

REVIEW OPEN ACCESS

Racemisation of Amino Acids: From Synthetic Challenge to Biological Significance

Othman Al Musaimi^{1,2,3,4} 

¹School of Pharmacy, Newcastle University, Newcastle upon Tyne, UK | ²Translational and Clinical Research Institute, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, UK | ³Department of Chemical Engineering, Imperial College London, London, UK | ⁴Orthogonal Peptides Limited, London, UK

Correspondence: Othman Al Musaimi (othman.almusaimi@newcastle.ac.uk)

Received: 30 March 2026 | **Revised:** 18 May 2026 | **Accepted:** 21 May 2026

Keywords: Alzheimer disease | cataract | forensic science | protein ageing | racemisation

ABSTRACT

Racemisation has traditionally been regarded as an undesirable side reaction that must be minimised or eliminated during chemical synthesis. However, racemisation is also a naturally occurring process that amino acids undergo as part of normal biological and evolutionary dynamics. Increasing evidence suggests that this stereochemical conversion plays important roles in biological systems and has been used to investigate cellular growth, ageing and protein turnover. While maintaining stereochemical purity is essential during synthetic processes, often achieved through milder coupling conditions and controlled reaction environments, it is important to recognise that racemisation can occur even under relatively mild, physiological conditions, without the need for elevated temperatures or extreme pH values. This review examines racemisation from both synthetic and biological perspectives, together with its biological applications. Particular attention is given to spontaneous racemisation as a contributing factor in ageing, where disruption of chiral balance and the accumulation of irreversible post-translational modifications (PTMs) are frequently observed. We discuss the mechanisms and factors that trigger this stereochemical conversion, strategies for minimising it during synthesis, and the potential benefits of understanding and exploiting racemisation. Applications such as age estimation in forensic science and insights into neuronal development are also highlighted.

1 | Introduction

Amino acid racemisation is one of the most detrimental side reactions in chemical peptide synthesis. It refers to the partial inversion of a stereogenic centre in a chiral molecule, resulting in the formation of a mixture of L- and D-amino acid enantiomers. Certain amino acids exhibit a particularly high propensity for racemisation through multiple mechanisms. Consequently, the incorporation of these residues during peptide synthesis necessitates careful optimisation and screening of reaction conditions to minimise racemisation.

Chirality is the property of a molecule that renders it non-superimposable on its mirror image, typically arising from a

stereogenic centre such as a carbon atom bonded to four distinct substituents. This gives rise to enantiomers with distinct spatial configurations (e.g., L/D or R/S). In biological systems, chirality is critical, as biomolecules such as amino acids and sugars exist predominantly in single enantiomeric forms. It refers to the absolute three-dimensional distribution of the groups linked to an sp³ hybridised carbon atom. The prerequisite for a centre to be chiral is to be sp³ hybridised, tetrahedral hybridisation, and four different groups are linked to the carbon. Based on this definition, we can say that all the proteinogenic amino acids are chiral except Gly, where it has two identical groups on its α -carbon (H, H).

Chirality is an important aspect as it is directly linked to the biological activity of molecules as it governs the way it interacts with

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2026 The Author(s). *ChemBioChem* published by Wiley-VCH GmbH.

biological targets (receptors and enzymes) [1, 2]. Hence, any change in that three-dimensional arrangement will impact the proposed interaction with the target and could, therefore, change the outcome, it could either become more potent, less potent, or even completely void, and in the worse scenario, the activity could become toxic [3, 4]. Furthermore, various changes in biological processes seem to be directly linked to the change in the chirality, where loss in functionality or diseases are the results of this alteration.

Non-equilibrium state (NE) of biomolecular ensemble is always under the impact of thermodynamic entropy which pushes towards the high-entropy state of equilibrium [5]. It is always referred to human as entropy generating machines. This definition is closely related to the concept of biochirality, entropy, biological information processing and ageing [6]. Unfolded proteins have high entropy and the homochirality of nascent proteins is transferred to all levels of their structural hierarchy during spontaneous condensation to native functional state (NS) [5]. The spontaneous protein folding will result in entropy loss, rendering a stable conformation, however, it is prone for additional spontaneous aberrant conformational changes such as aggregation triggered by amino acid racemisation [7, 8]. Protein ageing can be related to the trade-off between maintaining the prevalent of the homochirality and the spontaneous racemisation of the amino acids involved which is connected with the stress response system [5]. Post-translational modification (PTMs) contributing to protein ageing include oxidation, nitration, glycation and irreversible racemisation [9, 10].

The continuous flow of energy help in maintaining the chirality longer than the spontaneous racemisation which can result from any compromise to the source of energy [11]. While living organisms often have L-AA in their ribosomal protein machinery [12, 13], some portions as D-AA are also included, in addition, PTMs could incorporate D-AA as well [14].

This review discusses the mechanisms and factors driving stereochemical conversion, along with strategies to minimise racemisation during synthesis. It also explores the potential advantages of understanding and harnessing racemisation, highlighting applications in areas such as age estimation in forensic science and insights into neuronal development.

2 | Racemisation Pathways

Three distinct pathways can be identified for the racemisation, where mainly the α -proton is abstracted:

2.1 | Racemisation Through Azlactone Intermediates [5(4H)-Oxazolones] Formation

Intense activation of the carboxyl group in amino acids or peptides can lead to intramolecular cyclisation, forming an oxazol-5(4H)-one intermediate (#1) when the corresponding amino group is acylated. The formation of this intermediate is considered a major pathway responsible for racemisation during peptide synthesis (Figure 1).

In this mechanism, a peptide or *N*-acylated amino acid is first activated at its carboxylate group by a coupling reagent, generating a reactive intermediate. If this activated species (#1) does not rapidly undergo aminolysis with an incoming unprotected amino acid, due to factors such as low nucleophilicity, steric hindrance, low temperature or dilute conditions, it may instead undergo intramolecular cyclisation to form a reactive intermediate while retaining configuration at the α -carbon. Importantly, the formation of this cyclic intermediate does not inherently compromise optical purity, as it remains configurationally stable. When coupling proceeds efficiently, the incoming amino acid can react before any racemisation occurs. However, the extended lifetime of this intermediate, stabilised by ring resonance, increases its susceptibility to base-induced deprotonation at the α -position. This generates a resonance-stabilised anionic species, which upon reprotonation leads to loss of stereochemical integrity and the formation of a racemic mixture [15].

Because racemisation can proceed more rapidly than aminolysis under certain conditions, coupling often occurs with a partially racemised intermediate, ultimately leading to the formation of diastereomeric peptide products [16]. A key requirement for this pathway is the presence of an acyl substituent on the amino group of the activated residue. Although certain protecting groups can reduce the likelihood of deprotonation and suppress racemisation, complete prevention, particularly for residues with a high intrinsic tendency to racemise, remains challenging in practice.

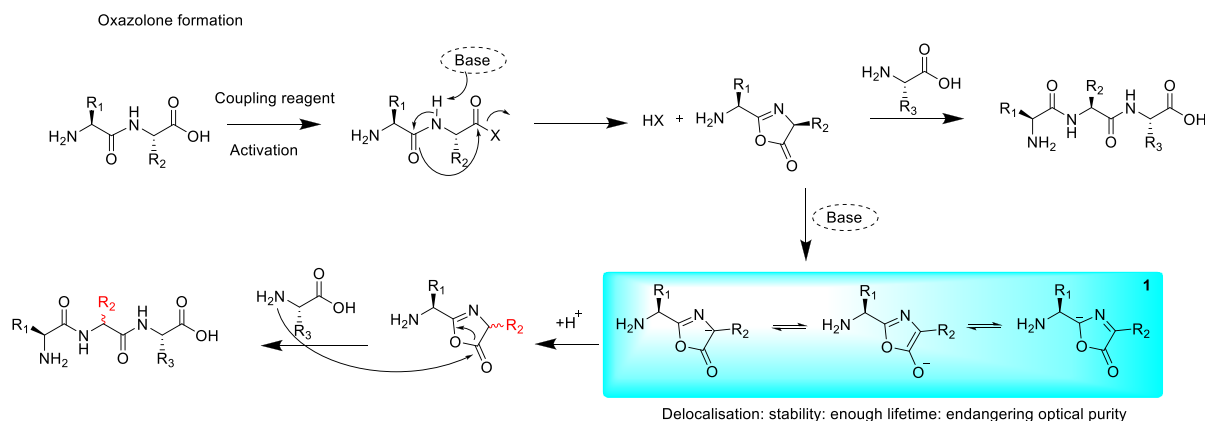


FIGURE 1 | Azlactone intermediates [5(4H)-oxazolones] delocalisation and racemised product. Red colour shows the change in optical purity.

During classical solid-phase peptide synthesis (SPPS), racemisation is minimised by protection of the α -amino group with the Fmoc carbamate, thereby avoiding the strongly acidic conditions required for Boc deprotection. This reduced propensity for racemisation arises from several factors. Carbamate protecting groups (e.g., Fmoc, Boc, Cbz) are less electron-withdrawing than amide-type protections (e.g., formyl or acetyl), which decreases the acidity of the α -proton and disfavours formation of the resonance-stabilised intermediate required for racemisation. In addition, the steric bulk of the Fmoc group hinders base-mediated deprotonation. Furthermore, the carbamate nitrogen is less nucleophilic than that of an amide, reducing the rate of intramolecular cyclisation to the oxazolone intermediate, a key species in racemisation pathways [17].

2.2 | Racemisation via Enolate Formation

During coupling, the amino acid or peptide is initially activated at its backbone carboxylate group by a coupling reagent, forming a highly reactive intermediate. This intermediate can undergo deprotonation at the α -position, leading to the formation of an enolate-type species. Subsequent intramolecular proton transfer results in a zwitterionic intermediate, which can further rearrange into a more stabilised enol form (#2) through intramolecular hydrogen bonding (Figure 2). These transformations facilitate the loss of stereochemical integrity at the α -carbon, ultimately resulting in racemisation during the coupling process.

The two racemisation pathways can be distinguished by their rate-determining steps: 5(4H)-oxazolone formation does not involve α -C-H bond cleavage, whereas enolate formation route is limited by α -hydrogen abstraction. Accordingly, deuterium isotope effects in α -deuterated substrates can differentiate the mechanisms, no isotope effect is expected for the oxazolone pathway, while a measurable effect is anticipated for enolisation, assuming oxazolone racemisation is fast relative to ring opening [18].

2.3 | Racemisation via Direct Abstraction of the α -Proton

Racemisation of amino acids during peptide synthesis can also occur via direct abstraction of the α -proton by a base, particularly

for residues in which this proton is relatively acidic [19]. This pathway may be considered an extension of enolate-mediated racemisation. Deprotonation at the α -position generates an anionic intermediate (#3), which upon reprotonation by the solvent or other proton sources results in loss of stereochemical integrity.

This mechanism is generally less significant when the carboxyl group remains in its free acid form, as the corresponding carboxylate can hinder α -proton abstraction through electrostatic effects. However, when the carboxyl group is converted into a more activated derivative, such as an ester during coupling, susceptibility to base-induced deprotonation and subsequent racemisation increases (Figure 3).

In comparison to cyclisation-mediated pathways, racemisation via direct α -proton abstraction is less prevalent and typically limited to particularly sensitive residues. These include amino acids bearing side chains capable of stabilising the resulting anion, such as aromatic groups or electron-withdrawing substituents [20]. Additionally, certain electron withdrawing side chains, such as Cys, can facilitate intramolecular proton transfer, further promoting racemisation [21]. Basic conditions, including those encountered during ester hydrolysis, may also enhance α -proton abstraction and contribute to racemisation at the stereogenic centre [22].

2.4 | Racemisation via Aspartimide Formation

Aspartimide formation represents a significant pathway leading to racemisation of aspartic acid and asparagine residues. Notably, the rate of racemisation associated with aspartimide-containing peptides has been shown to be substantially higher than that of their non-cyclised counterparts under comparable conditions [23]. This observation highlights that, beyond chemical synthesis, aspartimide formation and the resulting racemisation can also occur during peptide storage and biological ageing. Indeed, although the formation of D-isomers has been reported for various amino acids, the in vivo accumulation of D-aspartic acid is particularly pronounced, a phenomenon largely attributed to its propensity to form aspartimide intermediates [24–26].

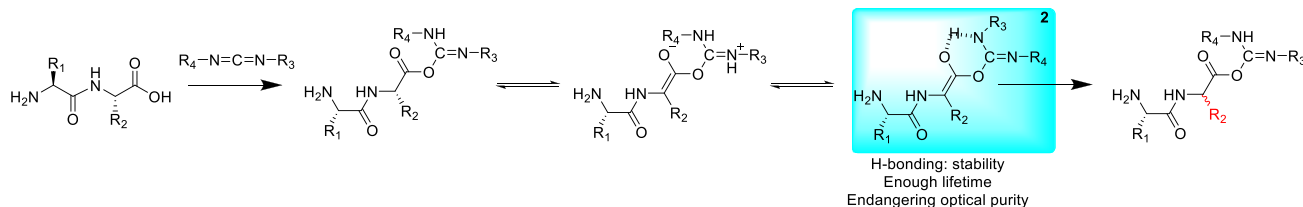


FIGURE 2 | Racemisation via enolate formation pathway.

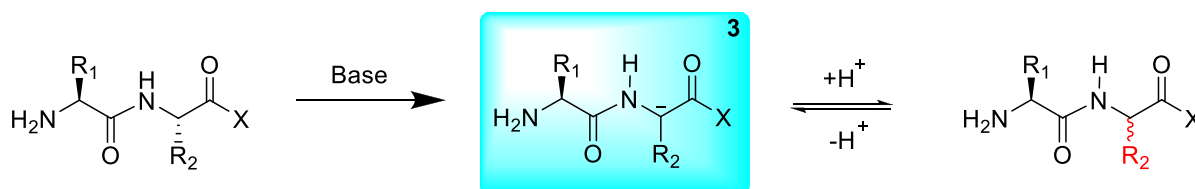


FIGURE 3 | Racemisation via direct abstraction of the α -proton pathway. Red colour shows the change in optical purity.

