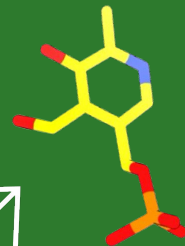
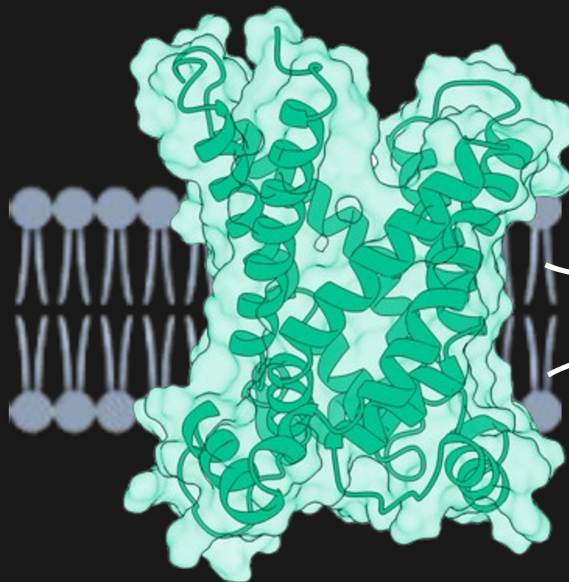


SLC25A38 binding of Pyridoxal 5'-Phosphate: A computational Study



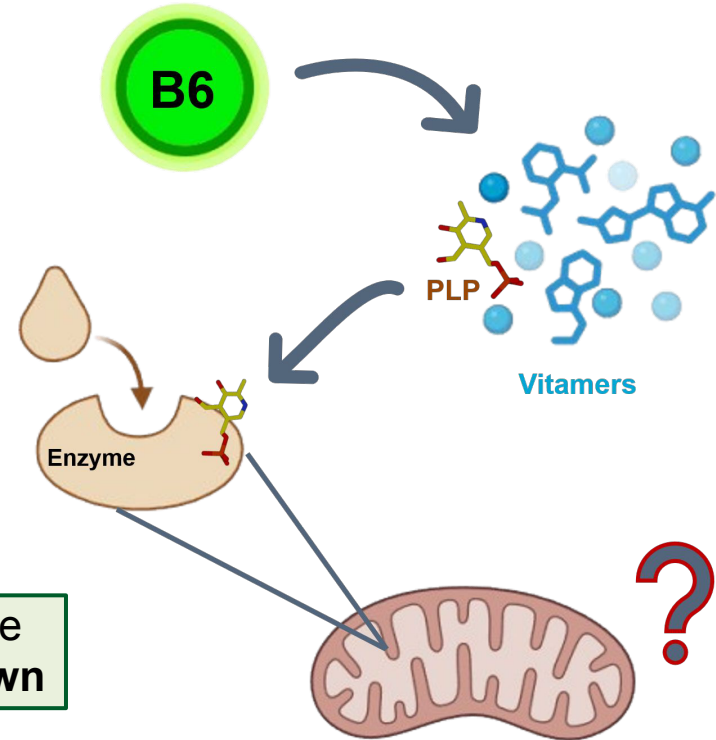
Miriana Quaranta

Sapienza University of Rome

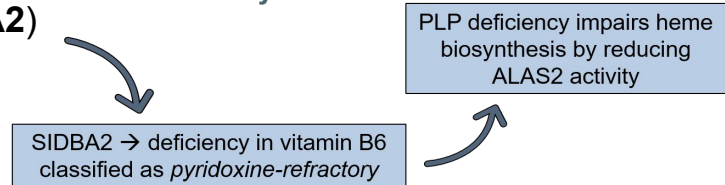
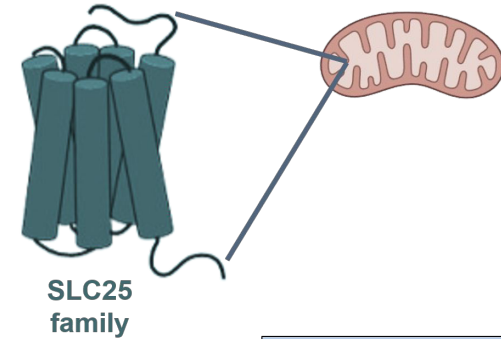


