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Review article

Insights into mechanism of ionic liquids for protein stability: Future implications for neurodegeneration treatment

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ABSTRACT

Neurodegenerative diseases are characterized by a gradual loss of neurons, cellular dysfunction, loss of intricate synaptic networks and brain damage, which are going to be the second leading cause of death in future. These proteinopathies are marked by abnormal amyloid fibril deposition, aberrant aggregation of misfolded proteins via polymerization, where protein aggregates serve as key pathological hallmarks in Alzheimer's, Parkinson's, and multiple system atrophy disorders. These toxic aggregates accumulate in the brain and disrupt neuronal function, targeting motor neurons, spinal cord, and ultimately leading to respiratory failure and death. As population ages, the prevalence of these neuronal disorders rises significantly, emphasizing to approach effective treatment for risk reduction. In the pursuit of developing effective anti-amyloidogenesis therapeutic agents, ionic liquids (ILs) continue to receive the least attention. ILs have emerged as promising substitute for conventional solvents, owing to their unique physicochemical properties that facilitate protein refolding, mitigate denaturation, amyloidogenesis, and prevent aggregation. This review critically addresses intricate IL-protein interactions, dictated by anions-cations composition of ILs, their polarity, hydrophobicity, kosmotropicity, chaotropicity, amphiphillicity, and network, which modulate protein behavior and support structural and functional integrity. This article also underscores the need for precision in IL selection, ensuring their properties align with the desired structural outcome. We showcase ILs as a promising therapeutic avenue for neurodegenerative diseases, demonstrating their potential to modulate pathological protein aggregation and enhance protein homeostasis. Lastly, this review of outstanding research works, account for current lacunae that will guide future perspectives for the rational designing of IL for protein stabilization and offers new strategies for addressing underlying mechanism of ageing disorders.

1. Introduction

Neurodegenerative diseases (ND) are characterized by progressive damage to neurons and the nervous system, leading to loss of coordination, strength, cognitive and motor decline in the brain; and premature death (Gao and Hong, 2008; Gadhave et al., 2024). These diseases have a chronic impact on global health. With rise in population and increase in life expectancy worldwide, ageing has become the primary risk factor for neural disorders susceptibility, and its prevalence increases with an increase in age (Vellingiri, 2024; Alrouji et al., 2024). To adopt a stable native state, proteins often pass through several intermediate states. In these sequential events protein get misfolded, which ultimately leads to their physical aggregation and contributes to the pathology of the above mentioned diseases (Holbrook et al., 2021; Wu et al., 2024). The proteinaceous deposits in neuronal tissues are primarily the hallmark of neurodegenerative diseases, causing incurable non-neuropathic systemic amyloidosis as represented in the Fig. 1 (Bashir et al., 2024). Early cognitive decline in patients having Alzheimer's show intracellular deposits composed of hyper-phosphorylated tau proteins in temporal and parietal brain regions in the form of neurofibrillary tangles, α-synuclein protein in Parkinson's, TAR DNA-binding protein-43 (TDP-43) in amyotrophic lateral sclerosis and amyloid-β peptides that are deposited as extracellular senile plaques, which cause dementia (Rawat et al., 2022; Ribarič, 2023). The accumulation of prion proteins cause fatal Creutzfeldt-Jakob disease and results in neuropathological spongiform alterations in the brain, where nearly 70% patients die within a year (Sitammagari and Masood, 2024; Jones and Mead, 2020). ND includes a wide range of symptoms such as

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significant impairment of judgement; loss of focus, memory and intellectuality; gradual destruction of brain neurons; and impairment of movements (Anon, 2025a; Emmady et al., 2022). With the anticipated rise in the population growth, aging and life expectancy, the global prevalence of neurodegenerative disorders is continuously increasing, also people affected with dementia is expected to rise globally to 152.8 million cases in 2050 (Nichols et al., 2022, 2019). Emerging studies highlight the pressing need for multifaceted strategies and the development of effective novel therapeutics to address underlying brain degenerative process and decelerate the anticipated global rise of aging disorders (Livingston et al., 2020; Khan et al., 2023).

1.1. Molecular orchestration of protein folding, aggregation and limitations of recent therapeutic approaches

Protein folding is driven by the encoded primary amino acid sequence and governed by intricate physiochemical interactions including, side-chain interactions, hydrogen bonding, solvent effects and hydrophobic clustering (Bellissent-Funel et al., 2016; Lopez and Mohiuddin, 2024). These forces guide the assembly of secondary and tertiary structures, which are stabilized by hydrogen bonds and van der Waals forces (Stollar and Smith, 2020). Despite large enthalpy (ΔH) and entropic (TΔS) disparities, optimal surface contacts favour the formation of native conformations (Heinz and Grubmüller, 2021; Zhou and Pang, 2018; Newberry and Raines, 2019). The folding paradigm adheres to the Anfinsen's principle, whereby a denatured protein can spontaneously refold upon removal of denaturants (Abaskharon and Gai, 2016). Molecular chaperones, notably the heat shock proteins (Hsp70, Hsp60, Hsp90), mitigates misfolding and aggregation via weak hydrophobic and electrostatic interactions, preventing premature misfolding (Hu et al., 2022). Protein aggregation arises from intrinsic folding disruptions driven by self-association of unfolded protein with exposed hydrophobic surfaces, intermediates like molten globule, genetic mutations, translational errors, and chaperone failure. Extrinsic environmental factors like proteotoxic stress, pH imbalance, temperature and molecular crowding may further enhance the formation of aggregates (Kikis, 2019; Verma et al., 2021; Chen et al., 2023). The seeding-nucleation model describes that misfolded species with exposed reactive termini act as nucleation points, which drive oligomer formation through rapid elongation, non-native cross-β stabilization via hydrogen bonding and hydrophobic interactions (Poothong et al., 2021; Sweeney et al., 2017). Alternative downhill polymerization describes amyloidogenesis through protofibril self-assembly and annular aggregate formation (Chen et al., 2020; Arya et al., 2015). Aggregation also presents a formidable challenge in pharmaceutical formulations, comprising protein therapeutics by inducing structural and functional loss (Akbarian and Chen, 2022; Shi and McHugh, 2023).

Preferential cation binding modulates aggregation pathways by influencing ionic strength, non-covalent interactions, structural modifications or site-directed mutagenesis, also excipients like sugars, polyols, amino acids, salts, surfactants, alter environmental properties (Singla and Bhattacharya, 2024, 2022; Jain et al., 2021). Pharmaceutical drugs that were based to target oxidative stress or neuro-inflammation, faced difficulties in drug delivery. To overcome this limitation, extracellular vesicles which were cell-derived or artificially generated have emerged to treat NDs, but their biogenesis was complex and exhibit extensive diversity in their conformation, composition and origin (Nuzzo et al., 2024). Another problem lies in the fact that most currently approved drug treatments are symptom-based, treating only psychological and behavioural symptoms rather than completely arresting the disease progression (Passeri et al., 2022). Even though nanotechnology appears as a good approach in therapeutics, but their toxicity is still a major challenge in nanomedicine (Harilal et al., 2019; Prabha et al., 2024). Immunotherapy are largely explored with monoclonal antibodies for anti-amyloid and pathological forms of 'tau', where the targeted amyloid plaques reduction was noticed but only at the early-stage, and also had adverse effects which limit their therapeutic value (Chundu et al., 2025). Antisense oligonucleotides-based therapy (short oligonucleotides that specifically binds to RNA and modify protein expressions) was studied to treat, SOD1 associated ALS and ACL6B-associated neurodevelopmental disorders. This application faced restricted delivery in brain region and also requires a deep knowledge in order to modify the protein expression (Lauffer et al., 2024). In CRISPR-based gene therapy for CNS, delivery of CRISPR cargos faced challenges (Kantor et al., 2025). Despite significant advances, the associated limitations highlight the need for alternative approaches, wherein novel systems such as ionic liquids have emerged as potent protein stabilizers, preventing misfolding and conferring thermal resilience, making them highly promising for biotechnological applications (Veríssimo et al., 2024; Uniyal et al., 2023).

1.2. Chemistry and types of ionic liquids and their modes of protein interaction

Ionic liquids (ILs) are salts entirely composed of paired large organic cations and organic/ inorganic anions, typically exhibiting a melting

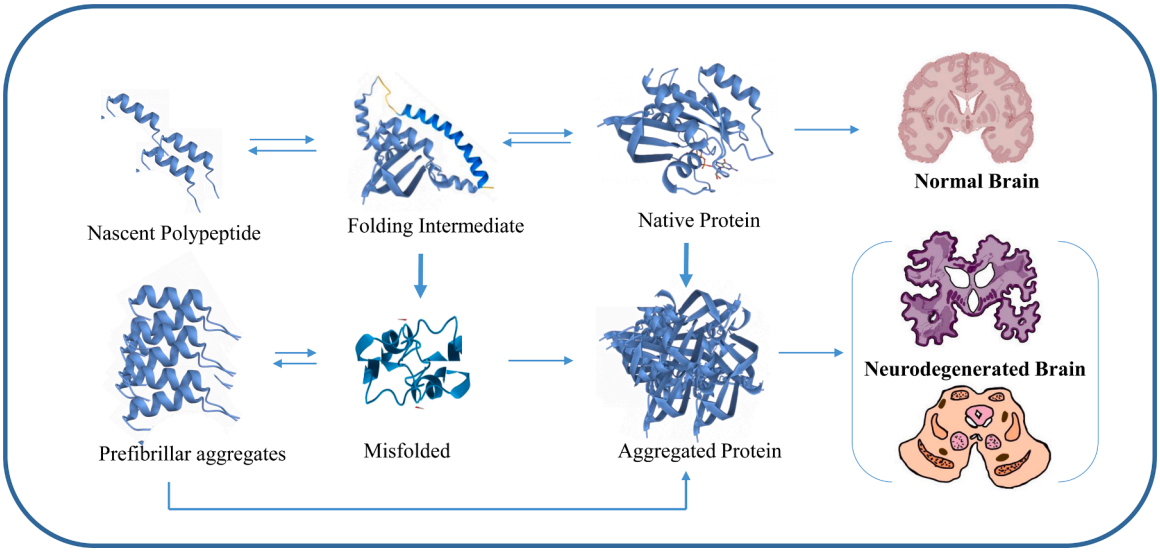

Fig. 1. Various steps of protein folding and possible pathways leading to neurodegeneration.

point below 100°C due to the presence of bulky and asymmetrical structure of ions (Kumar et al., 2024; Bhat et al., 2022). These cations are typically large and asymmetric, and in combination with diverse anions, they disrupt the crystalline lattice formation, resulting in low lattice energy and consequently a lower melting point (Sharma et al., 2024). They are molten salts exhibiting very low vapour pressure at room temperature, high ionic conductivity, self-assembly behaviour and low viscosity (Raouf Bhat et al., 2024), all these properties are summarized through Fig. 2**.** ILs offer a greater versatility as solvents or electrolytes because of their organic composition, which can be tailored by the pairing of ions together in different combinations (Anderson and Clark, 2018). Appropriately designed ILs serve as tunable and biocompatible solvents with promising applications in protein stabilization, as artificial chaperons, therapeutics and in amyloidogenesis research studies (Singh et al., 2017; Reslan and Kayser, 2018). The interfacial properties of ILs are deeply rooted by forces like, strong electrostatic forces; anisotropic and specific forces like dipole-dipole attraction and H-bonding; weak, isotropic interaction like van-der Walls and solvophobic forces (Fumino et al., 2009, 2011).