Mechanistically, peptides containing aspartic acid or asparagine residues can undergo intramolecular cyclisation initiated by nucleophilic attack of the backbone amide nitrogen from the adjacent C-terminal residue onto the side-chain carbonyl group. This process yields a cyclic imide intermediate that retains chirality initially but exhibits increased acidity at the α -position [26]. Deprotonation at this site generates a resonance-stabilised anionic species, which upon reprotonation leads to racemisation (Figure 4).

Subsequent hydrolytic ring opening of the racemised intermediate can occur at either of the imide carbonyl positions, producing a mixture of aspartic acid and isoaspartic acid residues in both enantiomeric forms. This pathway therefore contributes not only to racemisation but also to structural heterogeneity in peptides and proteins.

2.5 | Racemisation under Acidic Conditions

Peptide racemisation under acidic conditions is generally limited but can become significant in strongly acidic media or at elevated temperatures. While commonly used synthesis acids typically induce negligible racemisation, harsher conditions, such as 6 N HCl hydrolysis at 110°C for amino acid analysis, are known to promote this process [27, 28]. To distinguish D-amino acids formed during acidolysis from those generated through other pathways, specialised methods such as hydrolysis with deuterated HCl have been developed [29, 30]. In particular, amino acids

bearing hydroxylated side chains, such as 3-hydroxyvaline (OHVal), may exhibit enhanced susceptibility to racemisation under these conditions.

Mechanistically, acid-catalysed racemisation can proceed via multiple pathways. In some cases, enolisation contributes to stereochemical scrambling (Figure 2); however, under strongly acidic conditions, a carbocation-mediated mechanism is often invoked. This involves dehydration to form a cationic intermediate that can undergo hydration or lead to imine formation. Consistent with this, Ballard reported that, in acidic media, the rate of racemisation of mandelic acid exceeds that of deuterium incorporation in D₂O, supporting an acid-catalysed dehydration pathway. At higher pH, both processes occur at comparable rates, indicating a shift to a mechanism involving a shared enol intermediate [31].

Further insight has been provided by Broberg and co-workers, who demonstrated that residues such as 3-hydroxyvaline can form a stabilised tertiary carbocation following protonation and loss of the side-chain hydroxyl group as water [32]. Subsequent elimination of the adjacent α -proton and intramolecular cyclisation, involving nucleophilic attack of the carbonyl oxygen, leads to a proposed oxazoline intermediate. Reversal of this process can regenerate the amino acid with either retention or inversion of configuration [32]. Such carbocation-driven racemisation is therefore likely to occur in peptides containing 3-hydroxyvaline or other residues capable of forming stabilised C-3 carbocation intermediates during acidic hydrolysis (Figure 5).

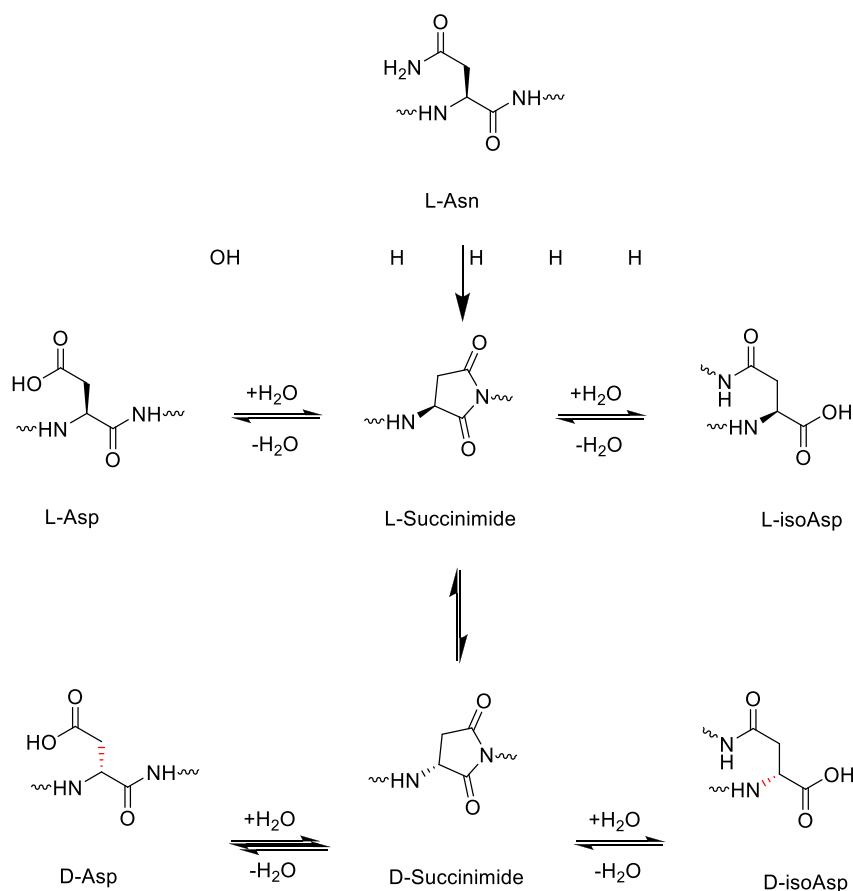


FIGURE 4 | Racemisation and isomerisation of L-Asn and L-Asp. Red colour shows the change in optical purity.

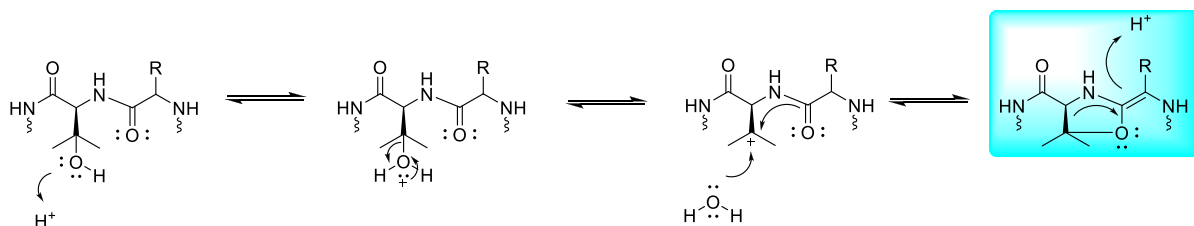


FIGURE 5 | Suggested mechanism for the generation of oxazolone intermediates in peptides containing 3-hydroxyvaline residues. Adopted from [32].

2.6 | Racemisation Prone Amino Acids

Amino acids with electron-withdrawing groups on side chain β -position such as Cys are more prone to racemisation as they stabilise the intermediate after the abstraction of the α -proton, the same applies for derivatives with resonance such as phenylglycine.

2.6.1 | Histidine (His)

Histidine (His) is among the most racemisation-prone residues in peptide synthesis, in both Boc- and Fmoc-based strategies [22, 33–35]. Beyond the common oxazol-5(4H)-one-mediated pathway, His racemisation arises from its imidazolyl side chain, which contains two nitrogens ($N\tau$ and $N\pi$) capable of intramolecular base catalysis. Modern His building blocks typically protect $N\tau$, leaving $N\pi$ partially reactive. The unshielded $N\pi$ can catalyse α -proton abstraction during activation, forming cationic intermediates that tautomerise to enolates or reprotonate to yield racemates (Figure 6A) [22].

Unprotected His derivatives may also undergo intramolecular nucleophilic attack of $N\pi$ on activated backbone carboxylates, generating cyclic carbonyl imidazolides prone to racemisation (Figure 6B). These mechanisms, in addition to oxazolone formation, make His highly susceptible to racemisation. Effective strategies to mitigate these pathways, including appropriate protecting group selection, are critical for preserving optical integrity in peptide synthesis and are discussed in subsequent sections.

2.6.2 | Cysteine (Cys) and Serine (Ser)

Cysteine (Cys) derivatives are inherently prone to racemisation during peptide synthesis, regardless of the sulfhydryl protecting group. This susceptibility is largely due to direct α -proton abstraction from N, S-protected Cys active esters in the presence of base [36]. Studies show that Cys activation in the absence of base or with minimal base significantly reduces racemisation [37, 38]. Racemisation is further exacerbated when Cys derivatives are immobilised on hydroxyl-type resins [39], particularly with DMAP catalysis [40]. SPPS via Cys esters can also induce racemisation during repetitive piperidine treatments for Fmoc removal [40]. In contrast, Cys residues anchored via amide bonds or incorporated at non-C-terminal positions exhibit minimal racemisation [21, 41]. These insights highlight the critical role of activation conditions and anchoring strategy in preserving Cys stereochemistry during peptide synthesis.

IR spectroscopy showed no evidence of 5(4H)-oxazolone formation during racemisation of *N*-(carbobenzoxyglycyl)-*S*-benzyl-L-cysteine active esters, indicating that α -hydrogen abstraction is the rate-determining step. The unusual racemisation behaviour of Z-Gly-Cys(Bzl)-ONp is attributed to increased α -hydrogen acidity induced by the *S*-benzyl side chain, allowing direct enolisation without oxazolone formation. Model studies suggest that proximity between sulphur and the enolate oxygen enables orbital overlap, stabilising the enolate relative to the oxazolone. This stabilisation may occur via sulphur d-orbital participation or, alternatively, through transient formation of an O-thiolactone

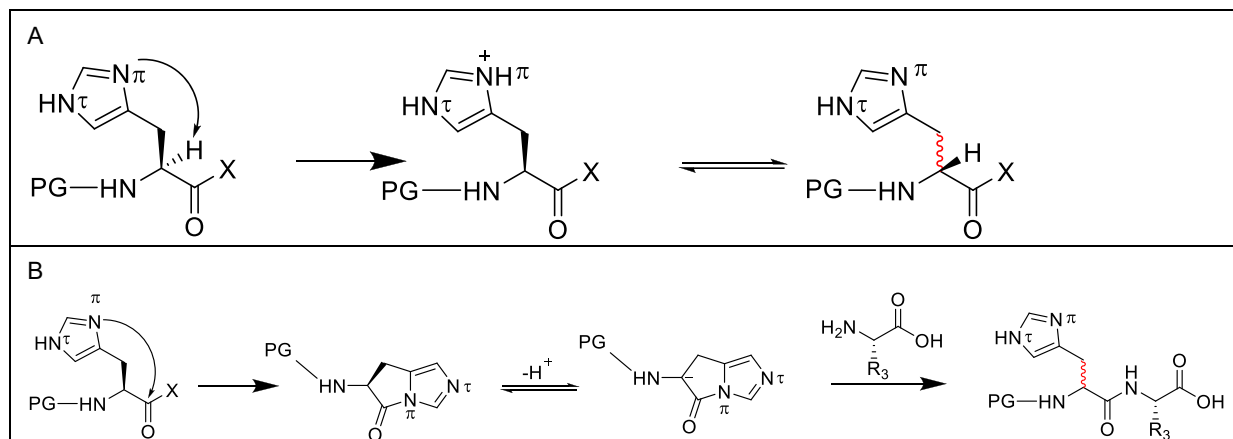


FIGURE 6 | α -Proton abstraction by: (A) The lone pair of imidazole ring and (B) oxazolone like formation. Red colour shows the change in optical purity.

sulfonium ion that opens to a ketene intermediate, leading to racemisation [18].

In serine, d-orbital or π -orbital overlap is not possible; consequently, racemisation proceeds predominantly via 5(4H)-oxazolone formation. The minor α -proton abstraction pathway can be rationalised where the serine side-chain oxygen participates in formation of a β -lactone oxonium intermediate that opens to a ketene, leading to racemisation. The ketene then rapidly reacts with the OPcp group to form the DL-peptide active ester [18].

2.6.3 | Tryptophan (Trp)

Trp has been proven to be the most racemisation-prone amino acid, exhibiting a higher susceptibility to chiral inversion under physiological and experimental conditions compared to other amino acids. Csapó and co-workers showed that racemisation of tryptophan is minimal under acidic to neutral conditions (pH 3–7) [42]. Under alkaline conditions (pH 9–11), prolonged heating leads to a slight increase in the D-enantiomer, although conversion remains below 1% after 12 h [42]. After 24 h, a modest decrease in L-tryptophan is observed, with total losses reaching 2%–5% upon extended heating. These losses are not solely due to racemisation, as oxidative degradation of the indole ring also contributes [42].

Racemisation of Trp occurs more readily under alkaline conditions than in acidic environments, although Trp is unstable under both extremes. Alkaline conditions facilitate enolisation at the α -carbon, promoting stereochemical inversion, while also increasing susceptibility to oxidative degradation. Elevated temperatures further accelerate these processes: under strongly basic conditions, partial to complete racemisation has been reported between 150°C and 185°C. For example, complete racemisation of free tryptophan occurs within 8 h at 151°C and 2 h at 185°C, whereas only ~11% conversion is observed after 18 h at 100°C [43]. Under milder alkaline conditions (pH 9, 83°C), racemisation proceeds slowly, with an inversion rate constant of approximately $2.7 \times 10^{-8} \text{ s}^{-1}$ [43].

Thermal treatment also leads to the formation of degradation products: at temperatures above 200°C, α - and γ -carboline are detected, although in lower amounts compared to β -carboline. In contrast, under acidic conditions such as those used in standard protein hydrolysis, racemisation of tryptophan occurs more slowly than for many other amino acids. This reduced rate is attributed to the electron-withdrawing nature of the indole side chain, which decreases the propensity for α -carbon proton abstraction and subsequent racemisation [43].

Griehl and co-workers developed an HPLC method to monitor racemisation during the coupling of a protected Ala-Trp derivative with a valine ester using various approaches [44]. Their results indicated that, although Trp was not the most prone residue overall, it exhibited a relatively high degree of racemisation, with a D-enantiomer content of 24.3% [44]. In another study, Riester and co-workers investigated racemisation during SPPS using capillary electrophoresis with 18-crown-6 tetracarboxylic acid as a chiral selector. Consistently, Trp was identified as one of the most highly racemisation-prone residues [45]. Musaimi et al. investigated racemisation across 18 amino acids,

identifying Trp as the most susceptible residue, with a racemisation level of 7.8%. This was followed by His (5%), Cys (1.0%) and Ser (0.9%) [46].

