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# Insights into ionic liquid-enhanced membrane protein stability through machine learning and molecular simulations

Ju Liu,<sup>a</sup> Guiming Zhang,<sup>bc</sup> Cheng Song,<sup>d</sup> Yanlei Wang,<sup>id</sup>\*<sup>b</sup> Jing Ren\*<sup>e</sup> and Hongyan He<sup>id</sup><sup>a</sup>

Protein stability plays a critical role in structural elucidation, enzyme activity, and the storage of protein drugs, where ionic liquids (ILs) have emerged as promising protein stabilizers due to their exceptional biocompatibility and superior solubility. However, the underlying mechanisms by which ILs modulate protein stability, particularly through the regulation of hydrogen bonding and interfacial structures, remain inadequately understood. Herein, a machine learning-based framework, integrating molecular docking, unsupervised learning, molecular dynamics simulations and correlation analysis, is applied to clarify the mechanism of ILs enhancing membrane protein stability. It is found that ILs form clusters that are adsorbed on the protein surface, with ILs entering the hydration layer of the protein and forming intermolecular hydrogen bonds with the protein surface, thereby improving stability, consistent with experiments. Furthermore, a predictive model for protein stability is established by supervised learning and verification of the mechanism through interpretability analysis. Our framework quantitatively reveals the influence of hydrogen bonds and interface structures on membrane protein stability. Overall, these quantitative results not only deepen our understanding of the interactions between ILs and protein but also shed light on the rational design of protein stabilizers.

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## 1. Introduction

The structural analysis of membrane proteins remains a pivotal challenge in the life sciences.<sup>1–3</sup> Although advanced prediction tools like AlphaFold have achieved remarkable accuracy in determining the three-dimensional structure of proteins,<sup>4,5</sup> experimental validation is still needed to establish the structure–function relationships.<sup>6</sup> However, a major obstacle is that membrane proteins tend to lose their native conformation during extraction and purification,<sup>7</sup> which necessitates optimized methods to preserve their stability. Ionic liquids (ILs) have emerged as effective agents in the extraction and purification of membrane proteins,<sup>8–11</sup> owing to their excellent

solubility, biocompatibility, and thermal stability.<sup>12,13</sup> ILs not only enhance extraction efficiency and increase protein yields but also act as stabilizers.<sup>12,14</sup> For instance, Brogan *et al.* demonstrated *via* circular dichroism spectroscopy that the half-denaturation temperature of myoglobin increased by 55 °C in pyrrole-based ILs, even surpassing water's boiling point.<sup>15</sup> Similarly, Banerjee *et al.* found that insulin maintained its secondary structure in the presence of choline and ILs at 4 °C for up to 4 months.<sup>16</sup> These results highlight the significant role of ILs in the field of protein stabilization.

Understanding the stabilization mechanism of proteins by ILs involves investigating two major factors: hydrogen bonding and protein–IL interface structure (Fig. 1). Some studies, such as those by Phillips *et al.*, demonstrated using wide-angle X-ray scattering that imidazolium ILs primarily disrupt internal hydrogen bonds within the protein, thereby reducing the structural stability of proteins.<sup>17</sup> In contrast, Ghanta *et al.* showed through molecular dynamics (MD) simulations that ILs predominantly break the hydrogen bonds between the protein and water, while forming new hydrogen bonds with the protein surface to enhance its stability.<sup>18</sup> Moreover, while certain studies using UV-visible absorption spectroscopy and far-UV circular dichroism suggested that ILs mainly affect the hydration layer of proteins by replacing water molecules,

<sup>a</sup> Center of Ionic Liquids and Green Energy, Beijing Key Laboratory of Solid State Battery and Energy Storage Process, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, China

<sup>b</sup> School of Chemistry and Life Resources, Renmin University of China, Beijing, 100872, China. E-mail: ylwang17@ruc.edu.cn

<sup>c</sup> College of Chemistry and Life Science, Beijing University of Technology, 100124, China

<sup>d</sup> School of Population and Health, Renmin University of China, Beijing, 100872, China

<sup>e</sup> Department of Plastic and Reconstructive Surgery, the First Medical Center, Chinese PLA General Hospital, Beijing 100853, China. E-mail: eggy119@163.com

