

Improved Anisotropic Network Models for Membrane Protein Dynamics and Mechanosensitive Ion Channels

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Mechanosensitive (MS) ion channels are crucial for translating mechanical stimuli into cellular responses; however, their dynamic properties pose significant challenges for experimental investigations. This study introduces two enhanced anisotropic network models (ANMs) that incorporate membrane contact probability (MCP) to more effectively capture the influence of the cell membrane on protein dynamics. MCP-ANM and MCP-multiscale ANM (mANM) outperform traditional ANM models that do not explicitly consider membranes when predicting the flexibility of various types of membrane proteins. Furthermore, by integrating MCP-mANM with perturbation response scanning, we are able to efficiently simulate the conformational changes of MS proteins subjected to different mechanical forces. This methodology allows for the examination of various gating mechanisms, including force-from-lipids and force-from-tether, across multiple MS ion channels such as no mechanoreceptor potential C, mechanosensitive channel of small conductance, and Piezo-type mechanosensitive ion channel. The calculated mechanosensitivity of the MS channels aligns well with the experimental findings. These results demonstrate the effectiveness of MCP-incorporated ANM in linking mechanical stimuli to protein dynamics, providing a reliable and efficient framework for understanding the mechanotransduction and gating mechanisms of MS ion channels.

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I. INTRODUCTION

Mechanosensitive (MS) ion channels constitute a distinct group of membrane proteins characterized by their ability to react sensitively to mechanical forces, including membrane tension, cell compression, and pressure. These stimuli facilitate a variety of physiological processes, such as touch sensation, hearing, and the regulation of cellular volume [1,2]. These MS channels, including well-known examples such as mechanosensitive channel of small conductance (MscS), TWIK-related K^+ channel (TREK), Piezo-type mechanosensitive ion channel (PIEZO), and hyperosmolality-gated calcium-permeable channel (OSCA), undergo conformational changes under mechanical stimuli to regulate ion flux, thus maintaining cellular homeostasis under external stress [2]. The dysfunction of MS channels is involved in various diseases, including hypertension, osteoporosis, and neuropathic pain, highlighting their importance as therapeutic targets [3–5].

Despite their significance, the study of MS ion channels remains challenging due to their dynamic and force-sensitive nature. Techniques such as patch-clamp electrophysiology

provide detailed insights into ionic currents; however, they face difficulties in accurately correlating mechanical forces with gating behaviors at the molecular level [6]. Structural biology methods, including x-ray crystallography and cryo-electron microscopy (cryo-EM), provide information on static conformational states but often fail to capture the dynamic transitions that are essential for mechanosensation [7].

Computational methods such as molecular dynamics (MD) simulations [8] have been widely used as a powerful tool to explore the dynamics of the MS channel at the atomic level, revealing insights into conformational changes and activation mechanisms. For example, Reddy *et al.* used MD simulations to explore the gating mechanism of the MscS channel, illustrating how membrane tension triggers structural rearrangements that open the pore [9]. More recently, Britt *et al.* have shown that MscS is modulated by lipid interactions, highlighting the importance of membrane composition in channel behavior [10]. Similarly, Rogacheva *et al.* used unbiased MD simulations to uncover an asymmetric opening mechanism in the MscL channel under tension, which involves sequential conformational shifts [11]. Clausen *et al.* applied atomistic MD simulations to show that membrane-mediated structural changes couple the proximal C-terminus to the selectivity filter in TREK-2, thereby regulating its mechanosensitive gating [12]. For the OSCA channel, Zhang *et al.* resolved its high-resolution structure using cryo-EM and used MD simulations to gain initial insight into its gating behavior [13]. Building on this, Han *et al.* integrated cryo-EM with MD simulations to reveal that mechanical stress induces the formation of a lipid-lined pore, emphasizing the role of

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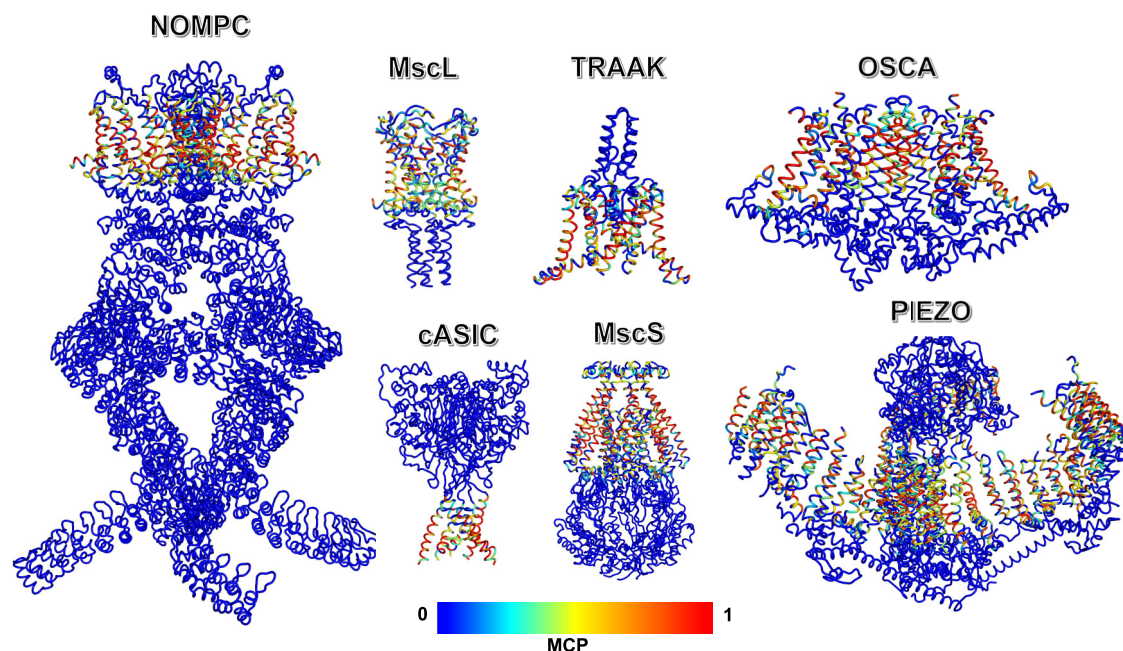


FIG. 1. Structural representations of MS ion channels colored according to the predicted MCP. The protein structures are depicted as tube models, with predicted MCP highlighted using the jet color map (blue to red). The color gradient represents the likelihood of the region being in contact with the membrane, with blue indicating lower contact probability and red indicating higher contact probability.

lipid-protein interactions [14]. For the no mechanoreceptor potential C (NOMPC) channel, Wang and Duan *et al.* employed MD simulations to show that compression and rotation of the anchor repeat domain drive a unique “push-to-open” and “twist-to-open” gating mechanism [15,16]. However, MD simulations remain computationally intensive, particularly for large MS proteins such as PIEZO, which require extended simulations to observe full gating events [17–19]. Consequently, MD simulations are impractical for high-throughput analyses across a diverse range of MS proteins.

Coarse-grained models, such as elastic network models (ENMs), offer a computationally feasible alternative by focusing on large-scale functional conformational changes without the detailed resolution of atomistic MD [20]. Such methods can successfully capture low-frequency motions that characterize large conformational shifts in proteins [21]. However, despite the advantages of traditional ENM approaches, they encounter limitations in incorporating environmental factors critical for protein function, particularly in the context of membrane environments for membrane proteins. Previous adaptations of the anisotropic network model (ANM, an extension of ENM that considers motion direction [22]), such as the implicit membrane anisotropic network model (imANM) [23], attempt to account for the influence of the membrane by adjusting the spring force constants in ANM according to the positioning of the membrane. These adaptations have improved the computational analysis of membrane proteins to a certain extent [23]. However, these methods typically assign stiffer spring constants in the xy plane (membrane plane) compared to the z direction to simulate membrane confinement, which may not accurately reflect the full complexity of biological membranes, where the stiffness in the z direction could be greater or the overall situation may be more intricate due to the complex interactions between proteins and membranes.

On the other hand, the explicit membrane anisotropic network model (exANM) offers a more detailed representation of the membrane environment by explicitly including membrane atoms or particles in the model [23]. However, exANM is computationally more intensive due to the increased number of nodes. More importantly, the performance of exANM is highly sensitive to the composition of the membrane, the level of coarse graining, and the shape and size of the membrane. It is also difficult to justify the proteinlike effects of the explicit membrane nodes, which should be more fluidlike. These problems pose challenges in standardizing the model and hinder its generalizability across different systems.

To address these limitations, we propose membrane contact probability– (MCP) based ANM models, MCP-ANM, and MCP-multiscale ANM (mANM). These models incorporate the predicted MCP [24,25], as illustrated in Fig. 1 to dynamically adjust the spring force constants employed in the ANM, based on deep learning predictions of the physical contact probability between protein residues and membranes. Our MCP-ANM models provide a more accurate representation of the complex system of membrane proteins embedded in an anisotropic membrane environment, thereby avoiding the use of simplistic membrane boundary conditions. Consequently, these models are better suited for capturing the influence of the membrane on protein dynamics.

To further explore the gating mechanisms of MS ion channels, we combined MCP-mANM with perturbation response scanning (PRS), a method that applies systematic perturbations to residues and evaluates the resulting conformational responses [26]. By extending PRS to incorporate MCP, we applied targeted mechanical forces to membrane-contacting or tethered residues of MS ion channels. This approach accurately simulates various gating mechanisms, including those induced by “force from lipids” and “force from tether.” This

integration enables a comprehensive analysis of the structural and functional dynamics of MS proteins within various mechanical environments. Our results highlight the efficacy of MCP-based ANM models in capturing the dynamic properties of MS ion channels and their potential to advance the understanding of mechanosensation at the molecular level.

