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REVIEW

Computational studies of Piezo1 yield insights into key lipid–protein interactions, channel activation, and agonist binding

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Abstract

Piezo1 is a mechanically gated ion channel responsible for converting mechanical stimuli into electrical signals in mammals, playing critical roles in vascular development and blood pressure regulation. Dysfunction of Piezo1 has been linked to several disorders, including hereditary xerocytosis (gain-of-function) and generalised lymphatic dysplasia (loss-of-function), as well as a common polymorphism associated with protection against severe malaria. Despite the important physiological roles played by Piezo1, its recent discovery means that many aspects underlying its function are areas of active research. The recently elucidated cryo-EM structures of Piezo1 have paved the way for computational studies, specifically molecular dynamic simulations, to examine the protein's behaviour at an atomistic level. These studies provide valuable insights to Piezo1's interactions with surrounding membrane lipids, a small-molecule agonist named Yoda1, and Piezo1's activation mechanisms. In this review, we summarise and discuss recent papers which use computational techniques in combination with experimental approaches such as electrophysiology/mutagenesis studies to investigate Piezo1. We also discuss how to mitigate some shortcomings associated with using computational techniques to study Piezo1 and outline potential avenues of future research.

Keywords Piezo1 · Channel activation · Computational techniques

Introduction

Mechanosensation, or the conversion of mechanical stimuli such as touch and proprioception into electrical signals, is a ubiquitous yet poorly understood phenomenon (Cox et al. 2019; Kefauver et al. 2020). In mammals, this process is mediated by the flow of cations through membrane-embedded mechanically gated channels. One of the first clearly identified examples of these in mammals are the Piezo channels, Piezo1 and Piezo2 (Coste et al. 2010). Of these, Piezo1 is the most well-studied and plays essential roles in vascular development (Ranade et al. 2014; Li et al. 2014), blood pressure regulation (Zeng et al. 2018), and osmotic homeostasis (Martins et al. 2016). Piezo1 has been shown to respond to a variety of mechanical stimuli, including shear force (Li et al. 2014) and cell stretching (Gudipaty et al. 2017). Studies have shown that Piezo1 channels are inherently mechanosensitive: they can sense forces directly transmitted through the bilayer without the need for cytoskeletal elements (Cox et al. 2016; Syeda et al. 2016), although this may be modulated by the cytoskeleton or extracellular matrix (Romero et al. 2020; Bavi et al. 2019; Gottlieb and Sachs 2012; Cox et al. 2016).

Constitutive knockout of Piezo1 leads to embryonic lethality in mice due to defects in vascular development, highlighting its physiological importance (Ranade et al. 2014; Li et al. 2014). Malfunction of Piezo1 is linked to diseases such as hereditary xerocytosis (Zarychanski et al. 2012; Glogowska et al. 2017) and generalised lymphatic dysplasia (Fotiou et al. 2015). In addition, a Piezo1 gain-of-function mutation found in one-third of the African population has recently been shown to confer protection from malaria (Ma et al. 2018). Despite the important physiological roles played by Piezo1 and its homologue Piezo2, their recent discovery means that the precise factors underlying their activation, interactions with lipids and small molecules, and permeation mechanisms remain areas of active research (Wang et al. 2021).

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Recently, the structure of Piezo1 in a non-conducting state was solved by three independent groups using cryo-electron microscopy (cryo-EM) (Ge et al. 2015; Guo and MacKinnon 2017; Saotome et al. 2018; Zhao et al. 2018). The mechanically gated channel consists of three subunits, forming a triskelion shape with curved propellers extending out from a central pore towards the extracellular space (Fig. 1A). In detergent and when reconstituted into liposomes, Piezo1 adopts a bowl-shaped conformation which induces local curvature within the bilayer (Guo and MacKinnon 2017). Each propeller contains nine transmembrane helical units (THUs), six of which are resolved in the cryo-EM structures of Piezo1 (Fig. 1B). Each THU is formed by a bundle of four transmembrane (TM) helices, which are remarkable in their structural similarity given that there is no identified sequence repetition between them. In addition to the 36 TM helices in each propeller, TM 37 (outer helix, OH) and TM 38 (inner helix, IH) from each monomer trimerise to line the pore of Piezo1 together with the C-terminal extracellular domain (CED), the anchor domain, and the C-terminal