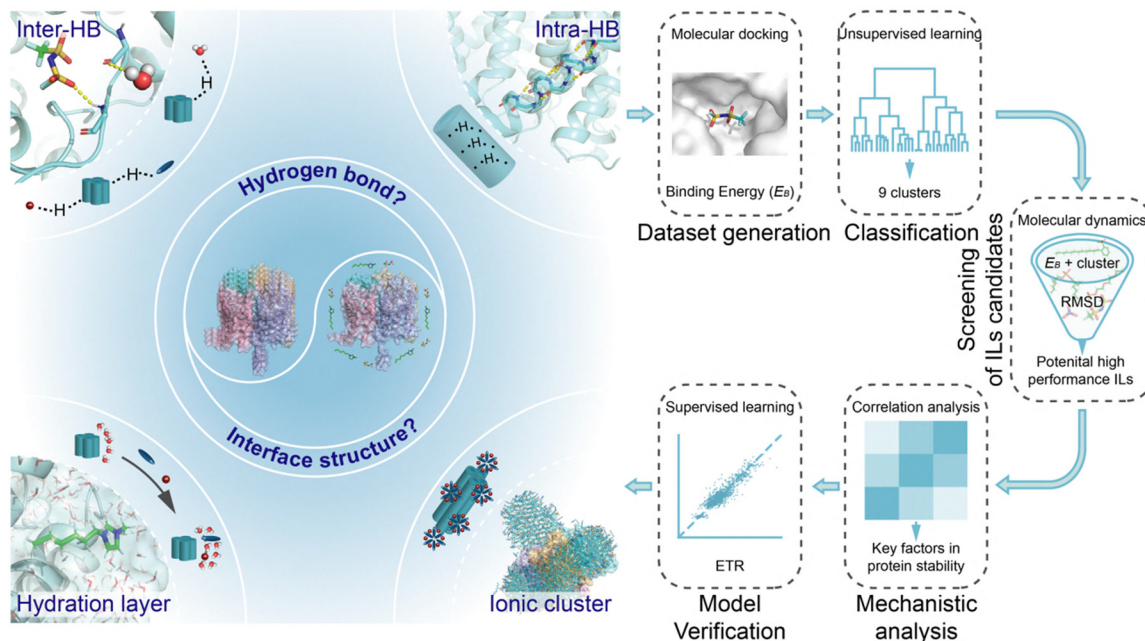


Fig. 1 The dominant stability mechanism of protein in IL-containing aqueous solution is considered from two main perspectives: hydrogen bonding and interface structure. The general workflow includes five major steps: (i) dataset generation, (ii) classification, (iii) screening of IL candidates, (iv) mechanistic analysis, and (v) model verification.

thereby enhancing protein stability,<sup>19–22</sup> other investigations proposed that the aggregation of cations into ionic clusters encapsulates the protein surface, thereby reducing structural fluctuations.<sup>8,9,23,24</sup> The interplay between the internal and interfacial hydrogen bonds, along with the various protein–IL interfacial interactions, critically determines overall protein stability. As those interactions can enhance or disrupt structural integrity, developing a unified quantitative framework to explain IL-mediated stabilization remains a significant challenge.