Interestingly, in a study conducted in collaboration with Eli Lilly and Company, Al Musaimi's laboratory determined hydrophobicity indices for both protected and unprotected amino acids [47]. Trp was identified as the most hydrophobic residue, which may account for its high propensity for racemisation [47]. Hydrophobicity can influence both the rate and selectivity of racemisation in chemical and biological systems, as hydrophobic environments may facilitate the formation of key intermediates, such as carbanion species, thereby promoting stereochemical inversion [48].

3 | Strategies to Suppress Racemisation

3.1 | $N\alpha$ Protecting Group

As oxazol-5(4H)-one formation from activated $N\alpha$ -protected amino acids represents a major pathway for racemisation during peptide synthesis, the nature of the $N\alpha$ -protecting group plays a critical role in determining the susceptibility of the amino acid to racemise during carboxylate activation [15]. $N\alpha$ -acyl-protected amino acids are highly susceptible to intramolecular cyclisation, forming oxazol-5(4H)-one intermediates during activation of the backbone carboxylate. As a result, the use of $N\alpha$ -acyl protecting groups such as formyl, acetyl, and benzoyl has become obsolete and is no longer employed in modern peptide synthesis.

Zhou and co-workers reported a thiol-labile α -amino protecting group for SPPS, 2,4-dinitro-6-phenylbenzene sulfenyl (DNPBS), which is cleaved rapidly under near-neutral conditions using p-toluenethiol/pyridine. DNPBS significantly reduces common side reactions associated with conventional SPPS. Although less efficient than Fmoc for long peptide synthesis, its orthogonality to Fmoc enables combined strategies to access otherwise challenging peptide sequences [49].

DNPBS-based strategies have been reported to further improve synthetic fidelity compared to conventional approaches. DNPBS (as a sterically demanding, electron-modulating protecting/activating system) reduces the prevalence of common SPPS side reactions by simultaneously limiting α -proton acidity and disfavours reactive intermediates that lead to epimerisation. In particular, its steric bulk and electronic properties hinder base-mediated deprotonation at the α -carbon, thereby suppressing racemisation pathways associated with enolate or oxazolone formation. In addition, reduced nucleophilicity of the protected amine decreases the likelihood of intramolecular cyclisation to oxazolone intermediates, which are key contributors to epimerisation during coupling steps. Collectively, these effects also mitigate secondary side reactions often observed in conventional SPPS, such as aspartimide formation, backbone rearrangements, and incomplete coupling due to steric congestion at the reactive centre. Overall, DNPBS-based protection/activation strategies provide a complementary advantage to Fmoc-based SPPS by reinforcing stereochemical integrity while reducing a broader spectrum of synthesis-related by-products.

3.2 | Coupling Regents

Racemisation is initiated upon activation of the amino acid backbone carboxylate, making the activation strategy a key determinant of its extent. Careful selection of coupling reagents is therefore essential for its control. While efficient activation can promote rapid aminolysis and reduce racemisation by quickly consuming reactive intermediates, excessive activation may instead enhance racemisation by facilitating pathways such as cyclisation or α -proton abstraction. Consequently, optimal suppression requires a balance between reactivity and stability through fine-tuning of activation conditions. As both racemisation pathways and susceptibility are highly dependent on the amino acid and peptide sequence, broad generalisations regarding coupling reagents and their effectiveness in minimising racemisation should be made with caution. Table 1 presents a comparison of amino acid activation strategies and their impact on racemisation.

3.3 | Coupling Strategies

3.3.1 | Pseudoproline

$N\alpha$ -urethane-protected proline exhibits a low tendency toward racemisation during carboxylate activation due to its inability to form oxazolone intermediates, a property that has inspired the development of pseudoproline derivatives from β -hydroxyl-

or β -sulfhydryl-containing amino acids such as Ser, Thr and Cys (Figure 7).

Incorporation of pseudoproline units into peptide chains can disrupt secondary structures, such as β -sheet-driven aggregation, thereby reducing conformational rigidity and improving coupling efficiency. Typically introduced as preformed dipeptides, these derivatives are acid-labile and can be readily converted back to the parent amino acids under standard cleavage conditions. Beyond improving solubility and synthesis efficiency, pseudoprolines expand the scope of peptide segment condensation by reducing racemisation at the C-terminus, allowing residues such as Ser, Thr and Cys to function as junction points in place of Gly or Pro. Overall, the pseudoproline strategy enhances synthetic flexibility and efficiency, particularly in the assembly of large or structurally challenging peptides [58].

3.3.2 | Native Chemical Ligation

Native chemical ligation (NCL) provides a complementary strategy to conventional peptide segment condensation for the synthesis of large peptides. In NCL, a notable advantage is that the C-terminal residue of the N-terminal fragment exhibits minimal racemisation owing to the high nucleophilicity of cysteine at neutral pH, with the ligation proceeding under mild, aqueous, near-neutral pH conditions. This eliminates the requirement for glycine or proline at this position, as confirmed by studies demonstrating compatibility with all proteinogenic amino acids,

TABLE 1 | Coupling strategies and racemisation in peptide synthesis.

Category	Reagent/Strategy	Key features	Effect on racemisation	Limitations/Notes	Ref.
Amino acid azides	Azide activation	Mild activation of carboxylate; reduces oxazolone formation	Generally low racemisation	Can increase with excess/strong base; base type and amount critical	[50, 51]
Amino acid halides	Acid fluorides	High reactivity; less prone to oxazolone formation; can react with little/no base	Low racemisation	Preparation complexity; better than acid chlorides	[52]
	Acid chlorides (in situ)	Generated in situ (e.g., BTC-mediated); useful for difficult couplings	Low racemisation when controlled	Solvent-sensitive (avoid NMP); high intrinsic racemisation if not controlled	
Halophosphonium salts	BroP, PyBroP	Effective for N-alkyl amino acid couplings	Low racemisation in N-alkyl systems	Can increase racemisation in standard couplings	[53–55]
	HATU	High efficiency and strong racemisation suppression	Very low racemisation	Expensive; safety concerns	
Uronium salts	HCTU	Balanced performance; better than HBTU	Good suppression	Moderate use	[56, 57]
	COMU, TOTU, HOTU (Oxyma-based)	Improved safety; strong coupling efficiency	Excellent suppression	Increasingly preferred	

Abbreviations: BroP, bromotris(dimethylamino)phosphonium hexafluorophosphate; COMU, 1-[(1-(Cyano-2-ethoxy-2-oxoethylideneaminoxy)dimethylaminomorpholino)]uronium hexafluorophosphate; HATU, Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium; HBTU, Hexafluorophosphate Benzotriazole Tetramethyl Uronium; HCTU, O-(1H-6-Chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOTU, O-[(Ethoxycarbonyl)cyanomethylenamino]-N,N,N',N'-tetramethyluronium hexafluorophosphate; PyBroP, Bromo-tris-pyrrolidino-phosphonium hexafluorophosphate; TOTU, O-[(Ethoxycarbonyl)cyanomethylenamino]-N,N,N',N'-tetra methyluronium tetrafluoroborate.

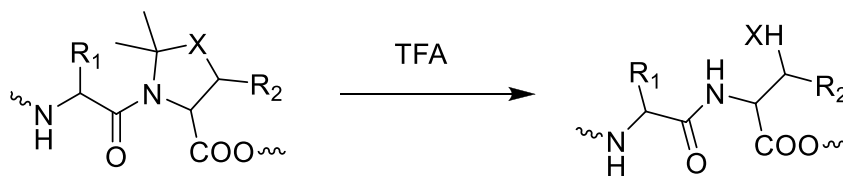


FIGURE 7 | Pseudoproline conversion mechanism. Ser (X = O, R = H), Thr (X = O, R = CH₃), and Cys (X = S, R = H).

although ligation rates may vary depending on steric effects. In NCL, a peptide thioester reacts chemoselectively with an *N*-terminal cysteine residue on a second fragment via reversible transthioesterification, followed by a rapid intramolecular S to N acyl shift to form a native peptide bond (Figure 8).

The reaction typically proceeds efficiently in aqueous buffered media with unprotected side chains, tolerates denaturing agents that enhance solubility, and is largely free of racemisation during ligation [59, 60]. However, epimerisation may occur during preparation of the peptide thioester, prompting the development of strategies such as intramolecular O to S acyl shifts and 'safety-catch' linker approaches in SPPS to minimise this limitation and broaden the applicability of NCL [61].

3.3.3 | Solvent

Racemisation during peptide synthesis is strongly influenced by solvent properties, particularly polarity, as these processes

proceed through charged intermediates. The choice of solvent therefore plays a critical role in both activation/coupling efficiency and the extent of racemisation, often with competing effects. Numerous studies have shown that incorporating dichloromethane (DCM) as a co-solvent with commonly used media such as DMF or NMP can significantly reduce racemisation across a range of coupling reagents. While non-polar solvents such as DCM or CHCl₃ are often suitable for solution-phase synthesis of short peptides, mixtures such as DCM/DMF are particularly effective in suppressing racemisation in sensitive transformations, including peptide segment condensation and cysteine activation [62]. However, the use of DCM may be limited by solubility issues of certain reagents. Additives such as fluorinated alcohols (e.g., TFE or HFIP) have also been shown to further mitigate racemisation in both solution- and solid-phase synthesis. Given the complex interplay between solvent effects and reaction kinetics, careful optimisation of solvent systems remains essential, especially for sequences prone to racemisation.

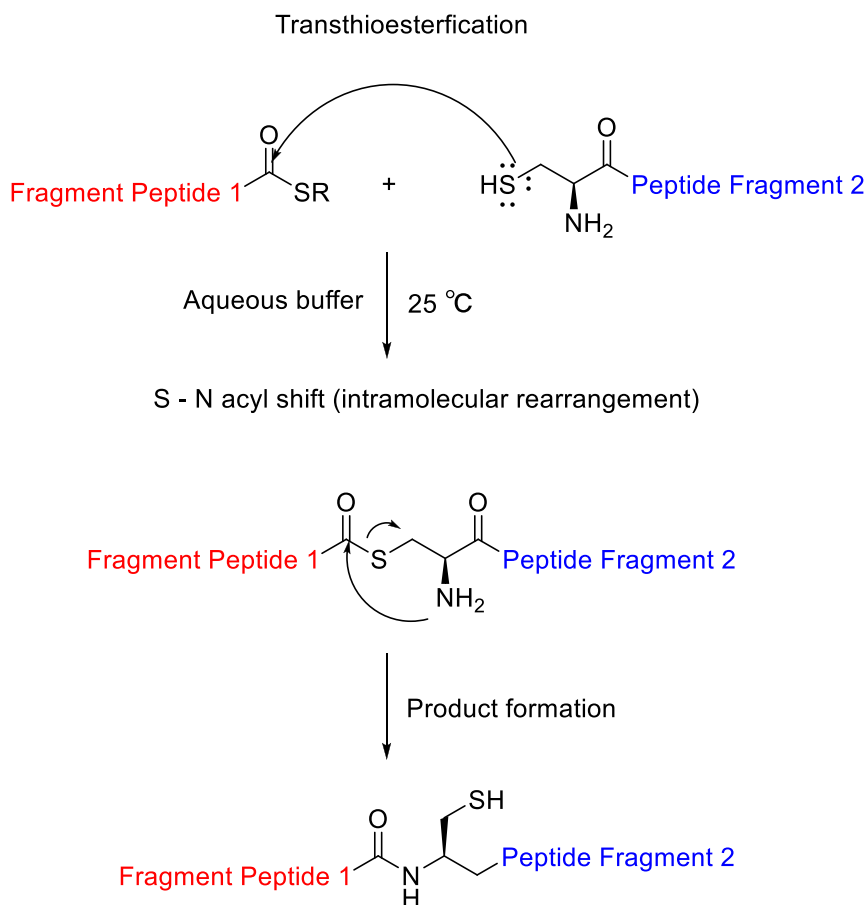


FIGURE 8 | Mechanism of native chemical ligation (NCL).

3.3.4 | Base

Tertiary organic bases are commonly employed in peptide coupling to facilitate amino group acylation and activate coupling reagents; however, they exert dual effects on the process. While they promote reaction efficiency, they can also enhance racemisation by facilitating pathways such as oxazolone formation and direct α -proton abstraction. Consequently, the choice and amount of base significantly influence the extent of racemisation. In general, sterically hindered and less basic tertiary amines are more effective in minimising racemisation. Although *N,N*-diisopropylethylamine (DIPEA) is widely used, alternative bases such as *N*-methylmorpholine (NMM) [46] or, more notably, 2,4,6-collidine [63], owing to its lower basicity and greater steric hindrance, have demonstrated superior performance in suppressing racemisation, particularly for sensitive residues and during peptide segment condensation. Additionally, reducing the amount of base can further limit racemisation. Overall, careful selection of the base, in conjunction with appropriate coupling reagents and solvent systems, is essential to minimise racemisation during peptide synthesis.

3.3.5 | Activation Mode

Amino acid preactivation is widely employed in peptide synthesis, particularly at large scale, to minimise *N*-terminal endcapping caused by excess coupling reagents. This approach consumes the reagents in advance, reducing undesired side reactions. However, preactivation prolongs the lifetime of activated amino acid species, thereby increasing the risk of racemisation through pathways such as cyclisation or direct α -proton abstraction [35]. In contrast, *in situ* activation shortens the lifetime of these intermediates and can reduce racemisation by promoting their rapid consumption [64]. While omitting preactivation is generally impractical in large-scale manufacturing, in cases involving highly racemisation-prone residues, *in situ* activation may be considered, provided that endcapping remains controlled.