II. RESULTS

A. Framework overview: MCP-based ANMs and integration with PRS

To incorporate the effects of the membrane environment into protein dynamics, we propose two modified ANMs, MCP-ANM and MCP-mANM. In MCP-ANM, the nodes are defined as the C_α atoms of the residues, and the edges are established between pairs of nodes within a defined cutoff distance. In MCP-mANM, each pair of residues is connected by an edge, using an exponential decay kernel function to represent residue interactions [27]. In both models, the uniform spring constant γ used in the vanilla ANM is replaced by direction-dependent spring constants based on the MCP for each residue. Residues with MCP values exceeding a defined threshold, which are considered membrane-contacting residues, are assigned modified spring constants to reflect the resistance exerted by the membrane during protein conformational changes (see Methods for details).

MCP is a predictive metric that quantifies the likelihood of direct interactions between amino acid residues and the acyl chains of lipid molecules [24,25]. It provides a complementary perspective to solvent accessibility, particularly for membrane proteins, whose surface hydrophobicity plays a crucial role. MCP values can be efficiently predicted from amino acid sequences using machine learning models trained on the MemProtMD datasets [28,29] or estimated by calculating the relative lipid accessibility from a known protein structure. Incorporating predicted MCP values into ANM not only introduces membrane-specific constraints but also captures the intrinsic mechanical anisotropy of the membrane environment. Consequently, MCP-based ANM models may provide a more realistic representation of the constraints imposed by the membrane, enabling accurate simulations of protein dynamics in lipid bilayers. This approach also allows for detailed investigations of membrane protein behaviors in diverse mechanical environments, as demonstrated in subsequent sections.

To investigate the gating mechanism of MS ion channels, we applied the MCP-based ANM framework in combination with the PRS method [26] to simulate mechanical stimulation. This integrated approach allowed a systematic analysis of how mechanical stress influences the structural dynamics and activation processes of MS channels. Furthermore, we employed the single exponential decay kernel in the MCP-mANM model, which reduces the stiffness of densely packed intraprotein connections [27]. This adaptation enables the simulation of greater flexibility in membrane proteins and better captures large-scale conformational transitions that are critical for mechanosensitive gating, such as pore expansion under mechanical stress. In contrast, MCP-ANM, although effective in predicting flexibility (as will be demonstrated in

the next section), maintains overly rigid internal connections, which can limit the conformational changes of the pores in response to PRS-applied forces. As our computational results indicated that MCP-mANM more effectively captured the mechanical gating behavior of the MS channels, it was selected for further investigation of the gating mechanisms of the MS channels. The overall workflow of this study is illustrated in Fig. 2.

Despite their structural and functional diversity, MS ion channels universally exhibit a gating mechanism that is activated by mechanical stimuli, leading to the opening of the pore and allowing for ion flux. Two primary models have been proposed to explain the gating mechanisms of MS ion channels:

(1) Membrane tension model (“force-from-lipids” model): This model proposes that mechanical tension or stress imbalance within the lipid bilayer itself triggers conformational changes in the embedded MS channel. When the bilayer is stretched, forces are transmitted directly to the channel through lipid-channel interactions, inducing structural rearrangements that open the pore.

(2) Tether model (“force-from-tether” model): In this model, MS ion channels are directly linked to intracellular or extracellular structural elements, such as the cytoskeleton or extracellular matrix, via springlike tethers. Mechanical forces acting on these tethers induce conformational changes that propagate through the channel, leading to opening of the pores.

Combining MCP-mANM with PRS, we conducted simulations to examine how these two distinct force-transmission mechanisms influence the gating behavior of various MS ion channels. Specifically, we applied forces that mimic lipid bilayer tension or exert forces along the tether to analyze the structural dynamics and transitions to the open state. The results indicate that both gating models can be simulated with our method, and the mechanosensitivity obtained is semiquantitatively consistent with experimental measurements.

B. Theoretical B-factor calculation

B-factors, also known as Debye-Waller factors, quantify the local flexibility of molecules and are commonly used to assess the dynamic properties of proteins [30,31]. For membrane proteins, B-factors obtained from x-ray crystallography reflect the crystalline environment, which often differs from that for soluble proteins due to the use of detergent micelles or lipid cubic phases that shield hydrophobic regions and partially mimic a membrane environment [32,33]. The detergent forms a highly anisotropic belt around the hydrophobic surfaces of membrane proteins rather than an isotropic environment [34–36], so the measured B-factors capture a combined effect of protein flexibility and the anisotropic matrix, effectively representing the impact of membranes. For cryo-EM structures, the absolute B-factor values can be influenced by factors such as map resolution, sharpening, and refinement protocols and thus may not directly correspond to atomic fluctuations [37]. Nevertheless, their relative distributions are generally reliable [38,39]. To ensure the applicability of cryo-EM B-factors in our analysis, we conducted a large-scale statistical comparison of over 1873 membrane proteins.

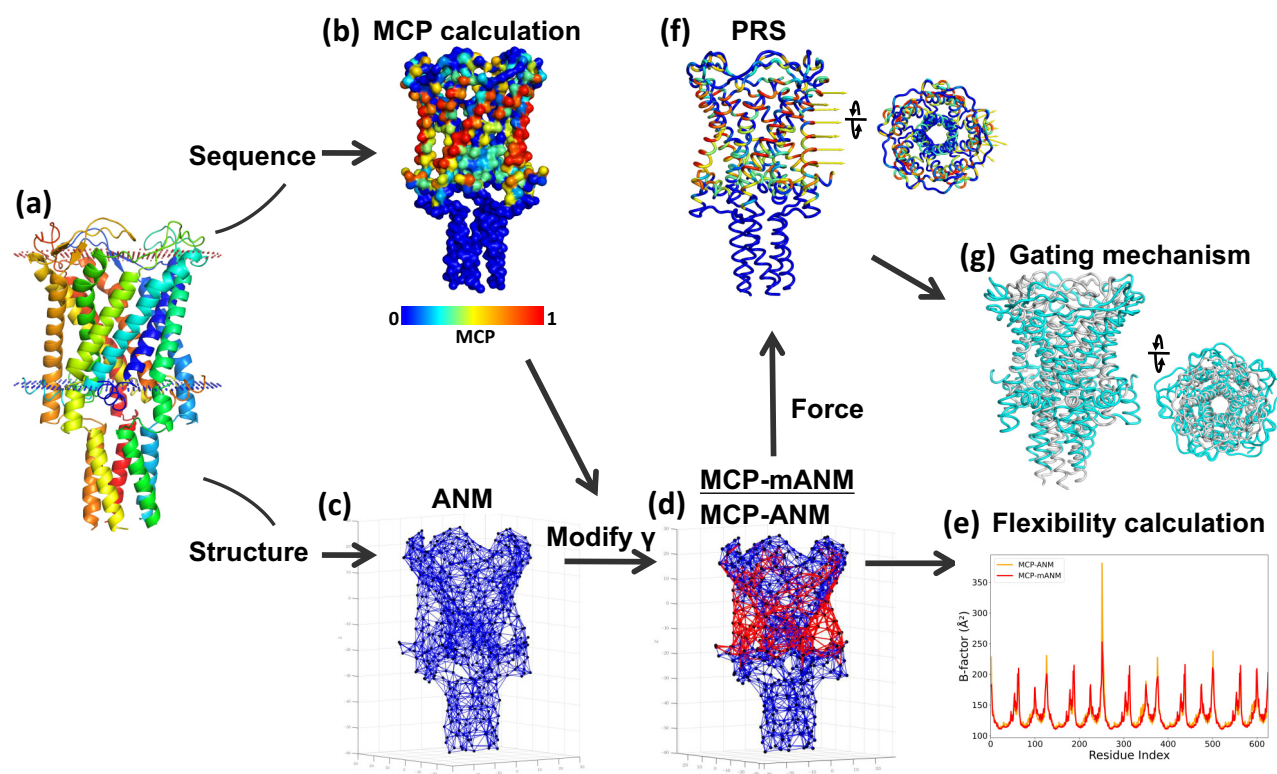


FIG. 2. Workflow of combining MCP-based ANM and PRS to investigate the gating mechanisms of mechanosensitive ion channels. (a) Experimental structure of a MS protein (MscL as an example). (b) Predicted MCP values represented on the protein structure surface. (c) The vanilla ANM model with uniform spring constants (blue network). (d) Transition to the MCP-based ANM model, where spring constants are adjusted based on MCP values. (e) Flexibility profiles calculated using both MCP-ANM and MCP-mANM approaches. (f) PRS analysis using the MCP-mANM model with forces (yellow arrows) simulating membrane tension. For clarity, only the forces on one subunit are shown. (g) The resulting structural transition from closed (gray) to open (cyan) state.

The results show that the distribution range of cryo-EM B-factors closely matches that from x-ray structures, with no systematic over- or under-estimation (see Fig. S1 of the Supplemental Material [40]). Therefore, both x-ray and cryo-EM B-factors are used for evaluating the performance of ANM-based models.