- Vitamin B6 includes biologically active **vitamers**
- Phosphorylated forms □ **Pyridoxal 5'-Phosphate (PLP)**
- Key enzymatic cofactor
- ~60 PLP-dependent enzymes
- One third of PLP-dependent enzymes localized in **mitochondria**

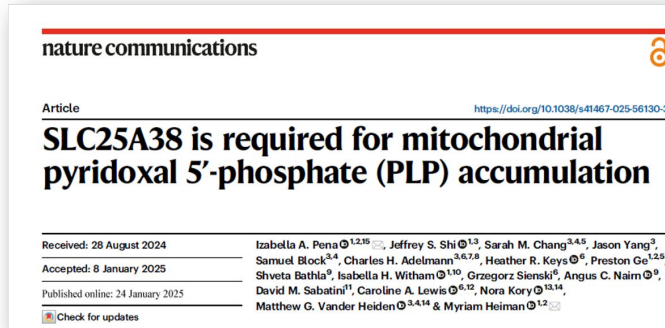
The **mechanisms** of PLP transport across the mitochondrial inner membrane remain **unknown**

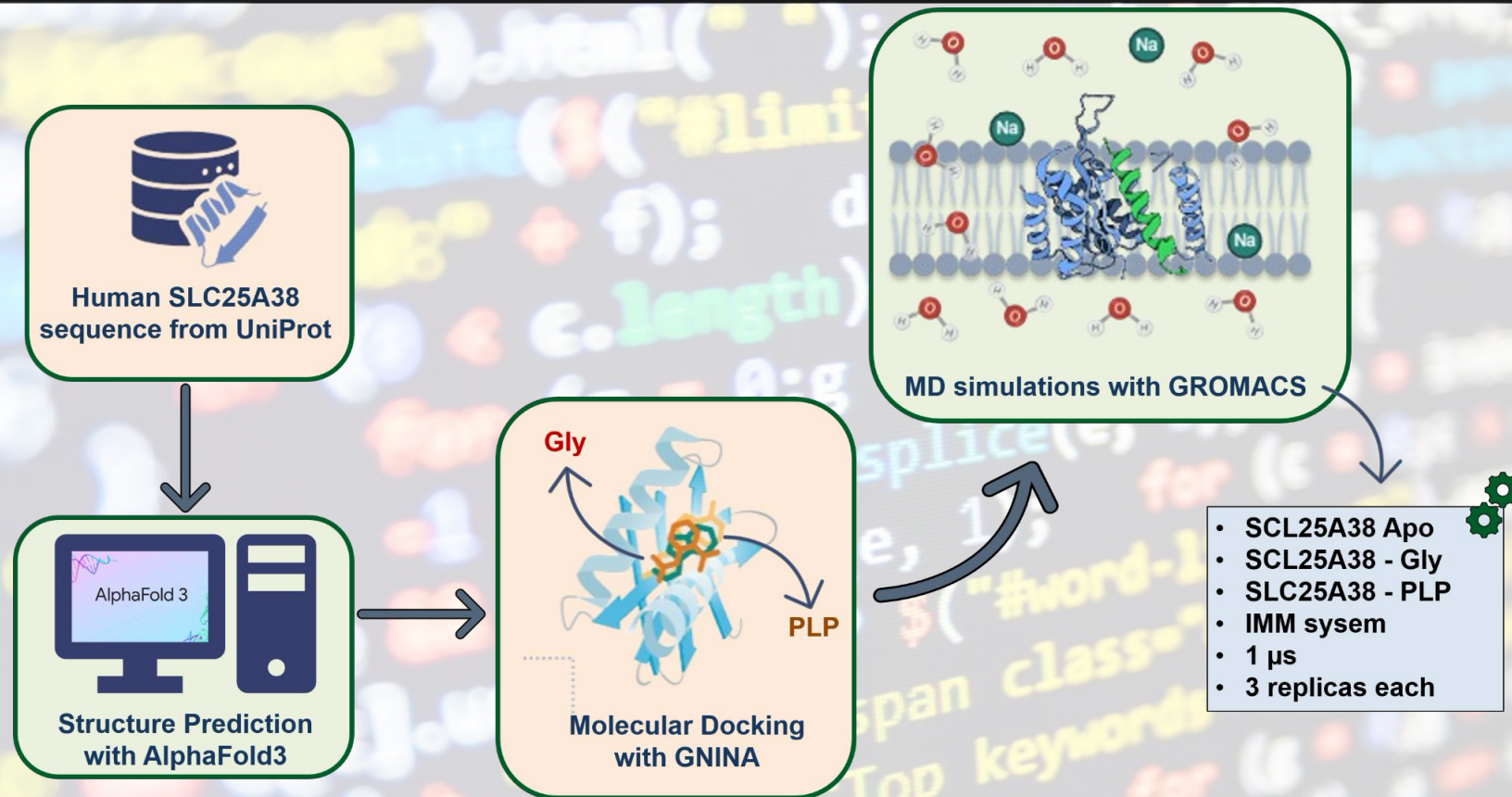


- SLC25 carriers □ mitochondrial transport
