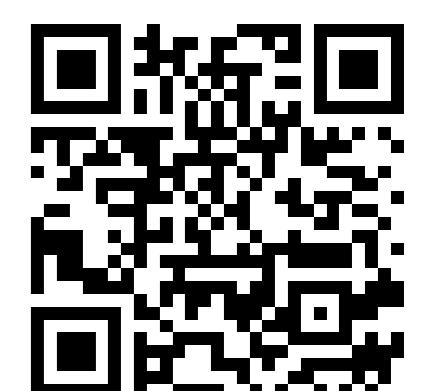


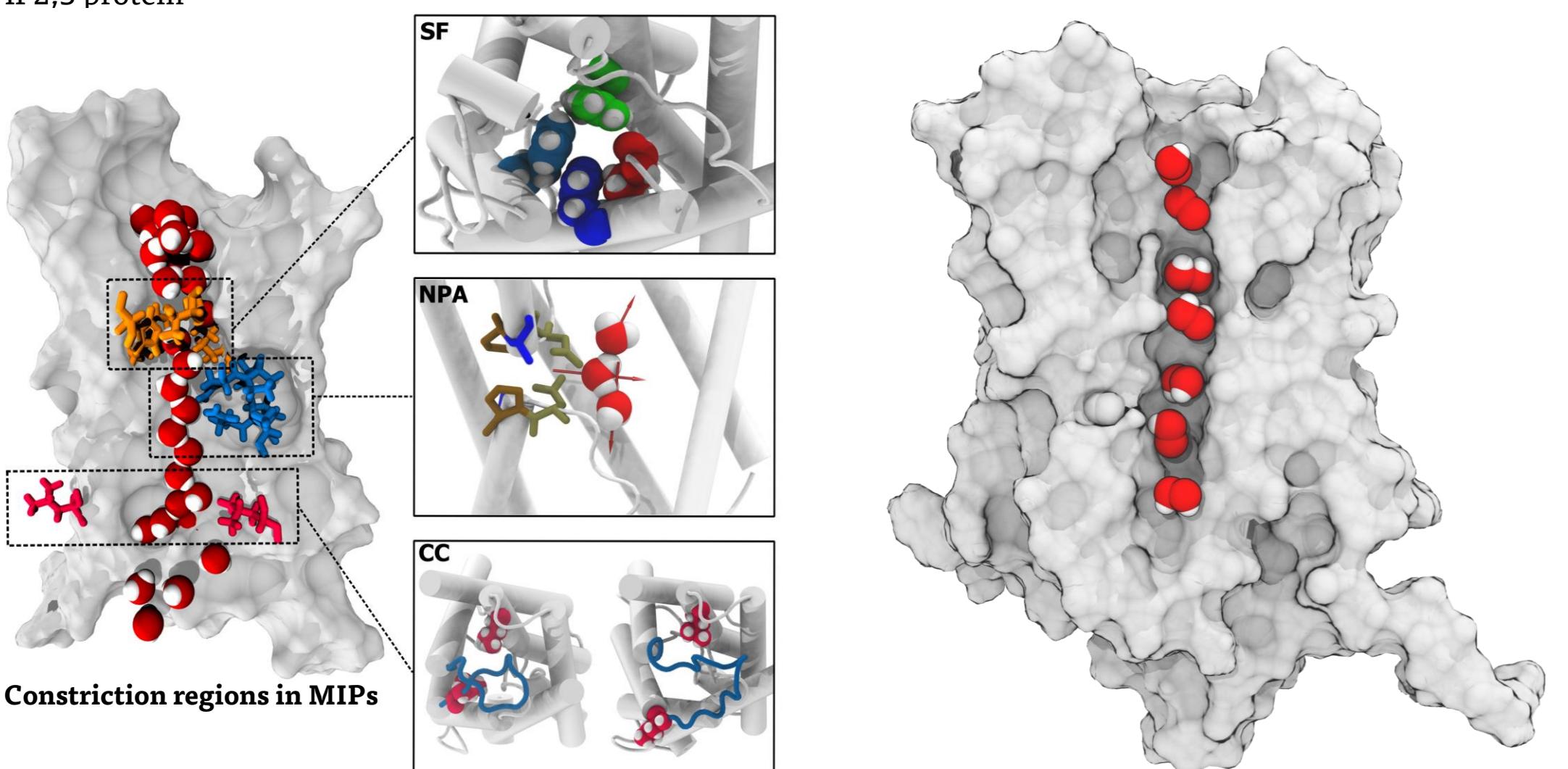


Conserved Peroxiporin Function in MIP Channels: Structural and Thermodynamical Insights.