3.3.6 | Temperature

Although peptide synthesis at elevated temperatures has been successfully applied, it is often associated with an increased risk of amino acid racemisation. While some racemisation pathways may show limited temperature dependence under specific conditions, extensive studies have demonstrated that higher temperatures generally promote racemisation. In cases where this side reaction is significant, performing amino acid activation at reduced temperatures can help mitigate stereochemical inversion. Similarly, conducting preactivation at lower temperatures may further suppress racemisation when it is pronounced under ambient conditions. Musaimi et al. minimised racemisation by coupling the first amino acid onto Wang resin at 0°C for 2 h, followed by an additional 2 h at room temperature to achieve complete loading [62].

3.3.7 | Cu(II) Additive

Cu(II) salts, such as CuCl₂, can be added to carbodiimide-mediated amino acid couplings to reduce racemisation, including highly susceptible *N*-methyl amino acids [65]. Preformed salts

like Cu(OBT)₂ or Cu(OAT)₂ are particularly effective, even during prolonged preactivation, and can be used with carbodiimide, phosphonium, or uronium reagents [66]. This approach has also been applied successfully in *N* to *C* peptide synthesis, which is prone to racemisation at the C-terminal activation step, although some yield loss may occur in certain cases.

4 | Biological Applications of D-Isomers

D-amino acids are stereoisomers of the naturally abundant L-amino acids and were once considered rare in biological systems [67]. However, growing evidence has shown that they occur widely across bacteria, plants, animals and humans, where they participate in diverse physiological and structural roles. Owing to their enhanced resistance to enzymatic degradation and distinct stereochemical properties, D-amino acids are increasingly utilised in biomedical and pharmaceutical applications, particularly in the design of stable peptides, antimicrobial agents, and molecular imaging probes.

Fernandez-Lopez and co-workers demonstrated that cyclic D, L- α -peptides exhibit selective antibacterial activity against Gram-positive and Gram-negative bacteria by disrupting membrane integrity, increasing permeability, and collapsing ion potentials, leading to rapid bacterial death. These D-isomer-containing peptides showed high efficacy against methicillin-resistant *Staphylococcus aureus* infection in mice, while also offering enhanced proteolytic stability and synthetic accessibility [68].

Wang and colleagues synthesised and evaluated the D-isomer of p-(2-[¹⁸F]fluoroethyl)-phenylalanine ([¹⁸F]FEP) alongside its L-isomer and previously reported D- and L-isomers of O-(2-[¹⁸F]fluoroethyl)-tyrosine ([¹⁸F]FET). Several studies have shown that D-amino acid analogues may exhibit superior imaging properties compared with their corresponding L-isomers. However, animal imaging studies of 9L tumours showed that D-[¹⁸F]FEP did not exhibit superior imaging properties compared to the corresponding L-isomer [69].

Ghadiri and colleagues demonstrated that cyclic peptides containing an even number of alternating L- and D-amino acids adopt a low-energy planar conformation that enables antiparallel stacking through intermolecular hydrogen bonding, forming β -sheet-like hollow nanotubular structures with outward-oriented side chains [70]. Building on this concept, Vilela-Picos and coworkers developed antimicrobial nanotube-forming cyclic peptides with alternating chirality, whose amphipathic and cationic properties promote selective interactions with anionic phospholipid-rich membranes [71].

We recently highlighted the key driving forces and mechanisms governing peptide self-assembly into functional nanomaterials, emphasising the roles of α -helical and β -sheet structures in determining nanomaterial architecture, properties, and biomedical applications. Potential uses in drug delivery, tissue engineering, and regenerative medicine were discussed alongside challenges including limited *in vivo* stability, proteolytic degradation and scalability. Emerging strategies such as rational design, sequence engineering and advanced fabrication techniques are being

explored to enhance predictability and reproducibility, advancing biomolecular self-assembly as a promising platform for next-generation biomaterials [72].

Selected biological applications of these important isomers have been discussed. However, for detailed discussions, readers are referred to the comprehensive reviews by Rodríguez et al. on the industrial applications of D-amino acids [73], and by Gu and colleagues, which covers the natural occurrence, biological functions, and analytical aspects of D-amino acids [74]. Furthermore, Abdulbagi and colleagues provided a comprehensive review summarising the occurrence of D-amino acid-containing peptides (DAACPs) and free D-amino acids, as well as their associations with disease development and progression. The review also highlights recent advances in analytical techniques that have significantly improved the detection, identification, and characterisation of both DAACPs and D-amino acids [75]. Khoronenkova and Tishkov reviewed the role of FAD-dependent D-amino acid oxidase (DAAO) in regulating D-amino acid levels, nervous system function, hormone secretion and disease development, and discussed its diagnostic and therapeutic potential [76]. Du and colleagues reviewed the important roles of D-amino acids in plants, invertebrates and vertebrates [77].

5 | Racemisation: A Key Driver of Protein Ageing and Pathology

Racemisation of L-Asp to D-Asp occurs much faster than in other amino acids, and its accumulation is age-dependent, resulting in alterations of protein structure and consequently affecting functionality [25]. This process is spontaneous and non-enzymatic, with the proportion of racemic mixtures increasing with age [25]. This non-enzymatic covalent modification leads to age-dependent accumulation of abnormal proteins in various human tissues, it is considered an autonomic process correlated to long-lived proteins [13]. Thus, it is considered an indicator of protein ageing, most importantly, that this change could result in function loss due to proteolysis or changes in molecular structure and identity [13]. It has been also discussed and correlated to various disease including degenerative disease and even cancer [13]. Certain proteolysis can detect and degrade altered proteins with altered Asp or Asn [23, 78]. In addition, conformational changes in protein lead to degradation due to instability in their structure due to altered Aso or Asn, which explains the decline in the concentration of bone osteocalcin [79] or of elastin in lung parenchyma and the artery wall with increasing age [80]. For example, IsoAsp formation can result in functional loss in proteins such as calmodulin, epidermal growth factor and ribonuclease, whereas others, like human growth hormone, remain unaffected [81]. Interestingly, repairing IsoAsp residues in affected proteins has been shown to restore their lost activity [82].

Isomerisation takes place in long lived proteins and especially where proteostasis is compromised, mainly in the context of ageing [13]. Isomerisation and racemisation at L-Asn and L-Asp residues proceed through the formation of a cyclic succinimide intermediate, generated via the loss of ammonia (in the case of Asn) or water (in the case of Asp), with the Asn pathway being irreversible [83]. This intermediate can undergo reversible

hydrolysis to yield either L-Asp or L-isoAsp [83]. In addition, the succinimide intermediate is susceptible to racemisation, forming the corresponding D-succinimide, which can subsequently hydrolyse to produce D-Asp or D-isoAsp [83]. The rates of these transformations are strongly influenced by neighbouring amino acid residues and the local structural environment within the protein [83]. IsoAsp occurs through a succinimide intermediate when the nitrogen atom on the next peptide bond attacks the side chain of asparagine or aspartic acid [25]. However, it is more favoured in the case of Asn, because amide favours succinimide ring formation over the carboxylate in Asp (Figure 4) [84]. Unsurprisingly, given that IsoAsp formation includes an addition of extra carbons to the protein backbone, where it acts as a β -linked amino acid, inserting an extra methylene group into the this will lead to some changes in their functionality, such as the loss of that activity within the catalytic domain of their target [13, 85].

The accumulation of D-Asp or D-Asn is known as protein-fatigue, where the de novo protein synthesis is not possible. As D-Asp disrupts the physiological conformation of proteins, thus it is considered a key player in age-related diseases [10]. It has been also related to the certain pathological disease, such as chronic haemolytic anaemia, hereditary spherocytosis and chronic renal failure and called aspartyl-related fatigue damage by Ingrosso and Perna [86]. D-Asp has been observed in various proteins leading to disrupting their native three-dimensional structures, such as amyloid β (A β) peptides that accumulate in the brains of patients with Alzheimer's disease [87], and crystallin proteins in aged eyes (Figure 9) [89]. It is to be mentioned that peptides and proteins containing D-AA are considered resistant toward proteases and association with prolonged half-lives [90, 91]. As peptides and proteins have complex structure as well as multiple functional groups, they are more enzyme-dependent stereo-chemical racemisation prone than free amino acids [14].

Fibrillar aggregates of β -amyloid peptide (A β) are major constituents of the senile plaques found in the brains of patients suffering

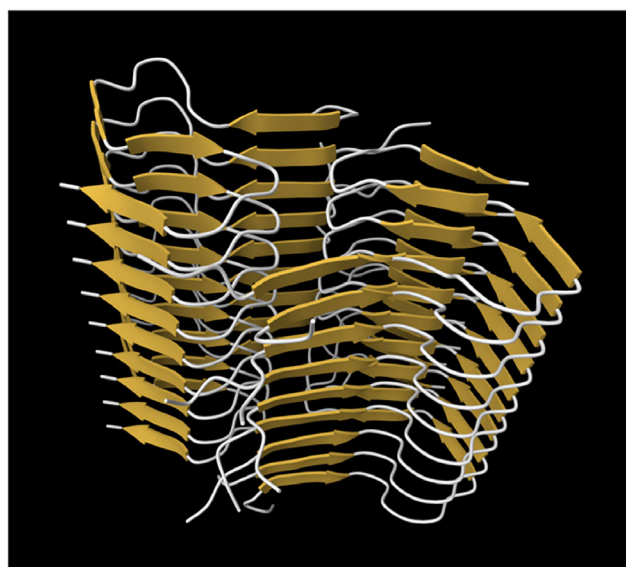


FIGURE 9 | Crystal structure of monomeric A β 42 amyloid fibrils; PDB ID 5KK3 [88].

from Alzheimer's disease (AD) [92]. Protein L-isoaspartyl O-methyltransferase (PIMT, EC 2.1.1.77) is a repairing enzyme converting L-isoaspartyl/D-aspartyl residues in damaged proteins to normal L-aspartyl residues. Jung and colleagues used Thioflavin-T (ThT) to investigate the capacity of PIMT to modulate the fibrillar aggregates formation (fibrillogenesis) in vitro via methylating the IsoAsp [92]. They conducted the study after ageing A β , and study confirmed the reduction in the fibrillogenesis as indicated by the slight increase in the fluorescence compared to the significant increase in the absence of PIMT [92]. Interestingly, the circular dichroism (CD) analysis also revealed the reduction in the β sheet secondary structure formation after the treatment with PIMT, which is otherwise increases proportionally with the amyloid fibrillogenesis [92]. Furthermore, transmission electron microscope (TEM) analysis proved the generation of amorphous structure of the A β and a rather reduction in the size of aggregates [92].

Hsiao and colleagues studied the effect of mutating position 23 and then 7 and 23 together [25]. They found that both synthesised peptides share the same double-stranded amyloid fibril structure, in addition to a triple-stranded amyloid fibril structure found in the peptide with both Asps 7 and 23 where swapped with D-Asp, being more predominant in this mutated peptide [25]. D-Asp was found aggregated in A β 42 at position 1 and 7 and tau protein in the brain of Alzheimer disease (AD) patients [25, 92].

For instance, Asp is found in three positions in A β 42 1, 7 and 23 which are near hot spots for familia AD and phosphorylation sites of Ser 8 and 26, meaning these structural alterations will increase the aggregation and nephrotoxicity of A β 42 (Figure 10). Synthetically, swapping L-Asp with D-Asp increased the aggregation propensity of A β 42, tau, and amylin, too. Interestingly, isoAsp, the intermediate between L-Asp to D-Asp was also found in AD patients, particularly at position 23 of A β 42 [25].

Neurodegenerative diseases are associated with the aggregation of proteins such as A β 42, tau, and α -synuclein [25, 93]. Although protein aggregates are inherently polymorphic, those observed in postmortem brain samples of patients are distinct from aggregates formed in vitro [25]. Hooi and Truscott reported that Ser residues also exhibit a high tendency to racemise, with nearly 4.5% of all Ser and over 9% of Asp/Asn residues racemised by the age of 70 [94]. This corresponds to approximately 2–3 amino acids per polypeptide chain, causing conformational changes and significant denaturation of long-lived proteins such as crystallins in aged lenses [94, 95]. Temperature plays a major role in racemisation, and elevated levels have been observed in cataractous lenses compared to normal lenses [94]. Similarly, Thr shows notable racemisation, whereas Glx generally exhibits lower rates

than Asp/Asn [94]. No significant racemisation was detected in Leu or Ala, while a slight increase was observed in Phe [94].

Fujii and colleagues documented deamidation of Asp and Gln in β B2-crystallin, the most abundant β -crystallin, from elderly donors [95]. They also observed inversion and isomerisation at Asp position 4, with a high proportion of D-Asp relative to L-Asp, which is considered inversion rather than classical racemisation [95]. Similar inversions were noted at Asp positions 58 and 151 in α A-crystallin from aged donors [95]. In addition to racemisation and inversion, other PTMs such as deamidation [96], phosphorylation [97], methylation [98], and truncation [99] can occur. Oxidation of Cys and Met is more pronounced in age-related nuclear cataract lenses [100, 101], but occurs to a much lesser extent in normal lenses [102, 103].

Interestingly, aspartic racemisation is also considered a good tool to estimate the age of tissue such as those in the tooth, where a relationship is evident between the elapsed time and the D-Asp content [104]. Arany and Ohtani utilised D-Asp to determine the age of blood stains. Previously, the age was used to be determined by looking at the colour change, solubility or enzyme activity, where all lack accuracy and they consume a lot of sample amount. Their argument is to use blood stains as an ex vivo experiment, where they are not prone to repair beyond their creation and yet their ageing begins instantaneously [104]. Unlike the study on blood cells which are prone to the repair of D-Asp/Asn at different maturation stages [104]. Such studies are very helpful in forensic science.

Demarchi and colleagues used amino acid racemisation to determine the chronological dating within shell midden deposits to provide relative age information [105]. Heating, induced by human could increase the degradation and racemisation of samples which leads to false age determination. Studies on shells are considered accurate as those shells are considered closed systems [105]. A mollusc shell contains both a mineral and a protein fraction, the protein undergoes diagenesis after the organism death, where they broke down in smaller peptide fragments by hydrolysis and single amino acids racemise and decompose [105]. Some protein In some molluscan, some proteins are entrapped in mineral crystals protecting them from external factors, hence it is a closed system [106]. Therefore, the diagenesis of those proteins is only ascribed to the thermal age of the fossil shells. The level of protein diagenesis is related to amino acid racemisation. As L amino acids exist in living organisms, after death they get converted into their D enantiomer, therefore that ratio reflects the time elapsed since their death [106, 107]. At equilibrium, a 1:1 of both enantiomers exists and the conversion stops at death, unless if the body is exposed to heat, humidity, pH and so on [107].

