

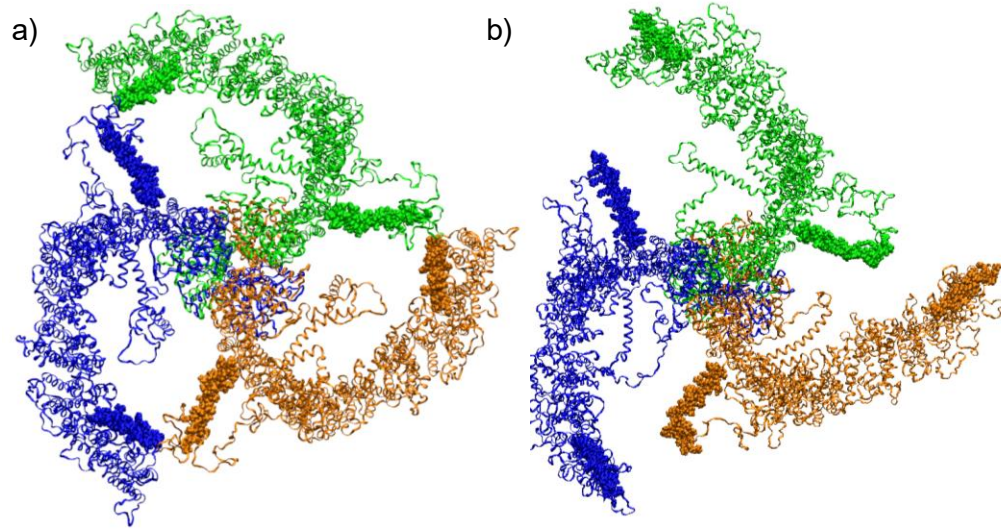
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## Background

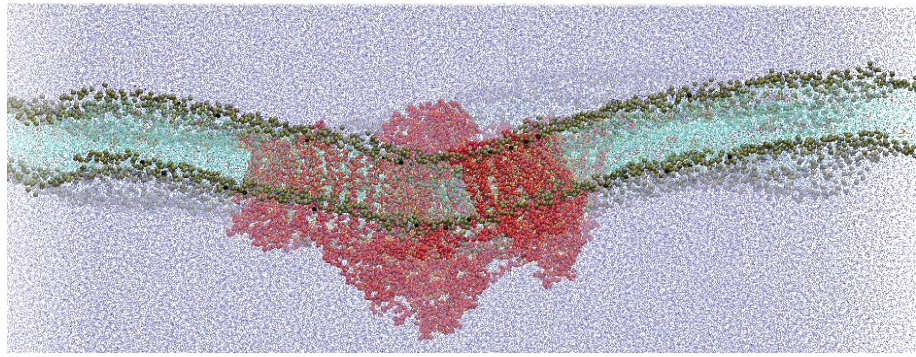
### PIEZO1 structure

PIEZO1 is a homotrimeric protein expressed in different tissues and mechanically activated by physical forces, such as pressure and shear stress. It plays a key role in processes like vascular development [1]. At the molecular level, interblade interactions determine the protein's shape, membrane induced curvature and pore structure. These interactions are mediated by PIP<sub>2</sub> lipids [2].



**Figure 1:** Bottom views of human PIEZO1 (hPIEZO1) structure with helices that are part of inter-blade interactions highlighted in van der Waals representation in a closed conformation (a) and in the open conformation (b).

## Methods



**Figure 2:** Simulation box containing hPIEZO1 (red) in a lipid membrane environment with water (violet spheres) and salt concentration of 150mM of NaCl.