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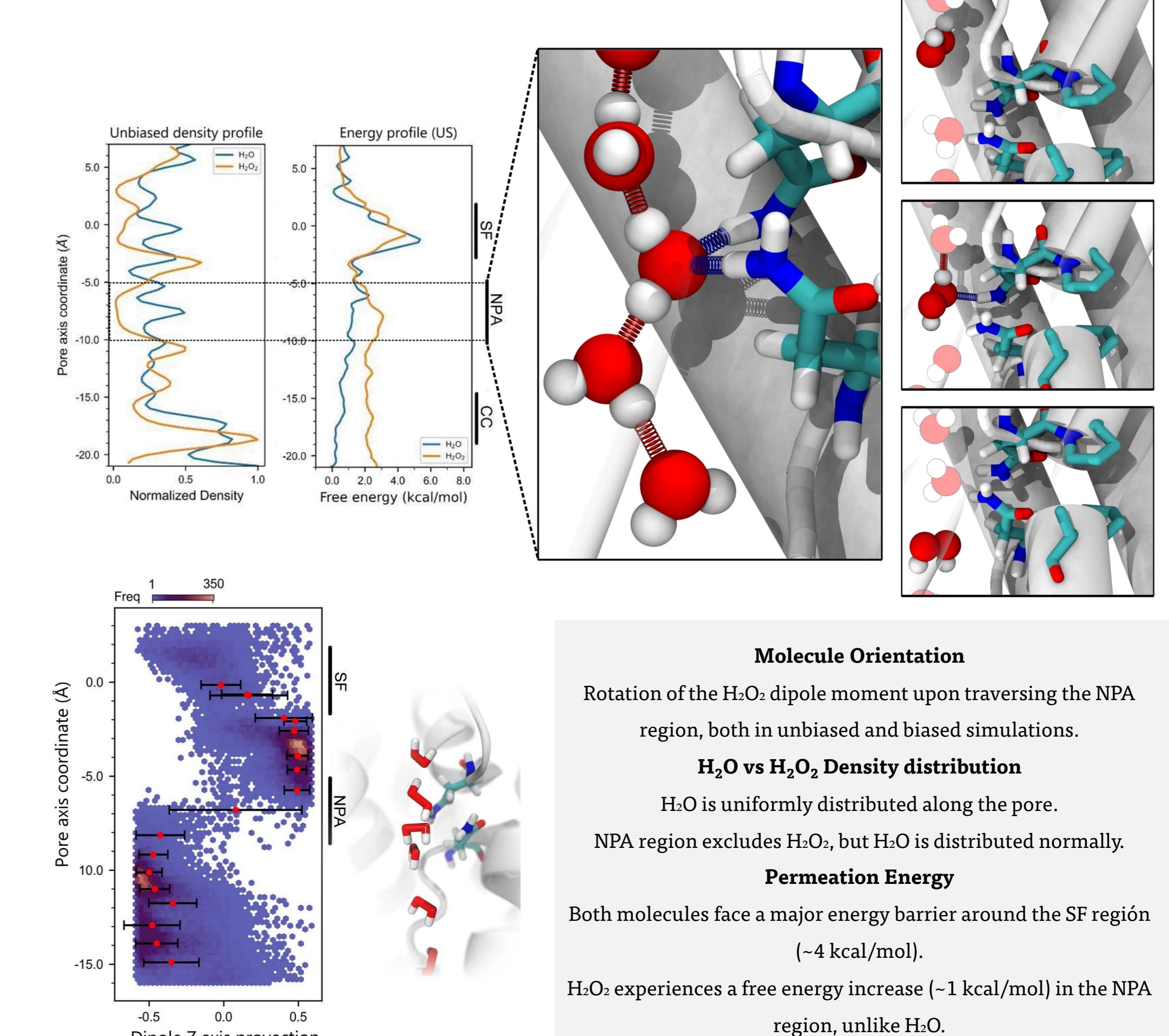
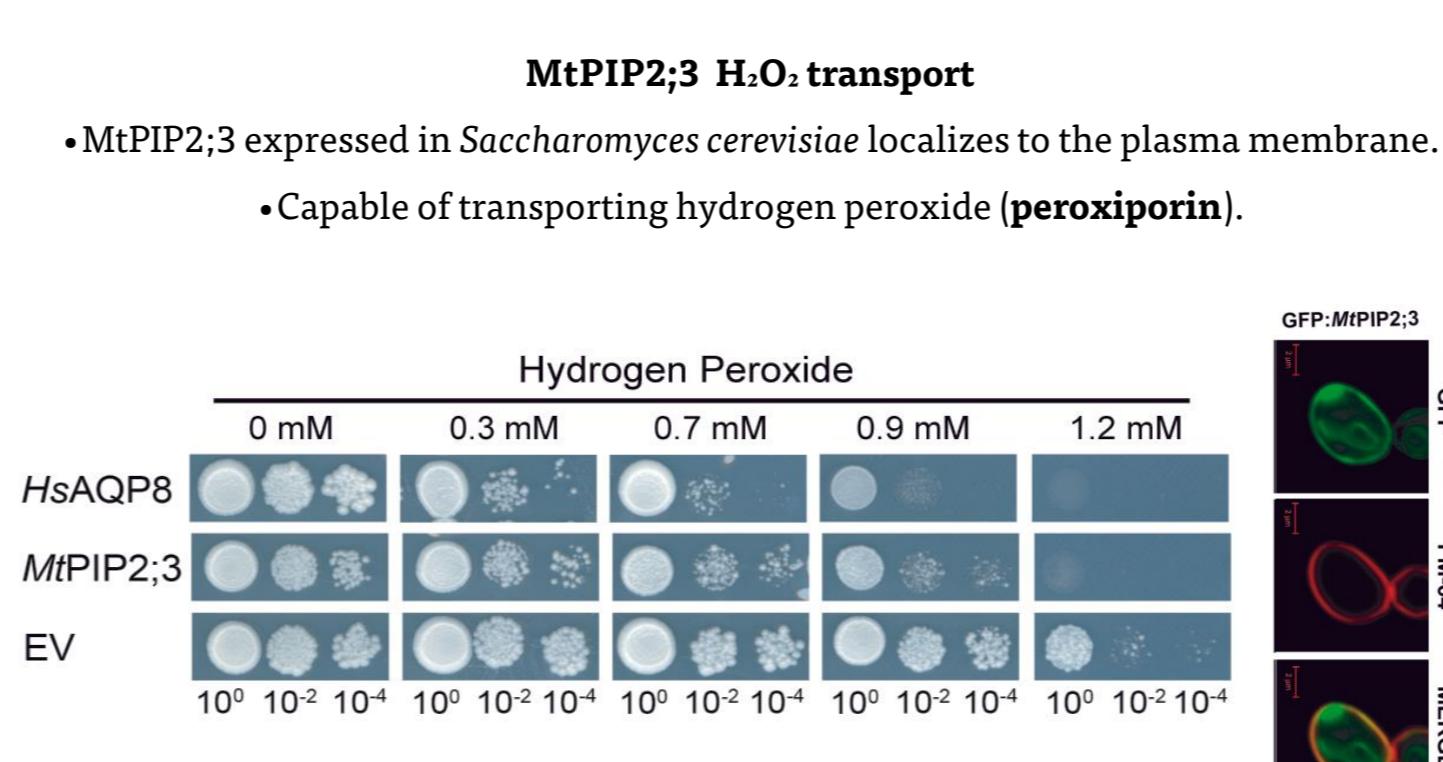
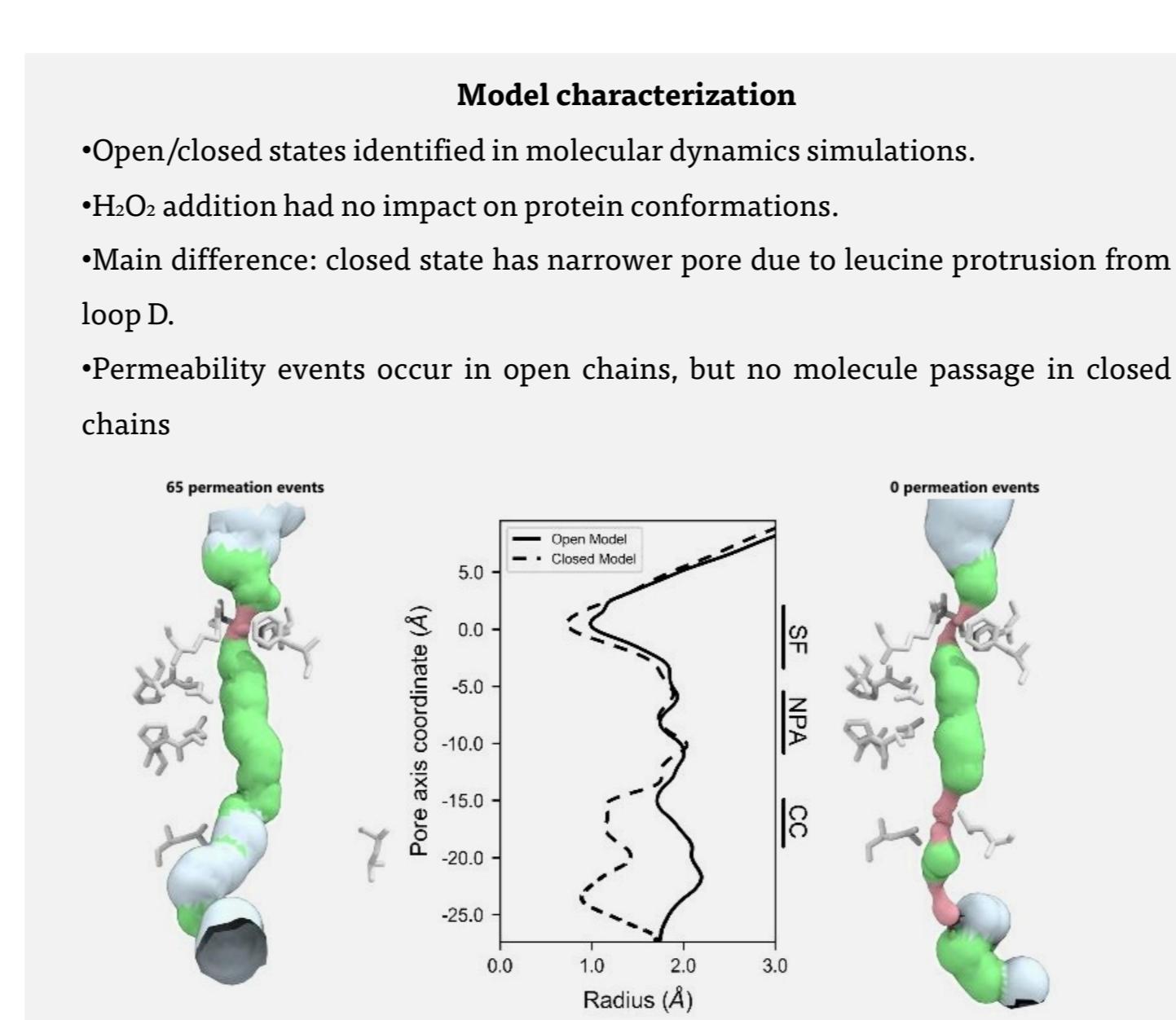
The Membrane Intrinsic Protein (MIP) family plays a crucial role in facilitating the transport of water and small solutes across biological membranes, including hydrogen peroxide (H_2O_2). However, the evolutionary origin of H_2O_2 transport within the MIP family remains unclear, with the possibility that this function could be an ancestral trait. MIPs that are capable of transporting H_2O_2 are referred to as **peroxiporins**. It is important to note that not all MIPs, even those that are closely related, exhibit H_2O_2 permeability. The molecular determinants that govern H_2O_2 permeation and selectivity are not yet fully understood. Our previous studies have provided valuable insights into the transport mechanism of the MtPIP2;3 protein.