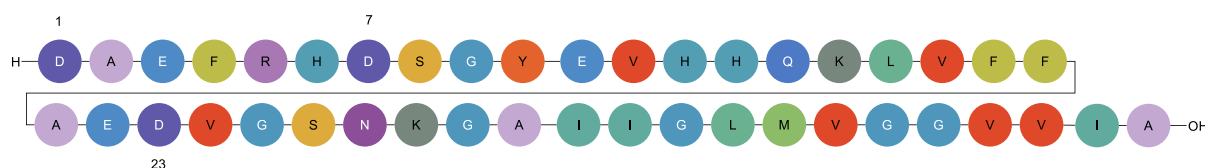


FIGURE 10 | Chemical structure of amyloid β peptide 1–42 (A β 42). Asp 1, 7 and 23 positions are shown.

Zapico and colleagues examined dentine from Spanish population (19–70-year-old) and utilised GCMS to determine the ratio between L- and D-Asp, their results were matching to previous studies, in which racemisation increases with age [108]. Interesting findings that the age estimation has some variabilities among different regions of Spain (Asturias, in the northwest of Spain, and Catalonia) which is clearly ascribed to the different environmental conditions which are known to influence racemisation extent [108].

γ S-crystallin is a major lens protein which can be modified with ageing. It was suggested by previous studies the identification of sites of isomerisation and racemisation of deamidated asparaginyl and aspartyl residues but were not proven due to complexity in detecting some post transitionally modified fragments. Halls and colleagues created a series of γ S-crystallin modified peptides including L-Asp, D-Asp, L-isoAsp and D-isoAsp at various positions as reference standards to identify tryptic digest of nuclear insoluble protein of a cataractous human lens (Figure 11) [83].

It is evident how conformational changes were introduced as a result if the increased deamidation to the human γ S-crystallin. While the overall tertiary structure folding is maintained, deamidated γ S variants form disulphide bonds more rapidly than wild-type γ S, destabilise protein structure and increase propensity for aggregation, and this is noticeable of the more ordered β strands in the deamidated variant in the N-terminal domain, like those seen in A β 42 amyloid fibrils (Figure 9), which contributes to the formation of higher-order, light-scattering aggregates (Figure 11).

Analysing the central nuclear region of a dissected cataractous human lens from 83-, 88- and 95-year old donors, using high-resolution mass spectrometry, the study confirmed that the isoforms contained L-isoAsp is prevalent [83]. Deamidation, isomerisation and racemisation are more abundant in the insoluble γ S than in the soluble form [83]. This study is very important as it

will aid in understanding the role of the of isoAsp in crystallin aggregation and cataract.

Mammalian D-Cys is a product of racemised L-Cys by Ser racemase, a pyridoxal phosphate (PLP)-dependent enzyme, which is the same enzyme that racemises Ser as well [110]. D-Cys plays an important role in the neuronal development because it inhibits the proliferation of neural progenitor cells (NPCs) via protein kinase B (AKT) signalling mediated by the FoxO family of transcription factors [110]. D-Cys binds to Myristoylated Alanine Rich C Kinase Substrate (MARCKS) and alters phosphorylation at Ser 159/163 and its translocation from the membrane (Figure 12) [110].

Interestingly, D-Ser and D-Asp appear to have neurotransmitters like properties but with much lower quantities than regular neurotransmitters (μ M), for instance, glutamate 20 mM [110]. Furthermore, unlike common neurotransmitters which are released from synaptic vesicles, D-Ser is released from the glia which is a transporter. Ultimately those D amino acids are consumed via oxidases [110].

Semenza and colleagues identified endogenous D-Cys in the brain of developing mice (embryonic day 9.5) where it regulates NPC proliferation via AKT signalling [111]. D-Cys is enriched in the embryonic brain reaching 4.5 mM, and then decreases as the development proceeds, reaching 40 μ M in the adults' brain [110]. Embryonic development represents a fine interplay between cell proliferation and its inhibition to maintain cellular homeostasis, thus, D-Cys plays a role in keeping this homeostasis [110]. Exogenous D-Cys can hydrolyse H₂S via mercaptopyruvate sulphotransferase and DAO [110], as it is involved in redox homeostasis [112].

Collectively, with the previously discussed studies, we land on an interesting hypothesis by Toxvaerd, published in BioSystems

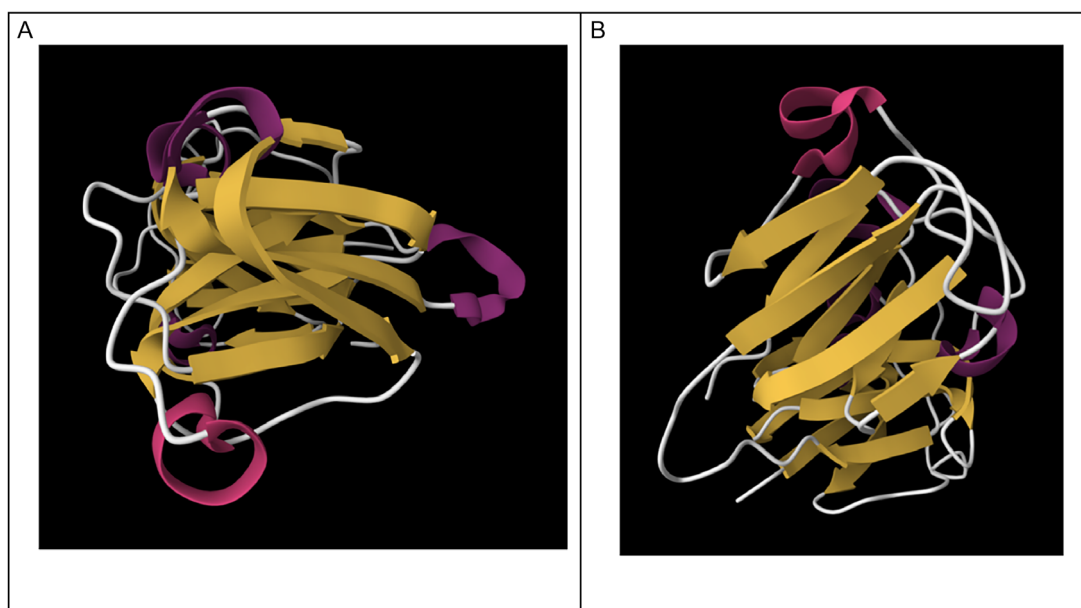


FIGURE 11 | Crystal structure of: (A) Wild-type human γ S-crystallin; PDB ID 7N36 and (B) 3-site deamidated variant of human γ S-crystallin; PDB ID 7N37 [109].

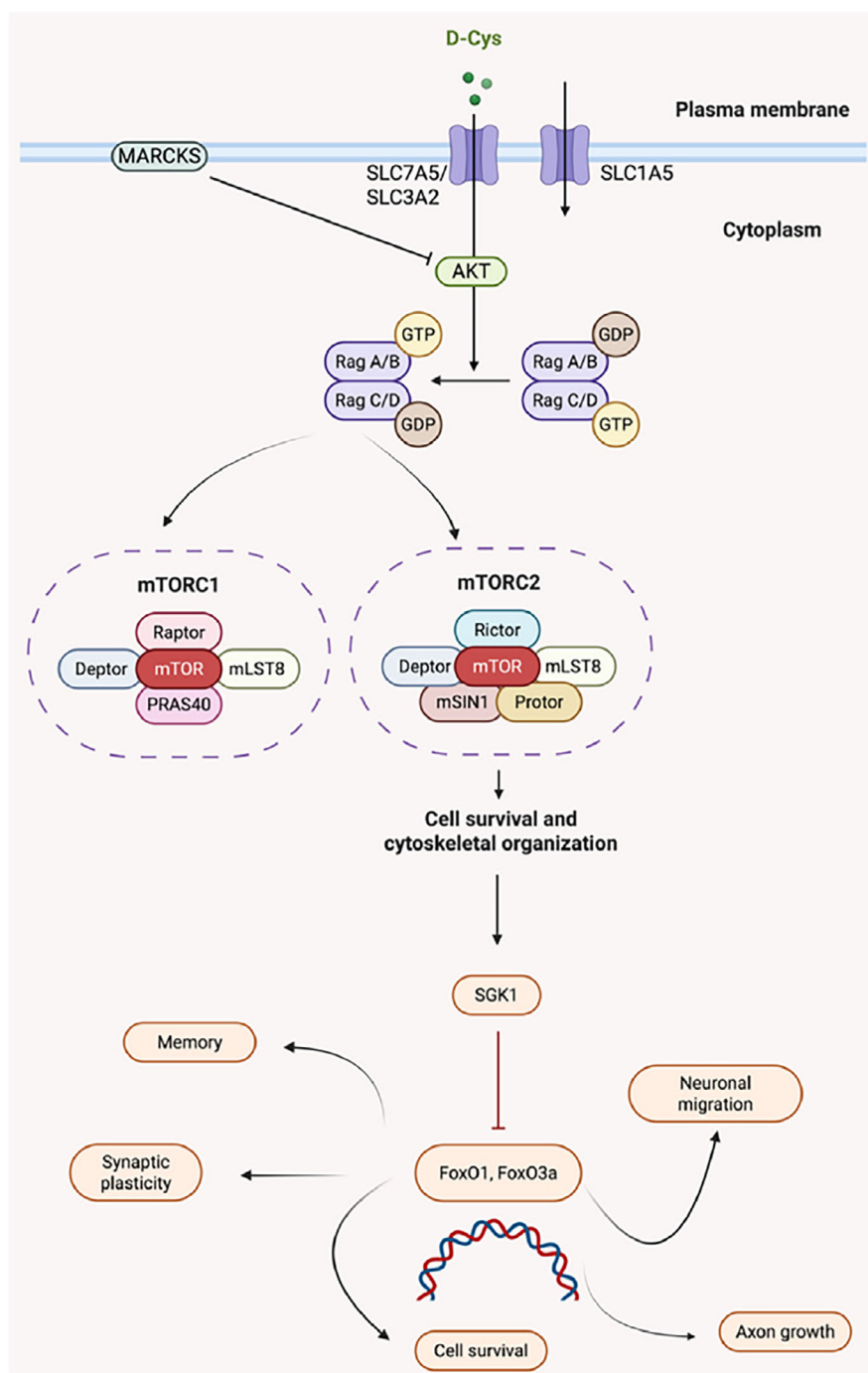


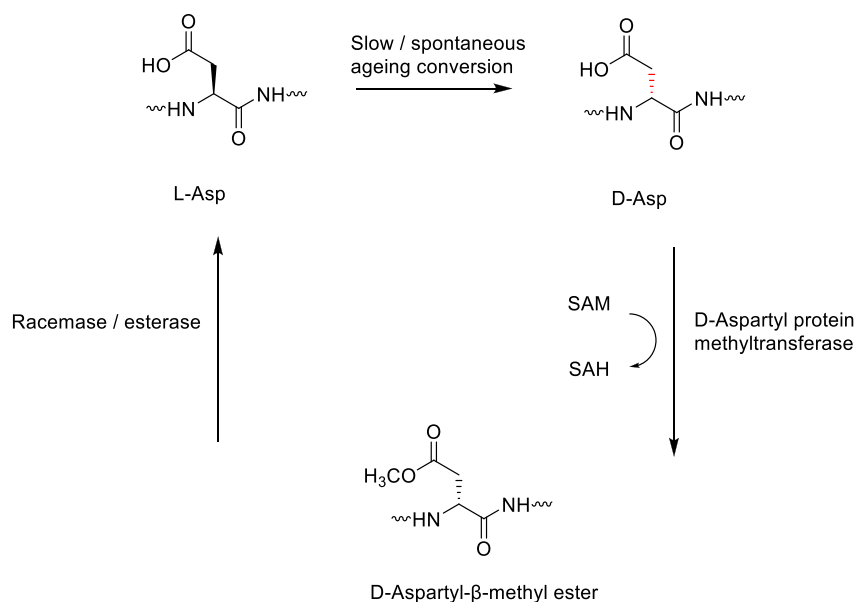
FIGURE 12 | Schematic representation of D-Cys downstream signalling and its inhibition of the proliferation of neural progenitor cells (NPCs). Created with www.biorender.com.

journal, the author argued that the long-time ageing of peptides in the cytosol leads to mortality of living organisms [113]. Basic bioreactions in cells are governed by stereospecific enzymes, importantly, the genetic codes in DNA and RNA are obtained by units with a stereospecific structure [113]. While the homochirality is important, the bottleneck here is amino acids are unstable, where in a solution of any amino acid we can notice the presence of racemic mixture ascribed to an active isomerisation kinetics [13, 114, 115]. Interestingly, while amino acid racemisation happens at extreme pH and temperatures, it could also take place at neutral pH and rather normal conditions [114].

5.1 | DNA Methylation Versus Asp Racemisation

While DNA methylation is an easy and quick procedure used for age estimation, Asp racemisation is more accurate, especially to determine the age at death. Of course, not to underestimate the sensitivity of Asp racemisation due to thermal and other damages than the DNA methylation, which is more stable to these conditions [107].

While racemisation does occur in other amino acids, such as Ala, Glu, Ile and Leu, but its rate in Asp is the fastest, hence, it is used



SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine.

FIGURE 13 | Repair pathway of mutated Asp residue in proteins. SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine. Red colour shows the change in optical purity. Adopted from [118].

in forensic to determine the death age [107]. Tissues with high metabolic rates can affect the accuracy of the results; therefore, the use of tissues with low metabolic rates are more common [116]. Those tissues are the hard tissues including bones and teeth. Teeth have proved to render accurate estimations, in particular the dentine part and this is ascribed to its being unaffected by diseases [107]. Therefore, the Asp acid racemisation methodology cannot be used for burnt samples or those that were exposed to high temperature. In addition, as its procedure is invasive, therefore, it can't be applied to living samples.

