala	Ile	Ile	Gly	Leu	Met	Val	Gly	Gly	Val	Val	Ile	Ala	
N-Terminal		D	A	E	F	R	H	D	**S**	G	Y	E	V	H	H	Q	K	L	V	F	F	A	E	**D**	V	G	**S**	N	K	G	A	I	I	G	L	M	V	G	G	V	V	I	A	C-Terminal
									*															**			*																	
									***															***			***																	

PTM: Enzymic (***) and Spontaneous (Racemization *, Izomerization **).

First, the pathological role of mitochondrial enzymes Ser proteases (SerPs) is attributed to neurodegenerative disorders such as AD and Parkinson's and disease [25–27]. The HtrA (high-temperature requirement) family represents a class of oligomeric SerPs [25]. Its members are classified by presence (in its AAs sequence) a catalytic triad contains His, Asp and Ser residues known as racemization prone.

Second, chaperone signaling complexes in AD involve a wide range of heat shock proteins (Hsp), including Hsp27, that are ingaged in protection against Aβ aggregation and toxicity [28]. Human Hsp27 is phosphorylated at three Ser residues (Ser15, Ser78 and Ser82), were Ser-78 and Ser-82 are the major phosphorylation sites [29,30]. It is evident that due to the stereo-specificity activity of both protein types (SerPs and Hsps) racemization ^{Sp} of Ser residues in each of them will contribute to the aberrant processing of substrates, including Aβ, inducing the cascade of aggregation, accompanied by neurodegeneration. The aggregation of protein and peptide indicates the decrease in the turnover rate. Accordingly, the previously short half-life-proteins are changing in the direction toward the long-lived one. Lowering turnover rate (i.e., protein aging) makes proteins the subject of the time-dependent PTMs ^{Sp}, including oxidation, nitration, glycation, isomerization and racemization [7]. The set of PTMs ^{Sp} and its effect on protein polymerization both are substrate specific. Tyrosine (Tyr) nitration, for example, significantly decreased the aggregation of Aβ1–40. [43].

3. Conclusions

In the manuscript, we assess the previous and current experimental results acquired in the specific areas of chiral proteomics—Aβ folding—from a broad perspective. For this purpose, we addressed the basic, fundamental and widely recognized facts and theories underlying the stereochemistry of Aβ. Due to progress in multidisciplinary fields, the view of the origin of biologic non-equilibrium chirality evolves from the physico-chemical nature of enantioselective autocatalytic reaction networks to a process that play an essential role in the pathogenesis of AD [44]. The phenomena of biochirality embrace two undivided branches of science biophysics and biochemistry. In 1990th, the nature of living organisms was associated with the absolute homochirality [50]. With the discovery of D-AAs in living organisms and the process of enzymic racemization, the concept of homochirality was replaced by the notion of prevalent chirality.

In the language of entropy, the transfer of protein/solvent system from the state of low-entropy (racemic mixture) to the high-entropy state (homochirality) is the order-disorder type transitions.

In terms of thermodynamics, this is the transition from the non-equilibrium to the equilibrium state. Accordingly, the enzymic PTMs, from a biophysical perspective, is the set of physiological non-equilibrium phase transitions. Enzymic racemization is an essential and necessary source of D-AAs in organisms. In contrast, the spontaneous racemization, as an aberrant PTMs, is the window for the irreversible transfer from non-equilibrium to equilibrium conditions.

In the words of proteomics, irreversible racemization is the conformation of protein from functional (physiological) to the dis-functional (inert or toxic) state of protein solvent, aggregates and depositions. The universal significance of the symmetry constraints is evident from the viral to the human proteome. The biologic significance of racemization-induced protein aggregation for the neuropathogenesis of AD was experimentally demonstrated as early as 1994 1994 [45]. Currently accumulated data about PTMs of many proteins and peptides, including Aβ are coherent with the silently accepted racemization hypothesis of protein aggregation (RHPA). Therefore, after "three decades of struggles, ACH [46] of the neurodegeneration should be complemented by the concept of PTMs ^{Sp} and non-equilibrium phase transitions (PhTs ^{NE}) [47–50].