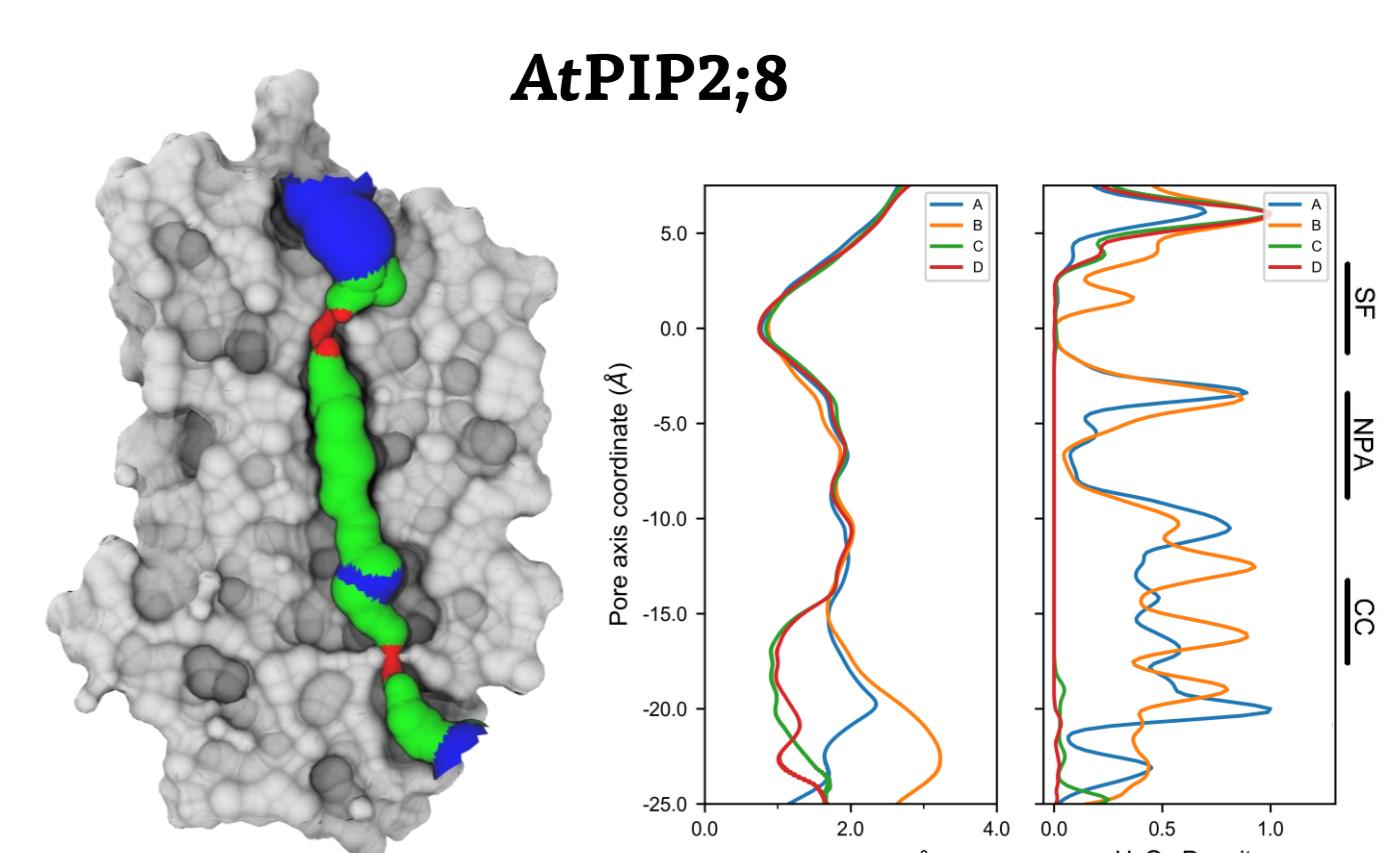
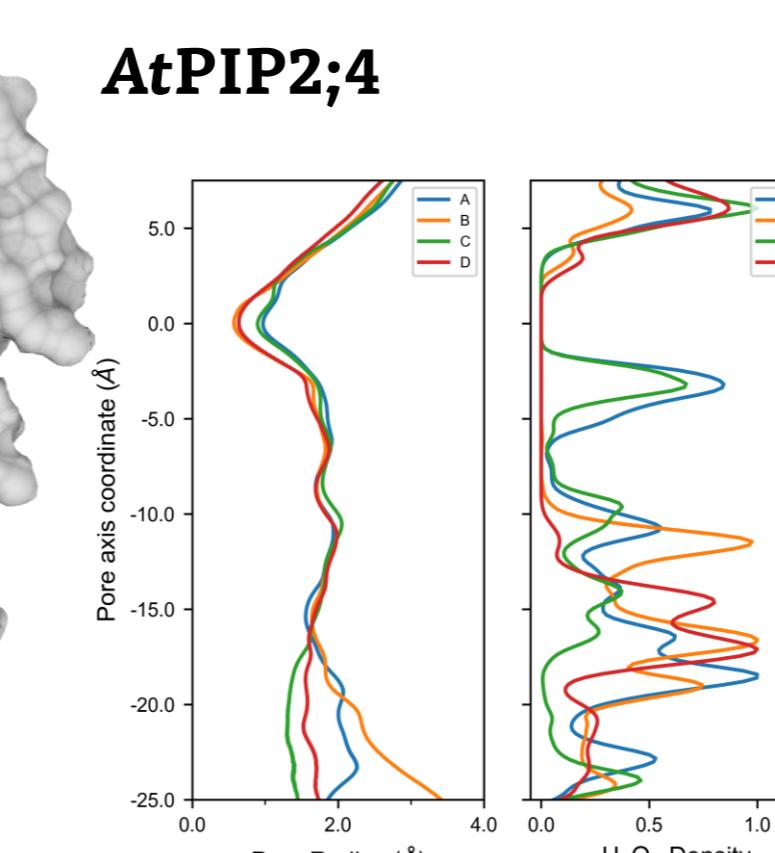
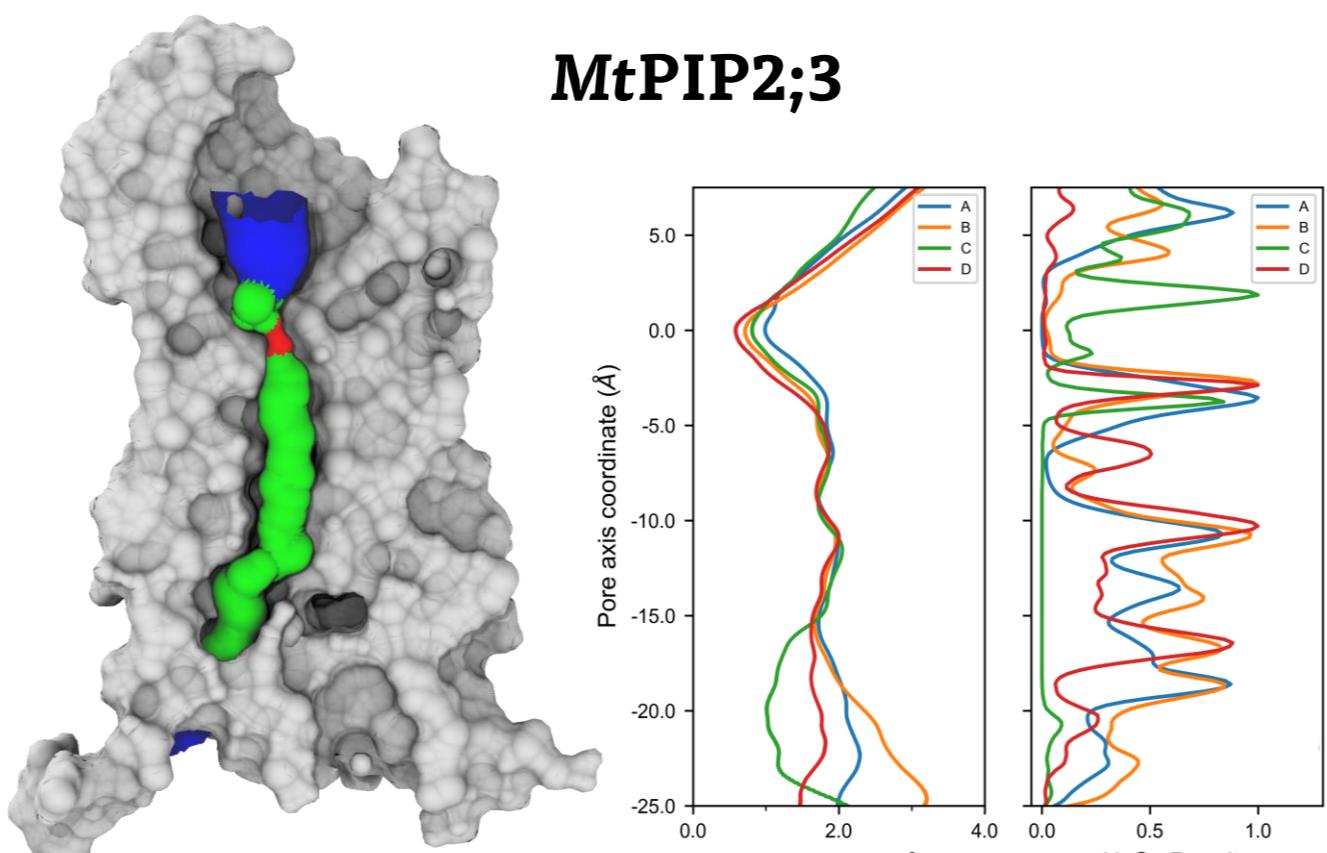
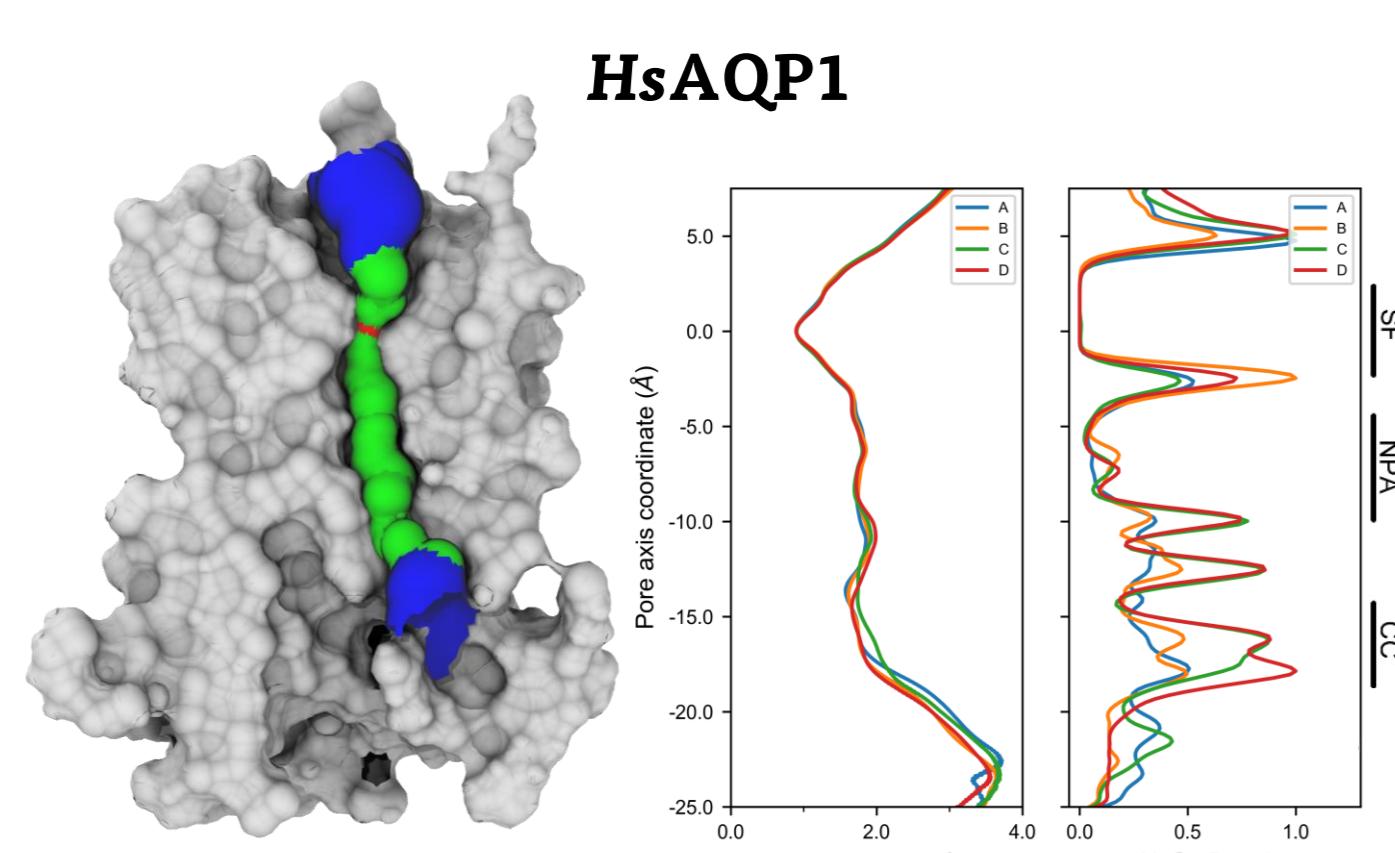
It is unclear whether peroxiporin activity is conserved across all MIP subfamilies or arose through lineage-specific diversification.