It is to be noted that the bone sex also affects the age determination. Given that the men's bones are less affected by disease, while the women's bones are affected especially after menopause where the lack of estrogen leads to bone resorption and increased osteoclast activity [117].

5.2 | Repair Pathways

Various defence mechanisms have been exploited by mammalian cells to revert isomerisation including isoaspartyl (Dasparyl) O-methyltransferase, which is an enzyme that reverts isomerised Asp back to L-Asp (Figure 13) [13, 118].

Another mechanism is D-aspartyl endopeptidase (DAEP) acts by cleaving the D-Asp from the carboxy terminal and boost degradation of abnormal protein-containing D-Asp [12]. This phenomenon affects protein with misfolding propensity, however, in amyloid β peptide 1–42 (A β 42), an opposing effect was observed. Ito and Utsunomiya-Tate identified mitochondrial serine β -lactamase-like protein (LACTB) as the first mammalian

protein with DAEP activity [12]. It is also considered as tumour suppressor, where its decreased expression was seen in various cancers as well as the poor overall survival in cancer patients. On the contrary, its overexpression is associated with an increased risk of Alzheimer's disease [119, 120], therefore the alteration in the DAEP of LACTB activity may cause the onset and progression of these diseases [12].

6 | Conclusion

Racemisation is an inherent and unavoidable process that affects the stereochemical integrity, purity, and biological activity of peptides and proteins, and is increasingly recognised as a contributing factor in various disease states. Although its extent can be influenced by external factors such as temperature and extreme pH, racemisation is fundamentally thermodynamically favoured and can occur under physiological conditions. While protein folding leads to a stable, low-energy conformation accompanied by a decrease in entropy, proteins remain susceptible to spontaneous structural alterations over time, including aggregation and misfolding, processes that can be exacerbated by amino acid racemisation.

D-amino acids have emerged as important biomolecular components with functions extending well beyond their classical perception as rare or non-proteinogenic residues. Advances in analytical methodologies have revealed their widespread natural occurrence across diverse biological systems, along with dedicated enzymatic pathways responsible for their synthesis, degradation, and regulation. Increasing evidence highlights their

involvement in physiological processes and their growing relevance in biotechnology and pharmaceutical applications, particularly in the design of more stable and functionally versatile peptide-based systems. Together, these findings position D-amino acids as key molecular building blocks for both understanding biological complexity and developing next-generation therapeutic and industrial materials.

Protein ageing may therefore be viewed as a balance between the maintenance of homochirality and the gradual accumulation of racemised residues, a process closely linked to cellular stress responses and functional decline. The susceptibility of amino acids to racemisation varies depending on their side-chain properties, particularly their ability to stabilise reaction intermediates, thereby prolonging their persistence and increasing the likelihood of stereochemical inversion.

Although biological repair mechanisms, such as the action of L-isopartyl O-methyltransferase, can partially reverse or mitigate these modifications, racemisation remains a cumulative process. Importantly, this phenomenon can also be exploited as a valuable analytical tool, particularly in age estimation of biological tissues such as bone and dentine, where a clear correlation exists between the extent of aspartic acid racemisation and chronological age.

Conflicts of Interest

The author declares no conflicts of interest.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

References

1. D. L. Lee, J. P. Powers, K. Pflegerl, M. L. Vasil, R. E. Hancock, and R. S. Hodges, "Effects of Single D-Amino Acid Substitutions on Disruption of Beta-Sheet Structure and Hydrophobicity in Cyclic 14-Residue Antimicrobial Peptide Analogs Related to Gramicidin S," *The Journal of Peptide Research* 63, no. 2 (2004): 69–84, <https://doi.org/10.1046/j.1399-3011.2003.00106.x>.
2. C. McInnes, L. H. Kondejewski, R. S. Hodges, and B. D. Sykes, "Development of the Structural Basis for Antimicrobial and Hemolytic Activities of Peptides Based on Gramicidin S and Design of Novel Analogs Using NMR Spectroscopy," *The Journal of Biological Chemistry* 275, no. 19 (2000): 14287–14294, <https://doi.org/10.1074/jbc.275.19.14287>.
3. M. C. Khosla, M. M. Hall, R. R. Smeby, and F. M. Bumpus, "Factors that Influence the Antagonistic Properties of Angiotensin II Antagonists," *Journal of Medicinal Chemistry* 16, no. 7 (1973): 829–832, <https://doi.org/10.1021/jm00265a018>.
4. L. H. Kondejewski, M. Jelokhani-Niaraki, S. W. Farmer, et al., "Dissociation of Antimicrobial and Hemolytic Activities in Cyclic Peptide Diastereomers by Systematic Alterations in Amphipathicity," *The Journal of Biological Chemistry* 274, no. 19 (1999): 13181–13192, <https://doi.org/10.1074/jbc.274.19.13181>.
5. V. V. Dyakin, N. V. Dyakina-Fagnano, L. B. McIntire, and V. N. Uversky, "Fundamental Clock of Biological Aging: Convergence of Molecular, Neurodegenerative, Cognitive and Psychiatric Pathways: Non-Equilibrium Thermodynamics Meet Psychology," *International Journal of Molecular Sciences* 23, no. 1 (2021): 285, <https://doi.org/10.3390/ijms23010285>.
6. K. Michaelian, "The Dissipative Photochemical Origin of Life: UVC Abiogenesis of Adenine," *Entropy (Basel)* 23, no. 2 (2021): 217, <https://doi.org/10.3390/e23020217>.
7. E. Faraggi, A. K. Dunker, R. L. Jernigan, and A. Kloczkowski, "Entropy, Fluctuations, and Disordered Proteins," *Entropy (Basel)* 21, no. 8 (2019): 764, <https://doi.org/10.3390/e21080764>.
8. J. L. Bada, "AMINO ACID RACEMIZATION DATING OF FOSSIL BONES," *Annual Review of Earth and Planetary Sciences* 13 (1985): 241–268, <https://doi.org/10.1146/annurev.ea.13.050185.001325>.
9. S. M. Morrow, A. J. Bissette, and S. P. Fletcher, "Transmission of Chirality through Space and across Length Scales," *Nature Nanotechnology* 12, no. 5 (2017): 410–419, <https://doi.org/10.1038/nnano.2017.62>.
10. C. Zucchi, G. Pályi, L. Caglioti, and G. Palyi, *Advances in BioChirality* (Elsevier Science, 1999).
11. T. R. Lambeth, D. L. Riggs, L. E. Talbert, et al., "Spontaneous Isomerization of Long-Lived Proteins Provides a Molecular Mechanism for the Lysosomal Failure Observed in Alzheimer's Disease," *ACS Central Science* 5, no. 8 (2019): 1387–1395, <https://doi.org/10.1021/acscentsci.9b00369>.
12. G. Ito and N. Utsunomiya-Tate, "Discovery and Biochemical Characterization of the D-Aspartyl Endopeptidase Activity of the Serine Protease LACTB," *The Journal of Biological Chemistry* 301, no. 6 (2025): 108549, <https://doi.org/10.1016/j.jbc.2025.108549>.
13. S. Ritz-Timme and M. J. Collins, "Racemization of Aspartic Acid in Human Proteins," *Ageing Research Reviews* 1, no. 1 (2002): 43–59, [https://doi.org/10.1016/s0047-6374\(01\)00363-3](https://doi.org/10.1016/s0047-6374(01)00363-3).
14. L. Grassi and C. Cabrele, "Susceptibility of Protein Therapeutics to Spontaneous Chemical Modifications by Oxidation, Cyclization, and Elimination Reactions," *Amino Acids* 51, no. 10 (2019): 1409–1431, <https://doi.org/10.1007/s00726-019-02787-2>.
15. M. Goodman and W. J. McGahren, "Mechanistic Studies of Peptide Oxazolone Racemization," *Tetrahedron* 23, no. 5 (1967): 2031–2050, [https://doi.org/10.1016/0040-4020\(67\)80037-1](https://doi.org/10.1016/0040-4020(67)80037-1).
16. C. Griehl, A. Kolbe, and S. Merkel, "Quantitative Description of Epimerization Pathways Using the Carbodiimide Method in the Synthesis of Peptides," *Journal of the Chemical Society* 11 (1996): 2525–2529, <https://doi.org/10.1039/P29960002525>.
17. G. B. Fields and R. L. Noble, "Solid Phase Peptide Synthesis Utilizing 9-Fluorenylmethoxycarbonyl Amino Acids," *International Journal of Peptide and Protein Research* 35 (1990): 161–214, <https://doi.org/10.1111/j.1399-3011.1990.tb00939.x>.
18. J. Kovacs and Y. Hsieh, "Racemization Mechanism of Cysteine Dipeptide Active Ester Derivatives," *The Journal of Organic Chemistry* 47, no. 25 (1982): 4996–5002, <https://doi.org/10.1021/jo00146a032>.
19. S. Duengo, M. I. Muhajir, A. T. Hidayat, W. J. A. Musa, and R. Maharani, "Epimerisation in Peptide Synthesis," *Molecules* 28, no. 24 (2023): 8017, <https://doi.org/10.3390/molecules28248017>.
20. M. Bodanszky and A. Bodanszky, "Racemization in Peptide Synthesis. Mechanism-Specific Models," *Chemical Communications* 12 (1967): 591–593, <https://doi.org/10.1039/C19670000591>.
21. Y. Han, F. Albericio, and G. Barany, "Occurrence and Minimization of Cysteine Racemization during Stepwise Solid-Phase Peptide Synthesis^{1,2}," *The Journal of Organic Chemistry* 62, no. 13 (1997): 4307–4312, <https://doi.org/10.1021/jo962274a>.
22. S. J. Harding, J. H. Jones, A. N. Sabirov, and V. V. Samukov, "Im-Trityl Protection of Histidine," *Journal of Peptide Science* 5, no. 8 (1999): 368–373, [https://doi.org/10.1002/\(SICI\)1099-1387\(199908\)5:8%3C368::AID-PSC210>3.0.CO;2-P](https://doi.org/10.1002/(SICI)1099-1387(199908)5:8%3C368::AID-PSC210>3.0.CO;2-P).