- **SLC25A38** was previously described exclusively as a glycine transporter and linked to **heme biosynthesis**
- Its dysfunction causes sideroblastic anemia type 2 (**SIDBA2**)



SLC25A38 mediates mitochondrial PLP transport; its loss reduces SHMT2 activity and consequently mitochondrial glycine synthesis

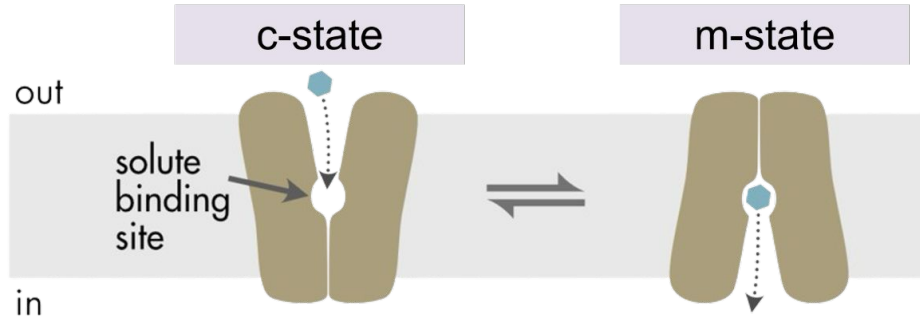




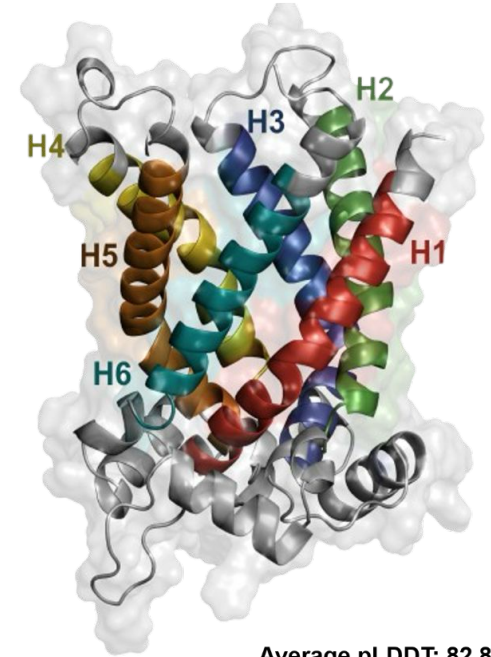
Results: Structural Modelling of SLC25A38