Depending on whether the base is quaternized by a proton (H^+) or an alkyl group (-R), ILs are categorised as protic ionic liquids (PILs) and aprotic ionic liquids (APILs) (Tien and Kayser, 2024). PILs are formed by proton transfer from a Bronsted acid (AH) to a Bronsted base (B), which produces a cation labile proton that can be donated or exchanged, whereas APILs possess no dissociable hydrogen atoms and are composed of cations without the labile protons. Charge balance of APILs is typically achieved through anion exchange, which takes place via any groups having higher complexity than a proton; such as alkyl or aryl chains, sulphonyl or carbonyl moieties and heterocyclic structures (Alreshidi et al., 2025). The respective physical properties of both types of ILs allow them to protonate or deprotonate in the aqueous media (Markusson et al., 2007; Angell et al., 2007). PILs have stronger Coulomb interactions and offer higher hydrogen bonding due to the proton-donating ability of the cation in comparison to their aprotic counterparts. They resemble water more closely and are inherently volatile in nature (Shukla and Mikkola, 2020). On the other hand, APILs exhibit higher melting temperature (Tm) and lower conductivity than their PIL counterparts. Owing to their smaller size of ions, high ionic conductivity and stability, they are widely used in electrochemical applications (Markusson et al., 2007). Zwitterionic ILs contain both cationic and anionic groups that are covalently linked within the same molecule, resulting in a neutral species that minimizes disruption of α-helix and β-sheets in proteins and so stabilizes their core structures (Hayes et al., 2015). Surface-active ionic liquids (SAILs) are another subgroup of ILs classified by ILs having long alkyl chain length substituents that exhibit surface activity and low partial charges. They are known for self-assembly into micelles, vesicles or other nano-scale aggregates, when introduced into the aqueous solutions. Recent research have explored SAILs for drug delivery applications while achieving sustained release of drugs (Zhen et al., 2024). Another type of ionic liquid is the dicationic or dianionic IL paired with the appropriate counterions, which have higher melting points and improved electrostatic energy, than their mono-cationic analogue. This property contributes to enhance hydrogen bonding with protein surfaces, specifically in bends and turns (Kuhn et al., 2020). Task-specific ILs are tailored accordingly with special functional groups, offers high selectivity, tunable hydrophobicity or hydrophilicity and they are able to mimic any ligand, stabilizing protein structure but with the less toxicity and better bioavailability (Bedrov et al., 2019; De Jesus and De Araújo Andrade, 2020). Numerous types of ILs have been thoroughly examined in recent studies, they usually contains a bulky cation like pyridinium, pyrrolidinium, cholinium, imidazolium, or its derivatives, and paired with a halide anion, such as tetrafluoroborate, hexafluorophosphate or another similar anions (Reslan and Kayser, 2018; Tarannum et al., 2022). In short, halide anions like [Cl^-], [Br^-] are strong hydrogen bond acceptors which stabilizes polar groups of protein and enhance their solubility, [BF_4^-] and [PF_6^-] contribute in increasing hydrophobicity of ILs which reduces the water solubility and hence stabilizes proteins' hydrophobic cores (Tuzimski and Petruczynik, 2024; Jamil et al., 2024). Furthermore, [NTf_2] imparts decreased viscosity of ILs upon hydrolysis; whereas [SCN^-], one of the most chaotropic ions in the Hofmeister series, disrupt the water structure. Amino acid-based anions like Asp and Ser, because of their side-chain chemistry mimic the biological interactions (McGrogan et al., 2024; Sarangi et al., 2023). Amino acid-based ILs represent a novel class of ILs that uniquely combine physiochemical properties of ILs with the inrinsic biocompatibility and chirality of

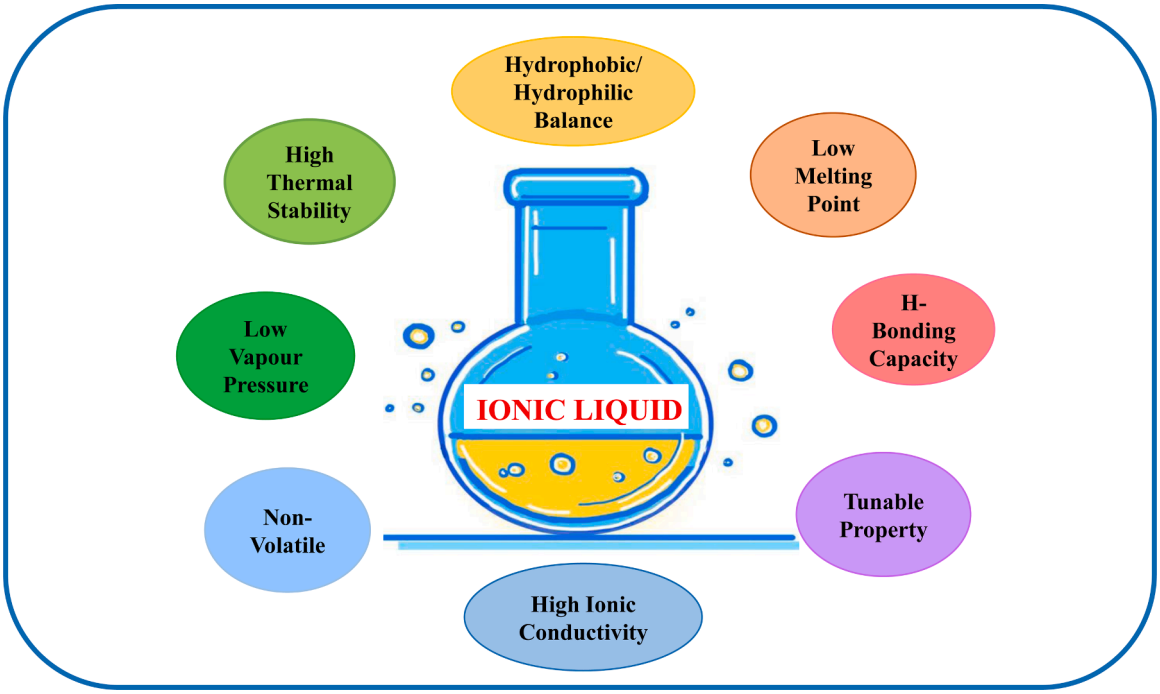

Fig. 2. Overview of properties of ionic liquids which makes them better alternative against conventional solvents.

amino acids. This synergy, offers control over stereospecific interactions, bonding behaviours, and also renders more green synthesis, which makes them eco-friendly and less toxic than traditional ILs (Le Donne and Bodo, 2021). Some commonly used cations and anions of IL compositions with structure are represented in Fig. 3. In protein systems, electrostatic forces between ion pairs and charged protein residues like lysine and aspartate, stabilize the loop regions of protein structure by increasing bend or turn contents (Silva et al., 2020; Duart et al., 2022). Similarly, in imidazolium-based ILs, H-bond donor cation and acceptor anion reduce the IL viscosity, increase conductivity, and further interaction with the polar groups enhances backbone stabilization (Elstone et al., 2023). ILs with longer alkyl chain lengths increase van der Waals interaction, forming non-polar domains, enhance hydrophobicity and reduce water solubility, which results in reduction of solvent-accessible surafce area and so stabilize the hydrophobic core of protein (Shukla et al., 2018; Pereira et al., 2024). Through the solvation effect of ILs, by forming a solvation shell around charged or polar residues, they stabilize the compact structure of protein and also resist the disruption of α-helix and β-sheet dynamics (Patel et al., 2022). ILs are used thorough different approaches like, as co-solvents, adjuvants, in conjugations as phase-forming components or as solvents in pure form (Basu et al., 2018). Additionally, ILs can dissolve mature fibrils, modulate protein folding and restore the biochemical function of proteins (Takekiyo and Yoshimura, 2018; Rawat and Bohidar, 2015). They display a higher viscosity which directly influence the dynamics of protein folding and aggregation kinetics (Szilagyi et al., 2014). Higher viscosity reduces the molecular diffusion of proteins and solvent molecules; also, water mobility is restricted. This leads to the stabilized hydration layer and a slower folding/unfolding rate, which could control aggregation kinetics. Controlled viscosity of solution can slow down protein-protein collision frequency, which will eventually supress the aggregation progression (Szilagyi et al., 2014; Papaneophytou et al., 2014).

1.3. ILs opening new horizon in enhancing protein refolding efficiency and aggregation dissociation

Traditional methods to balance correct protein folding have relied on, employing chaotropic agents, molecular chaperones, and chemical stabilizers but they often deliver limited stability, require higher concentration and are also found to act non-specifically (Yamaguchi et al., 2013; Yamamoto et al., 2017). To overcome this inefficiency, breakthrough of ILs came which were capable of modulating protein stability, reversing aggregation, and enhancing refolding yields through specific ion-protein interactions (Fujita, 2019).

During the process of refolding, the native structure of protein competes with the aggregation formation which often leads to irreversible inactivation. Due to this kinetic competition, effective refolding methods and additives must be developed having higher correct folding yield. Recent findings have explored ILs as biocompatible solvents with potential to refold to proteins and counteract denatured proteins (Kumar et al., 2019). The presence of ILs revoked the effect of the crowding agent dextran on HSA and showed positive results for protein refolding with choline bitartrate ([Ch][Bi]) and choline dihydrogen phosphate ([Ch][dhp]) (Bhakuni et al., 2021). Upon urea-induced BSA and HSA denaturation, application of ILs 1-ethyl-3-methyl imidazolium ethyl chloride ([EMIM][Cl]) and 1-ethyl-3-methyl imidazolium ethyl sulphate ([EMIM][ESO_4]), promoted tryptophan burial inside the core with increasing structural compactness and resulted in steering proteins back to their native conformation (Sindhu et al., 2020a). Amount of water in mixtures modulates the solvent characteristics of ILs, from conventional electrolyte solution-like behaviour to the molten salt-like behaviour (Ma et al., 2018). This phase transition enables concentration dependent protein stability modulation, however, this effect also follows more intricate mechanism. Studies on chicken egg white lysozyme in the presence of 1-butyl-3-methylimidazolium nitrate ([BMIM][NO_3]) revealed that higher concentration of IL promoted refolding of protein through fine-tuned solvation effects (Takekiyo et al., 2012).

A comparative study on IgG, β-lactoglobulin and BSA, showed that 1-hexyl-3-methylimidazolium chloride ([HMIM][Cl]) reduced the aggregate size from 800 nm to ~120 nm. It mimicked the traditional heparin's effects, although complete monomeric recovery was not achieved (Rawat and Bohidar, 2015). Table 1 provides the compilation of ionic liquids and proteins examined, with protein sizes measured in buffer and ILs. Choline based ILs like [Ch][Bi], choline acetate [Ch][OAc] and choline chloride [Ch][Cl] are found to be the most potent refolding agent and to re-establish the aromatic residues orientation in proteins (Bhakuni et al., 2021; Sindhu et al., 2020b). Researchers also turned to the hydrated ILs, which are prepared with controlled water content to fine-tune the protein interactions. On this principle, hydrated [Ch][dhp] showed significant dissolution and refolding of aggregated concanavaline A (Fujita et al., 2019).

In thermally aggregated cellulase 6 A from *Coprinopsis cinerea* (CcCel6A), phosphonium and ammonium-based hydrated ILs demonstrated enhanced solubilization and enzymatic activity recovery of aggregated protein (Fujita et al., 2023). On the same protein, [Ch][dhp] proved to be most effective in restoring both structural integrity and

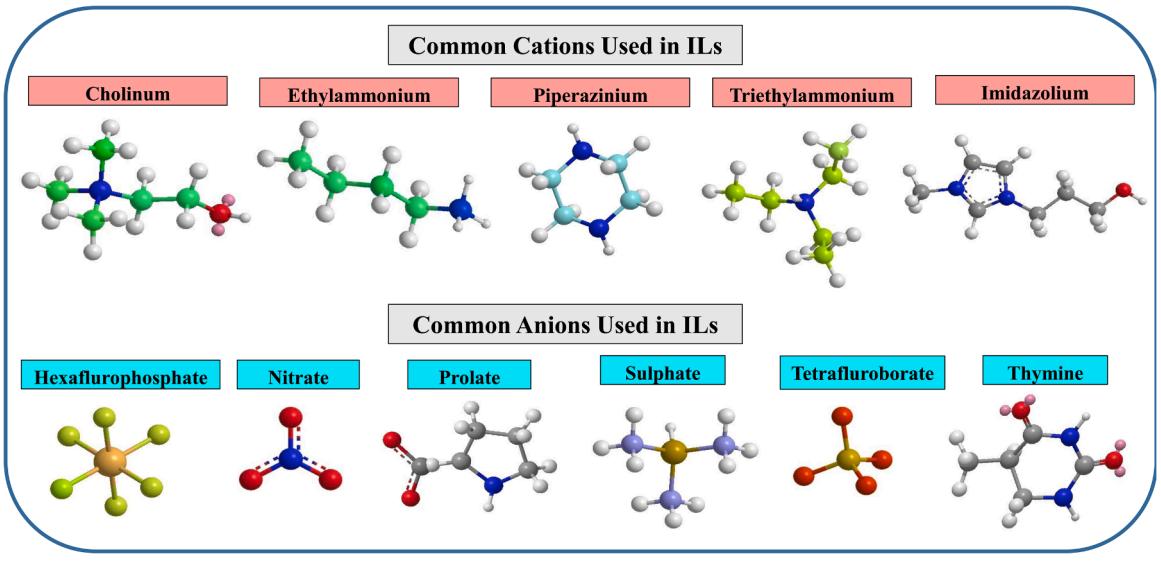

Fig. 3. Molecular structures of some of the most promising cations and anions of ionic liquids studied for both solubilization and stabilization of specific proteins.