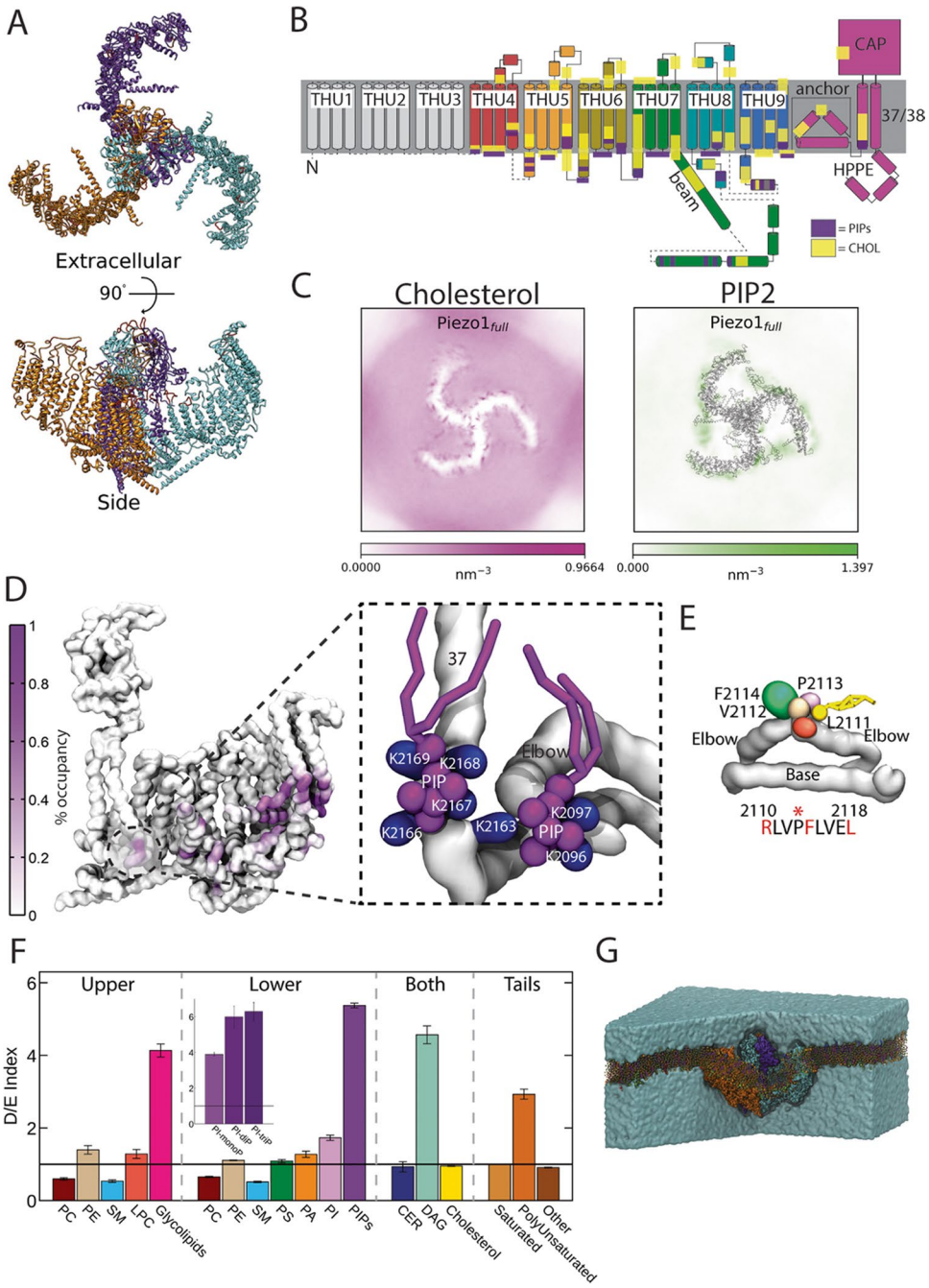

Fig. 1 Piezo1–lipid interactions and curvature of the membrane. **A** A top and side view of Piezo1's structure. Each monomer is coloured differently. **B** Schematic of Piezo1, with the unresolved regions in grey and each transmembrane helix unit (THU) in a different colour. Cholesterol interaction sites are yellow boxes, while PIP2 sites are purple boxes. The dark grey box in the background is the membrane. **C** Density plots of cholesterol (left) and PIP2 (right) around Piezo1 (D) PIP binding sites highlighted on one monomer (left), with a key PIP binding site corresponding to a Delta4K mutation (inset). **E** Cholesterol binding site corresponding to a site identified by experimental crosslinking. **F** Lipid depletion/enrichment indices highlighting which lipids are more likely to be present closer to Piezo1. **G** Curvature of the membrane induced by Piezo1. **A**, **C**, and **G** are adapted from Chong et al. (2021). **B**, **D**, **E**, and **F** are adapted from Buyan et al. (2020)

domain (CTD) (Zhao et al. 2016). A long helical beam of length 90 Å supports each propeller on the intracellular side, bridging the distal ends of the propeller to the central pore module.