We used Pearson's correlation coefficient (PCC) values between theoretical and experimental B-factors to evaluate the performance of multiple ANMs, including the vanilla ANM, the imANM previously developed by the Bahar group, and our MCP-ANM and MCP-mANM models. Diverse types of membrane proteins collected from the MemProtMD database [28], including ABC transporters, aquaporins, G protein-coupled receptors (GPCRs), ligand-gated ion channels, mechanosensitive ion channels, and outer membrane proteins, were used for evaluation. The results, presented in Figs. 3(a), 3(b), and 3(c), demonstrate that all three methods, MCP-ANM, MCP-mANM, and imANM, consistently outperform vanilla ANM in predicting B-factors across different types of membrane proteins. This suggests that accounting for membrane effects is crucial for accurately modeling the flexibility of membrane proteins. As shown in Fig. 3(d), MCP-ANM and MCP-mANM exhibit highly comparable performance in B-factor prediction, with MCP-ANM performing slightly better overall. Furthermore, Figs. 3(e) and 3(f) compare the performances of MCP-ANM and MCP-mANM with that of

imANM, illustrating the superior performance of MCP-based ANMs over imANM. These findings underscore that MCP-based ANMs not only capture the flexibility of membrane proteins more effectively than vanilla ANM and imANM but also demonstrate robustness and generalizability across diverse membrane protein types. By implicitly incorporating hydrophobic interactions at the lipid interface and the structural constraints imposed by membrane embedding, MCP-based ANMs provide a more accurate and comprehensive approach to modeling membrane protein dynamics compared to the vanilla ANM and imANM models.

As illustrated in Fig. 3(g), the most significant improvements are observed for GPCRs, which are characterized by seven transmembrane helices and play a fundamental role in a wide range of physiological processes [41,42]. Vanilla ANM models, treating proteins as systems in isotropic environments, fail to reproduce the anisotropic environmental effects present on membrane proteins, leading to poor or even negative PCC correlations with experimental B-factors. To further investigate the origins of such discrepancies and clarify the circumstances under which membrane constraints make the largest difference, we examined a representative GPCR system that exhibits pronounced improvement when the MCP-ANM was applied (Fig. 4). This representative case highlights how specific architectural features—such as the asymmetric arrangement of transmembrane helices and

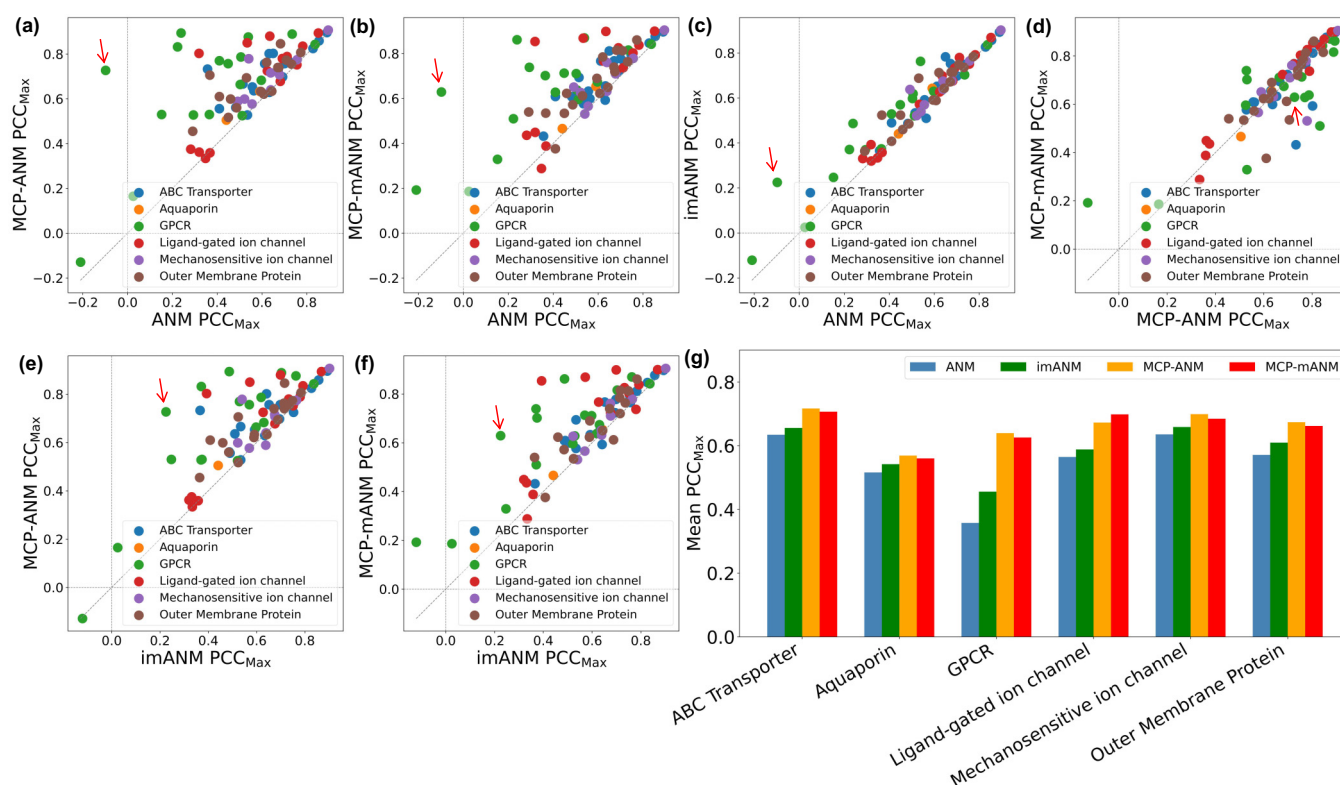


FIG. 3. Comparison of Pearson's correlation coefficients (PCCs) between experimental and theoretical B-factors obtained from four ANM models (ANM, imANM, MCP-ANM, and MCP-mANM) for different classes of membrane proteins. For each protein, the maximum PCC ($PCC_{\max}$) was selected, defined as the highest correlation achieved after systematically scanning the parameter space of each model. [(a)–(f)] Scatter plots comparing $PCC_{\max}$ values between pairs of models: (a) ANM vs MCP-ANM, (b) ANM vs MCP-mANM, (c) imANM vs MCP-ANM, (d) MCP-ANM vs MCP-mANM, (e) imANM vs MCP-ANM, (f) imANM vs MCP-mANM. (g) Bar chart showing the mean $PCC_{\max}$ values averaged over protein classes for the four models. Numerical values of the means are provided in Table S1 in the Supplemental Material [40].

extended extramembrane domains—render the vanilla ANM particularly inadequate, whereas incorporating membrane-contact information restores the expected flexibility patterns. The structure corresponds to the sphingosine 1-phosphate receptor 1, indicated by the red arrow in Figs. 3(a)–3(f) with its

structure shown in Fig. 4(a) (PDB ID: 7VIE). The PCC value obtained with the vanilla ANM was -0.098 , emphasizing the model's inadequacy in capturing membrane-protein interactions. imANM slightly improves the PCC to 0.255 but still falls short of reproducing the flexibility patterns reflected in

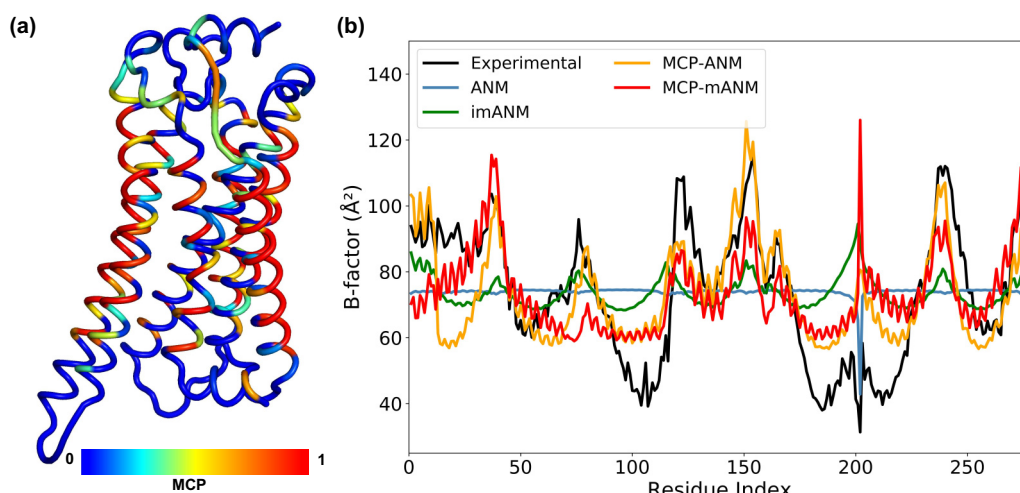


FIG. 4. (a) Structure of sphingosine 1-phosphate receptor 1 (PDB ID: 7VIE), colored according to the predicted MCP of residues. (b) The B-factor curves from experimental data and theoretical predictions calculated by four ANM models.

TABLE I. Comparison of $PCC_{\max}$ values from different ANM models for mechanosensitive ion channels. $PCC_{\max}$ represents the highest Pearson correlation coefficient obtained for each method. The best-performing model for each protein is shown in bold.

Name	PDB ID	ANM	imANM	MCP-ANM	MCP-mANM
MscL	2OAR	0.556	0.570	0.577	0.566
MscS	6PWN	0.639	0.639	0.640	0.632
cASIC1	4NYK	0.678	0.676	0.708	0.710
OSCA1.2	6MGV	0.896	0.902	0.907	0.905
NOMPC	5VQK	0.757	0.765	0.775	0.779
PIEZO1	5Z10	0.522	0.522	0.599	0.627
TREK1	6CQ6	0.492	0.638	0.589	0.651
TREK2	4XDJ	0.639	0.675	0.716	0.761
TRAAK	4WFF	0.541	0.540	0.779	0.531

the experimental B-factors. In contrast, MCP-based ANMs improved PCC to 0.727 (MCP-ANM)/0.629 (MCP-mANM) by incorporating membrane-specific constraints into the modeling process, leading to a more realistic representation of the dynamics of GPCR. Figure 4(b) compares the predicted B-factors from the vanilla ANM, imANM, MCP-ANM, and MCP-mANM models with experimental data. The blue curve represents predictions from the vanilla ANM, which remain nearly flat and do not reflect experimental fluctuations (black curve). The green curve from imANM shows some improvement, but it still lacks accuracy. In contrast, the orange and red curves from MCP-ANM and MCP-mANM predictions align closely with the experimental values, particularly in capturing the peaks and troughs corresponding to the protein's structural dynamics. This case study highlights the structural features that make certain systems benefit more strongly from membrane-constrained models. Importantly, the overall performance comparison across a large and diverse dataset is summarized in Fig. 3(g), demonstrating the general advantage of MCP-based ANMs over the vanilla ANM.