Table 1
Summary of the effects of ionic liquids on protein size; in buffer versus IL conditions.

Sr. no.	Protein	Protein size (R_h) in buffer	Ionic Liquid	Protein size in IL	Ref.
1.	Hemoglobin	6.4 nm	1-allyl-3-methylimidazolium chloride ([AMIM][Cl])	~ 6.4 nm	(Jha and Venkatesu, 2016)
2.	β-casein micelles	13 nm	1-dodecyl-3-methylimidazolium bromide ([DoMIM][Br])	6 nm	(Liu et al., 2013)
3.	BSA	3.4 nm	1-octyl-3-methyl imidazolium chloride ([OMIM][Cl])	5 ± 1 nm	(Rawat and Bohidar, 2012)
4.	Stem bromelain	6.22 nm	1-butyl-3-methylimidazolium hydrogen sulphate ([BMIM][HSO_4])	10 mM IL = > 5.2 nm 50 mM IL = > 7.9 nm	(Kumar et al., 2020)
5.	HSA	d_H =7.8 nm	1-ethyl-3-methyl imidazolium ethyl chloride ([EMIM][Cl]) and 1-ethyl-3-methyl imidazolium ethyl sulphate ([EMIM][ESO_4])	7.8 nm	(Sindhu et al., 2020a)
6.	Lysozyme	Native - 4.12 nm Fibril - 47.17 nm	Choline acetate ([Ch][OAc]) Choline dihydrogen phosphate ([Ch][dhp])	25 mM = > 8.9 nm 50 mM = > 12.2 nm 75 mM = > 9.85 nm	(Sindhu et al., 2022)
7.	β-lactoglobulin	d_H ~ 5.5 nm	Choline acetate ([Ch][OAc]) and Choline dihydrogen phosphate ([Ch][dhp])	~ 5.5 nm ~ 4 nm	(Sindhu et al., 2019)
8.	Avidin	R_g ~ 2.83 nm	Choline Aspartate ([Ch][Asp]) Choline hexanoate ([Ch][Hex]) Choline methionine [Ch][Met]	1.57 nm 1.56 nm 1.6 nm	(Shmool et al., 2021a)
9.	BSA	Native d_H = 8.9 ± 0.5 nm, with SDS d_H = 15.2 ± 0.5 nm	Tetrabutylammonium chloride (TBAC)	~ 9 nm	(Sahoo et al., 2024)
10.	Lysozyme	Fibrils = 450 nm	1-butyl-3-methylimdazolium bromide ([BMIM][Br])	50 mM IL = > ~ 200 nm 100 mM IL = > ~100 nm	(Kushwaha and Prabhu, 2024)

long-term stability, where the hydration level of IL played a pivotal role in optimizing refolding efficiency (Fujita et al., 2016a). Unlike conventional denaturants, EMIM-based ILs selectively interacted with the aromatic amino acids of recombinant plasminogen activator (rPA), enhancing its solubility without disrupting the protein backbone. This unique interaction suggested that, ILs stabilize proteins not only by solubilization but also by influencing side-chain interactions (Tischer et al., 2014). Furthermore, on horseradish peroxidase (HRP), a metalloenzyme reliant on Ca^{2+} and ferrous ion-containing heme group (hemin) based cofactors, here, adding [EMIM][Cl] to the refolding buffer not only increased the refolding efficiency but also enhanced the enzymatic activity, hinting at a synergy between ILs and essential metal cofactors (Bae et al., 2016). Further, *Yamamoto et al.* expanded repertoire of chaotropic IL, N-alkyl-N-methylpyrrolidinium chlorides and N-alkylpyridinium chlorides, where [EPyrr][Cl], [BPyrr][Cl], [BPy][Cl] being less hydrophobic, improved the refolding and prevented

Table 2
Schematic information of the influence of pyridinium and pyrrolidinium-based ionic liquids (with effective concentration) on various proteins defining stability (thermal, structural, refolding and fibrillation inhibition).

Sr. no.	Pyridinium - and pyrrolidinium-based ILs	Effective concentration of ILs	Studied Protein	Stability	Results	Ref.
1.	1-butyl-1-methylpyrrolidinium bromide ([BMPyrr][Br]), 1-butyl-4-methylpyridinium bromide ([BMPy][Br]) and 1-butyl-1-methylpiperidinium bromide ([BMPip][Br])	200 mM- 500 mM	Hen-egg white lysozyme (HEWL)	↑	IL slowed down the fibrillization of HEWL	(Kushwaha and Prabhu, 2024)
2.	N-methyl-N-butylpyrrolidinium hydrogen phosphate ([BMPyrr][HPO_4])	3 mM	Cytochrome C	↑	Thermal stability and long-term stability was achieved	(Fujita et al., 2007)
3.	N-alkylpyridinium chlorides ([EPyrr][Cl], [BPyrr][Cl]) and N-butyl-N-methylpyrrolidinium chlorides ([BMPyrr][Cl])	500 mM	Lysozyme	↑	Enhanced refolding and prevention of aggregation	(Yamamoto et al., 2011)
4.	1,4 methyl butyl pyrrolidinium dihydrogen phosphate ([MBPyrr][H_2PO_4])	3 mM	Cytochrome C	↑	IL enhanced stability and utility of proteins	(Fujita et al., 2006)
5.	N-butyl-N-methylpyrrolidinium dicyanamide ([BMPyrr][DCA])	80–90 wt%	Cytochrome C	↑	IL provided retention of secondary structure at extreme temperature	(Fujita et al., 2005)
6.	N-methyl-N-(ethoxycarbonylmethyl)pyrrolidinium bromide ([MECMPyrr][Br])	2–5 mM	BSA	↑	Exposed Trp towards hydrophobic environment	(Wang et al., 2012)
7.	1-butyl-1-methyl-2-oxopyrrolidinium bromide ([BMOxPyrr][Br])	4.15–29.20 mM	BSA	↓	increase in β-turn and β-antiparallel structure and	(Kumari et al., 2014a)
		0.0167–0.1044 M	HSA		decrease in α-helix structure	(Kumari et al., 2014b)
8.	2′,3′-epoxy-N-methyl-2-oxopyrrolidinium salicylate ([EMpyr][SAL])	0–2.7 μM	BSA	↓	IL induced conformational de-stability and denaturation by increase in hydrophobicity	(Arumugam et al., 2019)

aggregation of lysozyme, while more hydrophobic ILs completely suppressed the misfolding at lower concentrations (Yamamoto et al., 2011). Table 2 presents a comprehensive summary of various other pyridinium and pyrrolidinium-based ILs enhancing secondary structure stability and aggregation resistance.

Regain of native-like conformation with ammonium-based ILs, showed that hydrophobic cations stabilized the protein interactions, while the anionic component $[NTf_2]^-$ preserved the electrostatic integrity of the secondary structure, cementing the role of ILs as sophisticated molecular chaperones (Bisht et al., 2016). Enhanced oxidative refolding efficiency in lysozyme and antibody fragment ScFvOx, with their structural recovery was observed in the presence of IL, N′-substituted N-methylimidazolium chlorides. This positive effect of IL on renaturation of protein by suppressing the aggregation was influenced by the hydrophobicity of N-alkylated and hydroxylated imidazolium chlorides, where terminal hydroxylation improved the compatibility of molecules (Lange et al., 2005). Imidazolium chloride-based IL showed refolding efficiency in heat-aggregated cytochrome c (Cyt c), solubilizing tryptophan. This highlighted the hydrophobic impact of IL, where solubilizing ability increased with increase in the alkyl-chain length of IL (Takekiyo et al., 2019a). While misfolded proteins are troublesome, amyloid aggregates pose an even greater challenge. Researchers explored ILs for dissolving the amyloid fibrils, taking cues from their hydrogen bonding capacity and high protein affinity. Studies with 1-ethyl-3-methylimidazolium acetate [EMIM]OAc and N,N,N,N-tetramethyl guanidium propionate [TMGH]OAc unveiled new insights into cellulose aggregate dissolution and regeneration of proteins, demonstrating how IL's hydrotropy and hydrophobicity control the protein reassembly. This finding established that, the way ILs have dissolved aggregated by breaking hydrogen bond networks is mechanistically similar to how they can potentially disrupt the aggregates in NDs (Hauru et al., 2012). For insulin amyloid fibrils, ILs, 1-butyl-3-methylimidazolium thiocyanate [BMIM][SCN], interacted with Asn/Glu residues, while SCN^- bound to Lys/Arg specifically, offering a mechanistic glimpse into how ILs disrupt aggregation at the molecular level (Takekiyo et al., 2016). ILs, with their ability to fine-tune the hydrophobic, electrostatic, and hydrogen-bonding interactions, have emerged as a powerful alternative to traditional denaturants and stabilizers, thereby offering a good potential against neurodegenerative protein aggregates (Buchfink et al., 2010).

1.4. Ionic liquids as multifaceted regulators of protein structure, stability and functions

- ***conformational rigidity and backbone stabilization***

  ILs have emerged as strategic modulators of protein structure, influencing conformational integrity, altering intermolecular interactions and enhancing functional stability through a variety of mechanisms reported across diverse protein systems (Takekiyo and Yoshimura, 2018; Veríssimo et al., 2022). Investigation on Cyt c have demonstrated that choline threonine ([Ch][Thr]) and threonine nitrate ([Thr][NO_3]), enhanced the structural resilience of protein via preferential binding at terminal helices and loop regions. Here, the free energy values for binding between the protein and ILs ranged from 10 to 14 kJ/mol (Kumar Sahoo et al., 2020). Imidazolium-based IL (1-Cn-3-methylimidazolium chloride) stabilized the genetically modified super-folded protein (GFP), correlating with the enhanced hydrophobic sequestration of

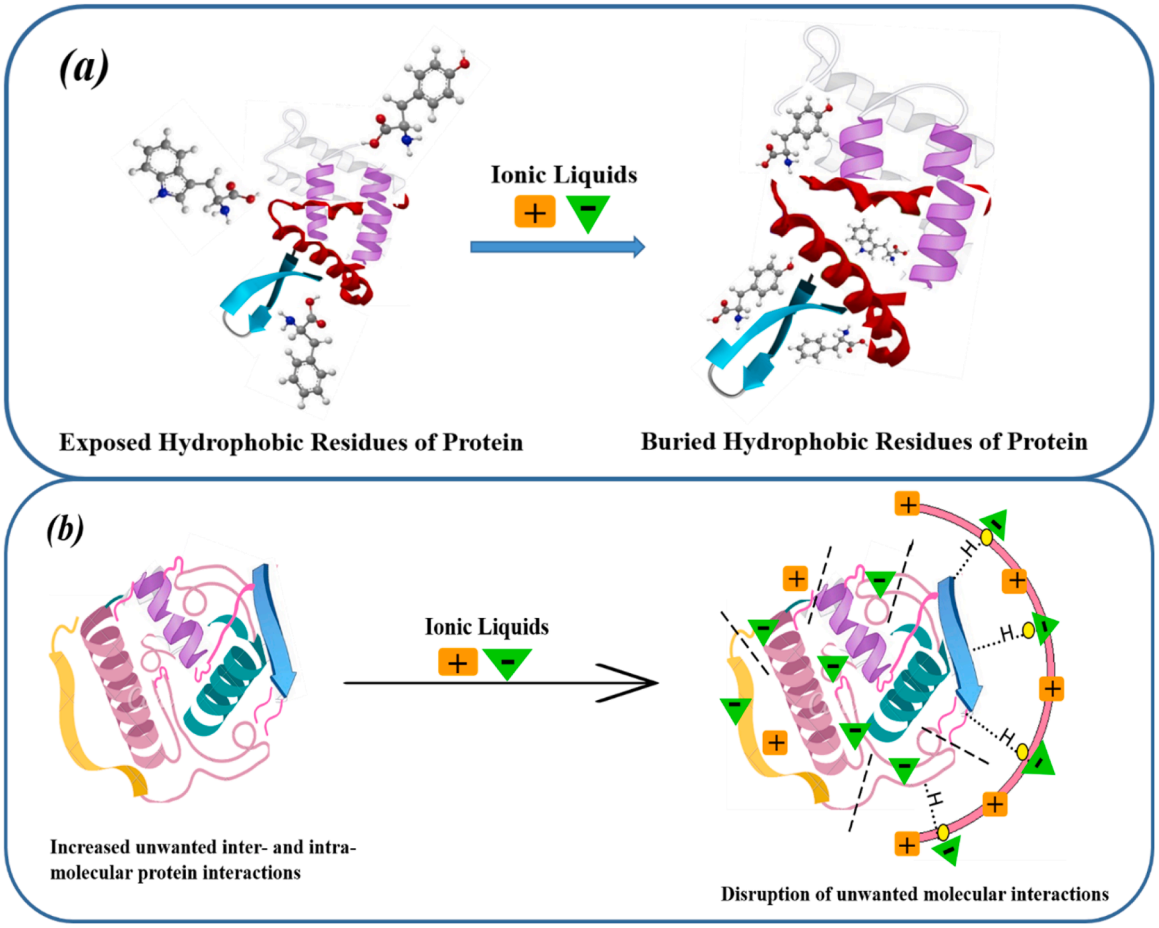

Fig. 4. (a) Schematic presentation of quenching effect occurred in hydrophobic residues of protein residues after addition of ionic liquid **(b)** Ionic liquids anions facilitate solvation interactions through hydrogen bonding and promoting disruption of unwanted molecular interactions.

solvent-exposed nonpolar residues of protein, thereby reducing their conformational flexibility and increasing the structural rigidity (Han et al., 2023a). This quenching effect by ILs is very commonly observed, where burial of hydrophobic residues inside protein core takes place, also illustrated in Fig. 4 **(a)**.