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- The transporters belonging to this family, typically alternates during solute transport between c- and m-states
- The c-state exposes the substrate-binding site to the cytoplasmic side



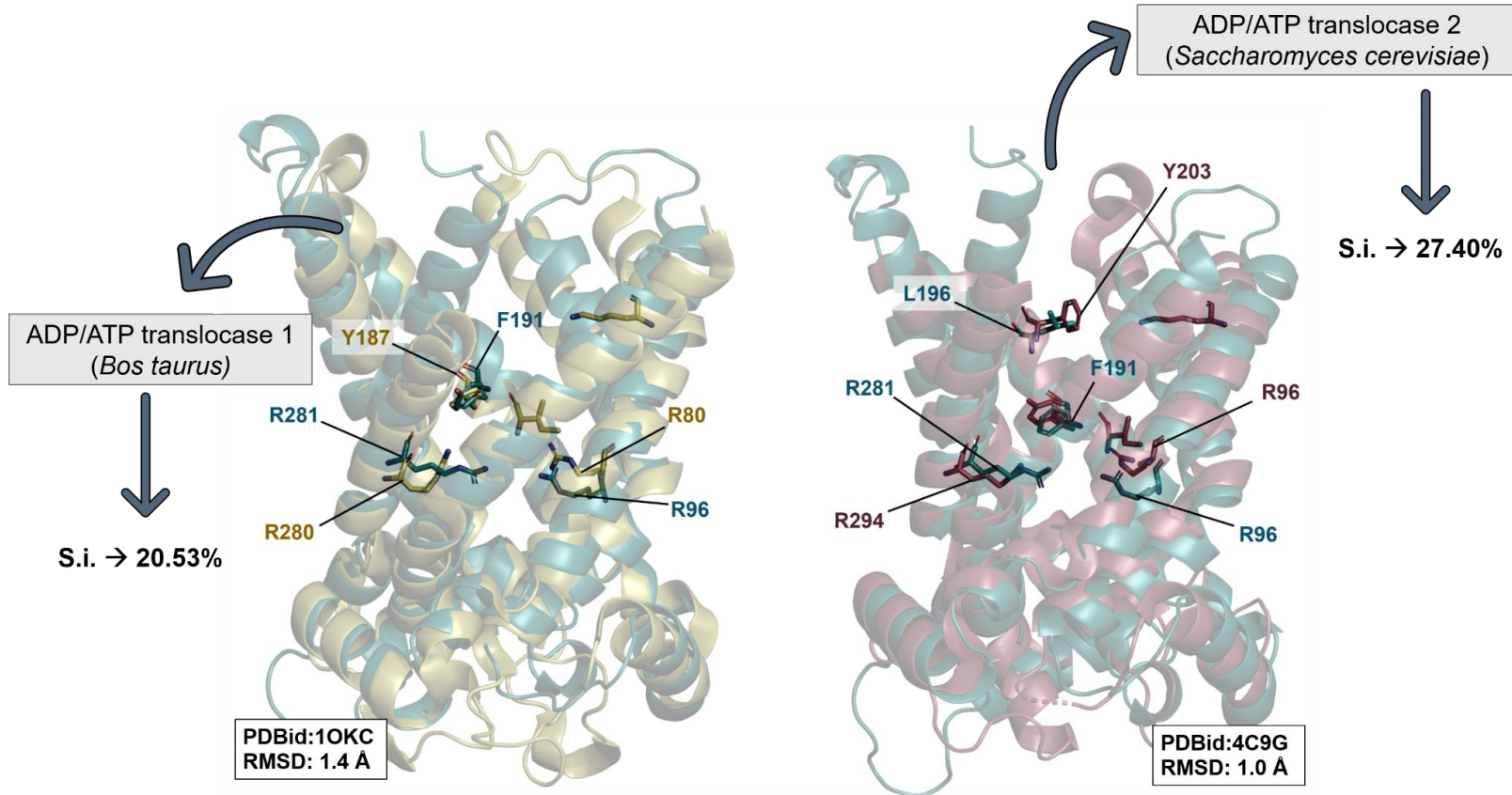
**Predicted Human SLC25A38
(c-state)**

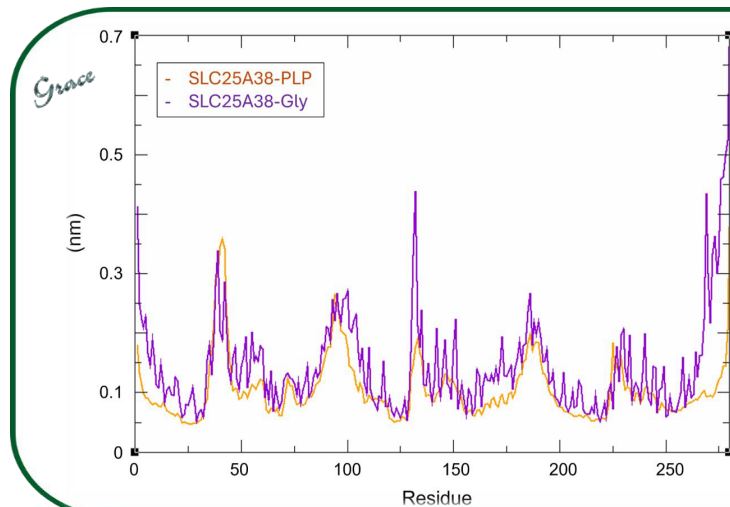


Average pLDDT: 82.88