For the MS ion channels of particular interest in this study, MCP-based ANM also shows improvements in the prediction of B-factor, as shown in Table I. The table summarizes the optimal PCC values for various MS ion channels modeled using four ANM models (the results including the optimal parameters and PCC for other membrane proteins are shown in Table S2 in the Supplemental Material [40]). For example, the PCC of TRAAK (PDB ID: 4WFF) increases dramatically from 0.541 with vanilla ANM to 0.779 with MCP-ANM. Similarly, the PCC of PIEZO1 (PDB ID: 5Z10) improves from 0.522 to 0.627 with MCP-mANM. These enhancements underscore the capacity of MCP-based ANMs to more accurately model the membrane environment by accounting for critical interactions between proteins and the membrane, an aspect that is inadequately addressed by vanilla ANM models. Compared to imANM, MCP-based ANMs also show superior performance in most cases (68 of 78 for MCP-ANM and 71 of 78 for MCP-mANM; Table I and Table S1 in the Supplemental Material [40]). This suggests that MCP-based ANMs are more effective than imANM in capturing the influence of membranes on the dynamics of membrane proteins.

By restricting unfavorable motions and incorporating membrane anisotropy, MCP-based ANMs enhance the accuracy of B-factor predictions and more effectively represent the dynamics of membrane proteins. However, the rigid internal connections present in MCP-ANM limit its applicability to large conformational changes induced by membrane tension. In contrast, the MCP-mANM offers a more flexible structure and a more accurate representation of large conformational changes and was therefore chosen for subsequent analyses of the gating mechanisms of MS ion channels.

C. Semiquantitative analysis of known MS ion channels

To validate the accuracy of our predictions regarding the gating behaviors of MS channels, we applied normalized force vectors to a subset of MS channels whose gating mechanisms are primarily driven by membrane tension-induced pore dilation. We then quantitatively evaluated changes in the diameter of the gate, a critical indicator of gating behavior. In our calculations, forces and tensions are expressed in arbitrary units (a.u.). According to the foundational work by Bahar and colleagues [22], one a.u. of force corresponds to approximately 70 pN in our calculations, and one unit of membrane tension corresponds to approximately 700 mN/m. Although this calibration enables comparisons with experimental gating thresholds in absolute terms (Table S4 in the Supplemental Material [40]), the primary focus should be on the relative sensitivities due to the semiquantitative nature of the model.

Figure 5 illustrates the progressive structural deformations of the MS channels in response to increasing membrane tension. Notably, under a certain membrane tension, PIEZO exhibits the most pronounced pore dilation, characterized by an outward displacement of the V2476 region, which aligns with its high mechanosensitivity. MscS exhibits moderate pore dilation, with an evident displacement of residue A102, while MscL shows relatively minor pore expansion, indicated by a slight displacement of residue L17. These findings highlight the varying mechanical sensitivities among the MS channels examined.

For a consistent comparative analysis of the dilated conformations of these channels, we selected a representative deformed structure for each system at the point where the change in pore diameter (ΔR) reaches 1.0 Å, as indicated by the magenta dashed line in Figs. 5(a)–5(c). This criterion allows a fair comparison across channels that differ in mechanical compliance. Specifically, the corresponding membrane tensions are 0.2 a.u. for PIEZO, 1.0 a.u. for MscS, and 3.0 a.u. for MscL. At these conditions, all channels exhibit evident conformational changes, establishing a clear relationship between applied membrane tension and gating behavior. Furthermore, the calculated ΔR values increase with tension, and the relative sensitivities—derived from the slopes of the corresponding ΔR —tension curves—follow the order $\text{PIEZO} > \text{MscS} > \text{MscL}$, consistent with experimental observations [43]. This agreement underscores the capability of our MCP-mANM model, combined with the PRS method, to capture the mechanical sensitivity and gating behavior of mechanosensitive channels.

To validate the specificity of our model, we used the bacterial potassium channel KcsA (K^+ channel of *Streptomyces*

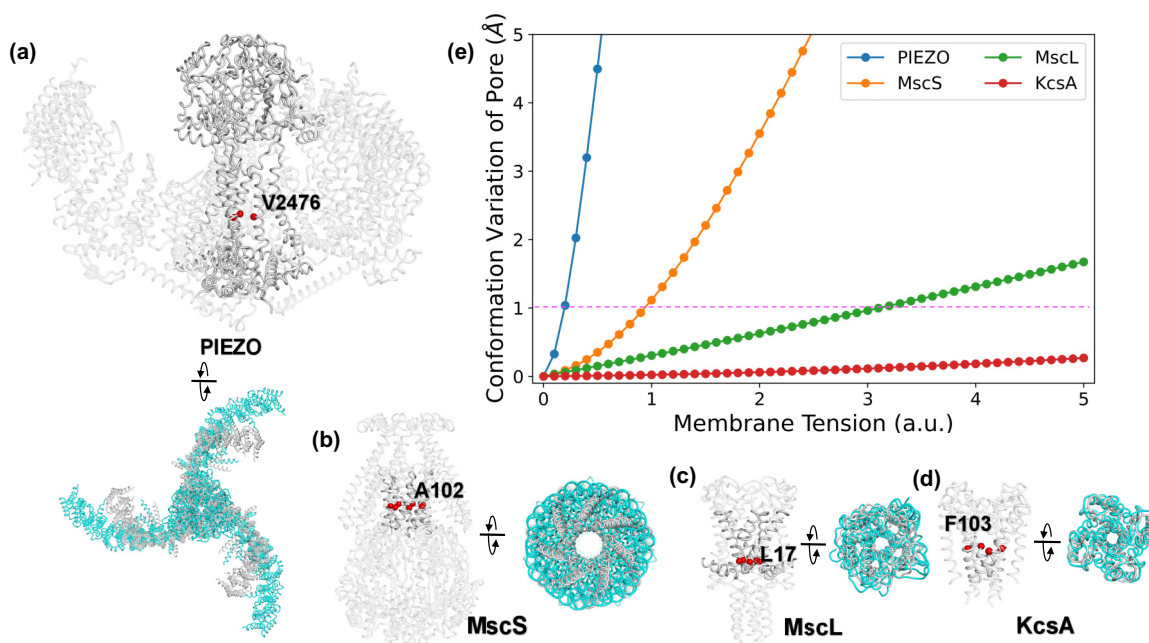


FIG. 5. Response of MS channels to applied tension. Gray structures indicate the initial state, and cyan structures show the conformation under membrane tension. For PIEZO, MscS, and MscL [(a)–(c)], the conformations correspond to the magenta dashed line in panel (e), where the pore diameter change (ΔR) reaches 1.0 Å. For KcsA (d), the conformation corresponds to a membrane tension of 5 a.u. Pore gate residues used for analysis are highlighted in red: V2476 (PIEZO), A102 (MscS), L17 (MscL), and F103 (KcsA). (e) Pore diameter variation under increasing membrane tension (a.u.) shows PIEZO (blue) with the largest response, followed by MscS (orange), MscL (green), and KcsA (red). Tension values are given in arbitrary units.

lividans, PDB ID: 1BL8) [44] as a negative control, as it is known to be insensitive to membrane tension. As illustrated in Fig. 5(e), KcsA exhibited negligible conformational variation in pore size across all levels of applied membrane tension. The structure shown in Fig. 5(d), representing the highest applied tension (5 a.u.), illustrates the minimal changes in distance at its narrowest constriction site near residue F103 [45]. The minimal conformational changes observed in KcsA, in contrast to the MS channels, supports that the combination of MCP-mANM with the PRS method specifically detects force-induced gating behaviors in MS ion channels.

However, it should be noted that because of the coarse-grained nature of MCP-mANM, atomic-level details, such as precise pore sizes, remain beyond our scope. Consequently, the analysis is semiquantitative and primarily focuses on the overall trends in conformational changes rather than absolute values.

D. Membrane tension-gated ion channels: Case studies on MscS and PIEZO

To observe the gating transitions of MS proteins under mechanical stimuli and further demonstrate the applicability of the MCP-mANM model within the membrane tension-gated model, we selected MscS [46] and PIEZO [47] channels as representative examples. Both of these MS ion channels exhibit distinct gating behaviors when subjected to membrane tension. For further analysis, we selected conformations that exhibit an approximate 1 Å increase in pore radius; that is, the PIEZO structure under a membrane tension of 0.2 a.u. and the MscS structure at 1 a.u., as shown in Fig. 5. This

selection ensures meaningful conformational changes while minimizing the risk of excessive structural distortion caused by overstretching.

MscS undergoes significant conformational rearrangements during gating in the presence of membrane tension [48]. Experimental studies, including cryo-EM, have identified two primary structural transitions that characterize MscS gating in response to mechanical stress [49]:

(1) Pore opening, which represents the conformational transition of the channel from a closed state to an open state, facilitating ion flow. This transition is highly dynamic and allows MscS to function as a sensor and regulator in cellular mechanotransduction pathways.