23. T. Geiger, and S. Clarke, "Deamidation, Isomerization, and Racemization at Asparaginyl and Aspartyl Residues in Peptides. Succinimide-Linked Reactions that Contribute to Protein Degradation," *The Journal of Biological Chemistry* 262, no. 2 (1987): 785–794.
24. J. L. Bada, "In Vivo Racemization in Mammalian Proteins," *Methods in Enzymology* 106 (1984): 98–115, [https://doi.org/10.1016/0076-6879\(84\)06011-0](https://doi.org/10.1016/0076-6879(84)06011-0).
25. L.-C. Hsiao, C.-H. Lee, K. Mazmanian, et al., "Impacts of D-Aspartate on the Aggregation Kinetics and Structural Polymorphism of Amyloid β Peptide 1-42," *Journal of Molecular Biology* 437, no. 12 (2025): 169092, <https://doi.org/10.1016/j.jmb.2025.169092>.
26. J. L. Radkiewicz, H. Zipse, S. Clarke, and K. N. Houk, "Accelerated Racemization of Aspartic Acid and Asparagine Residues via Succinimide Intermediates: An Ab Initio Theoretical Exploration of Mechanism," *Journal of the American Chemical Society* 118, no. 38 (1996): 9148–9155, <https://doi.org/10.1021/ja953505b>.
27. J. M. Manning and S. Moore, "Determination of D- and L-Amino Acids by Ion Exchange Chromatography as L-D and L-L Dipeptides," *The Journal of Biological Chemistry* 243, no. 21 (1968): 5591–5597.
28. K. Kaiser and R. Benner, "Hydrolysis-Induced Racemization of Amino Acids," *Limnology and Oceanography: Methods* 3, no. 8 (2005): 318–325, <https://doi.org/10.4319/lom.2005.3.318>.
29. R. Liardon, S. Ledermann, and U. Ott, "Determination of d-Amino Acids by Deuterium Labelling and Selected Ion Monitoring," *Journal of Chromatography A* 203 (1981): 385–395, [https://doi.org/10.1016/S0021-9673\(00\)80309-X](https://doi.org/10.1016/S0021-9673(00)80309-X).
30. H. Brückner and T. Westhauser, "Chromatographic Determination of L- and D-Amino Acids in Plants," *Amino Acids* 24, no. 1-2 (2003): 43–55, <https://doi.org/10.1007/s00726-002-0322-8>.
31. A. Ballard, S. Narduolo, H. O. Ahmed, et al., "Racemisation in Chemistry and Biology," *Chemistry – A European Journal* 26, no. 17 (2020): 3661–3687, <https://doi.org/10.1002/chem.201903917>.
32. A. Broberg, C. Nord, J. J. Levenfors, J. Bjerketorp, B. Guss, and B. Öberg, "In-Peptide Amino Acid Racemization via Inter-Residue Oxazoline Intermediates during Acidic Hydrolysis," *Amino Acids* 53, no. 3 (2021): 323–331, <https://doi.org/10.1007/s00726-021-02951-7>.
33. R. B. Merrifield, "Solid Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide," *Journal of the American Chemical Society* 85 (1963): 2149–2154, <https://doi.org/10.1021/ja00897a025>.
34. P. Sieber and B. Riniker, "Protection of Histidine in Peptide Synthesis: A Reassessment of the Trityl Group," *Tetrahedron Letters* 28, no. 48 (1987): 6031–6034, [https://doi.org/10.1016/S0040-4039\(00\)96856-4](https://doi.org/10.1016/S0040-4039(00)96856-4).
35. Y. Yang, L. Hansen, and A. Baldi, "Suppression of Simultaneous Fmoc-His(Trt)-OH Racemization and N α -DIC-Endcapping in Solid-Phase Peptide Synthesis through Design of Experiments and Its Implication for an Amino Acid Activation Strategy in Peptide Synthesis," *Organic Process Research and Development* 26, no. 8 (2022): 2464–2474, <https://doi.org/10.1021/acs.oprd.2c00144>.
36. J. Kovacs, G. L. Mayers, R. H. Johnson, R. E. Cover, and U. R. Ghatak, "Racemization of Amino Acid Derivatives. III. Rate of Racemization and Peptide Bond Formation of Cysteine Active Esters," *The Journal of Organic Chemistry* 35, no. 6 (1970): 1810–1815, <https://doi.org/10.1021/jo00831a021>.
37. H.-J. Musiol, F. Siedler, D. Quarzago, and L. Moroder, "Redox-Active Bis-Cysteinyll Peptides. I. Synthesis of Cyclic Cystinyl Peptides by Conventional Methods in Solution and on Solid Supports," *Biopolymers* 34, no. 11 (1994): 1553–1562, <https://doi.org/10.1002/bip.360341113>.
38. R. Behrendt, M. Schenk, H. J. Musiol, and L. Moroder, "Photomodulation of Conformational States. Synthesis of Cyclic Peptides with Backbone-Azobenzene Moieties," *Journal of Peptide Science* 5, no. 11 (1999): 519–529, [https://doi.org/10.1002/\(SICI\)1099-1387\(199911\)5:11%3C519::AID-PSC223>3.0.CO;2-3](https://doi.org/10.1002/(SICI)1099-1387(199911)5:11%3C519::AID-PSC223>3.0.CO;2-3).
39. P. Sieber, "An Improved Method for Anchoring of 9-Fluorenylmethoxycarbonyl-Amino Acids to 4-Alkoxybenzyl Alcohol Resins," *Tetrahedron Letters* 28, no. 49 (1987): 6147–6150, [https://doi.org/10.1016/S0040-4039\(00\)61832-4](https://doi.org/10.1016/S0040-4039(00)61832-4).
40. E. Atherton, N. L. Benoiton, E. Brown, R. C. Sheppard, and B. J. Williams, "Racemisation of Activated, Urethane-Protected Amino-Acids by p-Dimethyl-Aminopyridine. Significance in Solid-Phase Peptide Synthesis," *Journal of the Chemical Society* 7 (1981): 336–337, <https://doi.org/10.1039/C39810000336>.
41. B. H. Rietman, R. F. R. Peters, and G. I. Tesser, "On the Stability of S-(alkylsulfanyl) Cysteine Derivatives during Solid-Phase Synthesis," *Recueil Des Travaux Chimiques Des Pays-Bas* 114, no. 1 (1995): 1–5, <https://doi.org/10.1002/recl.19951140102>.
42. J. Csapó, É. Varga-Visi, K. Lóki, and C. Albert, "Analysis of the Racemization of Tryptophan," *Chromatographia* 63, no. 13 (2006): S101–S104, <https://doi.org/10.1365/s10337-006-0783-0>.
43. M. Friedman and J. L. Cuq, "Chemistry, Analysis, Nutritional Value, and Toxicology of Tryptophan in Food. A Review," *Journal of Agricultural and Food Chemistry* 36, no. 5 (1988): 1079–1093, <https://doi.org/10.1021/jf00083a042>.
44. C. Griehl, J. Weigt, and H. Jeschkeit, "Use of Tryptophan Peptides for an HPLC Racemization Test with Fluorescence Detection," *Journal of High Resolution Chromatography* 17, no. 10 (1994): 700–704, <https://doi.org/10.1002/jhrc.1240171004>.
45. D. Riester, K.-H. Wiesmüller, D. Stoll, and R. Kuhn, "Racemization of Amino Acids in Solid-Phase Peptide Synthesis Investigated by Capillary Electrophoresis," *Analytical Chemistry* 68, no. 14 (1996): 2361–2365, <https://doi.org/10.1021/ac9511511>.
46. O. Al Musaimi, A. El-Faham, A. Basso, B. G. de la Torre, and F. Albericio, " γ -Valerolactone (GVL): An Eco-Friendly Anchoring Solvent for Solid-Phase Peptide Synthesis," *Tetrahedron Letters* 60 (2019): 151058, <https://doi.org/10.1016/j.tetlet.2019.151058>.
47. V. Gavva, O. Al Musaimi, C. Bent, and D. R. Williams, "Determining the Hydrophobicity Index of Protected Amino Acids and Common Protecting Groups," *Separations* 10 (2023): 456, <https://doi.org/10.3390/separations10080456>.
48. M. Biler, R. M. Crean, A. K. Schweiger, R. Kourist, and S. C. L. Kamerlin, "Ground-State Destabilization by Active-Site Hydrophobicity Controls the Selectivity of a Cofactor-Free Decarboxylase," *Journal of the American Chemical Society* 142, no. 47 (2020): 20216–20231, <https://doi.org/10.1021/jacs.0c10701>.
49. Y. Zhou, H. Li, Y. Huang, et al., "Suppression of Alpha-Carbon Racemization in Peptide Synthesis Based on a Thiol-Labile Amino Protecting Group," *Nature Communications* 14, no. 1 (2023): 5324, <https://doi.org/10.1038/s41467-023-41115-x>.
50. H. Johansson, and D. S. Pedersen, "Azide- and Alkyne-Derivatized α -Amino Acids," *European Journal of Organic Chemistry* 2012, no. 23 (2012): 4267–4281, <https://doi.org/10.1002/ejoc.201200496>.
51. Y. S. Klausner and M. Bodanszky, "The Azide Method in Peptide Synthesis: Its Scope and Limitations," *Synthesis* 1974, no. 08 (1974): 549–559, <https://doi.org/10.1055/s-1974-23365>.
52. L. A. Carpino, M. Beyermann, H. Wenschuh, and M. Bienert, "Peptide Synthesis via Amino Acid Halides," *Accounts of Chemical Research* 29, no. 6 (1996): 268–274, <https://doi.org/10.1021/ar950023w>.
53. P. Li and J.-C. Xu, "1-Ethyl 2-Halopyridinium Salts, Highly Efficient Coupling Reagents for Hindered Peptide Synthesis Both in Solution and the Solid-Phase," *Tetrahedron* 56, no. 41 (2000): 8119–8131, [https://doi.org/10.1016/S0040-4020\(00\)00657-8](https://doi.org/10.1016/S0040-4020(00)00657-8).
54. J. J. Hans, R. W. Driver, and S. D. Burke, "Direct Transacylation of 2,2,2-Trihaloethyl Esters with Amines and Alcohols Using Phosphorus(III) Reagents for Reductive Fragmentation and In Situ

- Activation," *The Journal of Organic Chemistry* 65, no. 7 (2000): 2114–2121, <https://doi.org/10.1021/jo991711m>.
55. B. Castro, J.-R. Dormoy, B. Dourtoglou, G. Evin, C. Selve, and J.-C. Ziegler, "Peptide Coupling Reagents VII. A Novel, Cheaper Preparation of Benzotriazolyloxytris(dimethylamino)phosphonium Hexafluorophosphate (BOP Reagent)," *Synthesis* 1976, no. 11 (1976): 751–752, <https://doi.org/10.1055/s-1976-24189>.
56. J. N. deGruyter, L. R. Malins, L. Wimmer, et al., "CITU: A Peptide and Decarboxylative Coupling Reagent," *Organic Letters* 19, no. 22 (2017): 6196–6199, <https://doi.org/10.1021/acs.orglett.7b03121>.
57. V. Dourtoglou, B. Gross, V. Lambropoulou, and C. Zioudrou, "O-Benzotriazolyl- N,N,N',N'-tetramethyluronium Hexafluorophosphate as Coupling Reagent for the Synthesis of Peptides of Biological Interest," *Synthesis* 1984, no. 07 (1984): 572–574, <https://doi.org/10.1055/s-1984-30895>.
58. A. Isidro-Llobet, M. Álvarez, and F. Albericio, "Amino Acid-Protecting Groups," *Chemical Reviews* 109 (2009): 2455–2504, <https://doi.org/10.1021/cr800323s>.
59. V. Agouridas, O. El Mahdi, V. Diemer, M. Cargoët, J.-C. M. Monbaliu, and O. Melnyk, "Native Chemical Ligation and Extended Methods: Mechanisms, Catalysis, Scope, and Limitations," *Chemical Reviews* 119, no. 12 (2019): 7328–7443, <https://doi.org/10.1021/acs.chemrev.8b00712>.
60. S. Bondalapati, M. Jbara, and A. Brik, "Expanding the Chemical Toolbox for the Synthesis of Large and Uniquely Modified Proteins," *Nature Chemistry* 8 (2016): 407–418, <https://doi.org/10.1038/nchem.2476>.
61. S. Noki, H. Saneii, B. G. de la Torre, and F. Albericio, "Base-Labile Safety-Catch Linker: Synthesis and Applications in Solid-Phase Peptide Synthesis," *International Journal of Molecular Sciences* 26, no. 5 (2025): 2210, <https://doi.org/10.3390/ijms26052210>.
62. O. Al Musaimi, Y. E. Jad, A. Kumar, et al., "Greening the Solid-Phase Peptide Synthesis Process. 2-MeTHF for the Incorporation of the First Amino Acid and Precipitation of Peptides after Global Deprotection," *Organic Process Research and Development* 22 (2018): 1809–1816, <https://doi.org/10.1021/acs.oprd.8b00335>.
63. D'san M. M. Jaradat, O. Al Musaimi, and F. Albericio, "Advances in Solid-Phase Peptide Synthesis in Aqueous Media (ASPPS)," *Green Chemistry* 24, no. 17 (2022): 6360–6372, <https://doi.org/10.1039/D2GC02319A>.
64. S. R. Manne, A. El-Faham, B. G. de la Torre, and F. Albericio, "Minimizing Side Reactions during Amide Formation Using DIC and Oxymapure in Solid-Phase Peptide Synthesis," *Tetrahedron Letters* 85 (2021): 153462, <https://doi.org/10.1016/j.tetlet.2021.153462>.
65. I. Guryanov, A. Orlandin, I. De Paola, et al., "Copper (II) Lysinate and Pseudoproline Assistance in the Convergent Synthesis of the GLP-1 Receptor Agonists Liraglutide and Semaglutide," *Organic Process Research and Development* 25, no. 7 (2021): 1598–1611, <https://doi.org/10.1021/acs.oprd.1c00021>.
66. W. Van den Nest, S. Yuval, and F. Albericio, "Cu(OBT)₂ and Cu(OAT)₂, Copper(II)-Based Racemization Suppressors Ready for use in Fully Automated Solid-Phase Peptide Synthesis," *Journal of Peptide Science* 7, no. 3 (2001): 115–120, <https://doi.org/10.1002/psc.299>.
67. L. Pollegioni, N. Kustrimovic, L. Piubelli, E. Rosini, V. Rabattoni, and S. Sacchi, "D-amino Acids: New Functional Insights," *The FEBS Journal* 292, no. 17 (2025): 4395–4417, <https://doi.org/10.1111/febs.70083>.
68. S. Fernandez-Lopez, H. S. Kim, E. C. Choi, et al., "Antibacterial Agents Based on the Cyclic D,L-Alpha-Peptide Architecture," *Nature* 412, no. 6845 (2001): 452–455, <https://doi.org/10.1038/35086601>.
69. L. Wang, B. P. Lieberman, K. Plössl, W. Qu, and H. F. Kung, "Synthesis and Comparative Biological Evaluation of L- and D-Isomers of 18F-Labeled Fluoroalkyl Phenylalanine Derivatives as Tumor Imaging Agents," *Nuclear Medicine and Biology* 38, no. 3 (2011): 301–312, <https://doi.org/10.1016/j.nucmedbio.2010.10.004>.
70. M. R. Ghadiri, J. R. Granja, R. A. Milligan, D. E. McRee, and N. Khazanovich, "Self-Assembling Organic Nanotubes Based on a Cyclic Peptide Architecture," *Nature* 366, no. 6453 (1993): 324–327, <https://doi.org/10.1038/366324a0>.
71. M. Vilela-Picos, E. González-Freire, F. Novelli, et al., "Self-Assembling Cyclic Peptide Nanotubes for the Delivery of Doxorubicin into Drug-Resistant Cancer Cells," *ACS Applied Materials and Interfaces* 17, no. 36 (2025): 50191–50202, <https://doi.org/10.1021/acsami.5c05264>.
72. M. Fares and O. Al Musaimi, "Self-Assembly of Peptides and Biomolecular Systems Into Functional Nanomaterials," *Journal of Peptide Science* 32, no. 6 (2026): e70101, <https://doi.org/10.1002/psc.70101>.
73. S. Martínez-Rodríguez, A. I. Martínez-Gómez, F. Rodríguez-Vico, J. M. Clemente-Jiménez, and F. J. Las Heras-Vázquez, "Natural Occurrence and Industrial Applications of D-Amino Acids: An Overview," *Chemistry and Biodiversity* 7, no. 6 (2010): 1531–1548, <https://doi.org/10.1002/cbdv.200900245>.
74. S.-X. Gu, H.-F. Wang, Y.-Y. Zhu, and F.-E. Chen, "Natural Occurrence, Biological Functions, and Analysis of D-Amino Acids," *Pharmaceutical Fronts* 02, no. 02 (2020): e79–e87, <https://doi.org/10.1055/s-0040-1713820>.
75. M. Abdulbagi, L. Wang, O. Siddig, B. Di, and B. Li, "D-Amino Acids and D-Amino Acid-Containing Peptides: Potential Disease Biomarkers and Therapeutic Targets?," *Biomolecules* 11, no. 11 (2021): 1716, <https://doi.org/10.3390/biom11111716>.
76. S. V. Khoronenkova, and V. I. Tishkov, "D-Amino Acid Oxidase: Physiological Role and Applications," *Biochemistry (Moscow)* 73, no. 13 (2008): 1511–1518, <https://doi.org/10.1134/s0006297908130105>.
77. S. Du, M. Wey, and D. W. Armstrong, "d-Amino Acids in Biological Systems," *Chirality* 35, no. 9 (2023): 508–534, <https://doi.org/10.1002/chir.23562>.
78. J. Lowenson and S. Clarke, "Does the Chemical Instability of Aspartyl and Asparaginyl Residues in Proteins Contribute to Erythrocyte Aging? The Role of Protein Carboxyl Methylation Reactions," *Blood Cells* 14, no. 1 (1988): 103–118.
79. J. B. Lian and C. M. Gundberg, "Osteocalcin. Biochemical Considerations and Clinical Applications," *Clinical Orthopaedics and Related Research* 226 (1988): 267–291.
80. J. T. Powell, N. Vine, and M. Crossman, "On the Accumulation of D-Aspartate in Elastin and Other Proteins of the Ageing Aorta," *Atherosclerosis* 97, no. 2-3 (1992): 201–208, [https://doi.org/10.1016/0021-9150\(92\)90132-z](https://doi.org/10.1016/0021-9150(92)90132-z).
81. K. J. Reissner and D. W. Aswad, "Deamidation and Isoaspartate Formation in Proteins: Unwanted Alterations or Surreptitious Signals?," *Cellular and Molecular Life Sciences (CMLS)* 60, no. 7 (2003): 1281–1295, <https://doi.org/10.1007/s00018-003-2287-5>.
82. B. A. Johnson, E. L. Langmack, and D. W. Aswad, "Partial Repair of Deamidation-Damaged Calmodulin by Protein Carboxyl Methyltransferase," *The Journal of Biological Chemistry* 262, no. 25 (1987): 12283–12287.
83. V. S. Halls, L. L. David, K. D. Zientek, and K. J. Lampi, "Isomerized and Racemized Aspartyl and Deamidated Asparagine Residues Identified in γS-Crystallin," *ChemBioChem* 26, no. 16 (2025): e202500352, <https://doi.org/10.1002/cbic.202500352>.
84. J. Sun, T. Matsubara, T. Koide, K. J. Lampi, L. L. David, and T. Takata, "Characterization of Different-Sized Human αA-Crystallin Homomers and Implications to Asp151 Isomerization," *PLoS One* 19, no. 7 (2024): e0306856, <https://doi.org/10.1371/journal.pone.0306856>.
85. D. W. Aswad, M. V. Paranandi, and B. T. Schurter, "Isoaspartate in Peptides and Proteins: Formation, Significance, and Analysis," *Journal of Pharmaceutical and Biomedical Analysis* 21, no. 6 (2000): 1129–1136, [https://doi.org/10.1016/s0731-7085\(99\)00230-7](https://doi.org/10.1016/s0731-7085(99)00230-7).