The enzymatic stability of *Candida antarctica* lipase B (CalB) under low water conditions (2 % v/v) has been augmented by 1-ethyl-3--methylimidazolium bis (trifluoromethylsulfonyl) imide ([EMIM][NTf_2]) and butyltrimethylammonium bis (trifluoromethylsulfonyl) imide ([BTMA][NTf_2]). They promoted compact structural integrity and enzyme activity retention at 50°C through hydrogen bonding with polar residues, while shielding the nonpolar regions from solvent interactions (De Diego et al., 2005). ILs exert profound effects on protein folding landscapes, with significant implications in amyloidogenesis for neurodegenerative disorders (Kumar and Venkatesu, 2012). [BMIM][SCN] preferentially binds to the lysine residues in proteins such as Cyt c, myoglobin, lysozyme, and β-lactoglobulin (β-LG), thereby disrupting the intermolecular β-sheet assembly and precluding amyloid aggregation through binding to the key residues (Takekiyo et al., 2015).

ILs have proved to be a better replacement to the conventional excipients for stabilization and long-term storage of proteins (Bashir et al., 2021). [Ch][dhp] mediated stabilization to the conformational integrity of chimeric IgG4 antibodies by restricting Fab domain mobility and to the epidermal growth factor receptor monoclonal antibody (EGFR mAb) (Shmool et al., 2021b; Mazid et al., 2015). Choline proline ([Ch][Pro]) and proline nitrate ([Pro][NO_3]), preferentially bonded through electrostatic and hydrophobic interactions, spanning the inter-helical junctions and solvent accessible loops of Cyt C. In the presence of ILs, protein exhibited thermodynamically stabilized native conformation with estimated binding energies to be in the range of −9 to −17 kJ/mol (Sahoo et al., 2019). Condensed ethylammonium nitrate (EAN) and propylammonium nitrate (PAN) solutions exhibited site-specific electrostatic and hydrophobic interactions with insulin protein, which contributed to the pronounced monomeric insulin stabilization, underscoring their potential as protective excipients (Takekiyo and Yoshimura, 2016). Haemoglobin (Hb), a structurally labile protein under destabilizing conditions, exhibited increased resilience in the presence of choline glycine ([Ch][Gly]) and choline methionine ([Ch][Met]) ILs, through hydrogen bond stabilization of the non-native intermediates (Ahmed et al., 2020; Devi Tulsiyan et al., 2023). Additionally, lower concentration of 1-allyl-3-methylimidazolium chloride ([AMIM][Cl]) also promoted the native protein like stability by altering hydration dynamics (Jha and Venkatesu, 2016). Collectively, these findings established that, ILs can modulate protein structure and reinforce conformational stability through a variety of interaction forces, highlighting their emerging potential as promising candidate for therapeutic applications.

- ***enhancing solubility and aggregate dissolution***

An interesting application of ILs has emerged as a potent solvent to improve protein stability by enhancing their solubilization (Zhao, 2016; Russina et al., 2009). A hydrophobic solute surface can be penetrated more easily by cations or anions that have longer alkyl chains and they also affect physio-chemical properties (Schröder, 2017). For instance, tetramethylguanidium acetate was found to exclusively engage with the hydration layer of lysozyme, leading to the acceleration of water molecule relaxation dynamics that contributed to increase the protein stability. Study shows that acetate competes with internal H-bonds in water and thereby forms stronger hydrogen bonding with water (Pillai et al., 2022). Fig. 4 **(b)** illustrates how IL anions, with high hydrogen bond donor capacity, disrupt the undesirable inter and intramolecular protein interactions. Investigations into alkyl-1,2,4-triazolium trifluroacetates, such as 1-pentyl-3-methyltriazolium trifluroacetate (1-pent3HTTFA) and 1-hxyl-1,2,4-triazolium trifluoroacetate (1-hex3HTTFA), revealed their methanol-like polarity or solvating properties, which effectively modulated the microenvironment of proteins and contributed to enhanced protein stability through solvation effects (Selvam et al., 2024).

The refolding of aggregated recombinant protein CcCel6A from *Escherichia coli* was successfully facilitated by hydrated ILs such as [Ch][dhp], 1-ethyl-3-methylimidazolium tetrafluroborate ([EMIM][BF_4]), 1-ethyl-3-methylimidazolium methylphosphate ([EMIM][$MeO(H)PO_2$]). This direct dissolution of aggregated cellulose was influenced by ion composition of IL and number of water molecules paired with them (Fujita et al., 2016b). *Elgharbawy et al.*, demonstrated that incorporation of imidazolium-based longer alkyl chain length ILs resulted in the enhanced solubility, which improved the activity of enzyme. This effect was explained by the amplification of hydrophobic interactions; since the alkyl chain length increased for the cation, it increased for the anion too (Elgharbawy et al., 2018a).

In-silico investigation further elucidated the peptide conformational transitions between α-helix to the β-sheet in presence of EAN, ethylammonium mesylate (EAM) and triethylammonium mesylate (TEAM), along with the concentration-dependent solvation dynamics. Results established that hydration layer around the peptides increased with the increase in the concentration of IL and was more evident in the helical conformation (Dasari and Mallik, 2020a).

Similarly, imidazolium-based ILs in the presence of water demonstrated a pronounced enhancement in the ionic diffusion, reinforcing the role of hydration dynamics in dictating IL functionality (Schröder et al., 2000). Selective interaction of IL with the hydration shell of proteins, modulating water molecule dynamics, is illustrated in Fig. 5 **(a)**. Additionally, 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]) revealed an electrostatically governed amyloid-β ($A\beta_{42}$) peptide-IL interaction that restricted the water mobility at the interface. This shielding effect mitigated the hydrophobic collapse which led to effectively preventing fibril formation and stabilizing the native peptide conformation (Pal et al., 2023). All these studies establishes that, ILs acting as a co-solvent with water produce a synergistic stabilization effect, by reorganizing the protein-water interface. Under stress conditions theses modulated hydration layers, shields the aggregation prone regions and provide electrostatic balance, resulting in the reduced intermolecular contacts. It is well known that the aggregation rate of amyloid protein α-synuclein increases on reduction of water mobility, which is influenced by water hydration shell (Stephens et al., 2023). Given this, ILs have that potential to directly oppose the dehydration-driven nucleation by reinforcing the hydration shell.

1.5. Ionic liquid mediated modulation of protein environment and aggregation behaviour

- ***pH and solvation dynamics***

The isoelectric point (pI) of the protein and the pH of the solution are well recognised crucial factors that influence protein aggregation (Smirnova et al., 2015). IL addition alters the hydrogen bonding with anions which leads to burying of hydrophobic groups and enhance hydrophobic interactions with desirable salts and stabilize water-protein interface, also form a solvation layer around proteins (Han et al., 2021; Badgujar et al., 2022; Reslan and Kayser, 2018). A study showed that choline-based ILs with dicarboxylate, dihydrogen phosphate, and glutarate anions, preserved the activity and stability of Cyt C, where pH was adjusted using these ILs containing exchangeable acidic protons, [Ch][OH], and carboxylic acids (Bisht et al., 2017). Hydrophobic and electrostatic interactions from ILs contribute to protein stabilization by modulating pH balance (Zhu et al., 2025). Interaction of HSA with 1-butyl-3-methylimidazolium octylsulphate [BMIM][OSU] across pH 3–11.4 revealed that the IL's anionic component enhanced the solubility and structural integrity of protein through pH-dependent effects in the

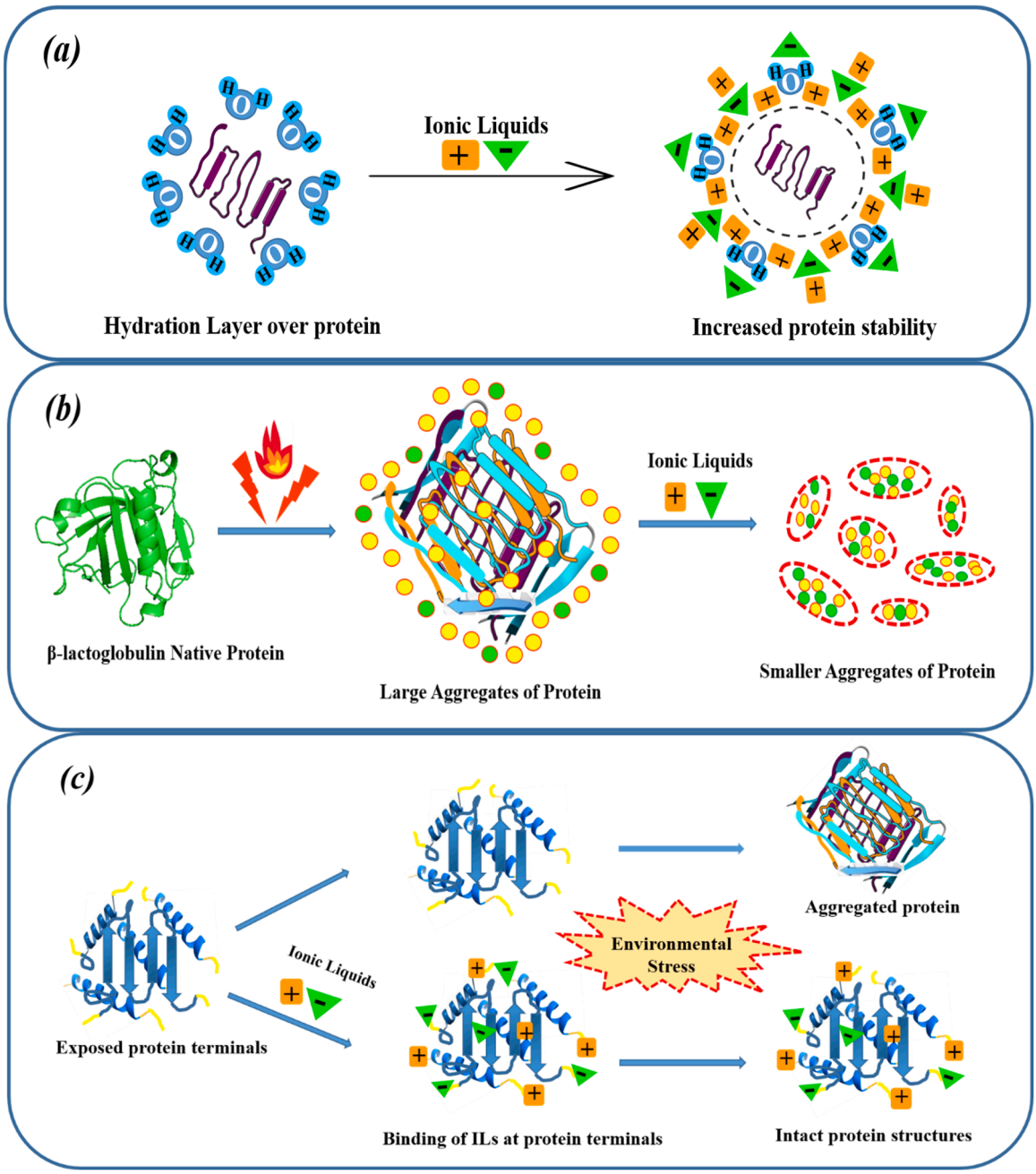

Fig. 5. (a) Mechanistic illustration of hydrophobic ILs forming a protective solvent cage around proteins leading to, accelerating water relaxation dynamics and stabilizing them against denaturation (b) Ionic liquid-mediated disaggregation of larger protein aggregates into smaller and more soluble structures (c) Ionic liquid binding at exposed terminals of protein structure providing them rigidity and protection from aggregation.