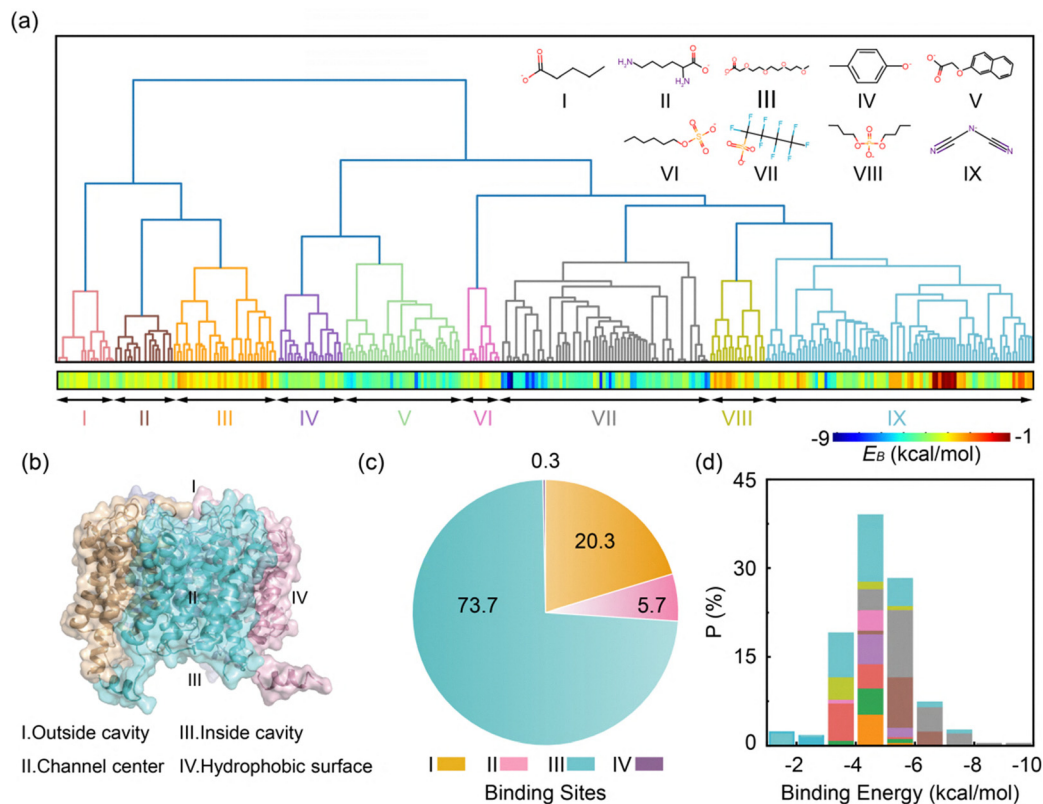
In this study, the membrane was not included in the MD simulations, as our focus was on the storage process of the protein after extraction from the membrane. Nevertheless, our previous studies demonstrated that ionic liquids preferentially insert at the protein–lipid interface while preserving protein structural stability.<sup>9,22</sup> Building on these results, we develop a machine learning (ML)-based framework to quantitatively elucidate how ILs enhance membrane protein stability (Fig. 1). Our approach encompasses five major steps: (i) dataset generation: we compile a dataset of 317 different anions paired with imidazolium cations and perform molecular docking simulations with aquaporin-2 (AQP<sub>2</sub>) and sodium channel protein (Nav) to calculate binding energies between anions and protein ( $E_B$ ). (ii) Classification: an unsupervised learning model is used to classify the anions into nine clusters, integrating their structural features with the  $E_B$  data to construct a ML model. (iii) Screening of IL candidates: we sample and evaluate ILs using more rigorous MD simulations based on  $E_B$  and clusters. In contrast to every sample investigated, we screened by focusing on anions with the highest  $E_B$  and representative candidates from each cluster. (iv) Mechanistic analysis: key

descriptors related to hydrogen bonding and interface structure are extracted from the trajectories of MD simulations, and correlation analysis is performed to identify the key factors influencing protein stability. (v) Model verification: finally, we verify the proposed mechanism by constructing a supervised learning model utilizing an extra trees regressor, with interpretability provided by the Shapley additive explanation (SHAP) analysis.

## 2. Results and discussion

### 2.1. Dataset for ionic liquids and binding energies with protein

Initially, we compiled a dataset of 317 anions, including their SMILES codes and structural files (Fig. S1), obtained from the ionic liquid database.<sup>25</sup> We then selected aquaporin 2 (AQP<sub>2</sub>) and sodium channel protein (Nav) as our model proteins because they play crucial roles in cellular water and ion transport (Fig. S2), making their stability essential for both physiological functions and biomedical research. Subsequently, we performed molecular docking studies of these anions with the target proteins AQP<sub>2</sub> and Nav to obtain binding energies ( $E_B$ ) and identify the corresponding binding sites,<sup>26</sup> thereby establishing a comprehensive dataset (Fig. 2a and Fig. S3). This dataset includes the SMILES codes of the anions, their respective PDB structural files,  $E_B$ , and the corresponding binding sites. Anions that exhibit stronger binding energies to AQP<sub>2</sub> also display stronger binding energies to Nav. Accordingly, we selected AQP<sub>2</sub> for further analysis. Given that the  $E_B$  is closely related to the specific binding site,<sup>27</sup> an analysis of these sites



**Fig. 2** (a) Hierarchical clustering dendrogram. The leaf nodes show the position where all anions are partitioned into 9 clusters, marked as I–IX from left to right and distinguished by different colors, and representative structures of each cluster are displayed in the blank spaces. Mapping the dendrogram to the binding energy reveals the structure–property relationships of the anions. (b) Structure of AQP<sub>2</sub>, where I, II, III, and IV represent the most stable binding sites for anions. (c) Probability distribution of binding sites. (d) Binding energy distribution of anions within each cluster; bar colors correspond to cluster categories in (a).