To assess the conservation of the H_2O_2 permeation mechanism observed in MtPIP2;3 and to investigate the structural determinants of H_2O_2 permeability, we extended our analysis to a diverse set of MIP channels representing the four major eukaryotic evolutionary groups (AQP1-like, AQP3-like, AQP8-like, and AQP11-like). This comparative analysis included both experimentally validated H_2O_2 transporters and non-transporters, as well as their human and plant homologs.

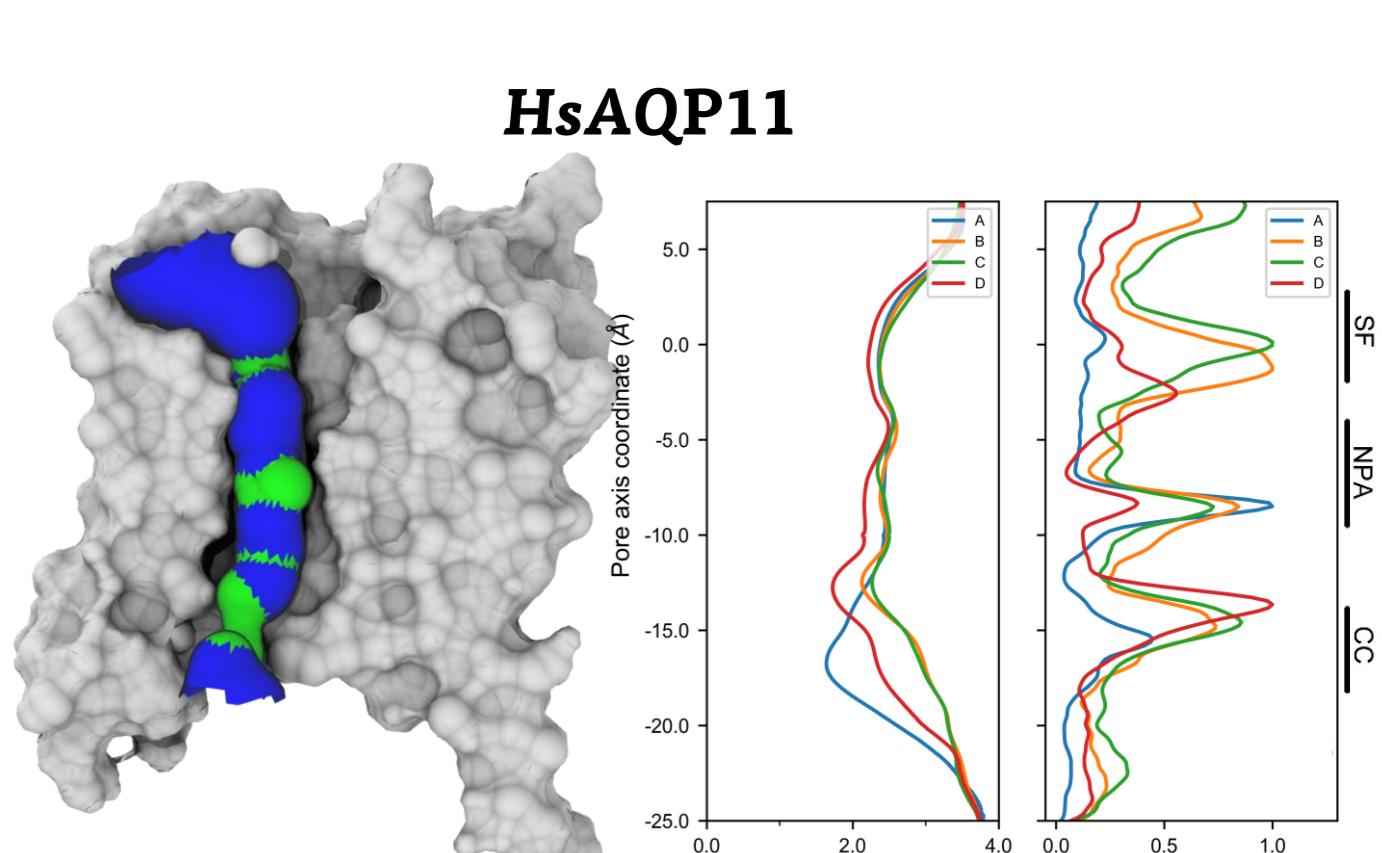
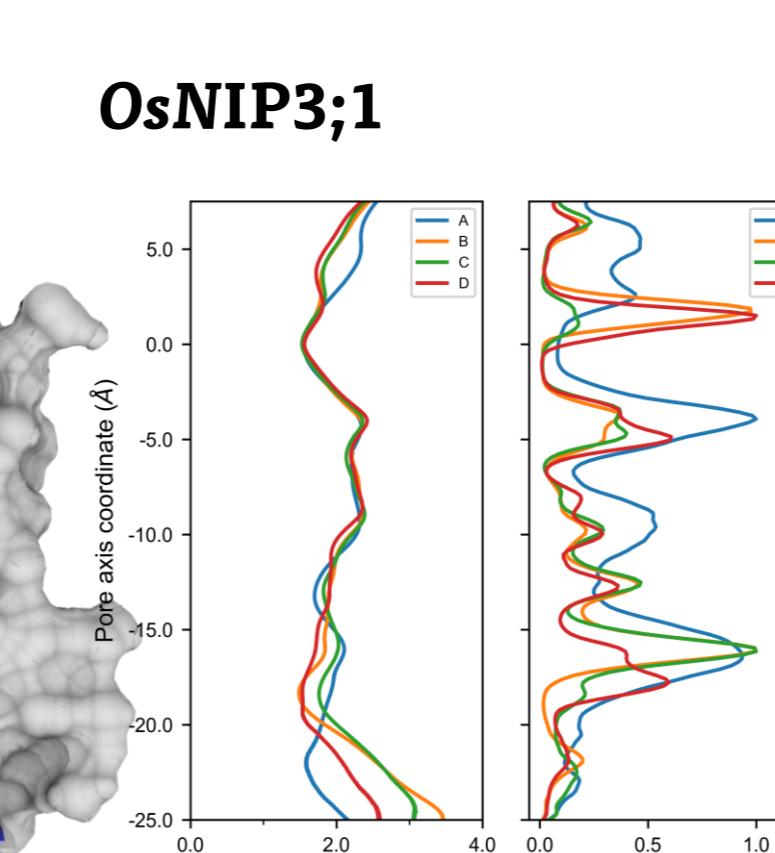
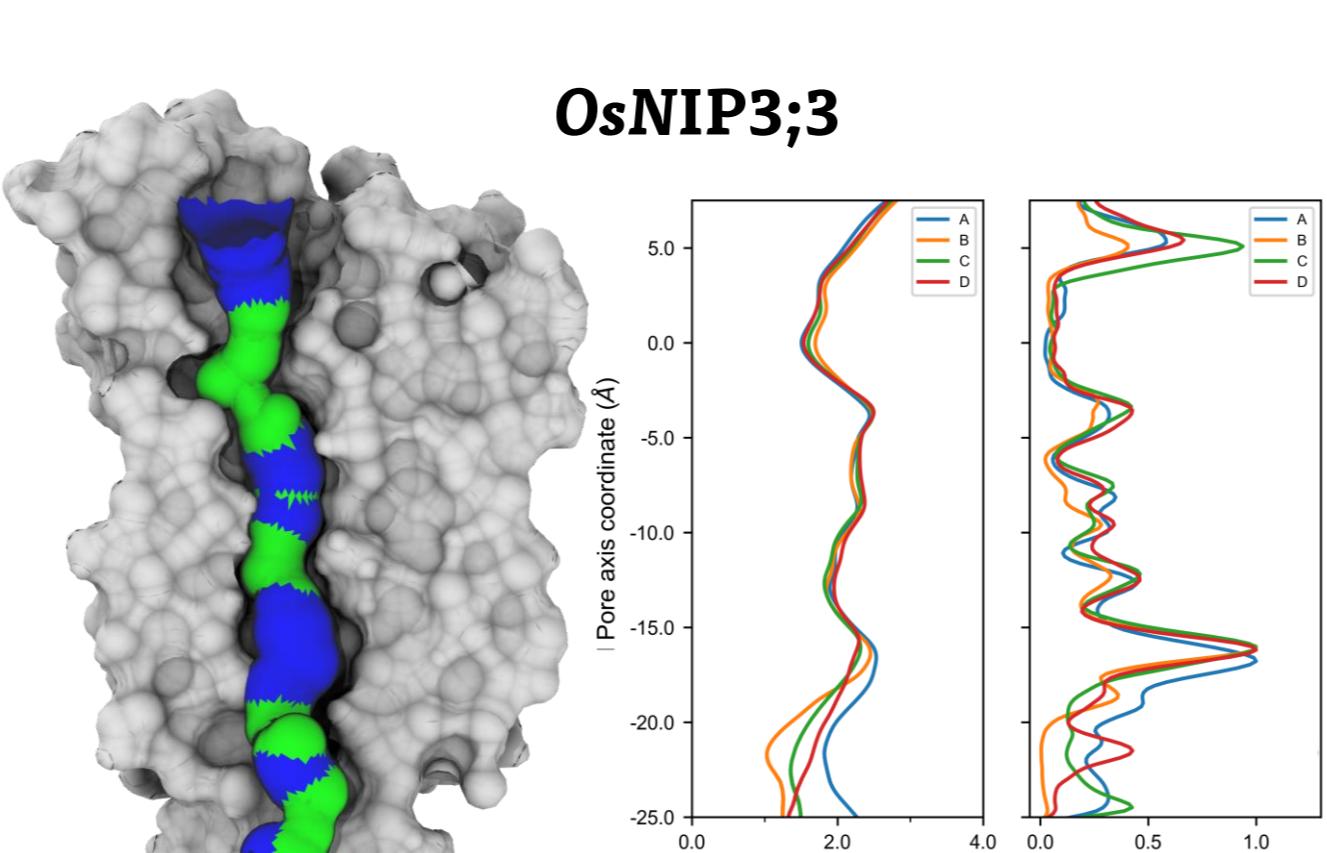
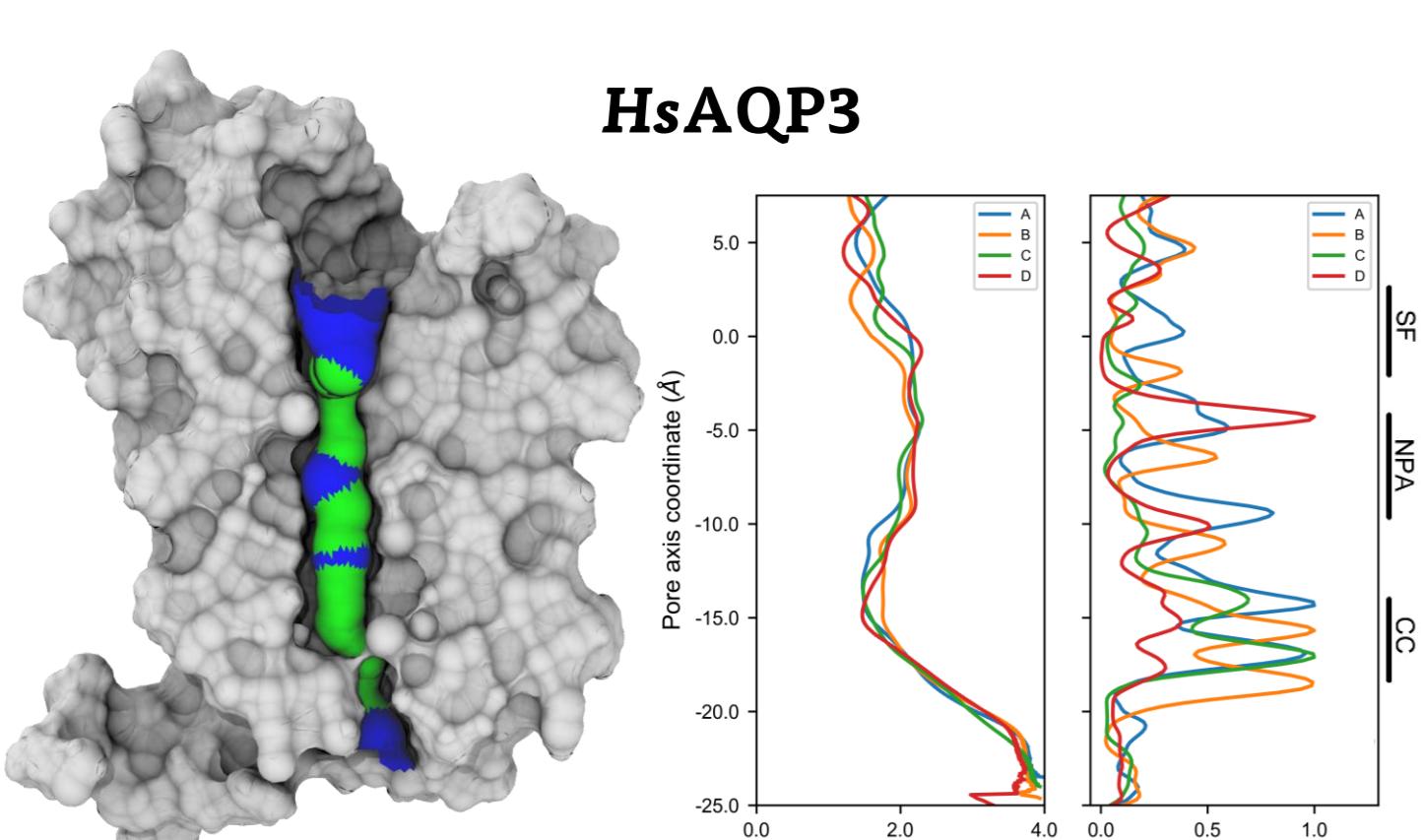
Case study: MtPIP2;3