aqueous media (Ravikanth Reddy et al., 2023). 1-butyl-3-methylimdazolium bromide ([BMIM][Br]) and 1-decyl-3-methylimdazolium bromide ([DMIM][Br]), showed improvement in HSA stability through hydrogen bonding, electrostatic interactions and π-π stacking (El-Hady et al., 2015). Similarly, super-folder green fluorescent protein (sf-GFP) exhibited enhanced stability and decreased aggregation in the IL-water mixtures, highlighting IL's potential in protein formulation. Mechanism behind this effect was that, ILs established the weak H-bond with sf-GFP at its surface, leading to the least exposure of hydrophobic group from water molecules, which resulted into stabilized water-protein interface and enhanced hydration (Veríssimo et al., 2022). *Burkholderia cepacia* Lipase (BCL) modified with [HOOCEPEG$_{350}$Im]-BCL-[BF_4] showed highest activity at pH 8, attributed to the synergistic effects of the imidazolium cation, PEG functional group and strong chaotropic anion (BF_4) (Xu et al., 2018). Furthermore, lysozyme activity was improved in the presence of [BMIM][BF_4], [BMIM][Cl], [BMIM][Br], and dimetylimidazolium iodide [DMIM][I] across pH 4.30–8.32, attributed to the stabilization of its native conformation. This effect was specifically attributed to the conformational change at Trp62 and Trp108 triggered by the addition of IL and so the optimum pH value for lysozyme activity was increased (Li et al., 2022a). *Candida rugosa* lipase (CRL) modified with [HOOCEPEG$_{350}$Im][H_2PO_4] showed increased activity from pH 6.0–7.0 at 40 °C, in comparison to native CRL. This stabilizing effect is linked to the cationic part of the imidazolium ring and PEG functional group, which enhanced the pH adaptability of protein (Li et al., 2015). The ability of ILs to modulate pH either through direct acid-base buffering or indirect interactions makes them highly versatile in the protein formulations, particularly beneficial in pharmaceutical industry. The

extremes of charged residues are neutralised by ILs around the proteins and it modulates solvation dynamics which maintain the delicate balance between electrostatic and hydrophobic forces and so enhance the protein stability (Piccoli and Martínez, 2024a). Also, ions of ILs shield the charged residue from excessive protonation or deprotonation which stabilizes H-bonds and create protective shells that limit aggregation, where buffer alone fails (Han et al., 2023b). This property of ILs can also be translated to stabilize neuronal proteins, which under the pH stress (induced by pathological conditions) gets oligomerized and fibrils are formed (Tien and Kayser, 2024). ILs through their buffering activity can minimize the charge imbalance and reduce the exposure of aggregation-prone hydrophobic patches and so can potentially preserve the native structure of amyloidogenic proteins such as α-synuclein, tau or Aβ-peptides and can also slow down the pathways linked to ND.

- ***micellar properties and self-aggregation***

Some proteins exhibit micellar properties,i.e., self-assembling into micelle-like structures in the presence of surfactants or other molecules due to their amphiphilic characteristics, which is crucial to maintain protein's structure and function (Mustafai et al., 2023). It protects protein aggregation by folding in a way by sequestering hydrophobic residues internally and exposing hydrophilic residues to the solvent (Duerasch et al., 2020; Rajan and Matsumura, 2023).

The hydrophobic nature of long alkyl chain ionic liquids enhances phase polarity, promoting the formation of aqueous two-phase systems (ATPS) consists of two conjugated immiscible aqueous phases having different density (Grilo et al., 2016; Nie et al., 2022). This role of ILs is extensively studied in colloidal and interfacial sciences, used for protein separation and purification, as they provide biocompatible environment with high water content and mild separation conditions. Additionally, solvation studies with [AMIM][Cl] and 1-butyl-3-methylimidazolium hexaflourophosphate ([BMIM][PF_6]) showed increased critical micellar concentration (CMC) and viscosity, suggesting that higher viscosity leads to lower solvation but promotes spontaneous micellization (El-Dossoki et al., 2022). At higher concentrations, 1-hexyl-3-methylimidazolium dodecylsulphate [HMIM][$C_{12}OSO_3$] (cmc=0.9) and 1-dodecyl-3-methylimidazolium bromide [DoMIM][Br] (cmc=10.2) promoted micelle formation of β-LG protein. Results indicated a shift from monomeric complexes to aggregates, driven by the IL's hydrophobicity and reduced micropolarity. Saturation further led to free micelle formation and fragmentation of larger aggregates of protein into smaller ones (Chabba et al., 2018). Fig. 5 **(b)** clearly illustrates this phenomenon of IL-mediated disaggregation of β-lactoglobulin into more soluble structures.

Co-solvent IL induces electrostatic and hydrogen bonding interactions, influencing charge distribution and facilitating micellization (Sharma et al., 2024; Buettner et al., 2022). Fluorinated ILs (FILs) such as choline ((2-hydroxyethyl) trimethylammonium) dihydrogen phosphate ([Ch][dhp]), 1-ethyl-3-methylimidazolium perfluorobutanesulfonate ([EMIM][Ms]) and choline perfluorobutanesulfonate ([Ch][Ms]) maintained lysozyme's globular folded structure through promotion of micelle assembly and slight occupancy at protein binding cleft, which mediated encapsulation (Alves et al., 2023). 1-dodecyl-3-methylimidazolium chloride ([DoMIM][Cl]) and its derivatives with functionalized amide and ester induced the transitions, which led to the formation of ordered self-assembled BSA structures like spherical, β-sheet rich or long helical fibres, demonstrating an IL-mediated protein refolding into their ordered structures (Singh and Kang, 2015). Imidazolium-based ILs have been reported to modulate protein stability by affecting structural integrity, refolding dynamics, and fibrillation kinetics. Table 3 provides a detailed overview of modulatory effects of imidazolium-based ILs across a range of protein systems. Data indicates that within this class, cations such as EMIM and BMIM combined with anions such as halides or NO_3^- exhibit more pronounced protein stabilizing effects among other ion combinations. A study explored the impact of hydrophobic IL with long alkyl chain length, where it showed that [DoMIM][Br]'s hydrophobic tail formed micelle-like smaller aggregates of β-casein micelles (β-CM) by inducing the collapse of N-terminal, which ultimately enhanced the structural compactness, hydrophobicity and a significant decrease in the polarity of protein environment (Liu et al., 2013). This shows that hydrophobic interactions are very crucial in IL-mediated micellization of protein structure. Furthermore, ILs thermodynamically favoured the spontaneous self-assembly of stable micellar structures, resulted by more negative value of Gibbs free energy and enthalpy of micellization (Anon, 2025b). [DoMIM][Cl] and 1-decyl-methylimidazolium chloride [DMIM][Cl] demonstrated a three-step model of complex formation between the cationic alkyl chain of IL and the hydrophobic sites of protein, where at lower concentration of IL protein begins to self-assemble, while the higher concentration led to the free micelle formation (Raw et al., 2023). It can be concluded that ILs influence protein micellization through multiple molecular interactions, acting as co-solvent, and collectively modulate protein stability. This can prevent the uncontrolled protein clustering that drives neurodegenerative pathology, thereby offering a mechanistic basis for developing new therapeutic strategies.

1.6. Enhancing protein stability and resilience under stress using ionic liquids

- ***mitigation of thermally induced protein denaturation***

Proteins subjected to elevated temperatures undergo destabilization due to the disruption of non-covalent intra-molecular interactions, leading to structural denaturation (Camilloni et al., 2016; Engelking, 2015). Thermo-protective effect of ILs was demonstrated in CALB enzyme with betaine-based ILs, where C16-betaine showed superior resistance to denaturation at 70°C, attributed to hydrophobic interface formation and increased structural rigidity through covalent interactions (Xue et al., 2022). Additionally, Tm of myoglobin increased from 70°C to 160.1°C in a solvent-free IL medium. This effect was attributed to the IL-induced entropic stabilization of the folded state, thereby mitigating the temperature-induced conformational collapse (Brogan, 2021). The increase in protein's melting temperature indicates an enhanced thermal stability, as the protein will require more energy to unfold.

Cryoprotective and refolding capabilities of ILs were seen in Cyt C with [BMIM][SCN] and EAN, where after the dialysis process more than 90 % recovery of structure and function of protein was observed. This effect was attributed to a metastable glassy phase at 77 K, that mitigated conformational shifts and aggregation, outperforming the effect of DMSO (Takekiyo et al., 2017a). Porcine pancreatic lipase (PPL) modified with [HOOCBMIM][Cl] exhibited a six-fold increase in the thermostability at 60°C. A systematic investigation of kosmotropic cations and chaotropic anions revealed that the stabilization trends were dependent on the alkyl chain length, with chaotropic anion PF_6^-, providing the highest thermal resistance (Elgharbawy et al., 2018b; Jia et al., 2013). Even at elevated temperatures, lysozyme microspheres retained their enzymatic activity, with optimal biocompatible IL [Ch][dhp] having concentrations (5 % by weight), which further enhanced the enzyme functionality (Qian et al., 2014). These effects are not only the marker of stabilization but also carries the functional importance by providing prolonged shelf-life to proteins and retained activity for longer duration even after undergoing in stress conditions like, heating, processing or storage. Novozyme 435 (immobilized CALB), demonstrated significant thermo-stability in the presence of hydrophobic ammonium-based ILs [$CH_3OCH_2CH_2$-MeN-t-BuOH][TF_2N] at 50°C-70°C for 24–48 h maintaining 52 % residual activity, underscoring IL-mediated stabilization under prolonged thermal exposure (Zhao and Toe, 2020). Thermodynamic analysis of BSA with IL revealed that IL's hydrophobicity plays a dominant role in reinforcing protein structures, along with changes in the spontaneous binding parameters like, (-ΔG),

Table 3
Schematic information of effect of imidazolium-based ionic liquids (with concentration) on various proteins, defining increasing stability (thermal, structural, refolding and fibrillation inhibition).