(Fig. 2b) revealed four distinct regions: the outside cavity, channel center, inside cavity, and hydrophobic surface. Notably, the outside and inside cavities of AQP<sub>2</sub> are characterized by predominantly carrying negative and positive charges, respectively.<sup>28</sup> Among these, the inside cavity accounts for 73.7% of all binding sites (Fig. 2c), which can be attributed to the fact that under electrostatic interactions, anions tend to bind to the positively charged inside cavity of AQP<sub>2</sub>.

We next performed hierarchical clustering analysis on the 317 anions using the Ward algorithm, resulting in 9 distinct clusters containing 19, 19, 34, 21, 38, 13, 68, 18, and 87 anions, respectively (Fig. S1). Notably, anions grouped within the same cluster exhibit similar structural motifs, indicating a rational basis for this classification. When correlating these structural features with their corresponding  $E_B$ , we found that anions within the same cluster exhibit comparable  $E_B$  values, indicating that we have established a robust structure–activity model that validates the rationality of our classification.

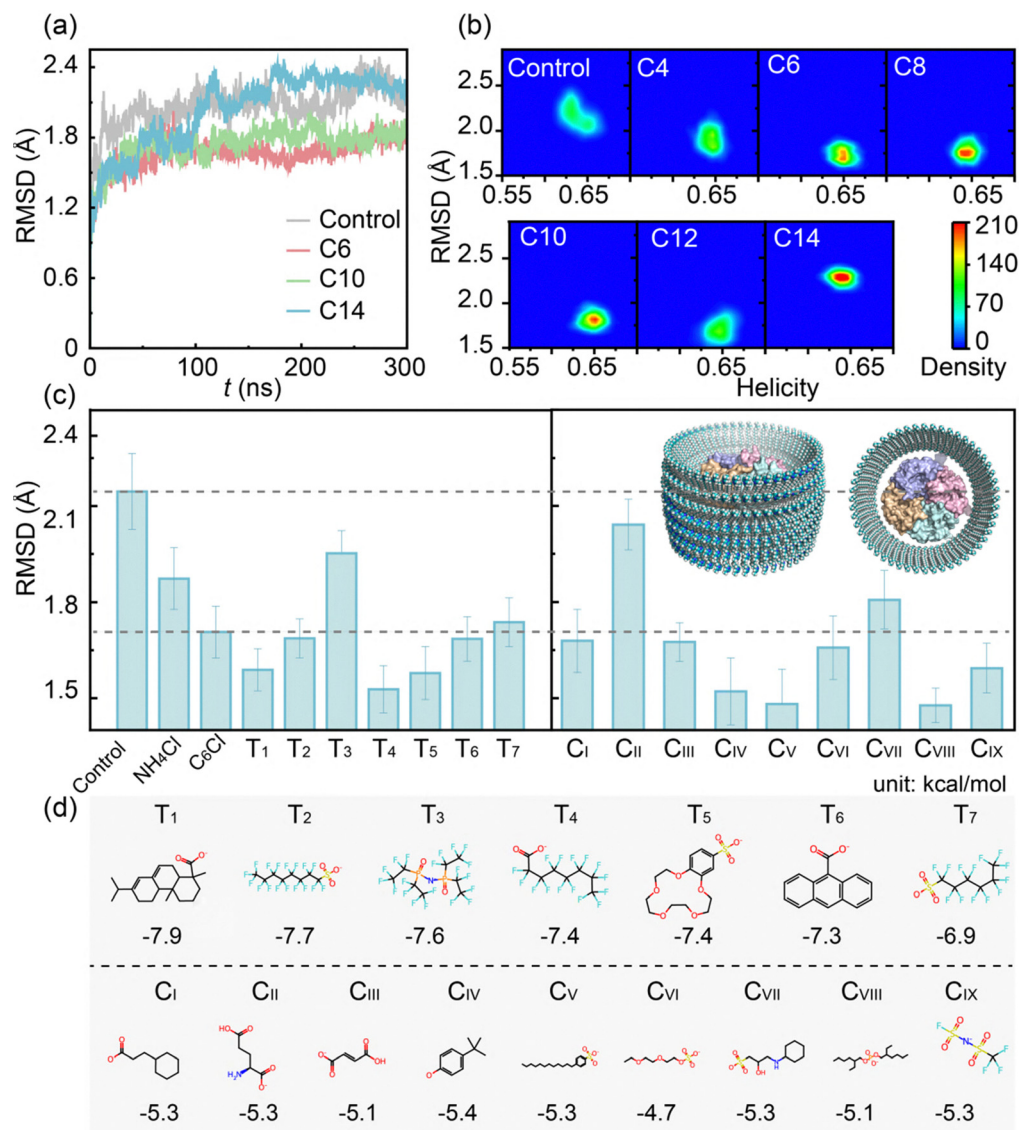
To further refine the structure–activity model of the anions, we analyzed both the functional groups of the anions and the distribution of their  $E_B$  values (Fig. 2d). Anions in clusters I to III all contain carboxyl groups but differ in their overall composition.