Abbreviations

ACH	Amyloid cascade hypothesis
PTMs	post-translational modifications
PTMs Enz	enzymic PTMs
PTMs Sp	spontaneous PTMs
PhTs NE	non-equilibrium phase transitions
Aβ	amyloid beta
RHPA	racemization hypothesis of protein aggregation
Ala	Alanine
Asn	Asparagine
Ser	Serine
Asp	aspartic acid
Glu	Glutamate
Ile	Isoleucine
Tyr	Tyrosine
Pro	Proline

References

1. Schreiner, E.; Trabuco, L.G.; Freddolino, P.L.; Schulten, K. Stereochemical errors and their implications for molecular dynamics simulations. *BMC Bioinform.* **2011**, *12*, 190. [CrossRef] [PubMed]

2. Dunlop, D.S.; Neidle, A.; McHale, D.; Dunlop, D.M.; Lajtha, A. The presence of free D-aspartic acid in rodents and man. *Biochem. Biophys. Res. Commun.* **1986**, *141*, 27–32. [CrossRef]

3. Jamasbi, E.; Separovic, F.; Hossain, M.A.; Ciccotosto, G.D. Phosphorylation of a full-length amyloid-β peptide modulates its amyloid aggregation, cell binding and neurotoxic properties. *Mol. BioSyst.* **2017**, *13*, 1545–1551. [CrossRef] [PubMed]

4. Yokoyama, H.; Mizutani, R.; Noguchi, S.; Hayashida, N. Structural and biochemical basis of the formation of isoaspartate in the complementarity-determining region of antibody 64M-5 Fab. *Sci. Rep.* **2019**, *9*, 18494. [CrossRef]

5. Moro, M.L.; Collins, M.J.; Cappellini, E. Alzheimer's disease and amyloid β-peptide deposition in the brain: A matter of 'aging'? *Biochem. Soc. Trans.* **2010**, *38*, 539–544. [CrossRef]

6. Roher, A.E.; Kokjohn, T.A.; Clarke, S.G.; Sierks, M.R.; Maarouf, C.L.; Serrano, G.E.; Sabbagh, M.S.; Beach, T.G. APP/Aβ structural diversity and Alzheimer's disease pathogenesis. *Neurochem. Int.* **2018**, *110*, 1–13. [CrossRef]

7. McCudden, C.R.; Kraus, V.B. Biochemistry of amino acid racemization and clinical application to musculoskeletal disease. *Clin. Biochem.* **2006**, *39*, 1112–1130. [CrossRef]

8. Radkiewicz, J.L.; Zipse, H.; Clarke, S.; Houk, K.N. Accelerated racemization of aspartic acid and asparagine residues via succinimide intermediates: An ab initio theoretical exploration of mechanism. *J. Am. Chem. Soc.* **1996**, *118*, 9148–9155. [CrossRef]

9. Helfman, P.M.; Bada, J.L.; Shou, M.Y. Considerations on the role of aspartic acid racemization in the aging process. *Gerontology* **1977**, *23*, 419–425. [CrossRef]

10. Takahashi, O.; Kirikoshi, R.; Manabe, N. Academic editor Mihai V. Putz. Racemization of the succinimide intermediate formed in proteins and peptides: A computational study of the mechanism catalyzed by dihydrogen phosphate ion. *Int. J. Mol. Sci.* **2016**, *10*, 1698. [CrossRef]

11. Kubo, T.; Kumagae, Y.; Miller, C.A.; Kaneko, I. Beta-amyloid racemized at the Ser26 residue in the brains of patients with Alzheimer's disease: Implications in the pathogenesis of Alzheimer's disease. *J. Neuropathol. Exp. Neurol.* **2003**, *62*, 248–259. [CrossRef] [PubMed]

12. Madeira, C.; Lourenco, M.V.; Vargas-Lopes, C.; Suemoto, C.K.; Brandão, C.O.; Reis, T.; Leite, R.E.P.; Laks, J.; Jacob-Filho, W.; Pasqualucci, C.A. D-serine levels in Alzheimer's disease: Implications for novel biomarker development. *Transl. Psychiatry* **2015**, *5*, e561. [CrossRef] [PubMed]