Sr. no.	Imidazolium-based ILs	Effective concentrations of ILs	Studied Protein	Results	Ref.
1.	1-hexyl-3-methylimidazolium dodecylsulphate ([HMIM][SDS]) and 1-dodecyl-3-methylimidazolium chloride ([DoMIM][Cl])	0.59 mM and 0.26 mM	Hemoglobin	Stronger complex formation and increased structural rigidity	(Vashishat et al., 2017)
2.	1-octyl-3-methyl imidazolium chloride ([OMIM][Cl])	-	BSA	Secondary structure enhanced and aggregation reversion in BSA	(Rawat and Bohidar, 2015)
3.	1-ethyl-3-methyl imidazolium chloride ([EMIM][Cl])	-	β-lactoglobulin & Immunoglobulin	Increased aggregate dissociation & aggregation reversion of proteins	
4.	Butyltrimethylammonium bis (trifluoromethylsulfonyl) imide ([BTMA][NTf_2]) and 1-ethyl-3-methylimidazolium bis (trifluoromethylsulphonyl) imide ([EMIM][NTf_2])	2 % v/v	*Candida antartica* Lipase B	Increased structural compactness of protein	(De Diego et al., 2005)
5.	1-butyl-3-methylimidazolium thiocyanate ([BMIM][SCN])	> 10 mol%	Myoglobin, Cytochrome c, Lysozyme, β-lactoglobulin and Ribonuclease A	Prevented protein aggregation and solubilization of amyloid aggregates of proteins	(Takekiyo et al., 2015)
6.	1-allyl-3-methylimidazolium chloride ([AMIM][Cl])	0.01–0.10 M	Haemoglobin	Increased thermal and structural stability of Hb	(Jha and Venkatesu, 2016)
7.	1-dodecyl-3-methylimidazolium bromide ([HMIM][Br])	0.6 mM	β-casein micelles	Increased protein's hydrophobicity and compactness	(Liu et al., 2013)
8.	1-butyl-3-methylimidazolium acetate ([BMIM][OAc]), 1-buty-l-3-methylimidazolium trifluoroacetate ([BMIM][TFA]) and 1-butyl-3-methylimidazolium dicyanamide ([BMIM][DCA])	0.3 M	Monomeric Insulin	Increased thermal denaturation temperature of Insulin	(Todinova et al., 2016)
9.	[$HOOCEPEG_{350}$IM]-BCL-[BF_4]	1 mM	*Burkholderiacepacia* Lipase	Improved catalytic performance and thermostability	(Xu et al., 2018)
10.	1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]) and 1-butyl-3-metylimidazolium bromide ([BMIM][Br])	75 mM	Lysozyme	Increased lysozyme's activity	(Li et al., 2022a)
11.	1-ethyl-3-methylimidazolium tetrafluroborate ([EMIM][BF_4]), 1-ethyl-3-methylimidazolium methylphosphate ([EMIM][$MeO(H)PO_2$])	20 mM	Recombinant protein Cellulase from Coprinopsis cinerea (CcCel6A)	Dissolution of aggregates, induced refolding and recovery of activity	(Fujita et al., 2016b)
12.	1-ethyl-3-methylimidazolium acetate ([EMIM][OAc])	1–11 mM	Cellulose	Increased solubility of protein	(Kakuchi et al., 2017)
13.	1-hexyl-3-methyl imidazolium chloride ([HMIM][Cl]) and 1-octyl-3-methyl imidazolium chloride ([OMIM][Cl])	< 3 mM	β-LG, Gelatin A and B, BSA, HSA and IgG	Stability of protein dispersions	(Rawat and Bohidar, 2012)
14.	1-ethyl-3-methylimidazolium tetrafluoroborate ([EMIM][BF_4])	1.4–1.6 M	Mycobacterium tuberculosis PE1 and PE2 protein	Retained the folded structure of protein and increased structural stability and esterase activity	(Divya and Guruprasad, 2020)
15.	3-(2-carboxymethyl)-1-butylimidazolium chloride ([HOOCBMIM][Cl])	500 mM	Porcine pancreatic lipase (PPL)	Increased thermostability of PPL	(Jia et al., 2013)
16.	1-hexyl-3-methylimidazolium chloride ([HMIM][Cl]), 1-butyl-3-methylimidazolium chloride ([BMIM][Cl]) and 1-ethyl-3-methylimidazolium chloride ([EMIM][Cl])	25 % (w/v)	Horseradish peroxidase	Increased enzymatic activity	(Machado et al., 2014)
17.	1-ethyl-3-methyl-imidazolium ethyl sulphate ([EMIM][ESO_4])	0.2–1 M	BSA	Increased thermal stability	(Satish et al., 2017a)
18.	1-butyl-3-methylimidazolium nitrate ([BMIM][NO_3]), 1-butyl-3-methylimidazolium hydrogen sulphate ([BMIM][HSO_4]) and 1-butyl-3-methylimidazolium acetate ([BMIM][OAc])	0.10–1.5 M	Cysteine proteinase enzyme - stem bromelain (BMN)	Increased thermal and structural stability of BMN	(Kumar et al., 2020)
19.	Ethylammonium nitrate (EAN) and 1-butyl-3-methylimidazolium nitrate ([BMIM][NO_3])	< 15 mol%	Bovine milk β-lactoglobulin	β-sheet to α-helix transition	(Takekiyo et al., 2013)
20.	1-octyl-3-methylimidazolium chloride ([OMIM][Cl]), 1-ethyl-3-methylimidazolium ethyl sulphate ([EMIM][ESO_4]), 1-ethyl-3-methylimidazolium chloride ([EMIM][Cl]) and 1-butyl-3-methylimidazolium chloride ([BMIM][Cl])	0.5–1.35 mM	Lysozyme	Enhanced conformational and thermal stability of protein	(Rather et al., 2020)
21.	1-ethyl 3-methyl imidazolium chloride ([EMIM][Cl]) and 1-ethyl 3-methyl imidazolium ethyl sulphate ([EMIM][ESO_4])	10^{-4} M 10^{-5} M	HSA BSA	Refolding and preservation of secondary structure	(Sindhu et al., 2020a)
22.	1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4])	-	$A\beta_{42}$ monomers	Provided structural rigidity	(Pal et al., 2022)
23.	1-ethyl-3-methylimidazolium tetrafluoroborate ([EMIM][BF_4]), 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]), and 1-ethyl-3-methylimidazolium bis (trifluoromethanesulfonyl) imide ([EMIM][NTf_2])	-	*Desulfovibrio vulgaris* Cytochrome c 3 (DvH Cyt C3)	Provided structural stabilization to the enzyme	(Ciaccafava et al., 2011)

(*continued on next page*)

Table 3 (*continued*)

Sr. no.	Imidazolium-based ILs	Effective concentrations of ILs	Studied Protein	Results	Ref.
24.	1-butyl-3-methylimidazolium thiocyanate ([BMIM][SCN])	20 mol%	Cryopreserved bovine insulin amyloid	Increased dissolution of amyloid and refolding achieved	(Ishikawa et al., 2018)
25.	1-hexadecyl-3-methylimidazolium chloride ([HeMIM][Cl]), 1-dodecyl-3-methyllimidazolium chloride ([DoMIM][Cl]) 1-methyl-3-octylimidazolium chloride ([OMIM][Cl])	0.08 mM, 0.29 mM 5.8 mM	HSA and BSA	Inhibition of protein fibrillation	(Kundu et al., 2017)

(+ΔH) and (ΔS) that are governed by these hydrophobic interactions. A study analysed that thermal stabilization followed the order, [EMIM][ESO_4] > [EMIM][Cl] > [BMIM][Cl], also anion played an important role here. Thermodynamic parameter (ΔG) for BSA-IL interaction was −3.03 kJ/mol in [EMIM][ESO_4], −3 kJ/mol in [EMIM][Cl] and −1.37 kJ/mol in [BMIM][Cl], with positive values of enthalpy change (Satish et al., 2017a). At higher temperature, nitrile hydratase (NHase) exhibited substantial fluctuations at its N-terminal, whereas in the presence of hydrophobic IL, 1-ethyl-3-methylzolium bis(trifluromethylsulphonyl) imide [EMIM][NTf_2], structural rigidity was gained at the terminals of protein structure, and fluctuations were significantly reduced, demonstrating ILs' ability to suppress the conformational mobility (Housaindokht et al., 2013). These observations, highlighting the enhanced terminal conformational restraint and diminished dynamic movement are depicted in Fig. 5 **(c)**.

1-octyl-3-methylimidazolium chloride [OMIM][Cl], [EMIM][ESO_4], [EMIM][Cl] and [BMIM][Cl], induced both single and biphasic unfolding transitions, where Tm of lysozyme increased. Electrostatic, hydrophobic, and H-bonding interactions between IL cations and negatively charged lysozyme residues were primary stabilizing factors, also IL increased the hydrophobicity of environment around the tryptophan residue of protein (Rather et al., 2020; Satish et al., 2016). Choline-based ILs increased the Proteinase K (PK) stability, elevating Tm from 74.4°C to 87.7°C. Here, kosmotropic anions structured the surrounding water molecules, which reduced the solvent-induced destabilization through preferential exclusion mechanism (Li et al., 2022b). The

Table 4
Schematic information of influence of Choline-based ionic liquids (with effective concentration) on enhancing stability metrics, refolding efficiency and inhibition of fibrillation.

Sr. no.	Choline-based IL	Effective Concentration of ILs	Studied Proteins	Results	Ref.
1.	Choline bitartrate ([Ch][Bi]), Choline dihydrogen phosphate ([Ch][dhp]) and Choline chloride ([Ch][Cl])	-	Lysozyme	Increased structural stability and inhibited fibril formation of protein	(Sindhu et al., 2022)
2.	Choline ((2-hydroxyethyl) trimethylammonium) dihydrogen phosphate ([Ch][dhp]) and Choline perfluorobutanesulfonate ([Ch][Ms])	1.8 % v/v and 1.2 % v/v	Lysozyme and BSA	Provided structural stability	(Alves et al., 2023)
3.	Choline threonine ([Ch][Thr])	1–5 mM	Cytochrome C	Provided structural and conformational integrity	(Kumar Sahoo et al., 2020)
4.	Choline glycine ([Ch][Gly]) and Choline methionine ([Ch][Met])	10–50 mM	Haemoglobin	Induced formation of more stabilized intermediate structure	(Devi Tulsiyan et al., 2023)
5.	Choline dihydrogen phosphate ([Ch][dhp])	20–40 % w/v	Lysozyme	Increased α-helical content and Tm of protein	Weaver et al., (2011)
		0.5–12 % w/v	Recombinant human interleukin-2 (rHIL-2)	Improved the secondary order structure content	Bisht et al., (2017)
		33 % w/v	Cytochrome C	Increased catalytic activity and long-term storage	Mazid et al., (2015)
		55 % w/v	Monoclonal antibody and epidermal growth factor receptor	Stabilized proteins against fragmentation	
6.	Choline phenylalaninate ([Ch][Phe])	0.05 M	Candida antarctica lipase B	Improved enzymatic activity	(Chan et al., 2024)
7.	Bis(2-hydroxyethyl) trimethylammonium L-asparaginate ($[Ch]_2$[Asp]) and (2-hydroxyethyl) trimethylammonium L-glutaminate ([Ch][Glu])	2.6 mM	Porcine pancreas insulin	Increased thermal stability	(Guncheva et al., 2019)
8.	Hydrated choline dihydrogen phosphate (Hy[Ch][dhp])	70 % wt	Cytochrome C	Increased structural stability of protein	(Fujita and Ohno, 2010)
9.	Choline dihydrogen phosphate ([Ch][dhp])	20 % v/v	HSA	Stabilized tertiary structure	(Akdogan et al., 2011)
		3 mM	Cytochrome C	Provided thermal stability and long-term stability	(Fujita et al., 2007)
10.	Choline bitartrate ([Ch][Bi]) and Choline dihydrogen phosphate ([Ch][dhp])	20–60 mg/ml	β-lactoglobulin	Increased thermal stability of protein	(Sindhu et al., 2019)
		10–50 mg/ml	HSA	Enhanced refolding and Tm of HSA	(Bhakuni et al., 2021)
11.	Choline hexanoate ([Ch][Hex]) and Choline methioninate ([Ch][Met]), Choline geranate ([Ch][Ger])	10 % wt	Avidin	Increased structural compactness by decreasing Rg and increased α-helical content	(Shmool et al., 2021a)
12.	Tricholinium citrate ($[Ch]_3$[Cit])	12.5–100 mM	α-chymotrypsin	Increased thermal and structural stability	(Sindhu et al., 2021)
13.	Choline proline ([Ch][Pro])	5 mM	Cytochrome c	Increased thermal and structural stability	(Sahoo et al., 2019)

corresponding overview of choline-based ILs, influencing various aspects of proteins stability metrics like thermal, structural, inhibition of fibrillation is given in Table 4. Analysis of available studies indicates that, within the class of choline-based ILs, those with 'dihydrogen phosphate' anion demonstrated more pronounced and consistent effects as compared with other choline-based formulations, also amino-acid based combinations showed good potential along with biocompatibility.

[Ch][Bi], [Ch][Cl] and [Ch][dhp] showed exceptional thermostability towards β-lactoglobulin. It was concluded that due to IL, aromatic residue of this protein was exposed to more hydrophobic microenvironment which provided them stability (Sindhu et al., 2019). From all these studies we noticed that raising the Tm was improved by strengthening intra and internal forces, including H-bonding or electrostatic balance. Since aggregation in neurodegenerative proteins has similar events like all here proteins discussed, ILs can be reasonably expected to have potential for neuro-protective activity. ILs have demonstrated unparalleled versatility in protein science, offering thermal stabilization, enzymatic enhancement, and structural protection.