Specifically, cluster I anions are primarily monocarboxylic compounds, exhibiting  $E_B$  values ranging from  $-3$  to

$-5$  kcal mol<sup>-1</sup>. In contrast, cluster III anions not only contain carboxyl groups but also incorporate other functional groups such as ether, amine, and hydroxyl groups. Particularly, cluster VII anions generally exhibit higher  $E_B$ , ranging from  $-4$  to  $-10$  kcal mol<sup>-1</sup>. This cluster mainly comprises fluorinated anions with sulfonic acid groups, which can form hydrogen bonds with the protein (Fig. S4), suggesting the significant role of hydrogen bonding in these interactions.<sup>7</sup> Additionally, cluster IX anions, characterized by a wide structural diversity that includes sulfonate groups, imidazole rings, azides, halogens, *etc.*, exhibit a broad distribution of  $E_B$  values spanning from  $-1$  to  $-8$  kcal mol<sup>-1</sup>.

## 2.2. High-throughput screening through molecular dynamics simulations

To investigate the effects of ILs on protein stability through MD simulations, we selected imidazolium cations with varying alkyl chain lengths  $[C_n\text{mim}]^+$  ( $n = 4\text{--}14$ )<sup>13,29</sup> and chloride as the counter ion due to its high water solubility.<sup>8</sup> As shown in Fig. S5, with the increase in alkyl chain length, the adsorption state of ILs on the protein surface evolves from disordered adsorption to membrane-like adsorption, while the clustering structures in solution gradually transition from free ions to large clusters. Protein stability was characterized by root-mean-square



**Fig. 3** (a) Time evolution of the RMSD of the protein in aqueous solutions containing  $[C_n\text{mim}]\text{Cl}$ . (b) Effects of cationic alkyl chain length on protein stability, reflected by  $\alpha$ -helicity and RMSD. (c) Average RMSD of the protein in solutions with  $[C_6\text{mim}]^+$  based ILs containing different anions, selected based on top  $E_B$  and varying clusters of the same  $E_B$ . The inset plots represent the model of the MD simulation, with the protein positioned at the center. The  $[C_6\text{mim}]^+$  are orderly arranged around the protein, while the anions are randomly distributed and not shown. (d) Structures of representative anions corresponding to the conditions in (c).

deviation (RMSD) and  $\alpha$ -helix content; as shown in Fig. 3a and b, the two-dimensional plot of  $\alpha$ -helicity and RMSD shifts from the center to the bottom-left corner, before returning to the center as the side chain of the cation elongates. This trend suggests an initial enhancement in stability, followed by a decline, peaking at  $[C_6\text{mim}]^+$ . Consequently,  $[C_6\text{mim}]^+$  was chosen for further investigation.

Subsequently, representative ILs were selected for MD simulations based on  $E_B$  and classification. The criteria involved selecting the anion with the highest  $E_B$  across all structures, followed by gradient selection of anions with varying  $E_B$  within the same cluster, and choosing anions with identical  $E_B$  from different clusters. Firstly, we chose the top 7 anions with the highest  $E_B$  (Fig. 3, named T<sub>1</sub> to T<sub>7</sub>), all exceeding

$-6.9 \text{ kcal mol}^{-1}$ . These anions typically have large molecular masses and contain fluorine and oxygen groups, and were paired with  $[C_6\text{mim}]^+$ . As shown in Fig. 3c, the ILs with the highest  $E_B$  all enhanced protein stability, with RMSD values of 1.53, 1.95, and 2.15 Å for T<sub>4</sub>, T<sub>3</sub>, and control groups, respectively. Simulation snapshots reveal that these ILs form large clusters and adsorb on the protein (Fig. S6). Additionally, due to the hydrophilic sulfonic acid groups and hydrophobic fluorinated alkyl chains present in T<sub>2</sub>, T<sub>4</sub>, and T<sub>7</sub> anions (Fig. 3d), they resemble phospholipid structures,<sup>30</sup> promoting the formation of an ordered membrane-like structure around the protein and further stabilizing it.