Results: Structural Modelling of SLC25A38

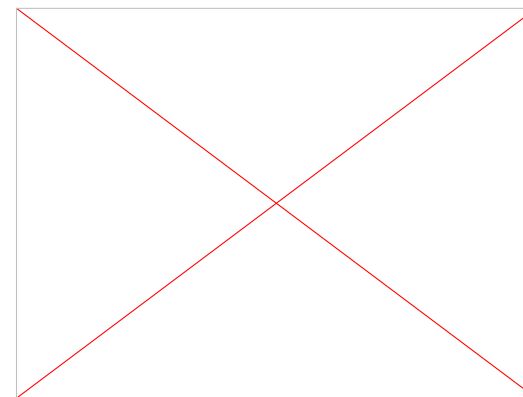
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SLC25A38-PLP

SLC25A38-Gly

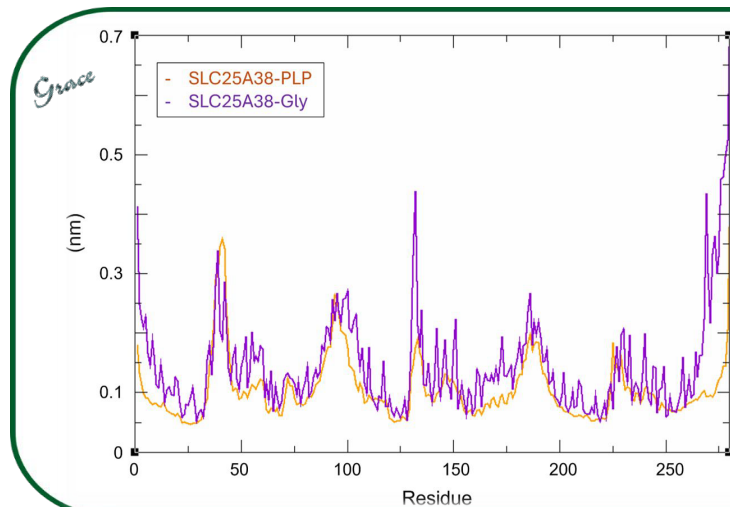


- **Lower residue flexibility** in presence of **PLP**
- **Glycine** remains bound only for the first **~10 ns**
- The **PLP-bound** system shows **reduced energy** fluctuations