Elucidating the structure of Piezo1 has paved the way for studies to examine protein behaviour at the atomistic level using computational techniques such as molecular dynamics (MD) simulations. While static snapshots of Piezo1 are key to understanding its unique structure, they cannot be used to explain the dynamics of the protein that allow it to carry out its physiological functions. To achieve this, we turn to MD simulations, which use static protein structures as a starting point and model their atomistic level movements using Newton's laws of motion. This generates a trajectory of the protein's motions over time, which can be analysed to help determine lipid and ligand binding sites, and conformational changes in response to activation or small-molecule binding.

MD simulations can capture biological events with high spatiotemporal resolution and as such have been widely applied to study the dynamics of biomolecules over the past few decades (Dror et al. 2012). Recent advances in simulation methodology, including the development of coarse-grained (CG) force fields which reduce the number of particles in a simulation box by grouping together multiple atoms into a single interaction site, have expanded the timescales and system sizes accessible via MD (Marrink et al. 2007). Due to the large size of the Piezo1 protein, many studies have naturally turned to this coarse-grained approach. When combined with automated tools for MD system set up (e.g. CHARMM-GUI) (Jo et al. 2008) and generation of complex and physiologically relevant lipid bilayers (Wassenaar et al. 2015), MD techniques are uniquely suited for studying the behaviour of large membrane-embedded proteins such as Piezo1.

To date, several studies have used MD simulations to address gaps in our understanding of Piezo1 function. Topics of investigation include the in silico activation of Piezo1 (De Vecchis et al. 2021; Jiang et al. 2021), characterising Piezo1's interactions with the surrounding lipid environment (Buyan et al. 2020; Chong et al. 2021) and agonist Yoda1 (Botello-Smith et al. 2019) and exploring how the interplay between multiple Piezo1 proteins affects channel kinetics (https://www.biorxiv.org/content/10.1101/2021.04.16.440217v1, https://www.biorxiv.org/content/10.1101/787531v1). In this review, we seek to summarise and analyse the key findings of several recent computational studies of Piezo1 and discuss how MD simulations has been combined with electrophysiology experiments to yield insights into Piezo1 behaviour. We will also address some potential shortcomings of using MD to study Piezo1, discuss how they can be mitigated, and outline potential avenues for future research into Piezo1 using computational techniques.

Lipid–Piezo1 interactions, membrane curvature, and clustering

The importance of the lipid bilayer in modulating membrane protein activity is being increasingly appreciated (Cordero-Morales and Vásquez 2018). This can happen in at least two ways: (i) lipids such as cholesterol can alter the physical properties of the membrane itself and thus alter the behaviour of the protein (Ridone et al. 2020; Gimpl et al. 1997; Zocher et al. 2012; Poveda et al. 2017; Taberner et al. 2015; Romero et al. 2019; Shi et al. 2020), or (ii) lipids can directly interact with the protein to modulate activity (Mitchell et al. 1990; Manna et al. 2016; Grouleff et al. 2015).

As Piezo1 is a membrane protein, it is both affected by and affects its lipid membrane environment. Lipids and the membrane environment have been shown to play a role in regulating Piezo1 activity. Piezo1 can also be activated in the absence of cytoskeletal elements (Syeda et al. 2016; Cox et al. 2016) and only activates in the presence of asymmetric membranes containing negatively charged lipids in the cytoplasmic leaflet (Syeda et al. 2016). Phosphatidylinositol bisphosphates' (PIP2, negatively charged lipids) depletion from the membrane via the activation of TRPV1 inhibits Piezo1 (Borbiro et al. 2015). An electrophysiology study has shown that varying the types of saturated, as well as polyunsaturated fatty acids (PUFAs) in the membrane, allows one to "fine-tune" the (in)activation properties of Piezo1 (Romero et al. 2019). For example, margaric acid (a saturated fatty acid found in the milkfat of ruminants) inhibits Piezo1 activation, while the addition of eicosapentaenoic acid (EPA, a PUFA found in fish oil supplements) speeds up the inactivation time of WT Piezo1 (Romero et al. 2019). In addition, cholesterol has been heavily implicated in promoting Piezo activation, both involving a direct protein–lipid interaction with Piezo1 shown via sterol crosslinking (Hulce et al. 2013), as well as altering membrane properties (Ridone et al. 2020; Qi et al. 2015). Sphingomyelin and ceramide have also been implicated in modulating channel inactivation kinetics in primary cell types (Shi et al. 2020).