To assess the influence of anions from the same cluster with different  $E_B$ , 7 anions with different  $E_B$  from cluster IX ( $E_1$  to  $E_7$ )

were selected, as shown in Fig. S7. The  $E_B$  values for  $E_1$ ,  $E_5$ , and  $E_7$  were  $-2.7$ ,  $-5.3$ , and  $-7.4$  kcal mol $^{-1}$ , respectively, with the corresponding protein RMSD values of 1.71, 1.59, and 1.58 Å (Fig. S8), indicating that the effect of anions from the same cluster on protein stability is not linearly correlated with the change of  $E_B$ . In systems containing  $E_1$  to  $E_3$ , ILs limited adsorption on the protein surface (Fig. S9), resulting in minimal stabilization effects. However, ILs in systems with  $E_4$  to  $E_7$  form clusters around the protein, leading to marked improvements in stability. Therefore, anions with higher  $E_B$  within the same cluster significantly enhance protein stability.

Since  $E_5$  with a binding energy of 5.3 kcal mol $^{-1}$  demonstrated the most significant enhancement in protein stability, we further selected anions with  $E_B$  values around 5.3 kcal mol $^{-1}$  from different clusters ( $C_I$  to  $C_{IX}$ ), as shown in Fig. 3d. Among these, anions from clusters  $C_{VIII}$ ,  $C_V$ , and  $C_{IV}$  exhibited the lowest RMSD values of 1.48, 1.48, and 1.52 Å, respectively. These ILs form clusters adsorbed on the protein surface (Fig. S10), contributing to enhanced stability. To further evaluate their performance, the five screened ILs with the lowest RMSD were compared to three conventional protein stabilizers: polysorbate 20 (Tw20),<sup>31</sup> polyethylene glycol (PEG),<sup>32</sup> and Nonidet P-40 (NP-40).<sup>12</sup> As shown in Fig. 3c and Fig. S11 and S12, the screened ILs outperformed commercial stabilizers, reducing RMSD by 12–18%. We further evaluated structural stability using root mean square fluctuation (RMSF) and solvent accessible surface area (SASA) of the hydrophobic core. As shown in Fig. S13a, the  $C_{VIII}$  system shows a markedly reduced RMSF relative to the control, with fluctuations ranging from 10 to 40 Å for the control and 0 to 10 Å for  $C_{VIII}$ . Consistently, the hydrophobic core is less exposed in  $C_{VIII}$ , with a SASA of 12 953 Å $^2$  versus 24 005 Å $^2$  for the control (Fig. S13b). These metrics corroborate that the candidate ILs enhance the structural stability of the membrane protein. Additionally, these ILs exhibited low synthetic accessibility score<sup>33</sup> (SAscore) (Fig. S14), with the  $C_V$  anion scoring 2.19, indicating favorable synthetic feasibility. Consequently, these ILs are potential protein stabilizers.

### 2.3. Machine learning revealing the mechanism of ionic liquids stabilizing membrane proteins

To elucidate the mechanism by which ILs enhance protein stability, two categories of descriptors were extracted from the simulation results: hydrogen bond and interface structure. As shown in Fig. 4a, hydrogen bond descriptors include the number of internal hydrogen bonds within the protein ( $HB_{pro}$ ), the number of hydrogen bonds between the protein surface and ILs ( $HB_{pro-IL}$ ), and the number of hydrogen bonds between the protein and water ( $HB_{pro-wat}$ ). Interface structure descriptors include the number of water molecules in the first and second hydration layers ( $C_{I-wat}$ ,  $C_{II-wat}$ ), anions ( $C_{I-an}$ ,  $C_{II-an}$ ), cations ( $C_{I-ca}$ ,  $C_{II-ca}$ ) near the protein surface, as well as the overall radius of gyration of the ILs ( $R_{IL}$ ). Through correlation analysis, the Pearson correlation coefficients ( $r$ ) between these descriptors were calculated to quantify their influence on protein stability (Fig. 4b). The sign of  $r$  indicates the direction of

correlation: a positive sign for a positive correlation and a negative sign for an inverse correlation.