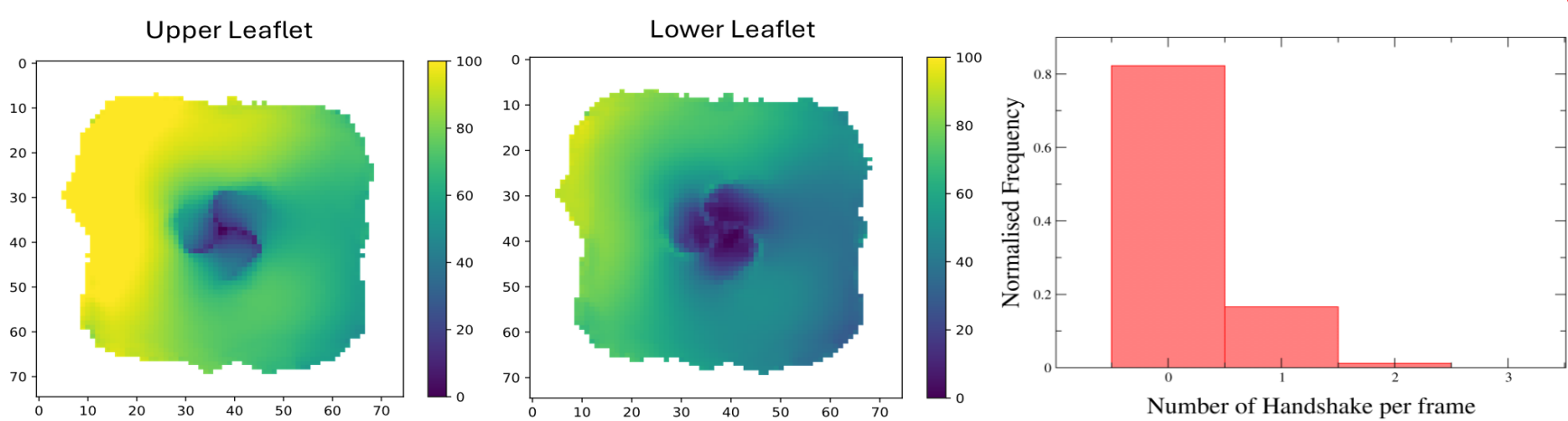
- Coarse-grained (CG) simulations were built using INSANE [3].
- Force field: MARTINI3 [4].
- Simulation and analysis: GROMACS v.2023-2 [5], CHAP [6] and in-house built analysis tools[3, 7].
- Cytoplasmic composition: 50% POPC, 20% POPE, 5% POPS, 20% Cholesterol and 5% PIP<sub>2</sub>. Extracellular membrane composition: 60% POPC, 20% POPE and 20% Cholesterol.
- All-atom (AA) simulation boxes built using the Backward method [9].

## Conclusions

- PIP<sub>2</sub> maintains strong interactions with the helices responsible for the handshake interactions resulting in PIEZO1 in different states when in the inactive curved conformation.
- Simulations performed under a lateral pressure of -30 bar promote flattening of PIEZO1 and the widening of the channel's central pore.