13. Ritz-Timme, S.; Collins, M.J. Racemization of aspartic acid in human proteins. *Ageing Res. Rev.* **2002**, *1*, 43–59. [CrossRef]

14. Roher, A.E.; Yablenson, J.D.; Clarke, S.; Wolkow, C.; Wang, R.; Cotter, R.J.; Reardon, I.M.; Ziircher-Neely, H.A.; Heinrikson, R.L.; Ball, M.J.; et al. Structural alterations in the peptide backbone of β-amyloid core protein may account for its deposition and stability in Alzheimer's disease. *J. Biol. Chem.* **1993**, *268*, 3072–3083.

15. Kuo, Y.M.; Webster, S.; Emmerling, M.R.; de Lima, N.; Roher, A.E. Irreversible dimerization/tetramerization and post-translational modifications inhibit proteolytic degradation of Aβ peptides of Alzheimer's disease. *Biochim. Biophys. Acta.* **1998**, *1406*, 291–298. [CrossRef]

16. Kumar, S.; Walter, J. Phosphorylation of amyloid beta (Aβ) peptides—A trigger for formation of toxic aggregates in Alzheimer's disease. *Aging* **2011**, *3*, 803–812. [CrossRef]

17. Szendrei, G.I.; Fabian, H.; Mantsch, H.H.; Lovas, S.; Nyéki, O.; Schön, I.; Otvos, L. Aspartate-bond isomerization affects the major conformations of synthetic peptides. *Eur. J. Biochem. FEBS* **1994**, *226*, 917–924. [CrossRef]

18. Xiao, Y.; Ma, B.; McElheny, D.; Parthasarathy, S.; Long, F.; Hoshi, M.; Nussinov, R.; Ishii, Y. Aβ (1–42) fibril structure illuminates self-recognition and replication of amyloid in Alzheimer's disease. *Nat. Struct. Mol. Biol.* **2015**, *22*, 499–505. [CrossRef]

19. Wildburger, N.C.; Esparza, T.J.; LeDuc, R.D.; Fellers, R.T.; Thomas, P.M.; Cairns, N.J.; Kelleher, N.L.; Bateman, R.J.; David, L.; Brody, D.L. Diversity of amyloid-beta proteoforms in the Alzheimer's disease brain. *Sci. Rep.* **2017**, *7*, 9520. [CrossRef]

20. Jiang, N.; Leithold, L.H.E.; Post, J.; Ziehm, T.; Mauler, J.; Gremer, L.; Cremer, M.; Schartmann, E.; Shah, N.J.; Kutzsche, J.; et al. Preclinical pharmacokinetic studies of the tritium labelled D-enantiomeric peptide D3 developed for the treatment of Alzheimer's disease. *PLoS ONE* **2015**, *10*, e0128553.

21. Malishev, R.; Arad, E.; Bhunia, S.K.; Shaham-Niv, S.; Kolusheva, S.; Gazit, E.; Jelinek, R. Chiral modulation of amyloid beta fibrillation and cytotoxicity by enantiomeric carbon dots. *Chem. Commun.* **2018**, *54*, 7762–7765. [CrossRef] [PubMed]

22. Zhou, Y.; Sun, Y.; Ma, Q.H.; Liu, Y. Alzheimer's disease: Amyloid-based pathogenesis and potential therapies. *Cell Stress.* **2018**, *2*, 150–161. [CrossRef] [PubMed]

23. Ravikirana, B.; Mahalakshmi, R. Unusual post-translational protein modifications: The benefits of sophistication. *RSC Adv.* **2014**, *4*, 33958–33974. [CrossRef]

24. Osna, N.A.; Carter, W.G.; Ganesan, M.; Kirpich, I.A.; McClain, C.J.; Petersen, D.R.; Shearn, C.T.; Tomasi, M.L.; Kharbanda, K.K. Aberrant post-translational protein modifications in the pathogenesis of alcohol-induced liver injury. *World J. Gastroenterol.* **2016**, *22*, 6192–6200. [CrossRef] [PubMed]