- ***reinforcing native structure and functional resilience***

IL influence spans electrostatic stabilization, Hofmeister-driven modulation, hydrophobic effects, kosmotropicity and refolding of proteins, thus conferring enhanced structural resilience and functional longevity to biomacromolecules under diverse physicochemical conditions (Singh et al., 2022; Kowalska et al., 2023).

The stabilization of proteins in IL environments often follows the Hofmeister series, where anions dictate the structural resilience in proteins (Kang et al., 2020). Trypsin, a serine protease, exhibited a clear anion-dependent stabilization hierarchy: sulphate > chloride > bromide > perchlorate. This trend was observed with N-benzoyl--L-arginine ethyl ester (BAEE), which underscores the role of electrostatic interactions in modulating enzyme rigidity and thermal resistance (Dušeková et al., 2022). The impact of anions of ILs, affect the protein stability via kosmotropic or chaotropic behaviour, like anions with high charge density strengthen the water structure and enhance hydration cells and promote intramolecular interaction. Whereas chaotropic ions disrupt the water structure and bind more directly to the hydrophobic patches or backbone sites of the proteins. So, effects like enhancing stability of protein, resisting their aggregation, increasing conformational flexibility varies specifically with the anion identity and its combination with cation part of IL (Piccoli and Martínez, 2024b; Kumar and Venkatesu, 2014).

Imidazolium-based ILs, [EMIM][ESO_4] and [EMIM][Cl] ($<10^{-3}$ M), facilitated α-helical content restoration and β-sheet reduction in urea-denatured BSA and HSA through disruption of hydrogen bonds between urea and the protein, which resulted in effectively reversing the protein denaturation (Sindhu et al., 2020a). Similarly, SDS-induced unfolding of BSA and HSA was mitigated using tetraalkylammonium ILs, including tetraethylammonium hydroxide (TEAOH), and tetrabutylammonium chloride (TBAC). These ILs restored the intrinsic fluorescence and α-helical content by scavenging SDS monomers and preventing further structural damage through robust ion-ion interactions (Sahoo et al., 2024). As we have discussed above, in various NDs proteins like α-synuclein, tau or TDP-43 lose their native α-helical and other secondary structures. Based on the above mechanism of actions, potential of ILs can be explored on these proteins to preserve their α-helical content or protection against unfolding transitions. Beyond electrostatic effects, ILs impact the protein solvation and hydration layer dynamics. The lysozyme protein's radius of gyration (Rg) increased from 14.3 Å (buffer) to 15.5 Å in EAN and 14.6 Å in ethylammonium formate (EAF), suggesting an enhanced hydration and a retained protein globular form in IL solutions (Han et al., 2023b). By stabilizing the protein's compact fold, ILs can be effective in the inhibition of transition to an extended aggregation-prone state of α-synuclein in PD.

Similar action of choline-based ILs mixtures was observed where, IL-mediated the facilitation of embedding of aromatic residue of β-LG within its cavity, thereby sustaining its micro-environmental stability and integrity of the internal structures (Sindhu et al., 2020b). A study unveiled an IL concentration-dependent contraction of Rg in avidin protein, highlighting an increase in compactness of structure. This effect was achieved via reduced solvent exposure of charged surfaces of protein and proportionately enhanced the coordination of IL cations with α- and β-motifs in protein (Shmool et al., 2021a). Further, robust stabilization of native folding patterns of HEWL was noticed during pH alterations and IL concentration variations. Here, findings highlighted the importance of the anionic part of the imidazolium based-IL, particularly its alkyl chain length for hydrophobicity which facilitated the interaction between HEWL and IL (Reddy et al., 2020).

Takekiyo et al. demonstrated the propensity of β-lactoglobulin to undergo β-to-α transitions with EAN and [BMIM][NO_3]. The molten salt-like properties of these ILs facilitated structural reorganization of β-LG, suggesting their potential utility in protein folding manipulations (Takekiyo et al., 2013). The corresponding influence of ammonium-derived ILs on protein stability and aggregation behaviour, across key structural and thermodynamic factors, is summarized in Table 5. From this compiled data, we concluded that ethylammonium-cation IL generally exhibited greater protein-stabilizing effects as compared to other cation-anion combinations within the ammonium-based IL family. All these studies provide insight about IL's multifaceted influence on protein architecture governed by ion-specific interactions, anion-dependent stabilization paradigm and kosmotropic/chaotropic balance. Based on similar framework in providing structural resilience under stress, sustaining compactness and refolding capacity to protein, their properties can easily be translated to supress aggregation of ND-linked proteins. By preserving native compactness, IL-mediated stabilization can reduce the population of aggregation-prone species and toxic oligomers which can further raise the kinetic barrier to that β-sheet nucleation and ultimately mitigate the downstream neurotoxic cascades which drives neurodegeneration.

1.7. Ionic liquids as emerging therapeutic agents for amyloid inhibition and fibril dissolution in neurodegeneration

Neurodegenerative diseases involve the pathological aggregation of amyloidogenic proteins into insoluble, β-sheet-rich aggregates, toxic fibrils that impair cellular functions (Chiti and Dobson, 2006; Knowles et al., 2014). Despite decades of research, an effective therapeutic intervention remains elusive. However, promising ILs are emerging as a potent force in the battle against amyloidogenesis (Takekiyo and Yoshimura, 2018; Fedunova et al., 2022).

α-synculin and IDP: α-synculin, an intrinsically disordered protein (IDP), undergoes helical-to-β-sheet transitions preceding amyloidogenesis (Chen et al., 2021). The influence of EAN on IDP aggregation was investigated, which revealed that IL induced structural compaction via charge masking at N-/C-terminal residues, it also induced the hydrophobic associations that facilitated fibril formation. These findings underscore the role of ILs in mediating amyloidogenic transformations through electrostatic screening and hydrophobic collapse (Takekiyo et al., 2020).

Aβ peptides: The role of ILs in mitigating amyloidogenic aggregation was assessed in Alzheimer's disease related $A\beta_{1-40}$ peptides. Protic IL triethylammonium methanesulfonate (TEAMs) facilitated α-helical reinforcement and prevented fibrillar assembly of protein showing IL's potential as neuro-protectant with implications in amyloid suppression (Debeljuh et al., 2011a). A breakthrough study by *A. Basu et al.* revealed that [BMIM][Br] effectively arrested amyloid fibrillation. Spectroscopic analysis demonstrated that, IL redirected the tryptophan residues toward a hydrophilic environment, preventing their entrapment within the fibrillar cores. Additionally, ILs disrupted the inter-residue interactions, reducing β-sheet content and stalling fibril elongation (Basu et al., 2018). Further investigations explored the protic ILs and their

Table 5
Schematic information of influence of ammonium-based ionic liquids (with effective concentration) on various proteins defining stability (thermal, structural, refolding and fibrillation inhibition).

Sr. no.	Ammonium-based ILs	Effective concentration of ILs	Studied Protein	Results	Ref.
1.	1-ethyl-3-methylimidazolium bis(trifluoromethylsulphonyl) imide ([EMIM][NTf_2]), butyltrimethylammonium bis (trifluoromethylsulphonyl)imide ([BTMA][NTf_2])	2 % v/v	Candida antarctica lipase B	Structural stability and compact enzyme conformation was achieved	(De Diego et al., 2005)
2	Triethylammonium mesylate (TEAM)	70 % v/v	Amyloid-β (16–22) peptide	Induction of α-helical structure was observed	(Dasari and Mallik, 2020b)
		25 wt%	Amyloid-β (16–22) peptide	Inhibition of amyloid aggregation	(Lee et al., 2024)
3.	Propylammonium nitrate (PAN), Ethylammonium nitrate (EAN) and Methylammonium nitrate (MAN)	40 % mol 0–30 mol%	Cytochrome C Bovine ribonuclease A, chicken lysozyme, bovine myoglobin, bovine rhodanese and bovine insulin	Increased solubility of proteins	(Takekiyo et al., 2019b)
4.	[$CH_3OCH_2CH_2$-Me_2N-t-BuOH] [Tf_2N]	-	Candida antarctica lipase B	Recovery of enzymatic activity of CALB	(Zhao and Toe, 2020)
5.	Triethylalkyl ammonium bromides (TEAB)	2 μM to 2 M	BSA	Refolding of BSA was achieved	(Satish et al., 2017b)
6.	Tetra butyl ammonium chloride (TBAC)		BSA and HSA	Refolding of proteins	(Sahoo et al., 2024)
7.	Ethylammonium formate (EAF)	1 % mol	Lysozyme	Maintained globular structure and protein activity	(Han et al., 2023b)
8.	Triethylammonium phosphate (TEAP) and Triethylammonium acetate (TEAA)	50 % (v/v)	α-Chymotrypsin	IL induced higher structure stabilization and refolding of α-CT	(Attri et al., 2011)
9.	Ethylammonium nitrate (EAN)	20 mol% - 50 mol%	Bovine milk β-lactoglobulin	Induced α-helical content	(Takekiyo et al., 2013)
10.	Triethylammonium methane sulfonate (TEAMs)	70–80 wt%	Aβ (1−40) peptide	Induced α-helical content	(Debeljuh et al., 2011a)
11.	Butyltrimethylammonium trifluromethylsulfonyl imide ([BTMA][NTf_2]) and ethyldimethylpropylammonium trifluromethylsulfonyl imide	1 % v/v	Lysozyme	Promoted refolding and structure stabilization	(Bisht et al., 2016)
12.	Ethylammonium nitrate (EAN) and propylammonium nitrate (PAN)	0 – 24 M	Insulin	Suppression of amyloid formation and aggregates	(Takekiyo et al., 2016)
13.	Ethylammonium mesylate (EAM), diethylammonium mesylate (DAM) and triethylammonium mesylate (TEAM)	10 wt%	Monomeric amyloid β-(16–22)	Conversion of Aβ monomers to Aβ amyloid fibrils	(Debeljuh et al., 2012)
14.	Triethylammonium phosphate ([TEA][H_2PO_4]), Triethylammonium hydrogen sulphate ([TEA][HSO_4]), Triethylammonium trifluoroacetate ([TEA][TFA]), Triethylammonium lactate ([TEA][La])	90 % wt	Amyloid β-(16–22)	Enhanced amyloid fibrillation	(Debeljuh et al., 2011b)
15.	Ethylammonium nitrate (EAN)	0.25 mol%-33 mol%	Lysozyme	Increased solubility	(Han et al., 2023b)
		-	Cytochrome C	Induced folding-refolding and stabilization against denaturation	(Jaganathan et al., 2015)
16.	Methyl ammonium formate (MAF) and Ethyl ammonium formate (EAF)	50 −70 % v/v	Cytochrome C	IL provided maintenance of conformational stability and enzymatic activity	(Wei and Danielson, 2011)
17.	Triethylammonium acetate ([TEA][OAc]), Triethylammonium mesylate (TEAM), Triethylammonium trifluoroacetate ([TEA][TFA]), and Trimethylammonium sulphate ([TEA][HSO_4])	80 % wt	β-lactoglobulin	Higher degree of refolding, stabilization of non-native conformations with higher α-helical content was achieved	(BYRNE et al., 2013)
18.	N,N-dimethylethanolamine propionate ([DMEA][Pr])	5 mM −1 M	BSA	IL provided maintenance of structure and increase in extraction efficiency	(Chen et al., 2014)

influence on amyloid-β (Aβ) monomer-to-fibril conversion. A series of ILs containing EAM, diethylammonium (DEAM), and TEAM, demonstrated a concentration-dependent effect, while lower concentrations promoted the Aβ fibrillation, whereas at 70 wt% TEAM, fibrillation was completely inhibited and helical content increased from 8 % to 31 %, suggesting that hydrogen bonding variations between cations drove the amyloid suppression (Debeljuh et al., 2012). However, simulation studies corroborated that TEAM attenuated the aggregation of Aβ 16–22 peptides at elevated concentrations as the interaction between $[TEA]^+$ and phenylalanine side chains led to weakening of hydrophobic cores of aggregated structure (Debeljuh et al., 2011b; Lee et al., 2024). In Aβ peptide dimers (Aβ 33–42), TEAM stabilized α-helical states via van der Waals stabilization, whereas [BMIM][BF_4] suppressed peptide bond fluctuations, mitigating the formation of aggregation-prone intermediates (Dasari and Mallik, 2020b; Sahoo et al., 2023).