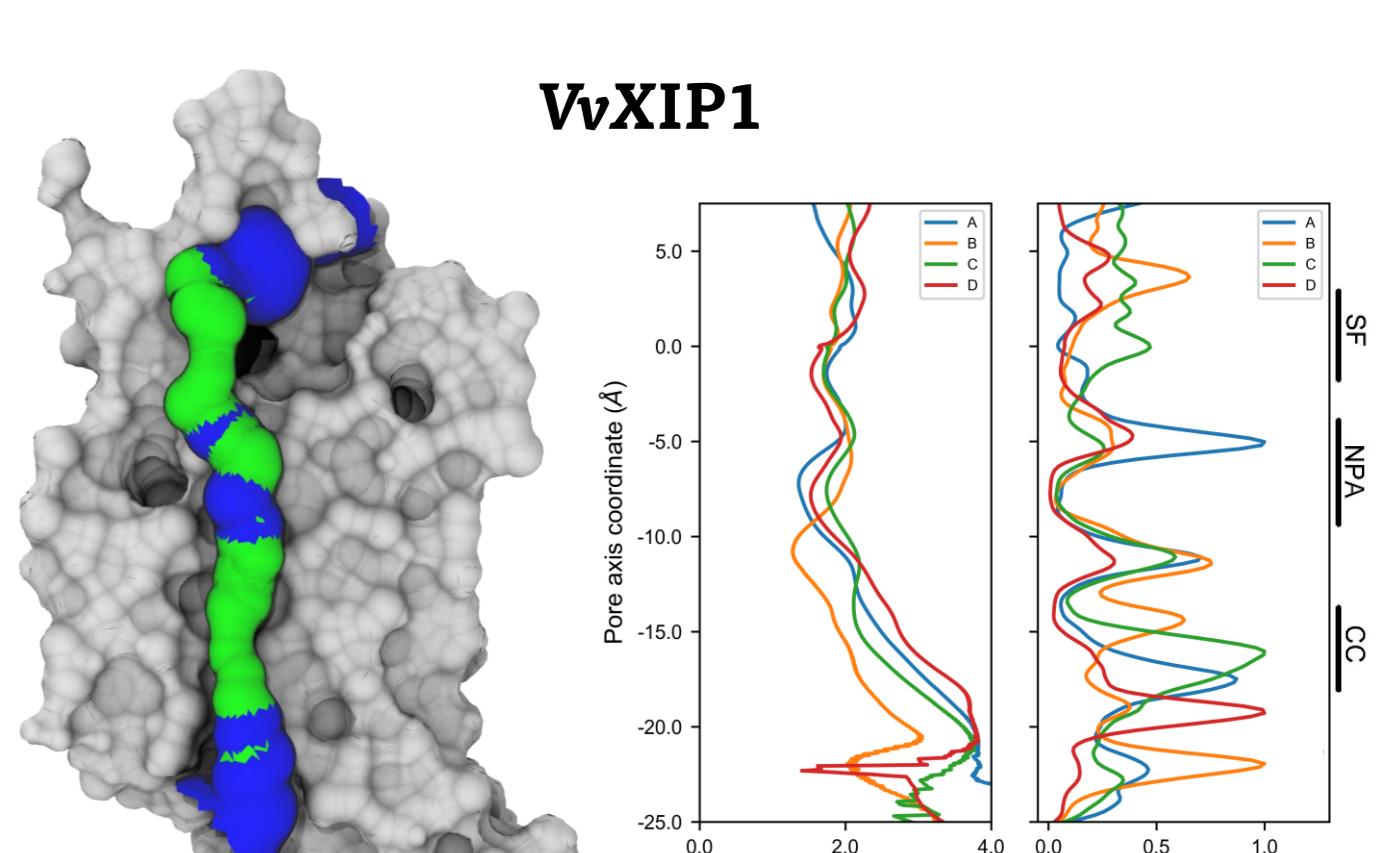
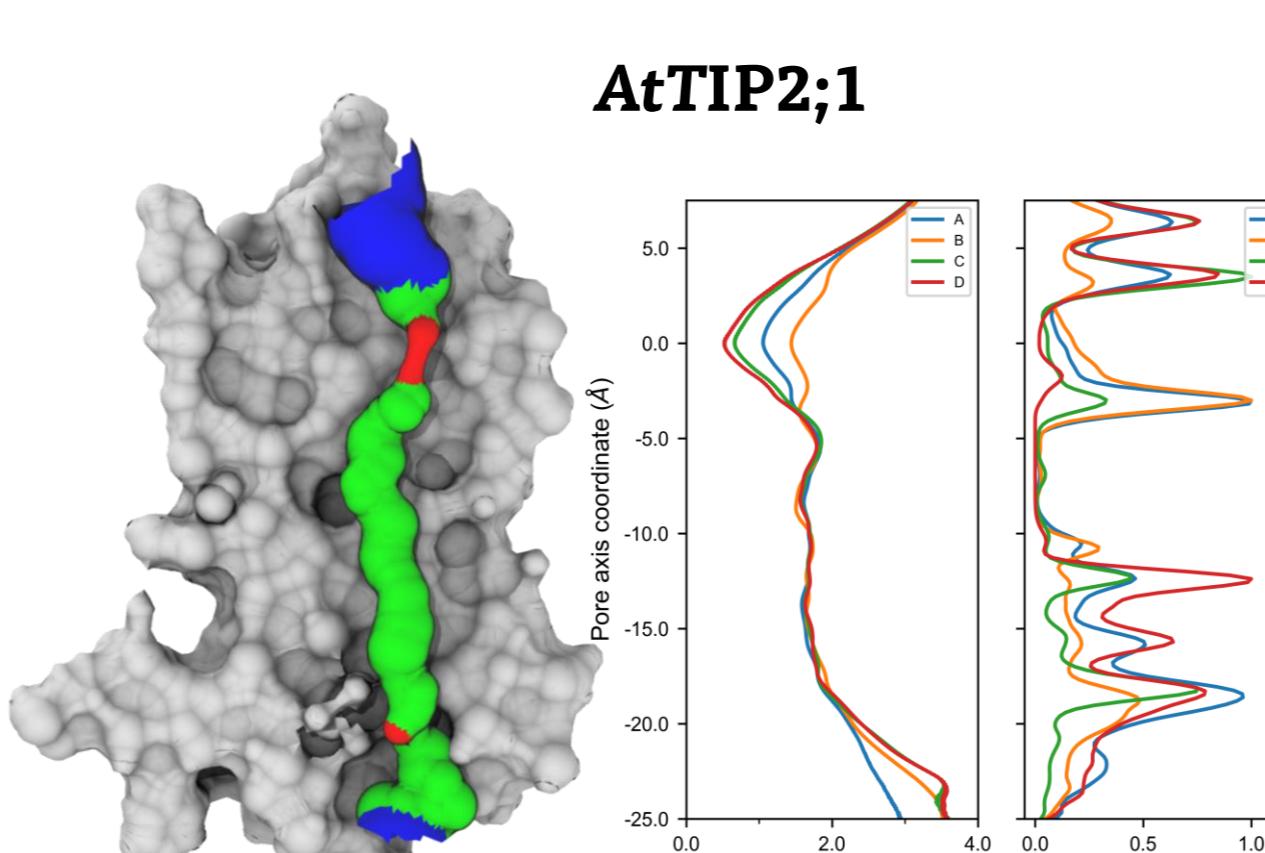