Due to the importance of lipids in determining Piezo1 function, there is increased interest in understanding the types of lipids that preferentially surround the protein, known as the lipid "fingerprint" (Corradi et al. 2018). Two CG MD studies have investigated the lipid fingerprint of Piezo1: one for a simple model membrane (Chong et al. 2021) and one with a complex mammalian membrane containing 63 different lipid types (Buyan et al. 2020). Both studies highlight the importance of cholesterol and PIP2's interaction with and activation of Piezo1 (see Fig. 1B and C). Chong et al. show a relatively even distribution of

cholesterol and PIP2 around Piezo1 (Fig. 1C), with many binding sites on the propellers. Buyan et al. found a number of relevant lipid binding sites, including a PIP2 site containing 4 lysines (K2181-K2185, mouse numbering) which, when deleted, removed Piezo1's ability to inactivate and suggests an integral role between PIP2 and Piezo1 inactivation (Fig. 1D). Other PIP2 lipid binding sites have been correlated with disease mutations, including R808Q and P(Δ4K) that lead to dehydrated hereditary stomatocytosis (Andolfo et al. 2013). Mutations causing hereditary xerocytosis that were found in cholesterol binding sites were R1943Q (Glogowska et al. 2017) and K2070Q (Picard et al. 2019). In addition, one cholesterol binding site found in the simulation (RLVP2130, mouse numbering) matched the location determined from chemical crosslinking and has been shown to be important for mechanical gating (Li et al. 2021) supporting the ability of CG simulations to locate lipid binding sites (Fig. 1E). Both studies found that PIPs were enriched around the protein (Fig. 1F). More recently, atomistic simulations of a truncated Piezo1 in a POPC/PIP2 bilayer identified 7 PIP2 binding sites, 6 of which were identified by Buyan et al. and Chong et al. (Jiang et al. 2021). These pioneering simulations have given us a first glimpse as to why PIPs and cholesterol are integral for Piezo1 function and highlight their potential involvement in disease-related mutations.

Piezo1 is also affected by the membrane on a macroscopic scale (Ridone et al. 2020; Qi et al. 2015; Romero et al. 2019) and exhibits the ability to gather in a "cluster"(Bae et al. 2013). The structure solved by Mackinnon et al. showed images of Piezo1 in simple liposomes, where it was seen to locally curve the liposome, and it has been suggested that this curvature is integral to the mechanism of force sensing by Piezo channels (Lin et al. 2019). The dome-shaped membrane curvature around the protein is observed in both CG (Chong et al. 2021; Buyan et al. 2020) and AA simulations (Jiang et al. 2021) (Fig. 1G), with two preprints delving into greater detail on this membrane deformation. The first preprint, by Buyan et al., (https://www.biorxiv.org/content/10.1101/787531v1) reproduces the curvature seen by Mackinnon et al. in simulations with Piezo1 in a complex membrane and demonstrates that the propeller domains are responsible for inducing curvature, something also reported by Chong et al. (2021). Buyan et al. also show that clustering of the proteins in cells overexpressing Piezo1 leads to tortuous structures in the plasma membrane the authors term "Piezo pits", though how many Piezo proteins this represents, and whether these structures are naturally occurring at native expression levels, remains unknown. The clustering of Piezo1 was further explored in a preprint by Wijerathne et al., (https://www.biorxiv.org/content/10.1101/2021.04.16.440217v1) who predict via electrophysiology that between 5 and15 Piezo, proteins are likely to associate in a single curved region, and this clustering of Piezo proteins is responsible for longer duration of electrical signals. They posit that open Piezo1 has a much less curved membrane region, thereby reducing the energy of association and allowing more proteins to aggregate and keep each other in an "open" state. A single MD replicate shows movement of open Piezo towards each other which is not seen for a closed channel. Though Wijeranthne et al. propose an elegant link between membrane curvature, clustering, and Piezo1 function, there is evidence that enhanced curvature can drive the clustering of many proteins (Chidlow and Sessa 2010; Nossal 2001; Peter et al. 2004) in contrast to the idea that flatter open channels more easily interact. In addition, a preprint by Lewis and Grandl (https://www.biorxiv.org/content/10.1101/2021.06.03.446975v1) indicates that at rest, channels do not open at a higher frequency when there is a greater density of channels.

Mechanical activation of Piezo1

Since its discovery in 2010, Piezo1 has been shown to be activated by a variety of mechanical stimuli, including fluid shear flow (Ranade et al. 2014), poking (Coste et al. 2010), and membrane stretching (Bae et al. 2013). Piezo1 can function in "reduced systems" such as reconstituted bilayers (Syeda et al. 2016) and membrane blebs (Cox et al. 2016), indicating that they are able to activate in the absence of cytoskeletal and extracellular elements. However, emerging functional and biochemical evidence points to a role of the cytoskeleton in Piezo1 gating, suggesting that they may be activated by both a force-from-lipid and force-from-filament mechanism (Romero et al. 2020; Bavi et al. 2019; Gottlieb and Sachs 2012; Cox et al. 2016).

To date, two computational studies have investigated the activation of Piezo1 in the absence of the cytoskeleton using distinct techniques. Recently, Jiang et al. (2021) described the in silico opening of the Piezo1 channel, which was achieved by using a small periodic box to mimic Piezo1 clustering. As each channel induces its own domed footprint, the proximity of the channels in a confined space tends to flatten the membrane, causing structural changes in the protein. To see this, CG simulations of a position restrained Piezo1 in a POPC: POPC/PIP_2 membrane was carried out for 12 μs to equilibrate the membrane and allow dome formation around the protein. Following this, the final snapshot was back mapped to an all-atom system and simulated for a further 2 μs, during which the channel undergoes a structural change that opens the central pore. The application of a large electric field generated ion currents consistent with the experimentally measured unitary conductance as well as the behaviour of conductance reducing mutants.