Dissolution of pre-formed amyloid aggregates: ILs, with their unique electrostatic and hydrophobic interactions, modulate protein aggregation by targeting specific amino acid residues (Bento et al., 2021). Their impact extends beyond aggregation inhibition; certain ILs can dissolve pre-formed amyloid aggregates, a feat that was traditionally achieved using harsh denaturants like guanidinium hydrochloride (GdnHCl) or dimethyl sulfoxide (DMSO), lipids, often at the cost of protein integrity (Byrne and Angell, 2009; Heldt et al., 2011). The property of low polarity and non-heterogeneity structure of ILs enable it to enhance hydrogen bonding in proteins and increase dissolution (Takekiyo et al., 2017b). Furthermore, interactions between dissolved insulin and IL components, including electrostatic forces and hydrogen bonding play a crucial role in stabilizing insulin monomers, preventing reformation of

amyloid structures (Vanik et al., 2023). A pivotal study on bovine insulin amyloids demonstrated the dissolution power of 1-butyl-3-methylimidazolium [BMIM] with SCN^-, NO_3^-, and Cl^- anions). Remarkably, IL treatment led to 80 % recovery of the protein's secondary structure, indicating successful refolding into its native monomeric form (Ishikawa et al., 2018).

SOD1 aggregation: Impact of ILs on SOD1 amyloidogenesis was studied with [Ch][Cl], tetrabutylammonium methanesulfonate (TBAMs) and tetrabutylammonium chloride (TBAC). Findings revealed that TBAMS and TBAC induced structural compaction in SOD1, reducing the surface hydrophobicity and aggregation propensity, also [Ch][Cl] inhibited amyloid formation but only at the lower concentration. This effect was attributed to stabilization of the electrostatic loop and Zn-binding loop of SOD1, which were critical in maintaining its native structure and preventing pathogenic misfolding (Mavadat et al., 2023; Kumari et al., 2022).

Lysozyme and serum albumin: Biocompatible ILs including [Ch][Ac], [Ch][dhp], [Ch][Bi] and [Ch][Cl] demonstrated amyloid inhibition in lysozyme, preserving secondary structure integrity (Sindhu et al., 2022). ILs exhibit anti-amyloidogenic properties, suggesting potential therapeutic applications for protein misfolding disorders (Hu et al., 2023). Studies on aromatic 1-butyl-4-methylpyridinium bromide ([BMPy][Br]), (BMIM)) and alicyclic 1-butyl-1-methylpyrrolidinium bromide ([BMPyrr][Br]), 1-butyl-1-methylpiperidinium bromide ([BMPip][Br]) ILs demonstrated the inhibition of fibril formation in lysozyme. Both IL categories effectively disrupted the amyloid formation, suggesting that stabilization was raised from a combination of hydrophobicity, charge interactions, and steric effects rather than the aromaticity alone (Kushwaha and Prabhu, 2024).

Similarly, surface-active ILs (SAILs) with varying alkyl chain length of imidazolium cations [OMIM][Cl], [DoMIM][Cl], [HeMIM][Cl]), used at concentrations below their cmc values (5.8, 0.29 and 0.08 mM), were found to suppress serum albumin fibrillization. This inhibitory effect was attributed to the easy insertion of aliphatic chain of SAIL insertion into the hydrophobic region of amyloid cores, which destabilized and subsequently dismantled the pre-formed aggregates (Kundu et al., 2017). Properties of IL, like low polarity and higher IL-amino acid interaction, showed potential for amyloid aggregation suppression in the field of protein engineering (Pillai and Benedetto, 2018). ILs exhibit dualistic behavior in amyloidogenesis, their function are modulated by electrostatic, hydrophobic, and hydrogen-bonding interactions (Bharmoria et al., 2024; Bui-Le et al., 2020). Strategic cation-anion tuning offers a promising avenue for precision control over amyloid inhibition and therapeutic intervention in neurodegenerative disorders. ILs after disrupting H-bond networks, supressing fibrillar growth and the aggregation will directly translate into therapeutic potential as promising alternatives for treating NDs (Manning et al., 2010; Bashir et al., 2020).

1.8. Paradoxical destabilization of protein conformational dynamics by ionic liquids

Among emerging modulators, ILs have drawn attention due to their ability to alter protein conformations through tailored cation-anion interactions (Schindl et al., 2019; Phani Kumar and Reddy, 2020). Recent investigations have revealed their paradoxical nature as; ILs can both preserve and disrupt secondary structures of proteins, depending on their chemical composition and interaction mechanisms (Khachatrian et al., 2023; Fedunova et al., 2022). Exposure of 1-butyl-3-methyl pyrrolidinium tetrafluoroborate [BMPyrr][BF_4], [EMIM]OAc and tetramethylguanidinium acetate (TMGA) and [BMIM][BF_4] lowered the unfolding energy barrier of myoglobin, destabilizing its native state in a concentration-dependent manner and highlighting the role of ionic strength in regulating protein conformation (Fedunova et al., 2022). A range of ILs exhibit dual effects on protein stability, influenced variably by their concentration and the nature of constituent cations and anions; representative examples are summarized in Table 6. Direct interaction of BMIM and EMIM with positively charged Cyt C chain significantly reduced the free energy of stabilization $\delta(\Delta G^{\circ})_{stability}$. Also, IL with longer alkyl chain length showed enhanced stability effect, reinforcing the role of hydrophobicity in the protein-solvent interactions, whereas disruption of same protein was observed with IL [BMIM][C_8OSO_3] (Singh et al., 2018). 1-ethyl-3-methylimidazolium tetrafluoroborate [EMIM][BF_4] led to amyloid formation of BSA at 37°C due to time--dependent hydrolysis of anions which altered the pH of solution; hence similar destabilization was observed in HSA (Singh et al., 2021; Wang et al., 2021). Interaction of haemoglobin with surface active 1-dodecyl-3-methylimidazolium chloride ([DoMIM][Cl]) (cmc=11 mM) and 1-hexyl-3-methylimidazolium dodecylsulphate ([HMIM][SDS]) (cmc=0.98 mM) demonstrated the 'heme' dissociation from their hydrophobic core. UV measurement indicated that, for Hb-[DoMIM][Cl] interaction ΔG = -13.4 kJ/mol and for Hb-[HMIM][SDS], it was −24.13 kJ/mol (Vashishat et al., 2017). A progressive reduction in Tm of β-LG with increasing alkyl chain length in the presence of IL alkylammonium nitrate, implicated that IL destabilized the native state of protein through hydrophobic and electrostatic perturbations (Yoshida et al., 2019). However, at higher IL concentrations, Hofmeister series deviations emerged, suggesting a non-classical solvation-dependent regulation of aggregation dynamics (Vanik et al., 2023; Okur et al., 2017). Imidazolium-based ILs markedly reduced the lag phase of nucleation, augmenting side-chain solvent accessibility, hydrophobic core destabilization, and extensive hydrogen-bonding networks, thereby enhanced the fibrillar conversion (Hwang et al., 2009). Collating data from multiple studies, it could be concluded that [EMIM] and [BMIM] displayed the most contrasting behaviour, tending to destabilize proteins more markedly than other classes of ILs. This suggests that specific ion-protein interactions can either suppress or promote aggregation, adding another layer of complexity to the IL-mediated amyloid regulation (Han et al., 2022).

2. Conclusion and future prospects

This review article critically analyses the possibilities regarding the application of various ILs including alkyl-substituted ammonium, cholinium, imidazolium, and pyridinium cations as protein stabilizers and aggregation inhibitors for neural proteins. The molecular basis of their efficacy lies in protein-IL interactions, low polarity, nano-heterogeneity and hydration dynamics. ILs offer a multifaceted approach to stabilize proteins' secondary structure against denaturation through electrostatic fortication, hydrophobic interactions, Hofmeister-driven modulation and hydration shell regulation. The alteration in Coulomb interaction, shielding of non-polar residues from solvent interaction and promotion of H-bonding contributed to native protein stabilization with enhanced function. Future studies should prioritize rational IL design by tailoring specific cation-anion combinations, chain length of cations and their concentration to improve efficacy. While compiling the data, we observed that most studies were done using [Ch][dhp] and reported improved thermal stability of proteins. These mechanism with mitigation of conformational collapse show a great potential in counteracting proteinopathies and age-related disorders.

Recent studies have established the disruption of nucleation and elongation phases of amyloid fibrils by selectively interacting with protein domains. However, their divergent effects on different protein systems necessitate precise tuning of IL composition, considering their ionic strength, hydrophobicity, and kosmotropic or chaotropic balance. The molecular insights obtained here suggest that ILs can selectively influence the folding–unfolding equilibrium of proteins, thereby offers next-generation therapeutics for neurodegenerative disorders.

Declaration of Competing Interest

declare that they have no competing interests.

Table 6
Schematic information of structure destabilizing effect of various ionic liquids highlighting its effective concentration on various proteins.

Sr. no.	Ionic Liquids	Effective concentration of ILs	Studied Protein	Results	Ref.
1.	1-ethyl-3-methylimidazolium diethylphosphate, ([EMIM][Et_2PO_4]) and 1-ethyl-3-methylimidazolium ethylsulphate, ([EMIM][$EtSO_4$])	17–75 wt%	Lysozyme	Reduced melting temperature	(Singh et al., 2020)
2.	1-ethyl-3-methylimidazolium nitrate ([EMIM][NO_3]), 1-ethyl-3-methylimidazolium acetate ([EMIM]OAc), 1-ethyl-3-methylimidazolium hydrogen sulphate ([EMIM][HSO_4]) and 1-ethyl-3-methylimidazolium tetrafluoroborate ([EMIM][BF_4])	25 mM	Insulin	IL promoted insulin amyloid fibrillization	(Vanik et al., 2023)
3.	Tetramethyl ammonium hydroxide (TMAH)	50 % v/v	Haemoglobin and Myoglobin	IL decreased thermal stability	(Jha et al., 2014)
4.	1-ethyl-3-methyl imidazolium tetrafluoroborate acetate ([EMIM]OAc) and 1-ethyl-3-methyl imidazolium tetrafluoroborate ([EMIM][BF_4])	0.5 %, 1 % and 5 % (v/v)	Lysozyme	IL accelerated amyloid fibrillization	(Fedunova et al., 2022)
5.	Choline malonate ([Ch][Mal])	12.5 mM to 100 mM	HSA and BSA	IL induced deformed tertiary structure but increased thermal stability	(Khachatrian et al., 2023)
6.	1-hexyl-3-methylimidazolium dodecylsulphate [HMIM][SDS]	12.3 mM	Hemoglobin	Aggregation of protein occurred	(Vashishat et al., 2017)
7.	1-butyl-3-methyl imidazolium tetrafluoroborate ([BMIM] [BF_4]), tetramethylguanidinium acetate (TMGA) and 1-butyl-3-methyl pyrrolidinium tetrafluoroborate ([BMPyrr][BF_4])	50–300 mM	Horse-skeletal myoglobin	IL destabilized the protein and accelerated unfolding	(Miller et al., 2016)
8.	[IM1–12][$B(CN)_4$]	20 mM	HSA	Decreased α-helical content	(Kowalska et al., 2023)
9.	1-butyl-3-methylimidazolium hydrogen sulphate ([BMIM][HSO_4]), 1-butyl-3-methylimidazolium nitrate ([BMIM][NO_3]) and 1-butyl-3-methylimidazolium acetate ([BMIM][OAc])	0.10 M – 1.5 M	Cysteine proteinase enzyme-stem bromelain	IL induced structure destabilization	(Kumar et al., 2020)
10.	1-butyl-3-methyl imidazolium tetrafluoroborate ([BMIM] [BF_4]) and tetramethylguanidinium acetate (TMGA)	50–300 mM	Horse-skeletol myglobin	Accelerated myoglobin unfolding kinetics	(Miller et al., 2016)
11.	1-hexyl-3-methylimidazolium chloride ([HMIM][Cl]), 1-ethyl-3-methylimidazolium chloride ([EMIM][Cl]) and 1-butyl-3-methylimidazolium chloride ([BMIM][Cl])	2 M	Horse heart Cyt C	Modulation of structural stability and conformational-fluctuations	(Garg et al., 2024)
12.	1-butyl-3-methylimidazolium nitrate ([BMIM][NO_3])	6 M	Chicken egg white lysozyme	Unfolding of protein compact structure	(Takekiyo et al., 2012)
13.	Methyl ammonium nitrate (MAN)	5 mol%	β-lactoglobulin	IL induced formation of amorphous aggregates	(Yoshida et al., 2019)

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