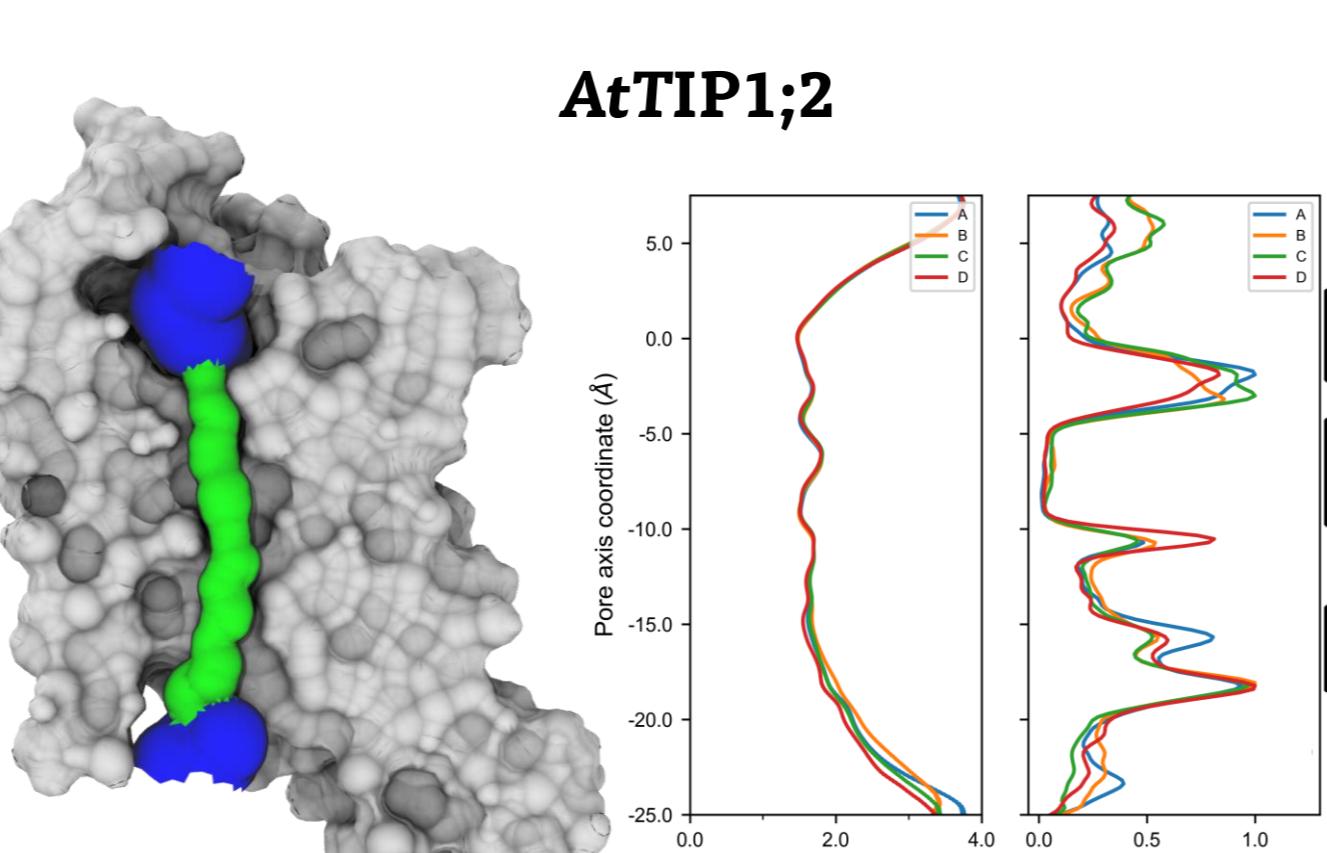
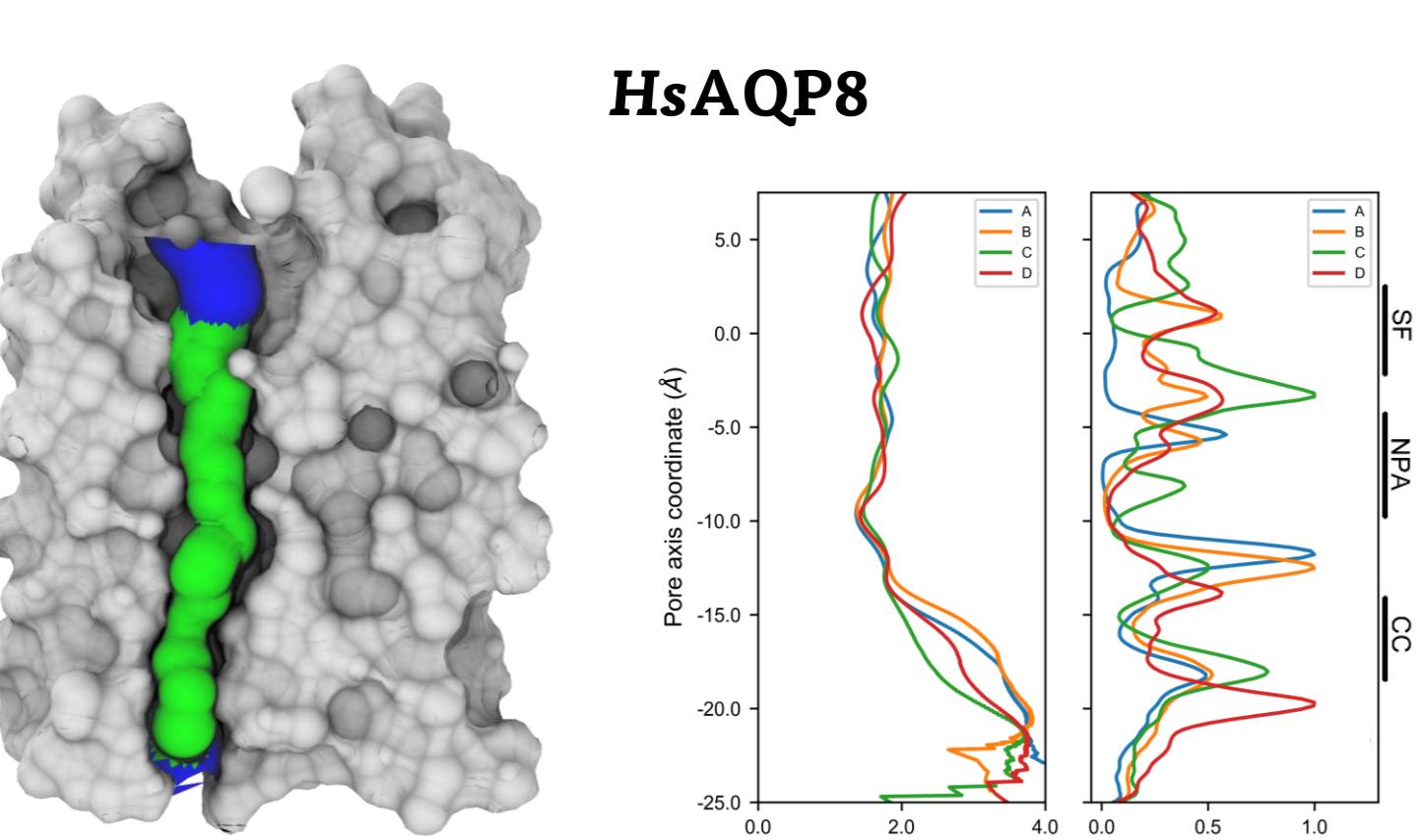
AQP1-Like