Shortly afterwards, De Vecchis et al. (2021) also simulated the activation of Piezo1 using a different approach: the application of lateral membrane tension. 500 ns CG simulations were also utilised here to equilibrate the protein in a six-component model endothelial membrane and facilitate dome formation. Following backmapping to atomistic resolution, several gradations of tension were applied to the bilayer plane for 50 to 100 ns, with the − 40-bar system (corresponding to a lateral pressure of ~ 68 mN/m) leading to channel opening within the simulation timeframe. In addition to detailing the conformational changes associated with channel opening, the authors also discuss the role of lipids in mediating an early inactivation state when further simulations are performed in the absence of membrane tension. The propellers are demonstrated to be critical for channel activation, with propeller-free constructs failing to activate in response to membrane tension, likely due to the absence of the membrane dome. Studies of these blade-free constructs invite the possibility of computationally removing other structural features of Piezo1 (e.g. beam, anchor, CED) to better understand their role in activation.

Despite the different techniques used to open the channel, both studies report similar conformational changes associated with pore opening (Fig. 2). Extension of the propellers

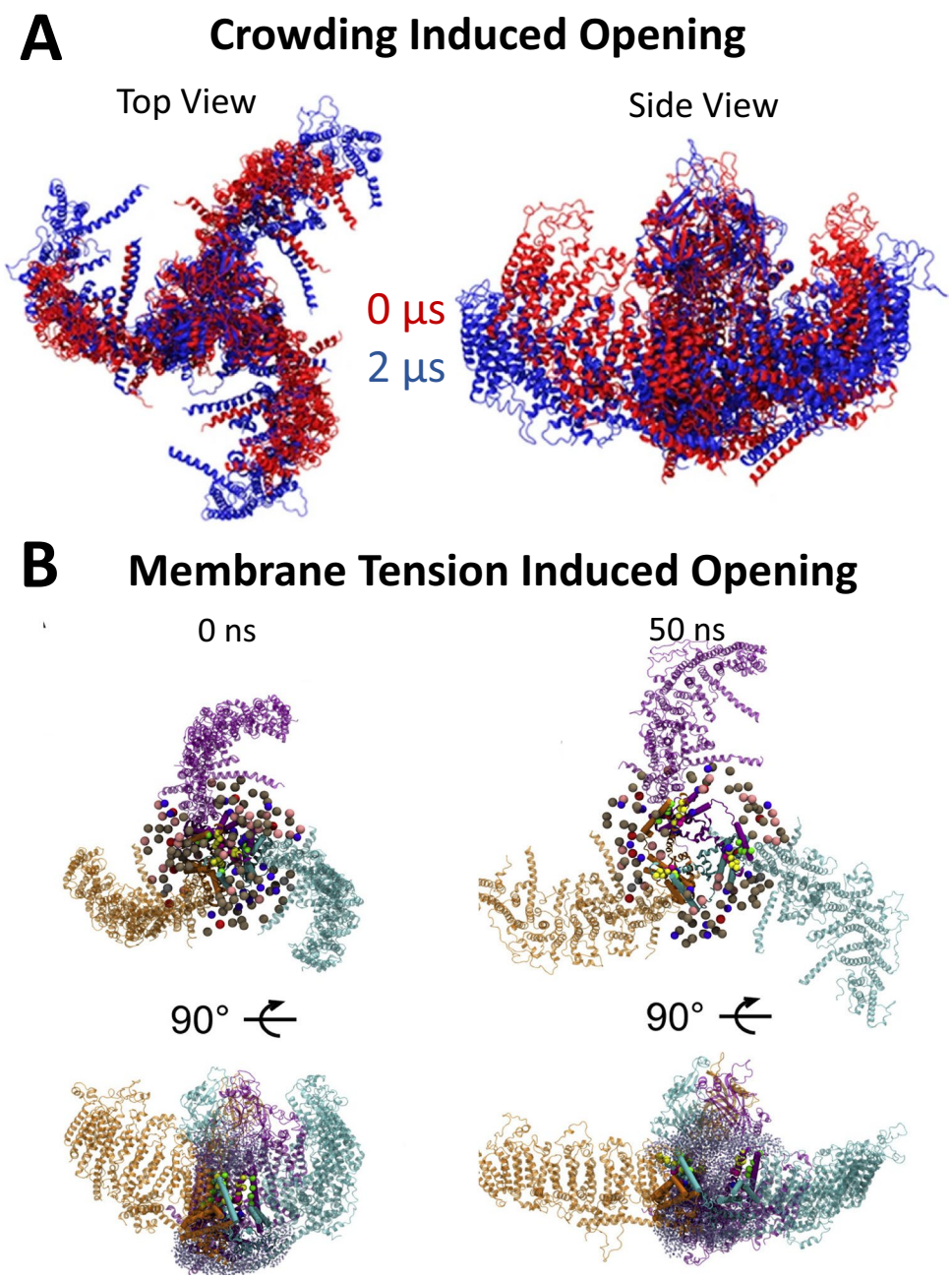

Fig. 2 Mechanical activation of Piezo1 in MD simulations: **A** crowding-induced opening — representative structures of Piezo1 in the closed (0 us, red) and open (2 us, blue) state (**B**). Membrane tension-induced opening — Piezo1 in the closed (0 ns, left) and open state (50 ns, right). **A** is De Vecchis et al. (2021) **adapted** from Jiang et al. (2021) under the Creative Commons Attribution 4.0 International Licence. **B** is adapted from **under** the Creative Commons Attribution 4.0 International Licence

is observed in response to mechanical stimuli, leading to the in-plane expansion of Piezo1 and concomitant dome flattening. This in turn leads to the outward tilting of the pore adjacent TM 37 and TM 38 helices which increases the radius of the pore. The beam below each propeller tilts in response to footprint flattening such that it is almost parallel with the membrane. Finally, upward displacement of the CED is also observed, leading to its greater exposure to the extracellular space. Both studies identify solvated intracellular and extracellular lateral fenestrations near the pore which form potential ion permeation pathways. Additionally, Jiang et al. (2021) note the cation-selective behaviour of these fenestrations which arise due to the negatively charged residues which line the entrance of each pathway, a finding consistent with experimental data (Zhao et al. 2016; Geng et al. 2020).