(2) Rearrangements of transmembrane helices, which are essential for accommodating mechanical strain induced by membrane tension. Specifically, structural studies and computational simulations showed that, upon the application of tension, the transmembrane helices of MscS rotate outward, causing pore dilation [49,50]. This expansion allows for a greater flow of ions through the pore.

Our simulations successfully captured the transmembrane helical rearrangements of MscS under tension. As shown in Fig. 6(a), the MscS structure is colored according to the MCP, highlighting regions with direct membrane interactions. Figure 6(b) illustrates the forces applied on one of the MscS chains, indicated by yellow arrows. Figure 6(c) provides a structural comparison of MscS before (gray) and after (cyan) the application of membrane tension, revealing significant conformational changes. Figures 6(d) and 6(e) further detail these changes: Figure 6(d) shows the rotation of the

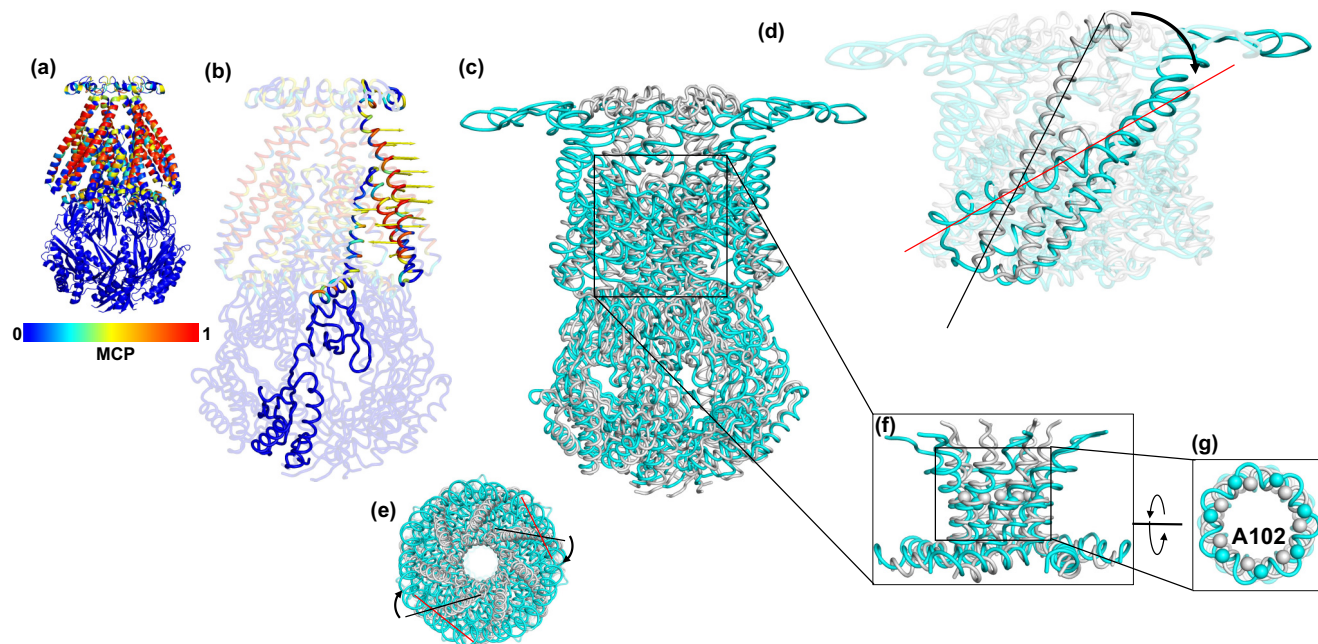


FIG. 6. Structural changes in MscS under membrane tension. (a) MscS structure colored according to MCP. (b) The applied force on MscS is represented by a yellow arrow on one of the chains. (c) Structural comparison of MscS before (gray) and after (cyan) the application of membrane tension in our calculation. (d) Rotation of transmembrane helices in response to applied tension. (e) Top view showing the rotation and structural changes of MscS upon membrane tension, with the N-terminal of 20 residues omitted for clarity of the helical movements. (f) Zoom-in on the pore-lining helices, showing their repositioning under force. (g) Top view of the pore region, illustrating the radial displacement and dilation of the channel. The spheres represent residue A102.

transmembrane helices in response to applied tension, while Fig. 6(e) offers a top view of the structural changes, highlighting the helical rearrangements and the dilation of the pore. Figure 6(f) presents a zoomed-in view of the pore-lining helices, emphasizing their repositioning under force, and Fig. 6(g) illustrates a top view of the pore region, showing the radial displacement and further dilation of the channel. These simulation results clearly demonstrate the rotation of the transmembrane helices, which is closely aligned with experimental cryo-EM data [49]. This finding validates the reliability and accuracy of our model in reproducing the gating dynamics of MS ion channels at the molecular level.

Another representative case is PIEZO, an MS ion channel that plays a pivotal role in the cellular response to mechanical stimuli. PIEZO channels are involved in various physiological processes, including touch sensation, blood pressure regulation, and organ development [51]. These channels are characterized by their unique structural design, which includes a large curved architecture that allows significant flexibility in response to mechanical forces [52]. Upon membrane tension, PIEZO undergoes large-scale conformational changes, facilitating its gating process and enabling ion permeation [47]. Unlike MscS, the gating of PIEZO involved much larger conformational changes. Upon application of membrane tension, the highly curved dome-shaped architecture of PIEZO flattens, transitioning to a more planar form [47]. This structural reorganization is accompanied by substantial rearrangements in the protein domains. Specifically, the caplike domains at the apex of the protein rotate outward, leading to the widening of the central pore [53]. This shape change is believed to facilitate the passage of ions, as the

widened pore allows greater ion permeability, consistent with experimental findings from cryo-EM studies [54]. Previous studies have provided snapshots of the PIEZO's gating process, revealing that membrane tension directly induces the flattening of the PIEZO structure, facilitating its transition from a closed state to an open state [47,55].

In our simulations, we successfully reproduced the large conformational changes observed in the gating of PIEZO. Figures 7(a) and 7(b) show the residues in contact with the membrane and the force applied to one of the PIEZO chains, respectively. Under simulated membrane tension, the PIEZO channel undergoes a gradual flattening process, with the top “cap” regions rotating outward, as indicated by the red arrows in Figs. 7(c) and 7(d). This flattening process is essential for the gating mechanism [54]. As shown in Fig. 7(c), the side view reveals the structural changes from a curved to a flattened state, as well as the outward of the cap regions, leading to the widening of the central pore. Figure 7(d) provides a top view that further demonstrates the complete domain rearrangements, particularly highlighting the rotational changes. In addition, Fig. 7(e) presents a zoomed-in view of the pore region, illustrating the conformational shift of pore-lining helices under tension, while Fig. 7(f) offers a top view that depicts the radial displacement and dilation of the channel. These structural changes indicate the opening of the pore in response to membrane tension, which is in good agreement with experimental observations and cryo-EM data [54]. These results highlight the accuracy and predictive capability of our computational approach in capturing the large-scale conformational transformations that are integral to the gating mechanism of PIEZO.

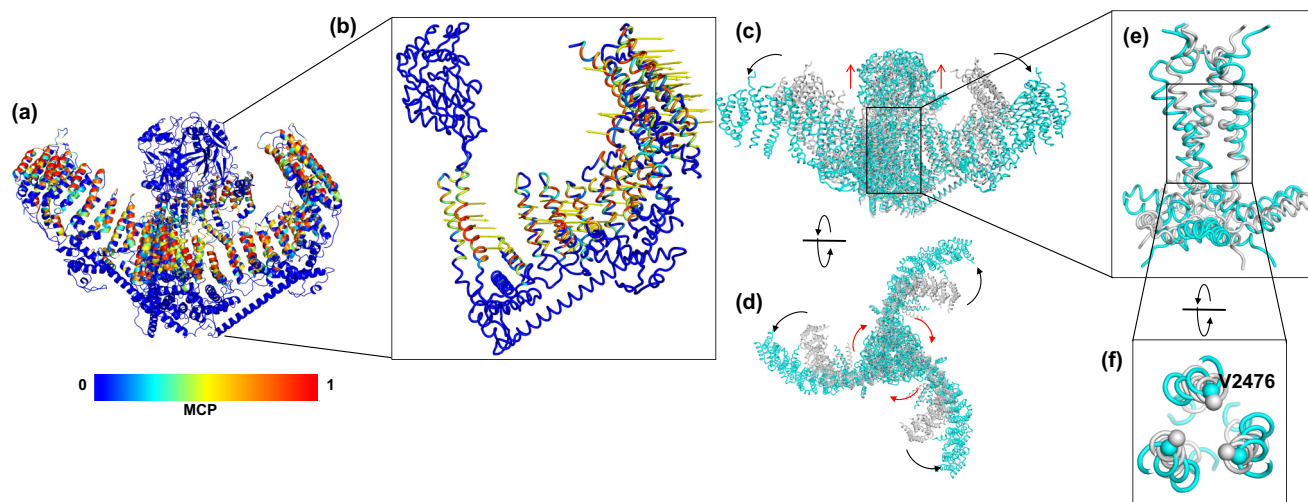


FIG. 7. Structural changes of PIEZO under membrane tension in our calculation. (a) PIEZO structure colored according to the predicted MCP. (b) The forces applied on PIEZO, depicted by yellow arrows on one chain. (c) Side view of the structural changes from a curved to a more flattened state and (d) corresponding top view. The red arrow indicates the changes of the top “cap.” (e) Zoom-in on the pore region, showing the conformational shift under tension. (f) Top view of the pore region, illustrating the radial displacement and dilation of the channel.