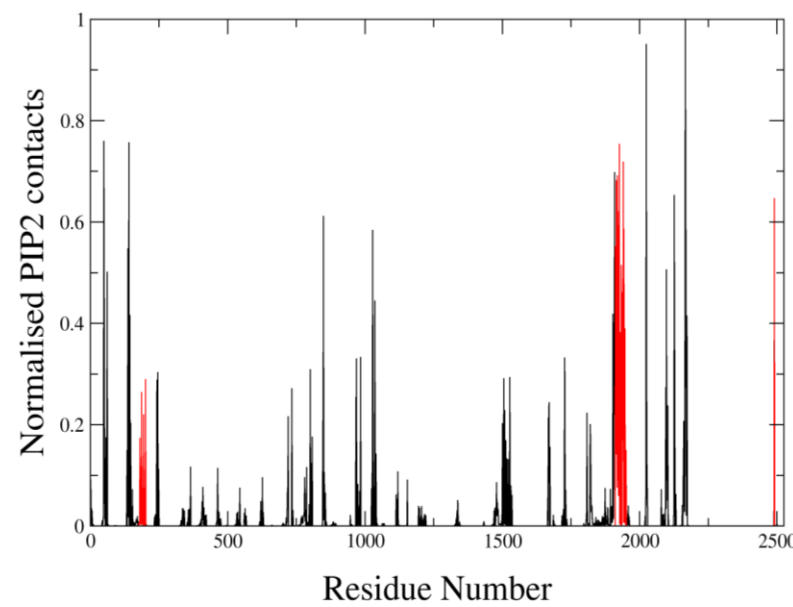
**Literature cited:** [1] Beech DJ and Kalli AC. *ATVB*. 39(11), 2228 (2019); [2] Smith *et al*, *Sci. Adv.* 11, eadt7046 (2025). [3] Wassenaar, T. A *JCTC*. 11, 2144 (2015). [4] P. C. T. Souza *et al*. *Nature Methods*, 18, 382 (2021). [5] de Jong *et al*. *JCTC*, 9, 1, 687 (2013). [6] Kleese *et al* *J. Mol. Biol.* 431(17), 3353 (2019). [7] Chong J *et al*. *Biophys.J.* 120, 1343(2021). **Acknowledgements:** HECBIOSIM, HPC Leeds, BB/Z517227/1.

## Inactive Curved hPIEZO1



**Figure 3:** Height maps of upper and lower leaflet of averaged over all replicas at CG simulations.

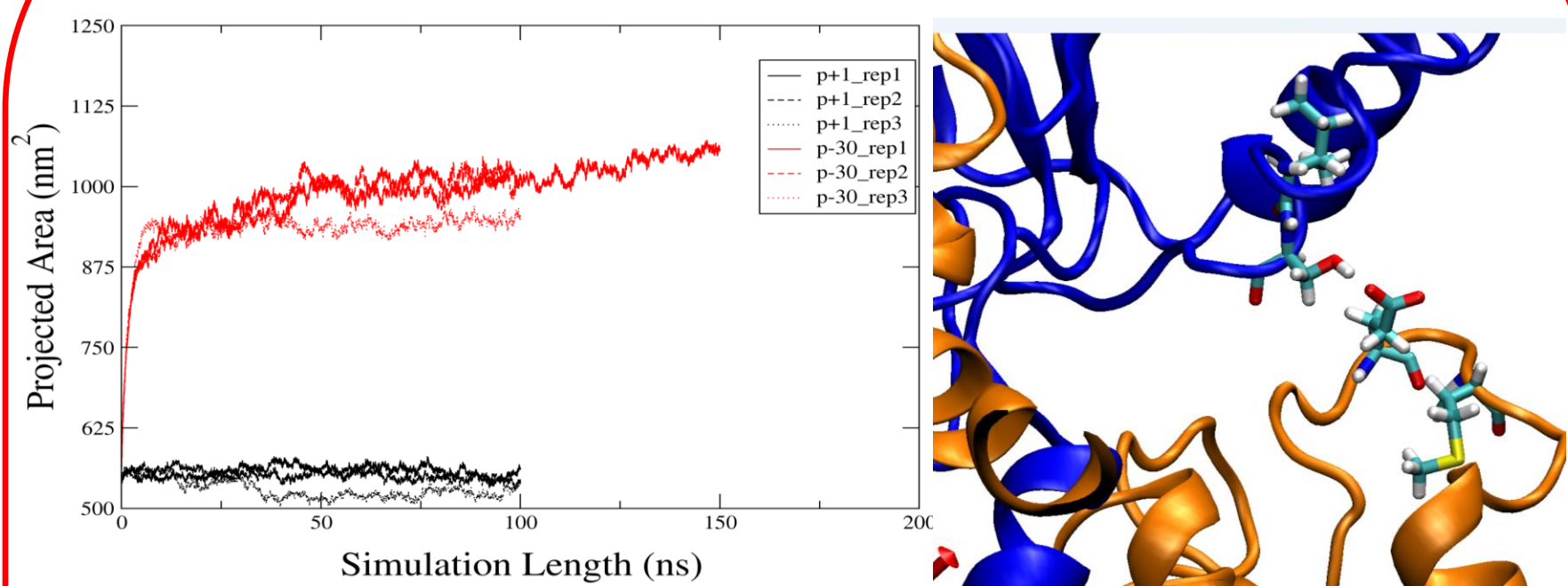
**Figure 4:** Frequency of Handshake (HS) interactions at all CG simulations.