As shown in Fig. 4b and c,  $HB_{pro-IL}$  exhibits a moderate negative correlation with RMSD ( $r = -0.39$ ), indicating that an increased hydrogen bonds between the protein surface and ILs is associated with enhanced structural stability. This aligns with previous experiments.<sup>34,35</sup> However,  $HB_{pro}$  has a negligible correlation ( $r = -0.05$ ), indicating that the ILs have limited influence on the internal hydrogen bonds of the protein and mainly affects surface interactions, consistent with reports that the structure of membrane proteins is stable and that ILs have difficulty disrupting their internal interactions.<sup>22</sup> On the other hand, for the interface structure, the  $C_{I-wat}$ ,  $C_{II-wat}$ ,  $C_{I-an}$ , and  $C_{II-an}$  have moderate correlations with RMSD, with all  $|r|$  values exceeding 0.36, indicating the importance of the hydration layer in protein stability. Notably,  $C_{II-an}$  is strongly negatively correlated with  $C_{II-wat}$  ( $r = -0.94$ ), suggesting that IL anions can stabilize proteins by replacing water in the hydration layer. Moreover, the correlation between  $C_{II-ca}$  and RMSD ( $|r| = 0.27$ ) is weaker than that of  $C_{II-an}$  ( $|r| = 0.39$ ), indicating that anions play a more significant role in stabilizing proteins, consistent with previous studies.<sup>36</sup> Additionally,  $R_{IL}$  shows a moderate positive correlation with RMSD (Fig. 4a) ( $r = 0.31$ ), implying that more compact IL structures are associated with greater protein stability.<sup>24,25</sup>

In addition, to comprehensively analyze the impact of hydrogen bonds and interface structures on protein stability, canonical correlation analysis was performed. The strength of correlations between the two descriptor sets was evaluated using the first canonical correlation coefficient ( $r_c$ ) in Fig. 4d, which ranges from 0 (no linear relationship) to 1 (perfect linear relationship). The hydrogen bond set ( $HB_{com}$ ) and the interface structure set ( $S_{com}$ ) exhibit moderate correlations with RMSD, with  $r_c = 0.44$  and 0.41, respectively, indicating that both contribute to protein stability, aligning with previous findings.<sup>7</sup> Additionally, a strong correlation was observed between  $HB_{com}$  and  $S_{com}$ , with  $r_c = 0.88$ , further emphasizing the close relationship between hydrogen bonds and interface structures. Based on the above analysis, the results suggest that ILs enhance protein stability by forming ionic clusters adsorbed onto the protein surface, penetrating the hydration layer, and establishing intermolecular hydrogen bonds with the surface. Moreover, the role of anions is more pronounced than that of cations.

To validate the proposed mechanism, supervised learning was employed to predict the RMSD of protein using the extra trees regressor. Descriptors selected from MD simulations and correlation analysis were used as input features. The model was optimized to minimize the root-mean-squared error (RMSE) between predicted and actual RMSD values. A Pearson correlation coefficient of 0.913 was achieved on the test set (Fig. 4e and Fig. S15), indicating high predictive accuracy and supporting the relevance of the selected descriptors to protein stability. SHAP analysis indicated that the top four most influential features are  $R_{IL}$ ,  $C_{II-wat}$ ,  $HB_{pro-IL}$  and  $C_{II-an}$  (Fig. 4f). Particularly, decreased  $R_{IL}$  and  $C_{II-wat}$ , along with increased  $HB_{pro-IL}$  and



protein hydration layer. These ILs form intermolecular hydrogen bonds with the protein surface, thereby stabilizing the protein. Finally, the model predicts the RMSD of the protein using an extra trees regressor, and SHAP values were utilized to identify the key explanatory variables influencing RMSD, which verified the mechanism. This work reveals the mechanism by which ILs improve protein stability, which is of great significance for understanding the interaction between ILs and proteins and developing new membrane protein extraction and storage systems.

## 4. Models and methods

### 4.1. Molecular docking

The molecular docking between aquaporin-2 (AQP<sub>2</sub>), sodium channel protein (Nav) and ILs was conducted using the AutoDock Vina program.<sup>37</sup> The crystal structure of AQP<sub>2</sub> (PDB: 4nef<sup>28</sup>) and Nav (PDB: 5vb8) were utilized, and the protein and ILs' input files were prepared by merging non-polar hydrogen atoms and adding charges. The target protein remained fixed. The docking results were visualized using PyMOL, while the protein–ligand interaction was calculated using the protein–ligand interaction profiler web service.<sup>38</sup>

### 4.2. Unsupervised learning

The dendrogram function in the SciPy package is used to implement agglomerative hierarchical clustering (AHC).<sup>39</sup> In this method, each sample begins as its own individual cluster and progressively merges with others based on similarity as it moves up the hierarchy. The result of AHC is a bottom-up hierarchical tree diagram. In this diagram, the Euclidean distance between branches serves as the measure of similarity, while the Ward algorithm is employed to calculate the dissimilarity between nodes.<sup>40</sup>