While these two studies have yielded valuable insight into the structural rearrangements required for channel opening, several uncertainties remain. Firstly, neither study accounts for the 3 N-terminal THUs that are unresolved in the cryo-EM structures. These domains are proposed to further extend the membrane footprint, and their inclusion would more accurately represent the conformational changes which occur on channel opening and shed light on the role of the distal ends of each arm in force sensing and Piezo1 activation. This could be achieved following examples in which the last 3 THUs of Piezo1 have been modelled using either Piezo1 THU 4,5,6 as a template (Chong et al. 2021) or using the recently elucidated cryo-EM structure of Piezo2 that resolves these domains (Wang et al. 2019). Secondly, the timescale of these simulations (~2 µs for Jiang et al. (2021) and ~50–100 ns for De Vecchis et al. (2021) is much shorter than the physiological duration of channel opening and may not capture the full conformational change. The large forces applied by De Vecchis et al. (2021) to induce channel opening within a brief time are above the membrane rupture limit and may lead to distortions in the protein structure which would not occur in a physiological context. However, such large tensions have been useful in modelling the behaviour of other channels such as MscL (Jeon and Voth 2008; Deplazes et al. 2012; Yefimov et al. 2008). The use of PBC conditions to mimic Piezo1 clustering is novel and may represent a gentler approach; however, it creates a dense cluster containing infinitely many channels which are all mirror images of one another, whereas the molecular nature of Piezo1 clusters, and their role in channel activation remains to be determined experimentally. Finally, both studies use simplified membranes, and the role of complex lipid composition on Piezo1 conformation changes remains to be determined. While the simulations use 6 and 2 lipid types, respectively, they both include PIP_2 driven by experimental evidence underlining its importance for Piezo1 activation (Borbiro et al. 2015). Interestingly, the lipids included all contain palmitoyl and oleoyl tails. These saturated/monounsaturated tails are the most common acyl chains in many mammalian membranes and as such are frequently used in model membranes for MD simulations (Goossens and De Winter 2018). It would be interesting to understand the role of lipids containing polyunsaturated tails (that are not in either model) on activation given their substantial (> 10%) concentration in endothelial cell membranes (Cansell et al. 1997), noted relative enrichment around Piezo1 (Buyan et al. 2020) and established role in modulating Piezo1 activity (Romero et al. 2019) (as discussed in the previous section of this review).

Overall, these two computational studies have elucidated the conformational changes which occur on Piezo1 channel activation in response to membrane flattening and highlighted the potential for MD simulations to investigate the dynamics of mechanically gated ion channels. Future studies could look to using these methods to study the mechanisms through which Piezo1 is activated by other mechanical stimuli, such as shear flow. Additionally, computational studies are well suited to address questions regarding the role of the cytoskeleton and other cellular components in Piezo1 activation.