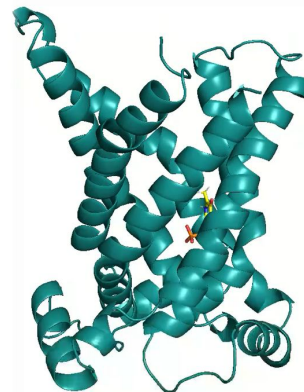
FAST. FLEXIBLE. FREE.
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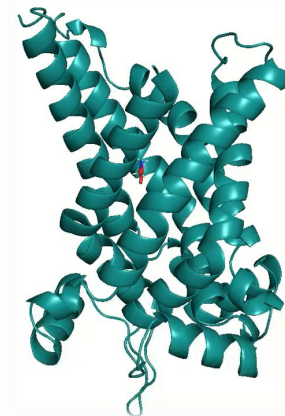
- Force field → **CHARMM General Force Field (CGenFF)**
- System → **Mitochondrial internal Membrane**
- Water model → **TIP3P**
- Length → **1 μ s** (triplicate)



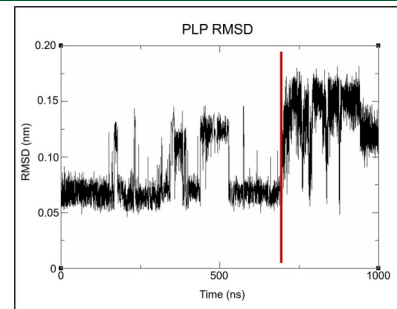
SLC25A38-PLP



SLC25A38-Gly



- **Lower residue flexibility** in presence of PLP
- **Glycine** remains bound only for the first ~10 ns
- The **PLP-bound** system shows **reduced energy fluctuations**



FAST. FLEXIBLE. FREE.
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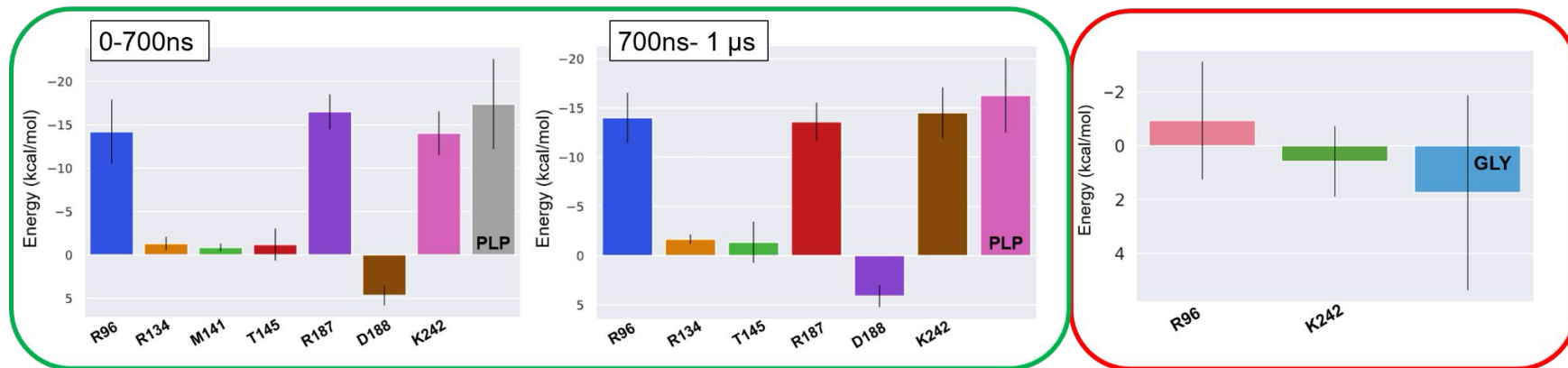
- Force field → CHARMM General Force Field (CGenFF)
- System → Mitochondrial internal Membrane
- Water model → TIP3P
- Length → 1 μ s (triplicate)