**Figure 5:** Normalised number of contacts between PIP<sub>2</sub> and hPIEZO1 residues per chain. Regions of handshake (HS) interaction mediated by PIP<sub>2</sub> in red.

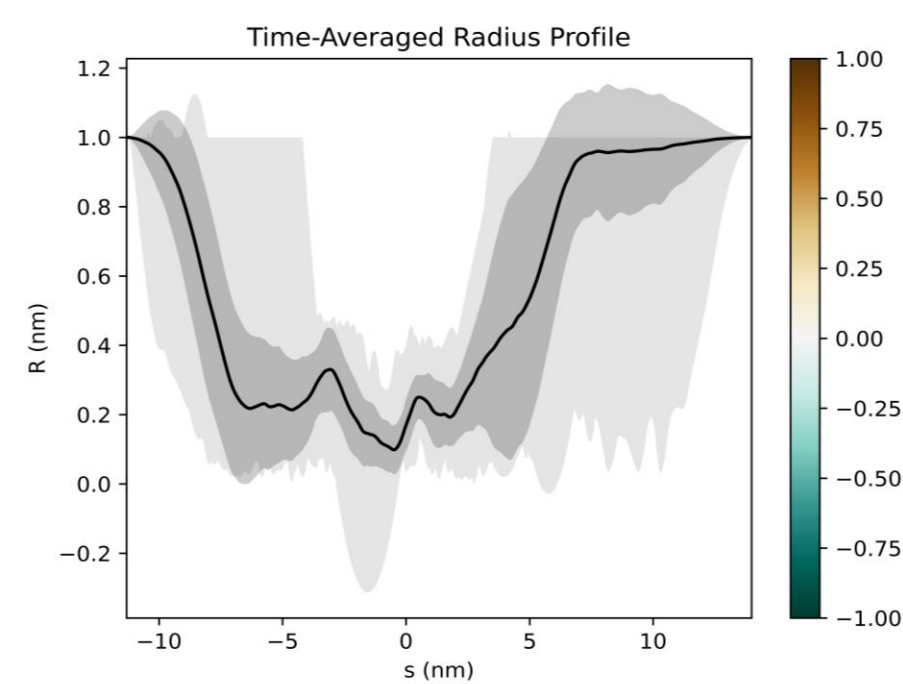
- In its curved and closed state, hPIEZO1 has a characteristic membrane curvature footprint (Figure 3).
- PIEZO1 forms intersubunit interactions, referred to as handshake (HS) interactions along the simulations (Figure 4).
- PIP<sub>2</sub> notably interacts with helices involved in the HS interactions (red regions in Figure 5), effectively shielding electrostatic repulsion within this region and mediating this interaction.

## Flattening hPIEZO1



**Figure 6:** hPIEZO1 projected area at different lateral pressures (3 replicas for each value).

**Figure 7:** Main interactions involving CED and THU9 during opening simulations.



**Figure 8:** Time-averaged radius profile for the hPIEZO1 channel at p=-30bar.

- Application of a lateral tension of -30 bar in the xy-plane promotes expansion of hPIEZO1 and induces membrane flattening (Figure 6).
- The loop regions between TM35 and TM37 (residues Glu2054 and Met2056) in hPIEZO1 interact with the CED (cap) domain, thereby preventing detachment of the cap (Figure 7).
- Pore dimension estimations (Figure 8) are consistent with those reported for open-channel stage ( $r > 0.15$  nm).