Small-molecule modulators of Piezo1

Using high-throughput drug screening, several chemical modulators of Piezo1 have been identified (Fig. 3A). The first discovered agonist of Piezo1 is a synthetic molecule named Yoda1. When applied to Piezo1, it increases cation flux through the channel by stabilising the open conformation and reducing the mechanical threshold for activation (Syeda et al. 2015; Lacroix et al. 2018). The potent and specific nature of Yoda1 renders it a useful research tool for studying Piezo1 activity without the need for application of mechanical force, which is cumbersome and requires specialist equipment. Two more agonists, Jedi1 and Jedi2, have since been discovered which activate Piezo1 via a different mechanism than Yoda1 (Wang et al. 2018). The discovery of these three agonists in addition to Dooku1, which antagonises Yoda1-evoked activation of Piezo1 (Evans et al. 2018), opens up the potential of chemically manipulating Piezo1's responses to mechanical forces. All identified chemical modulators of Piezo1 do not affect Piezo2 — despite simultaneously screening Piezo2, no agonists or antagonists of Piezo2 have been found. Unravelling the binding pathways and mechanism of action of these small molecules is of clinical interest due to the diverse and important roles that Piezo channels play in human physiology and pathology. A recent experimental study exploited the inability of Yoda1 to activate Piezo2 to identify regions key for Yoda1-mediated activation on Piezo1 by generating chimaeras of the two homologues (Lacroix et al. 2018). Through this, the authors

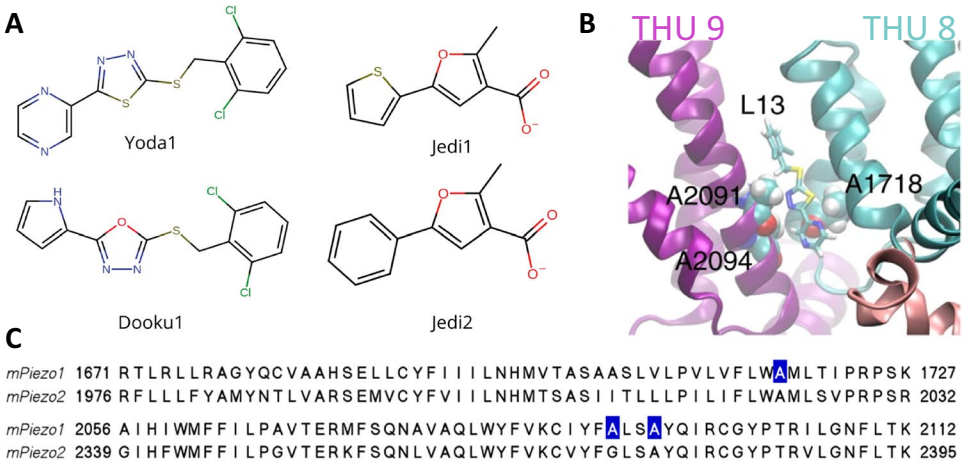

Fig. 3 Small molecules and Piezo1: **A** chemical structures of the 4 small-molecule modulators of Piezo1: Yoda1, Dooku1, Jedi1, and Jedi2. **B** Yoda1 in its putative binding site, between THU 8 and 9. **C** A sequence alignment (T-Coffee) of the mouse Piezo1 (UniProt ID: E2JF22) and mouse Piezo2 genes (UniProt ID: Q8CD54), showing the three Piezo1 residues (A1718, A2091, and A2094) which coordinate Yoda1 binding in Botello-Smith et al. (2019), are conserved/similar in Piezo2. **B** is adapted from Botello-Smith et al. (2019) under the Creative Commons Attribution 4.0 International Licence

identified a segment spanning residues 1961 to 2063 which is required for Yoda1-mediated activation.

To more clearly define the binding site of Yoda1 on Piezo1, one computational study performed all-atom flooding simulations where 20 Yoda1 molecules were added to a system containing Piezo1 embedded in a POPC bilayer (Botello-Smith et al. 2019). During a 4.8 μs simulation, one Yoda1 molecule was shown to enter a narrow hydrophobic pocket adjacent to the residues 1961–2063 previously identified to be required for Yoda1 activation of Piezo1 (Lacroix et al. 2018) (Fig. 3B). Yoda1 was shown to be stable in this site and on the application of membrane tension, acted as a molecular wedge to increase the mobility of the Yoda1-bound propeller. Botello-Smith et al. propose this hydrophobic pocket as the putative binding site of Yoda1 and support this using experimental mutagenesis studies conducted on three alanine residues (A1718, A2091, and A2094) purported to coordinate Yoda1 binding. Mutation of these residues to tryptophan abolished (A1718W) or diminished (A2091W and A2094W) Yoda1-mediated activation. However, this may be due to general disruption of Piezo1's mechanotransduction mechanism rather than only preventing Yoda1 binding. This is exemplified by the inability of the A1718W mutant to generate pressure-activated currents and lack of Jedi2 response in the A2094W mutant — both suggesting that not just Yoda1 binding is affected by these mutations. While this putative binding site for Piezo1 is proximal to the residues 1961–2063, it is not within this segment identified to be required for Yoda1-mediated activation of Piezo1. These three alanine residues are conserved (A1718 and A2094) or similar (A2091, glycine) in Piezo2, so if this site is correct, it remains to be reconciled with experimental evidence that Yoda1 does not activate Piezo2 (Fig. 3C). Additional replicates which increase the number of independent binding events observed would increase the confidence in the validity of the binding site. Additionally, these simulations may benefit from using a full-length structure of Piezo1 as it is possible that the binding site is contained in THU 1–3, and a complex membrane, given the possible interplay between bilayer components and the hydrophobic Yoda1.