In addition to the qualitative comparisons above, we further performed quantitative analyses for channels with both closed and open experimental structures (summarized in Table S3 and depicted in Figs. S2–S3 in the Supplemental Material [40]). For MscS, we compared the closed structure (6PWN) with the open state in nanodiscs (8DDJ). Following prior work [56], we quantified the tilt angles of the pore-lining helices TM1 and TM2 relative to the membrane normal, using the closed state as the 0° reference. Experimentally, TM1/TM2 tilt angles reach $45.97^\circ/43.89^\circ$ in the open state; our PRS-induced open state yields $48.52^\circ/35.25^\circ$, approaching the experimental magnitudes while deviating from the closed reference. For PIEZO, we compared the closed trimer (5Z10) with the tension-flattened conformation (7WLU), generally regarded as an openlike state [54]. We quantified (i) the projected membrane footprint radius and (ii) the blade rotation about the central hub (with the closed state set as 0°). The experimental open state shows a radius increase from $96.50 \text{ \AA}$ to $116.57 \text{ \AA}$ and a blade rotation of 7.11° ; our PRS-induced open state gives $123.00 \text{ \AA}$ and 7.43° , respectively, indicating a further flattening/expansion consistent with PIEZO gating signatures. In summary, the PRS-induced open conformations resemble the experimental open states in these quantitative metrics, supporting that our protocol not only drives gating-related transitions but also reproduces key structural signatures of the open conformations.

E. Tether-gated ion channels: NOMPC as an example

To evaluate the applicability of our method to the tethered mechanosensation model, we selected NOMPC, a member of the transient receptor potential (TRP) ion channel family [57], as a representative example. NOMPC is characterized by extended ankyrin repeat (AR) domains that serve as force sensors, linking external mechanical stimuli to the channel’s pore region. This structural configuration facilitates a “push-to-open” or “twist-to-open” gating mechanism,

wherein mechanical forces applied to the AR domains are transmitted through the TRP domain to the pore-lining helices (S6), thereby inducing channel opening [15,16,58]. Previous studies have shown that compressing the AR domains results in clockwise rotation of the TRP domain, leading to pore opening [15,16,58]. Based on this, we applied upward pushing forces to the AR1-8 domain of NOMPC and analyzed the resulting conformational changes.

As illustrated in Fig. 8, the application of an upward push force to the ankyrin repeat (AR1-8) domain of NOMPC resulted in a series of structural transformations that align with the tether-gating model. The structure shown in Fig. 8(a) correspond to the conformation obtained at a force magnitude of 0.4 a.u. , as indicated along the x axis in Figs. 8(d)–8(g). The initial mechanical stimulus induced compression within the AR domains [Figs. 8(a) and 8(d)], resulting in a cascade of deformations that propagated to the TRP domain. This force transmission triggered a clockwise rotation of the TRP domain about the channel’s central axis [Figs. 8(c) and 8(e)], which serves as a crucial intermediate step in converting linear mechanical force into rotational movement. The rotational motion of the TRP domain induces a progressive tilt in the S6 helices [Figs. 8(b) and 8(f)], which comprise the channel pore, resulting in an increase in pore diameter (the Ca-Ca distance of opposite I1554 residues that form the narrowest part of the gate) [Fig. 8(g)]. This sequential propagation of force and deformation, originating from the AR domains to the TRP domain and ultimately to the pore-lining helices, underscores the intricate compress-twist coupling mechanism that drives mechanosensitive gating in NOMPC.

These results underscore the critical role of AR domains in the gating mechanism of NOMPC. By functioning as initial force sensors, the AR domains transmit mechanical forces to the TRP domain, thereby facilitating pore opening. This supports the hypothesis that the coupling between AR and TRP domains is essential for NOMPC’s mechanosensitivity, especially regarding its structural adaptations in response to

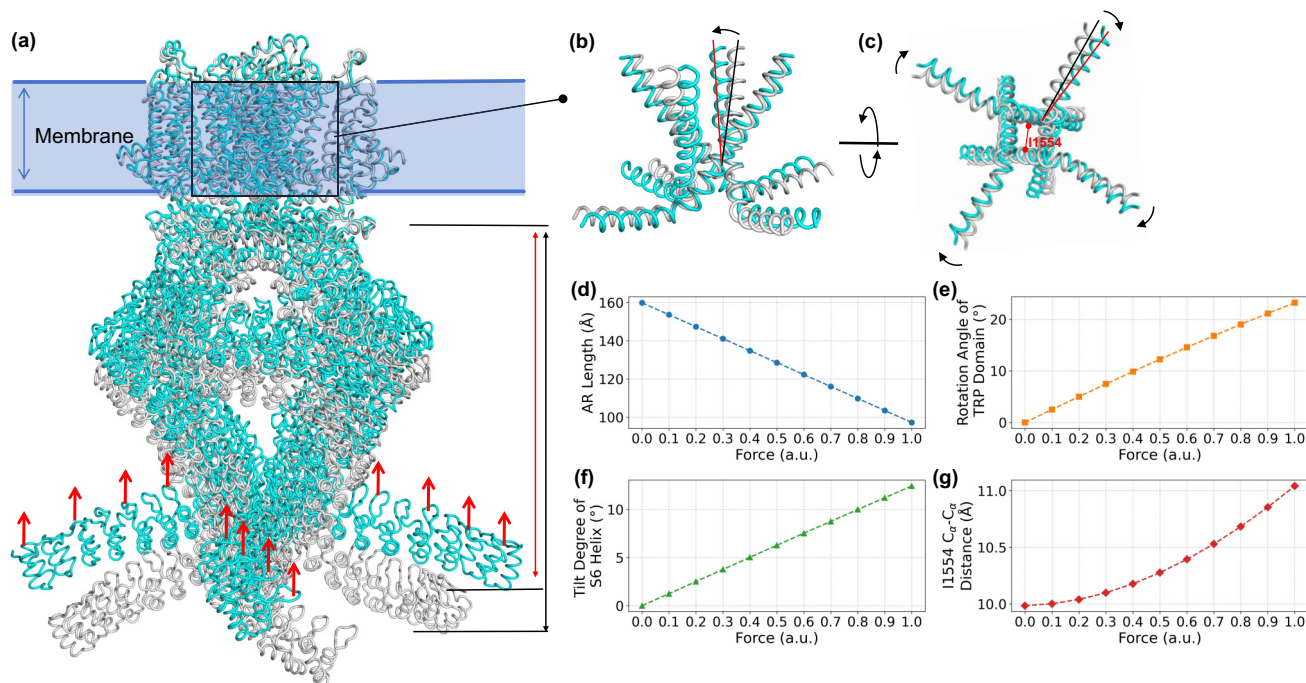


FIG. 8. Structural changes during the push-to-open gating process of NOMPC. (a) Overall structural changes with the initial state shown in gray and the deformed state in cyan. The cell membrane is represented by a light blue strip. The red arrows indicate the direction of the applied forces. (b) The S6 helix slightly tilts downward. (c) The TRP domain rotates clockwise (viewed from the intracellular side) under the torque generated by the pushing force. [(d)–(g)] The structural changes as a function of applied forces, illustrated by the length of the AR domain (d), the rotation angle of the TRP domain viewed from the intracellular side (e), the tilt degree of the S6 Helix (f), and the pore size represented by the distance between two C α atoms of the I1554 residues on the two opposite subunits (g).

mechanical stimuli [57]. Moreover, these results demonstrate the broader applicability of our approach, with both membrane tension- and tether-gating mechanisms being nicely accounted for.

III. DISCUSSION

The computational study of the large-scale dynamics of membrane proteins remains challenging, particularly when external stimuli such as mechanical forces are involved. Conventional coarse-grained methods often omit the influence of the membrane environment on proteins or build unrealistic models to account for the membrane effects, making it difficult to accurately capture the dynamic properties of membrane proteins within their membrane surroundings. By integrating predicted MCP values into ANMs, our method significantly enhances its predictive accuracy, establishing MCP-based ANMs as robust tools for investigating the dynamics of membrane proteins. This integration provides a more realistic representation of the behavior of membrane proteins under the influence of the membrane environment, enabling efficient analysis of their conformational flexibility.

The PRS method based on MCP-mANM introduces a new approach to quantifying the mechanical force responses of mechanosensitive ion channels. In contrast to previous applications of the PRS method, which perturbed all residue positions to identify those critical for overall protein function and conformation, our strategy begins with known functionally significant residues located on the proteins. By applying

perturbations or mechanical forces to these membrane-contacting or force-acted residues, we can efficiently simulate how these mechanical stimuli drive conformational changes. This approach shifts the focus from merely identifying key residues to exploring underlying functional mechanisms, providing new insights into the gating mechanisms of MS ion channels.

Our method's capacity to replicate actual membrane tension by applying forces to membrane-contacting residues enables it to capture the intrinsic dynamics of MS ion channels within their native environment. This capability is essential for accurately reproducing the gating mechanisms observed in experimental studies. For instance, our simulations of MscS and PIEZO channels successfully replicated their large-scale conformational changes in response to membrane tension, demonstrating the method's accuracy and predictive power. Meanwhile, for tether-gated MS ion channels such as NOMPC, our simulations also revealed the gating processes and detailed conformational changes, further validating the robustness of our approach.