25. Grau, S.; Baldi, A.; Bussani, R.; Tian, X.; Stefanescu, R.; Przybylski, M.; Richards, P.; Jones, S.A.; Shridhar, V.; Tim Clausen, T.; et al. Implications of the serine protease HtrA1 in amyloid precursor protein processing. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 6021–6026. [CrossRef] [PubMed]

26. Gieldon, A.; Zurawa-Janicka, D.; Jarzab, M.; Wenta, T.; Golik, P.; Dubin, G.; Lipinska, B.; Ciarkowski, J. Distinct 3D architecture and dynamics of the human Htra2(Omi) protease and its mutated variants. *PLoS ONE* **2016**, *11*, e0161526. [CrossRef] [PubMed]

27. Goo, H.G.; Rhim, H.; Kang, S. Pathogenic role of serine protease HtrA2/Omi in neurodegenerative diseases. *Curr. Protein Pept. Sci.* **2017**, *18*, 746–757. [CrossRef]

28. Singh, M.K.; Sharma, B.; Tiwari, P.C. The small heat shock protein Hsp27: Present understanding and future prospects. *J. Therm. Biol.* **2017**, *69*, 149–154. [CrossRef]

29. Landry, J.; Lambert, H.; Zhou, M.; Lavoie, J.N.; Hickey, E.; Weber, L.A.; Anderson, C.W. Human HSP27 is phosphorylated at serines 78 and 82 by heat shock and mitogen-activated kinases that recognize the same amino acid motif as S6 kinase II. *J. Biol. Chem.* **1992**, *267*, 794–803.

30. Katsogiannou, M.; Andrieu, C.; Rocchi, P. Heat shock protein 27 phosphorylation state is associated with cancer progression. *Front. Genet.* **2014**. [CrossRef]

31. Lowenson, J.D.; Clarke, S.; Roher, A.E. Chemical modifications of deposited amyloid-β peptides. *Methods Enzym.* **1999**, *309*, 89–105.

32. Takahashi, O.; Kirikoshi, R.; Manabe, N. Racemization of serine residues catalyzed by dihydrogen phosphate Ion: A computational Study. *Catalysts* **2017**, *7*, 363. [CrossRef]

33. Shapira, R.; Austin, G.E.; Mirra, S.S. Neuritic plaque amyloid in Alzheimer's disease is highly racemized. *J. Neurochem.* **1988**, *50*, 69–74. [CrossRef] [PubMed]

34. Kaneko, I.; Morimoto, K.; Kubo, T. Drastic neuronal loss in vivo by beta-amyloid racemized at Ser (26) residue: Conversion of non-toxic [D-Ser (26)] beta-amyloid 1–40 to toxic and proteinase-resistant fragments. *Neuroscience* **2001**, *104*, 1003–1011. [CrossRef]

35. Tomiyama, T.; Asano, S.; Furiya, Y.; Shirasawa, T.; Endo, N.; Mori, H. Racemization of Asp23 residue affects the aggregation properties of Alzheimer amyloid beta protein analogues. *J. Biol. Chem.* **1994**, *269*, 10205–10208.

36. Shimizu, T.; Fukuda, H.; Murayama, S.; Izumiyama, N.; Shirasawa, T. Iso-aspartate formation at position 23 of amyloid beta peptide enhanced fibril formation and deposited onto senile plaques and vascular amyloids in Alzheimer's disease. *J. Neurosci. Res.* **2002**, *70*, 451–461. [CrossRef]

37. Warmack, R.A.; Boyer, D.R.; Zee, C.T.; Richards, L.R.; Sawaya, M.R.; Cascio, D.; Gonen, T.; Eisenberg, D.S.; Clarke, S.G. Structure of A-β (20–34) with Alzheimer's-associated isomerization at Asp23 reveals a distinct protofilament interface. *Nat. Commun.* **2019**, *10*, 3357. [CrossRef]

38. Kumar, S.; Singh, S.; Hinze, D.; Josten, M.; Sahl, H.G.; Siepmann, M.; Walter, J. Phosphorylation of amyloid-β peptide at serine-8 attenuates its clearance via insulin-degrading and angiotensin-converting enzymes. *J. Biol. Chem.* **2012**, *287*, 8641–8651. [CrossRef]