Understanding the binding mechanisms of small-molecule modulators on the Piezo channels is key to leveraging them for pharmacological interventions. MD simulations offer unparalleled insight into ligand–protein interactions, as they can uncover ligand binding pathways and examine the role of membrane components involved, in addition to elucidating binding sites. Future studies could use computational methods to validate the putative binding site of Yoda1 and reveal the binding sites of Jedi1, Jedi2, and Dooku1 on Piezo1. These simulations could in turn inform the design of more potent and specific modulators of Piezo1, or alternatively, modification of these compounds to target activation or inhibition of Piezo2.

Outlook

Recent computational advances and the elucidation of the Piezo1 structure have allowed MD simulations to reveal detailed insights into the behaviour of the channel. In this review, we have summarised and discussed several studies which use MD simulations, usually in conjunction with mutagenesis and electrophysiology, to better understand

Piezo1 activity. Several recent papers have used MD to explore the interactions between Piezo1 and the lipid environment, revealing key PIP2 and cholesterol binding sites on the protein as well as the more macroscopic role that membrane curvature may play in Piezo1 clustering (Chong et al. 2021; Buyan et al. 2020; Jiang et al. 2021). While there is evidence for clustering of Piezo1 when overexpressed (Ridone et al. 2020), whether this occurs at native expression levels and whether it modulates mechanical sensitivity or channel activity (Haselwandter and MacKinnon 2018) remains to be determined. Two computational studies, using distinct methods, have examined the structural changes which trigger the opening of the Piezo1 channel and uncovered several ion permeable fenestrations adjacent to the pore (Jiang et al. 2021; De Vecchis et al. 2021). Additionally, a flooding simulation has revealed a putative binding site for the small-molecule agonist Yoda1 on Piezo1 (Botello-Smith et al. 2019).

Some broad limitations of using MD to study Piezo1 include the large size of the Piezo1 protein, which limits the timescale of MD simulations and introduces difficulties when examining phenomena which occur beyond the low microsecond timescale at atomistic resolution. These challenges have been addressed using CG force fields which reduce the number of particles in the simulation box. However, coarse graining leads to a loss of resolution and is unable to model large protein conformational changes, such as those associated with channel opening, due to the use of elastic networks to preserve the overall protein structure. Additionally, the high number of transmembrane helices in Piezo1 leads to difficulties in preserving the tertiary structure and trimeric symmetry of the protein. In CG simulations where an elastic network is implemented to constrain the tertiary structure of Piezo1, high elastic network distance cut-off values preserve the protein structure but may lead to dramatic membrane rippling during dome formation. Conversely, lower elastic network distance cut-offs sometimes permit unrealistic protein deformation. Inherent to the use of MD to study membrane proteins is the oversimplification of the model to comply with computational and methodological limitations. While the role of the cytoskeleton and accessory proteins in Piezo1 function is still being determined, it is likely that future simulations of Piezo1 may need to include these elements to properly model its physiological behaviour. Given the availability of a Piezo2 cryo-EM structure (Wang et al. 2019), and its similarities to Piezo1, MD simulations of Piezo2 may yield interesting insights into its relationship with the bilayer and show whether it also induces local membrane curvature. Additionally, simulations of Piezo2 could be used to elucidate its opening mechanisms and facilitate comparison with Piezo1.

While computational limitations restrict the timescales accessible by all-atom MD, and conventional CG MD cannot model large protein conformational changes, emerging techniques such as the Gō-MARTINI model could be used to more feasibly study Piezo1's structural rearrangements. Unlike conventional CG models, which implement a network of unbreakable harmonic bonds to maintain the tertiary structure of large proteins, Gō-MARTINI models use a Lennard–Jones potential based on a contact map of the native protein structure and hence can be used to investigate large protein conformational changes (Poma et al. 2017).

As progress continues towards improving parameters and increasing our computational capabilities, studies can look to probing the role of the extracellular matrix and/or the cytoskeleton in Piezo1 activation and examining the mechanisms through which Piezo1 is activated by other mechanical stimuli such as shear flow. Additionally, MD simulations are well suited to increasing our understanding Piezo1's interactions with small molecules which may in turn inform rational drug design.

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