This versatility underscores the potential of our methodology to investigate the gating mechanisms of other unknown or less-characterized MS channels, offering valuable insights into their functional regulation and role in cellular physiology. In addition to these advantages, our method is computationally efficient and significantly faster than atomistic as well as coarse-grained MD simulations. For example, all our computational experiments were conducted on a workstation equipped with an Intel Xeon W-2255 CPU operating at

3.70 GHz (10 physical cores and 20 threads) and 64 GB of RAM. To ensure accurate and consistent timing, computations were executed in a single-threaded environment. We evaluated the scalability of our MCP-mANM method using three protein structures of increasing size: MscL (PDB ID: 2OAR, 625 residues), MscS (PDB ID: 6PWN, 1960 residues), and NOMPC (PDB ID: 5VKQ, 5916 residues). The corresponding computation times were 1.45, 21.42, and 258.27 s for the MATLAB version, and 6.06, 76.21 and 722.28 s for the Python version, respectively. This speed enables the rapid elucidation of mechanosensitive proteins, particularly the gating mechanisms of MS ion channels, establishing it as a powerful tool for high-throughput investigations. All results for additional MS ion channels, along with a detailed comparison of wall-clock running times across different MCP-ANM models, are summarized in Table S5 in the Supplemental Material [40].

Despite its strengths, our MCP-based ANM method has several limitations. First, as a coarse-grained approach, it lacks atomistic details, which is a common drawback of ANM-based models. This limitation may restrict its ability to accurately capture fine-scale molecular interactions, such as the quantitative deformation of the pore or specific lipid-protein contacts. Second, the performance of the method is based on the accuracy of the MCP predictions. Although MCP predictions are generally reliable [24,25], errors in these predictions may still impact the overall results, particularly for proteins that exhibit atypical membrane interactions. This limitation can be mitigated by combining other methods, such as structure-based analysis and coarse-grained MD simulations. Nevertheless, MCP-based ANM remains a rapid and effective tool for elucidating the gating mechanisms of MS ion channels, providing valuable insights into the dynamics of membrane proteins. For comparison, we also evaluated the ability of imANM to reproduce membrane tension—and tethering force—induced conformational changes in the same set of mechanosensitive channels (see Figs. S4–S5 [40]). In general, imANM reproduced only limited or localized deformations, showing restricted motions in the transmembrane region and minimal pore expansion in MscS, MscL, and PIEZO due to excessive core rigidity, and markedly smaller conformational changes in NOMPC. These results reflect the overly rigid nature of the cutoff-based stiffness modulation in imANM. In contrast, MCP-mANM yielded more cooperative and experimentally consistent gating motions, indicating that it provides a more physically accurate framework by combining membrane contact with exponential decay kernels. This enables larger internal flexibility of proteins and more realistic membrane-mediated conformational changes.

Building upon these findings, the predictive nature of our MCP-based mANM framework opens the door for broader applications and validations. For example, our approach can be further extended and validated through large-scale proteomic analyses and experimental collaborations. We are currently applying our model, in combination with the AlphaFold structural database, to screen mechanosensitive membrane proteins across the human proteome. This integrative strategy enables systematic identification of potential mechanosensitive candidates and characterization of their structural and

dynamic properties. Promising targets will then be subjected to experimental validation to verify the predicted mechanical mechanisms. Such computational-experimental synergy holds great potential for bridging molecular modeling with experimental discoveries and advancing the functional annotation of mechanosensitive membrane proteins.

In summary, this study introduces a new computational framework for investigating the dynamic properties of membrane proteins. The integration of MCP data into ANM and the refinement of PRS significantly enhance the predictive capabilities of these methodologies, thereby advancing the field of membrane protein dynamics research. By providing a more realistic and efficient approach to studying MS ion channels, our methodology facilitates faster and deeper insights into their functional mechanisms, supports high-throughput MS protein investigation and offers potential applications in biomedical research.

IV. METHODS

A. Dataset

To evaluate the performance of MCP-based ANM for membrane proteins, we compiled a dataset of membrane proteins from the MemProtMD database [28], a database generated through molecular dynamics simulations of membrane proteins with known structures. These proteins were classified according to the MemProtMD scheme, with an additional category for MS proteins, resulting in a total of six distinct categories and 2090 membrane proteins. The membrane protein structures were then selected based on the following criteria: resolution < 4.0 Å and exclusion of structures derived from NMR data. Ultimately, we obtained a total of 1873 membrane proteins.

To minimize redundancy and enhance the diversity of the dataset, we conducted structure clustering using the Foldseek tool [59,60], ultimately selecting 78 representative structures. The clustering was carried out to ensure that the structural overlap among the chosen proteins did not exceed 90%, while the structural similarity (E-value) between the groups remained below 0.01. A comprehensive list of the final selected structures is provided in Table S2 in the Supplemental Material [40].

B. MCP prediction

The MCP predictor was employed to quantify the likelihood of residue-membrane contacts with 6 Å as the distance cutoff, which requires only the protein sequence in FASTA format as input [24,25]. For a given protein sequence, the MCP predictor outputs per-residue values on a [0,1] scale, with increasing values corresponding to stronger predicted contact probabilities with the lipid membrane. Recently, we developed a new version of the transformer-based MCP predictor, which uses language model embeddings as input features, offering significantly enhanced speed and accuracy [25]. In this study, we employ this updated version to perform the MCP predictions. The source code for our MCP predictor is publicly available [61]. An online computation server is available for user access [62].

C. ANM

The ANM is a simplified molecular dynamics approach utilized for the investigation of protein dynamics. In conventional ANM, a protein structure is represented as an elastic network composed of selected atoms (here C_α). In this network, pairs of atoms/nodes that are within a specified cutoff distance (r_c) are interconnected by springs, each characterized by a uniform force constant [20]. The total potential energy of the network can be written as follows:

$$V_{\text{ANM}} = \frac{1}{2}\gamma \sum_{i,j}^N (R_{ij} - R_{ij}^0)^2, \quad (1)$$

where R_{ij} and R_{ij}^0 refer to the instantaneous and equilibrium distances between node i and j , respectively. The dynamic properties of proteins are determined by a Hessian matrix $\mathbf{H}$, whose elements are 3×3 submatrices. The submatrix $\mathbf{h}_{ij}$ is calculated as the matrix of second-order derivatives of the potential with respect to the Cartesian coordinates of the nodes. When $i \neq j$, the corresponding $\mathbf{h}_{ij}$ is as follows:

$$\mathbf{h}_{ij} = \begin{pmatrix} \frac{\partial^2 V}{\partial X_i \partial X_j} & \frac{\partial^2 V}{\partial X_i \partial Y_j} & \frac{\partial^2 V}{\partial X_i \partial Z_j} \\ \frac{\partial^2 V}{\partial Y_i \partial X_j} & \frac{\partial^2 V}{\partial Y_i \partial Y_j} & \frac{\partial^2 V}{\partial Y_i \partial Z_j} \\ \frac{\partial^2 V}{\partial Z_i \partial X_j} & \frac{\partial^2 V}{\partial Z_i \partial Y_j} & \frac{\partial^2 V}{\partial Z_i \partial Z_j} \end{pmatrix}. \quad (2)$$

When $i = j$, the submatrix is $\mathbf{h}_{ij} = -\sum_{i \neq j} \mathbf{h}_{ij}$.

The dynamical properties can be determined using the pseudoinverse of the Hessian matrix, as detailed in the subsequent section regarding the calculation of B-factors.

D. Multiscale ANM

In the mANM approach, interactions across multiple scales are typically modeled using different exponential decay kernel functions to accurately capture both short- and long-range couplings [27]. In our study, we adopted a simplified variant that employs only a single exponential decay kernel function. While this reduction in complexity limits the capability to model hierarchical interactions, it proves advantageous in our MCP-mANM (refer to the next section). This simplification not only streamlines parameter tuning but also better preserves the essential internal dynamics relevant to mechanosensitive gating. Specifically, it alleviates excessive rigidity within the protein interior that results from dense local packing, thereby enabling more realistic conformational transitions in response to mechanical stress.

In mANM, the interaction strength between nodes i and j is defined by:

$$\Phi(R_{ij}, \eta, \kappa) = \exp\left(-\left(\frac{R_{ij}}{\eta}\right)^\kappa\right), \quad \kappa > 0, \quad (3)$$

where R_{ij} is the distance between the i th and j th nodes, and the parameters η and κ control the decay extent.

When $i \neq j$, the submatrix corresponding to the (single) kernel function is given by

$$[h^{\text{mANM}}]_{ij} = \Phi(R_{ij}, \eta, \kappa) h_{ij} \quad (4)$$

and for $i = j$, the diagonal element is computed as

$$[h^{\text{mANM}}]_{ii} = -\sum_{j \neq i} [h^{\text{mANM}}]_{ij}. \quad (5)$$

This formulation allows us to attenuate the contribution of strong near-range interactions directly through the kernel function, thereby reducing the stiffness of the network and enabling the simulation of large-scale conformational transitions that are essential for mechanosensitive channel gating.