39. Barykin, E.P.; Petrushanko, I.Y.; Kozin, S.A.; Telegi, G.B.; Cherno, A.S.; Lopina, O.D.; Radko, S.P.; Mitkevich, V.A.; Makarov, A.M. Phosphorylation of the amyloid-beta peptide inhibits zinc-dependent aggregation, prevents Na,K-ATPase inhibition, and reduces cerebral plaque deposition. *Front. Mol. Neurosci.* **2018**, *11*, 302. [CrossRef]

40. Rezaei-Ghaleh, N.; Amininasab, M.; Kumar, S.; Walter, J.; Zweckstetterc, M. Phosphorylation modifies the molecular stability of β-amyloid deposits. *Nat. Commun.* **2016**, *7*, 11359. [CrossRef]

41. Milton, N.G. Phosphorylation of amyloid-beta at the serine 26 residue by human cdc2 kinase. *Neuroreport* **2001**, *12*, 3839–3844. [CrossRef] [PubMed]

42. Kumar, S.; Wirths, O.; Stüber, K.; Wunderlich, P.; Koch, P.; Theil, S.; Rezaei-Ghaleh, N.; Zweckstetter, M.; Bayer, T.A.; Brüstle, O.; et al. Phosphorylation of the amyloid β-peptide at Ser26 stabilizes oligomeric assembly and increases neurotoxicity. *Acta Neuropathol.* **2016**, *131*, 525–537. [CrossRef] [PubMed]

43. Zhao, J.; Wang, P.; Li, H.; Gao, Z. Nitration of Y10 in Aβ1–40: Is it a compensatory reaction against oxidative/nitrative stress and Aβ aggregation? *Chem. Res. Toxicol.* **2015**, *28*, 401–407. [CrossRef] [PubMed]

44. Ribó, J.M.; Hochberg, D. Concept Paper. Chemical basis of biological homochirality during the abiotic evolution stages on Earth. *Symmetry* **2019**, *11*, 814. [CrossRef]

45. Mori, H.; Ishii, K.; Tomiyama, T.; Furiya, Y.; Sahara, N.; Asano, S.; Endo, N.; Shirasawa, T.; Takio, K. Racemization: Its biological significance on neuropathogenesis of Alzheimer's disease. *Tohoku J. Exp. Med.* **1994**, *174*, 251–262. [CrossRef]

46. Kasim, J.K.; Kavianinia, I.; Harris, P.W.R.; Brimble, M.A. Three decades of amyloid beta synthesis: Challenges and advances. *Front. Chem.* **2019**, *7*, 472. [CrossRef]

47. Dyakin, V.V.; Lucas, J. Non-equilibrium phase transition in biochemical—Systems. Chain of chirality transfer as Determinant of Brain Functional Laterality. Relevance to Alzheimer disease and cognitive psychology. In Proceedings of the Alzheimer's Association International Conference (AAIC-2017), London, UK, 16–20 July 2017.

48. Ornes, S. Core concept: How nonequilibrium thermodynamics speaks to the mystery of life. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, 423–424. [CrossRef]

49. Schrödinger, E. *What is Life? The Physical Aspect of the Living Cell*; Cambridge University Press: Cambridge, UK, 1994.

50. Nansheng, Z. The role of homochirality in evolution. In *Advances in BioChirality*; Zucchi, C., Caglioti, L., Palyi, G., Eds.; Elsevier Science: Amsterdam, The Netherlands, 1999.

51. Hsu, Y.H.; Chen, Y.-W.; Wu, M.-H.; Tu, L.H. Protein glycation by glyoxal promotes amyloid formation by Islet Amyloid polypeptide. *Biophys. J.* **2019**, *116*, 2304–2313. [CrossRef]

52. Zhang, A.; Qi, W.; Good, T.A.; Fernandez, E.J. Structural differences between Aβ (1–40) intermediate oligomers and fibrils elucidated by proteolytic fragmentation and hydrogen/deuterium exchange. *Biophys. J.* **2009**, *96*, 1091–1104.