86. D. Ingrosso and A. F. Perna, "D-Amino Acids in Aging Erythrocytes," *Exs* 85 (1998): 119–141, https://doi.org/10.1007/978-3-0348-8837-0_8.
87. A. E. Roher, J. D. Lowenson, S. Clarke, et al., "Structural Alterations in the Peptide Backbone of Beta-Amyloid Core Protein May Account for Its Deposition and Stability in Alzheimer's Disease," *The Journal of Biological Chemistry* 268, no. 5 (1993): 3072–3083.
88. M. T. Colvin, R. Silvers, Q. Z. Ni, et al., "Atomic Resolution Structure of Monomeric A β 42 Amyloid Fibrils," *Journal of the American Chemical Society* 138, no. 30 (2016): 9663–9674, <https://doi.org/10.1021/jacs.6b05129>.
89. P. M. Masters, J. L. Bada, and J. S. Zigler, "Aspartic Acid Racemization in Heavy Molecular Weight Crystallins and Water Insoluble Protein from Normal Human Lenses and Cataracts," *Proceedings of the National Academy of Sciences* 75, no. 3 (1978): 1204–1208, <https://doi.org/10.1073/pnas.75.3.1204>.
90. M. T. Englander, J. L. Avins, R. C. Fleisher, et al., "The Ribosome Can Discriminate the Chirality of Amino Acids Within Its Peptidyl-Transferase Center," *Proceedings of the National Academy of Sciences* 112, no. 19 (2015): 6038–6043, <https://doi.org/10.1073/pnas.1424712112>.
91. S. Raghunathan, K. Yadav, V. C. Rojisha, et al., "Transition between [R]- and [S]-Stereoisomers without Bond Breaking," *Physical Chemistry Chemical Physics* 22, no. 26 (2020): 14983–14991, <https://doi.org/10.1039/d0cp02918a>.
92. G. Jung, J. Ryu, J. Heo, S. J. Lee, J. Y. Cho, and S. Hong, "Protein L-Isoaspartyl O-Methyltransferase Inhibits Amyloid Beta Fibrillogenesis In Vitro," *Die Pharmazie* 66, no. 7 (2011): 529–534.
93. S. Minegishi, S. Ohtani, K. Noritake, et al., "Preparation of Dentin Standard Samples for Age Estimation Based on Increased Aspartic Acid Racemization Rate by Heating," *Legal Medicine* 38 (2019): 25–31, <https://doi.org/10.1016/j.legalmed.2019.03.005>.
94. M. Y. Hooi and R. J. Truscott, "Racemisation and Human Cataract. D-Ser, D-Asp/Asn and D-Thr Are Higher in the Lifelong Proteins of Cataract Lenses than in Age-Matched Normal Lenses," *Age (Dordr)* 33, no. 2 (2011): 131–141, <https://doi.org/10.1007/s11357-010-9171-7>.
95. N. Fujii, T. Kawaguchi, H. Sasaki, and N. Fujii, "Simultaneous Stereo-inversion and Isomerization at the Asp-4 Residue in β B2-Crystallin from the Aged Human Eye Lenses," *Biochemistry* 50, no. 40 (2011): 8628–8635, <https://doi.org/10.1021/bi200983g>.
96. P. G. Hains and R. J. Truscott, "Age-Dependent Deamidation of Lifelong Proteins in the Human Lens," *Investigative Ophthalmology and Visual Science* 51, no. 6 (2010): 3107–3114, <https://doi.org/10.1167/iovs.09-4308>.
97. A. Spector, R. Chiesa, J. Sredy, and W. Garner, "CAMP-Dependent Phosphorylation of Bovine Lens Alpha-Crystallin," *Proceedings of the National Academy of Sciences* 82, no. 14 (1985): 4712–4716, <https://doi.org/10.1073/pnas.82.14.4712>.
98. V. N. Lapko, D. L. Smith, and J. B. Smith, "Methylation and Carbamylation of Human Gamma-Crystallins," *Protein Science* 12, no. 8 (2003): 1762–1774, <https://doi.org/10.1110/ps.0305403>.
99. O. P. Srivastava and K. Srivastava, "BetaB2-Crystallin Undergoes Extensive Truncation during Aging in Human Lenses," *Biochemical and Biophysical Research Communications* 301, no. 1 (2003): 44–49, [https://doi.org/10.1016/s0006-291x\(02\)02975-3](https://doi.org/10.1016/s0006-291x(02)02975-3).
100. R. J. Truscott and R. C. Augusteyn, "Changes in Human Lens Proteins during Nuclear Cataract Formation," *Experimental Eye Research* 24, no. 2 (1977): 159–170, [https://doi.org/10.1016/0014-4835\(77\)90256-1](https://doi.org/10.1016/0014-4835(77)90256-1).
101. M. H. Garner and A. Spector, "Selective Oxidation of Cysteine and Methionine in Normal and Senile Cataractous Lenses," *Proceedings of the National Academy of Sciences* 77, no. 3 (1980): 1274–1277, <https://doi.org/10.1073/pnas.77.3.1274>.
102. M. C. Wells-Knecht, T. G. Huggins, D. G. Dyer, S. R. Thorpe, and J. W. Baynes, "Oxidized Amino Acids in Lens Protein with Age. Measurement of o-Tyrosine and Dityrosine in the Aging Human Lens," *The Journal of Biological Chemistry* 268, no. 17 (1993): 12348–12352.
103. R. J. Truscott, "Age-Related Nuclear Cataract-Oxidation Is the Key," *Experimental Eye Research* 80, no. 5 (2005): 709–725, <https://doi.org/10.1016/j.exer.2004.12.007>.
104. S. Arany and S. Ohtani, "Age Estimation of Bloodstains: A Preliminary Report Based on Aspartic Acid Racemization Rate," *Forensic Science International* 212, no. 1–3 (2011): e36–e39, <https://doi.org/10.1016/j.forsciint.2011.05.015>.
105. B. Demarchi, M. G. Williams, N. Milner, N. Russell, G. Bailey, and K. Penkman, "Amino Acid Racemization Dating of Marine Shells: A Mound of Possibilities," *Quaternary International* 239, no. 1 (2011): 114–124, <https://doi.org/10.1016/j.quaint.2010.05.029>.
106. K. E. Penkman, D. S. Kaufman, D. Maddy, and M. J. Collins, "Closed-System Behaviour of the Intra-Crystalline Fraction of Amino Acids in Mollusc Shells," *Quaternary Geochronology* 3, no. 1–2 (2008): 2–25, <https://doi.org/10.1016/j.quageo.2007.07.001>.
107. E. Prastyo, E. I. Auerkari, A. W. Suhartono, R. S. Pasaribu, A. G. A. Putra, and P. Auerkari, "Performance of Forensic Age Estimation by Aspartic Acid Racemization and DNA Methylation: A Systematic Review," *F1000Research* 13 (2024): 1368, <https://doi.org/10.12688/f1000research.147348.1>.
108. S. C. Zapico, and D. H. Ubelaker, "Application of Aspartic Acid Racemization for Age Estimation in a Spanish Sample," *Biology (Basel)* 11, no. 6 (2022): 856, <https://doi.org/10.3390/biology11060856>.
109. B. Norton-Baker, P. Mehrabi, A. O. Kwok, et al., "Deamidation of the Human Eye Lens Protein γ S-Crystallin Accelerates Oxidative Aging," *Structure* 30, no. 5 (2022): 763–776.e4, <https://doi.org/10.1016/j.str.2022.03.002>.
110. R. Roychoudhuri, "Mammalian D-Cysteine: A New Addition to the Growing Family of Biologically Relevant D-Amino Acids," *Chirality* 35, no. 9 (2023): 535–539, <https://doi.org/10.1002/chir.23555>.
111. E. R. Semenza, M. M. Harraz, E. Abramson, et al., "D-Cysteine Is an Endogenous Regulator of Neural Progenitor Cell Dynamics in the Mammalian Brain," *Proceedings of the National Academy of Sciences of the United States of America* 118, no. 39 (2021): e2110610118, <https://doi.org/10.1073/pnas.2110610118>.
112. C. E. Paulsen and K. S. Carroll, "Cysteine-Mediated Redox Signaling: Chemistry, Biology, and Tools for Discovery," *Chemical Reviews* 113, no. 7 (2013): 4633–4679, <https://doi.org/10.1021/cr300163e>.
113. S. Toxvaerd, "Origin of Homochirality in Peptides: The First Milestone at the Origin of Life," *Bio Systems* 253 (2025): 105479, <https://doi.org/10.1016/j.biosystems.2025.105479>.
114. J. L. Bada, "Racemization of Amino Acids," in *Chemistry and Biochemistry of the Amino Acids*, ed. G. C. Barrett (Springer, 1985), 399–414.
115. J. L. Bada, "Kinetics of Racemization of Amino Acids as a Function of pH," *Journal of the American Chemical Society* 94, no. 4 (1972): 1371–1373, <https://doi.org/10.1021/ja00759a064>.
116. M. Pillalamarri, R. Manyam, S. Pasupuleti, S. Birajdar, and S. T. Akula, "Biochemical Analyses for Dental Age Estimation: A Review," *Egyptian Journal of Forensic Sciences* 12, no. 1 (2022): 2, <https://doi.org/10.1186/s41935-021-00260-4>.
117. T. Monum, C. Jaikang, A. Sinthubua, S. Prasitwattanaseree, and P. Mahakkanukrauh, "Age Estimation Using Aspartic Amino Acid Racemization from a Femur," *The Australian Journal of Forensic Sciences* 51, no. 4 (2019): 417–425, <https://doi.org/10.1080/00450618.2017.1391330>.
118. P. N. McFadden and S. Clarke, "Methylation at D-Aspartyl Residues in Erythrocytes: Possible Step in the Repair of Aged Membrane Proteins," *Proceedings of the National Academy of Sciences* 79, no. 8 (1982): 2460–2464, <https://doi.org/10.1073/pnas.79.8.2460>.

119. A. P. Wingo, Y. Liu, E. S. Gerasimov, et al., "Integrating Human Brain Proteomes with Genome-Wide Association Data Implicates New Proteins in Alzheimer's Disease Pathogenesis," *Nature Genetics* 53, no. 2 (2021): 143–146, <https://doi.org/10.1038/s41588-020-00773-z>.
120. Y. H. Wang, P. P. Luo, A. Y. Geng, et al., "Identification of Highly Reliable Risk Genes for Alzheimer's Disease through Joint-Tissue Integrative Analysis," *Frontiers in Aging Neuroscience* 15 (2023): 1183119, <https://doi.org/10.3389/fnagi.2023.1183119>.

Biographies



Al Musaimi is a lecturer in the School of Pharmacy at Newcastle University, vice president at Orthogonal Peptides Limited, and an honorary research fellow at Imperial College London. His research specialises in advancing peptide synthesis, green chemistry and cutting-edge purification methodologies. With over 55 peer-reviewed publications and four patents to his name, his innovations have driven the creation of Orthogonal Peptides, a spin-out company focused on pioneering first-in-class peptide motifs (WO2024261319A1). Al Musaimi, is a recipient of prestigious grants such as an EPSRC Adventurous Manufacturing Award and Imperial College London's Deep Technology Prime, was recently named the winner of the Royal Society of Chemistry's Emerging Technologies Competition in the Change Makers category (2024). This accolade underscores his groundbreaking contributions to transformative pharmaceutical technologies. Additionally, he was honoured as a winner in the Unsung Heroes - Advancement of Knowledge category by C-DICE, the Centre for Postdoctoral Development in Infrastructure, Cities, and Energy.