AQP3-Like



AQP8-Like



MIPs exhibiting positive experimental H_2O_2 transport

MIPs exhibiting negative experimental H_2O_2 transport

Summary of H_2O_2 permeation events and experimental H_2O_2 Transport in studied MIPs

MIP	H_2O_2 transport activity	Reference	Permeation events by chain
AQP1-LIKE	MtPIP2;3	+	Chevriau et al., 2024
	HsAQP1	-	Almasalmech et al., 2014
	AtPIP2;4	+	Hooijmair et al., 2012
	AtPIP2;8	-	Hooijmair et al., 2012
AQP3-LIKE	HsAQP3	+	Miller et al., 2010
	OsNIP3;3	+	Katsuhara et al., 2014
	OsNIP3;1	-	Katsuhara et al., 2014
AQP8-LIKE	HsAQP8	+	Almasalmech et al., 2013
	AtTIP1;2	+	Laforenza et al., 2017
	AtTIP2;1	-	Bienert et al., 2007
	VvXIP1	+	Noronha et al., 2016
AQP11-LIKE	HsAQP11	+	Bestetti et al., 2019

Methods

Molecular Dynamics simulations

Molecular dynamics simulations were performed for homotetrameric assemblies of selected MIP, with homology models created with SWISS-MODEL online server, using SoPIP2;1 open state crystal 3D structure (2BF) as template, or by AlphaFold Server. Assemblies were embedded in a fully hydrated POPC bilayer, with explicit solvent (TIP3P water) and ions (NaCl 0.15M) using the membrane builder tool provided in the CHARMM-GUI website. ~300ns was added to the systems by replacement of water molecules, and 500ns unbiased molecular dynamics simulations were run with an NPT ensemble with full periodic boundary conditions, performed with AMBER18 MD package, using hydrogen mass repartitioning (HMR) and parameters from AMBER14SB and LIPID17 force fields.

Density probability calculation

The density probability function were calculated by extracting the XYZ positions of the geometric center of the H_2O_2 or H_2O molecules throughout the simulation time with the cptraj program of the AmberTools package. The origin of the Z coordinate of the system was centered in each simulation frame towards the geometric center formed by the Ar/R residues and the probability density along the pore was calculated by Kernel Density Estimation [KDE] using the software R.

Permeation events

To quantify permeation events, a custom Python script was developed. This script leverages molecular dynamics trajectories to track the movement of molecules through a defined pore region. Specifically, the script establishes reference points on either side of the pore and counts the number of molecules that traverse these points over the course of the simulation, regardless of the direction of permeation.

Pore Dimensions

To calculate pore dimensions along the Z-axis for each trajectory and perform statistical analysis, the HOLE module within the MDAnalysis package was employed, along with custom Python scripts.

Conclusions

- Spontaneous H_2O_2 permeation was observed in channels previously considered non-transporters (AtPIP2;8, OsNIP3;1, AtTIP2;1). This suggests pore structure alone doesn't fully explain H_2O_2 permeability, with other factors like post-translational modifications, lipid interactions, or experimental limitations playing a role. Negative results in yeast experimental assays may be due to methodological issues.

- H_2O_2 transport appears to be a characteristic shared across multiple MIP families, suggesting it could be an ancestral trait. The loss of H_2O_2 permeability in certain MIPs may not be due to changes in the pore itself, but rather to post-translational modifications or regulatory mechanisms acting outside the pore.

- The H_2O_2 restriction seen in MtPIP2;3 NPA region was also observed in other MIP families, indicating this may be a broader regulatory mechanism.

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