### 4.3. Molecular dynamics simulations

The IL–AQP<sub>2</sub> system was constructed as shown in Fig. 3c, [C<sub>*n*</sub>mim]<sup>+</sup> (*n* = 4–14) cations were orderly arranged around AQP<sub>2</sub>,<sup>41</sup> and anions were randomly placed using Packmol.<sup>42</sup> Water molecules surrounded the protein and ILs. The simulation box dimensions were set to 13.6 × 13.6 × 13.6 nm<sup>3</sup>. Periodic boundary conditions were applied in all three directions. The MD simulations were performed using Amber22 software.<sup>43</sup> The time step was 2 fs. The protein was described using the protein.ff14SB force field, while the second-generation General AMBER Force Field (GAFF2) was employed to describe the ILs,<sup>44,45</sup> which has been shown to reliably reproduce their structural and dynamical behaviors.<sup>8,46–48</sup> The TIP3P force field was used for water molecules.<sup>46</sup> Non-bonded interactions included electrostatic and van der Waals terms. The former was computed using the particle-mesh Ewald algorithm,<sup>47,48</sup> while the latter was described using the Lennard-Jones potential with a cutoff distance of 1.0 nm. The SHAKE algorithm was employed to constrain high-frequency vibrations of hydrogen atoms.<sup>49</sup> Temperature and pressure

were controlled using the Berendsen thermostat<sup>50</sup> and the Berendsen barostat,<sup>51</sup> respectively, with a coupling constant of 1.0 ps. The system was first equilibrated for 10 ns under the NPT ensemble at 310 K and 1 bar, followed by 300 ns of equilibration under the NVT ensemble at 310 K. The last 50 ns trajectory was used to analyze the impact of ILs on protein stability.

### 4.4. Correlation analysis and the supervised learning model

Correlation analysis was performed using the Statistical Package for the Social Sciences (SPSS)<sup>52</sup> to correlate the number of internal hydrogen bonds within the protein (HB<sub>pro</sub>), the number of hydrogen bonds between the protein surface and ILs (HB<sub>pro-IL</sub>), the number of hydrogen bonds between the protein and water (HB<sub>pro-wat</sub>), and the number of water molecules in the first and second hydration layers of the protein surface (C<sub>I-wat</sub> and C<sub>II-wat</sub>), the number of anions in the first and second hydration layers of the protein surface (C<sub>I-an</sub> and C<sub>II-an</sub>), the number of cations in the first and second hydration layers of the protein surface (C<sub>I-ca</sub> and C<sub>II-ca</sub>), and the overall radius of gyration of the ILs (R<sub>IL</sub>) for the root mean square deviation (RMSD) of protein. The above descriptors are extracted from the last 50 ns trajectory of each MD to build a dataset.

$$\text{RMSD} = \sqrt{\frac{1}{N} \sum_{i=1}^N (d_i)^2}$$

where  $d_i$  is the Euclidean distance between atom  $i$  in the two structures and  $N$  is the total number of atoms.

The model training was conducted using scikit-learn.<sup>53</sup> The dataset was randomly divided into two groups: 80% for model training and 20% for model testing. The extra trees regressor was used, the maximum depth of the trees is unrestricted, and each leaf node must contain at least one sample. The ensemble model consists of 100 trees, and its performance is measured using the root mean square error (RMSE) calculated by:

$$\text{RMSE} = \sqrt{\frac{1}{n} \sum_{i=1}^n (y_i - \hat{z}_i)^2}$$

where  $n$  represents the number of samples,  $y_i$  is the actual target value, and  $z_i$  is the predicted value.

## Author contributions

Yanlei Wang and Jing Ren conceived and led the project. Ju Liu conducted the simulations and drafted the manuscript. Ju Liu and Guiming Zhang conducted the research. Cheng Song, Yanlei Wang, and Hongyan He contributed to this work by analyzing the data and revising the manuscript. Yanlei Wang and Hongyan He supervised the project. All authors discussed the results and commented on the manuscript.

## Conflicts of interest

The authors declare no competing financial interests.

## Data availability

The data supporting this article have been included as part of the supplementary information (SI). Supplementary information: the relevant data on the enhancement of protein stability by ILs. See DOI: <https://doi.org/10.1039/d5cp02130h>.

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