E. MCP-ANM and MCP-mANM

We modified the vanilla ANM and mANM by replacing the uniform spring constant γ with direction-dependent spring constants $\gamma_x, \gamma_y, \gamma_z$. For residues with MCP values exceeding a certain threshold, the new spring constants were adjusted to reflect the influence of the membrane environment, as shown in Eq. (6).

$$\begin{aligned} \frac{\partial^2 V}{\partial X_i \partial X_j} &= \frac{-(X_j - X_i)(X_j - X_i)\sqrt{\gamma_x}\sqrt{\gamma_x}}{R_0^2} \\ \frac{\partial^2 V}{\partial X_i \partial Y_j} &= \frac{-(X_j - X_i)(Y_j - Y_i)\sqrt{\gamma_x}\sqrt{\gamma_y}}{R_0^2} \\ \frac{\partial^2 V}{\partial X_i \partial Z_j} &= \frac{-(X_j - X_i)(Z_j - Z_i)\sqrt{\gamma_x}\sqrt{\gamma_z}}{R_0^2}. \end{aligned} \quad (6)$$

This modification enables independent adjustment of force constants along each Cartesian coordinate, facilitating a more precise simulation of protein dynamics within the membrane environment. Specifically, the membrane resistance to protein motion was modeled through two scaling factors: $\gamma_x = \gamma_y = s_1 > 0$: A scaling factor for radial motions (in the x and y directions), which captures the membrane's resistance to lateral displacements of the protein, simulating the constraining effect of the lipid bilayer on the protein's movement within the plane of the membrane. $\gamma_z = s_2 > 0$: A scaling factor for motions normal to the membrane (along the z direction), which accounts for the additional resistance experienced by the protein when attempting to move across the lipid bilayer, as the membrane is more resistant to vertical movement.

These modifications enable us to model the perturbations in protein dynamics that result from the physical properties of the membrane, offering a more precise representation of the behavior of membrane proteins in their natural environment.

F. Calculation of B-factors based on ANMs

In ANMs, the dynamic properties can be expressed as

$$\langle \Delta \mathbf{R}_i \cdot \Delta \mathbf{R}_i \rangle = \frac{k_B T}{\gamma} (\mathbf{H}_{3i-2,3i-2}^{-1} + \mathbf{H}_{3i-1,3i-1}^{-1} + \mathbf{H}_{3i,3i}^{-1}). \quad (7)$$

The pseudoinverse of the Hessian matrix can be decomposed as

$$\mathbf{H}^{-1} = \sum_{k=7}^{3N} \lambda_k^{-1} \boldsymbol{\mu}_k \cdot \boldsymbol{\mu}_k^T, \quad (8)$$

where λ_k and $\boldsymbol{\mu}_k$ are the k th eigenvalue and eigenvector of the Hessian matrix, respectively. According to the Debye-Waller

theory, the theoretical B-factor for the i th node can be calculated using the following expression:

$$B_i^t = \frac{8\pi^2}{3} \langle \Delta \mathbf{R}_i \cdot \Delta \mathbf{R}_i \rangle. \quad (9)$$

The PCC was used to evaluate the degree of correlation between theoretical and experimental B-factors, which is given by

$$\text{PCC} = \frac{\sum_{i=1}^N (B_i^t - \bar{B}^t) \cdot (B_i^e - \bar{B}^e)}{\sqrt{\sum_{i=1}^N (B_i^t - \bar{B}^t)^2} \cdot \sqrt{\sum_{i=1}^N (B_i^e - \bar{B}^e)^2}}, \quad (10)$$

where B_i^t and B_i^e are the theoretical and experimental B-factors for the i th node, respectively, and $\bar{B}^t$ and $\bar{B}^e$ are their mean values. The PCC ranges from -1 to 1 , with a value of 1 indicating perfect correlation and a value of -1 indicating perfect anticorrelation.

It should be noted that for the vanilla ANM, imANM, MCP-ANM, and MCP-mANM models, optimized parameters were determined to construct the corresponding ANM models by maximizing the PCC value between the theoretical and experimental B-factors for each protein. We used our own reproduced code for these calculations and, to better account for the flexibility of each residue, the rotational translation block method [63] was not employed in the imANM model. During this optimization process, the cutoff distance was systematically varied within the range of [8 Å, 20 Å] with a step size of 1 Å. In the case of imANM, the model defined the spring constants in the xy plane as multiples of the spring constant in the z direction, which was fixed at a value of 1 by default in the program [23]. Consequently, the multiplier for the x - y spring constant (relative to the z direction) was varied across the values [1, 2, 4, 8, 16, 32, 64]. For the MCP-based ANM, the MCP cutoff parameter was systematically varied in the range of [0.1, 0.9] with a step size of 0.1, while the parameters s_1 and s_2 were varied in the range of [1, 2, 4, 8, 16, 32, 64]. For MCP-mANM, the parameter η varied in the range of [1, 8] and the parameter κ varied in the range of [1, 3] with a uniform step size of 1.

G. Perturbation response scanning approach

The PRS approach [26] based on linear response theory [64] was developed to elucidate the allosteric properties of proteins. This method enables the calculation of the response of a residue k to a perturbation applied to another residue i . The $3N$ -dimensional vector $\Delta \mathbf{R}$ of node displacements in response to the exertion of a perturbation ($3N$ -dimensional force vector $\mathbf{F}$) obeys Hooke's law $\mathbf{F} = \mathbf{H} \Delta \mathbf{R}$, where $\mathbf{H}$ is the $3N \times 3N$ Hessian matrix in the ANM theory. The force exerted on the residue i is written as follows:

$$\mathbf{F}^i = (000 \dots \Delta F_x^i \Delta F_y^i \Delta F_z^i \dots 000)^T \quad (11)$$

and the resulting response is as follows :

$$\Delta \mathbf{R}^i = \mathbf{H}^{-1} \mathbf{F}^i, \quad (12)$$

where $\Delta \mathbf{R}^i$ is a $3N$ -dimension vector describing the displacements of all residues away from their equilibrium positions in response to the exerted force $\mathbf{F}^i$, which are nonzero only for the three terms related to residue i .

The parameters of the ANM-based models (including ANM, imANM, MCP-ANM, and MCP-mANM) were optimized by maximizing the PCC between theoretical and experimental B-factors. While the PCC optimization ensures that the relative distribution of residue fluctuations is well reproduced, it does not guarantee the correctness of the absolute magnitude of these fluctuations. Since PRS-predicted displacements are directly proportional to the eigenvalues of $\mathbf{H}$, an unscaled Hessian matrix may yield displacement amplitudes that are physically unrealistic and not comparable across different proteins.

To address this, we defined a global scaling factor s following the standard procedure introduced by Bahar and coworkers [22,65,66]:

$$s = \frac{\sum_{i=1}^N B_i^e}{\sum_{i=1}^N B_i^t}. \quad (13)$$

Rather than applying *a posteriori* scaling to the PRS results, we optimized the construction of the elastic network itself. Specifically, for MS proteins, we searched for parameter sets that inherently yield a global scaling factor $s \approx 1$ (typically within the range of 0.9–1.1) while maximizing the PCC between theoretical and experimental B-factors. In this way, the Hessian matrix directly produces fluctuations and PRS-predicted displacements that are quantitatively consistent with experimental magnitudes, without requiring any additional normalization. This inverse-fitting strategy ensures that the amplitudes of motions are physically meaningful and that the responses obtained from different MS proteins are comparable on an absolute scale.

PRS systematically applies forces to individual residues and records the linear response of the entire protein. This response is quantified by analyzing both the magnitude of residue displacements and their directionality. In this study, we extend the PRS method to investigate the responses of MS proteins to mechanical forces by applying perturbation force vectors to membrane-contacting or force-acted residues. This extension facilitates precise control over the direction and magnitude of the applied forces, enabling the simulation of mechanical impacts on MS proteins. For MS proteins with different gating mechanisms, such as “force-from-lipids” and “force-from-tether,” we employ tailored strategies to ensure a comprehensive analysis of all types of MS proteins.

For MS proteins operating under the “force-from-tether” mechanism, upward or downward forces were directly applied to the tethered residues along the membrane normal (z axis) to simulate physiological pushing or pulling. To prevent global translation and ensure local transmission of these forces, the z -degree-of-freedom of transmembrane residues was subsequently constrained by assigning an extremely large stiffness in the Hessian matrix, effectively rendering them immobile. This constraint was applied after constructing the MCP-ANM Hessian, thereby preserving the MCP-ANM network topology while fixing the z direction of transmembrane residues to anchor them within the membrane. The approach immobilizes the transmembrane region along the membrane normal while retaining natural couplings in the xy plane, mimicking the physiological anchoring of transmembrane domains within the lipid bilayer.

For the “force-from-lipids” mechanism, forces are similarly applied to membrane-contacting residues. The applied force consists of two components:

(1) Surface normal vector: The tension was aligned with the normal vector of the surface at the location of each residue.

(2) Radial vector adjustment: To refine the direction of the force, the normal vectors were projected onto the xy plane. Subsequently, these vectors were further adjusted using radial vectors pointing away from the geometric center of the protein.

For membrane tension-gated MS ion channels, we applied the same magnitude of force to each membrane-contacting residue ($MCP > 0.9$) of the proteins examined, since this approximately generates a uniform tension for each protein, allowing for a direct comparison of conformational changes across different MS proteins under the same condition. Specifically, a uniform force F_{residue} was applied to each selected residue with $MCP > 0.9$. The magnitude of the surface tension is given by the following:

$$\tau = \frac{F_{\text{residue}}}{l}, \quad (14)$$

where l denotes the linear dimension of each membrane-contacting residue. We approximate l to be the same for all residues, and then τ is proportional to F_{residue} for all membrane proteins. Consequently, the same magnitude of force applied

to each selected residue generated the same surface tension. In our simulations, the magnitude of membrane tension τ varied within the range of $[0, 1.5]$ (arbitrary units) with a step size of 0.1. Following the convention introduced by Bahar and coworkers [22,67], we adopted the same parametrization as in the original ANM, where the uniform spring constant was set to $\gamma = 1 \text{ kcal mol}^{-1} \text{ \AA}^{-2}$. Under this convention, one arbitrary unit (a.u.) of force corresponds approximately to 70 pN, and one a.u. of membrane tension corresponds to about 700 mN m⁻¹.

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The authors declare no conflicts of interest.

DATA AVAILABILITY

The data that support the findings of this article are openly available [68], embargo periods may apply.

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