Results: Binding energetics & key residues

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System	Time window	ΔG (kcal/mol)
Protein-PLP	0 - 700 ns	-68.83 ± 0.32
Protein-PLP	700 - 1 μ s	-63.80 ± 0.44
Protein-Gly	0 - 11 ns	1.51 ± 0.69



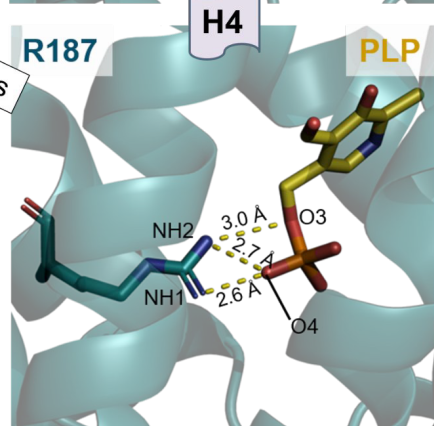
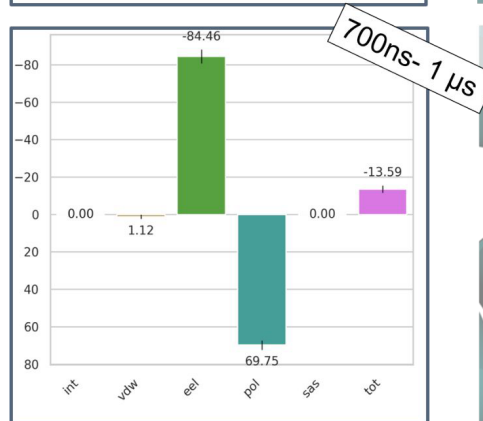
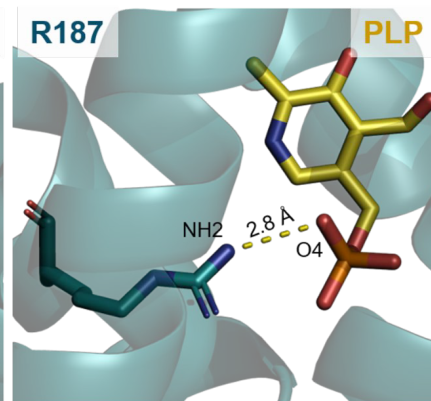
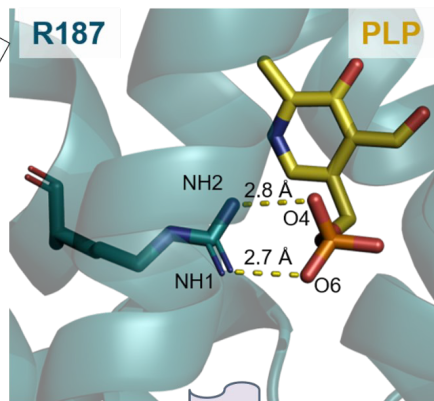
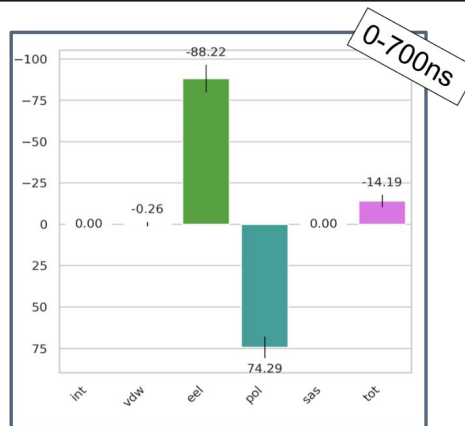
PLP binding is energetically **favorable**, driven by conserved charged residues

Results: PLP binding key residue Arg187

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LIGPLOT⁺

Absent from
glycine interaction
network



- Arg87 binding is driven by **electrostatic interactions**
- Stabilize PLP **phosphate** group
- It oscillates between interactions involving two oxygen atoms

- Int: internal energy
- Vdw: Van der Waals
- eel: electrostatic
- Pol: polar solvation

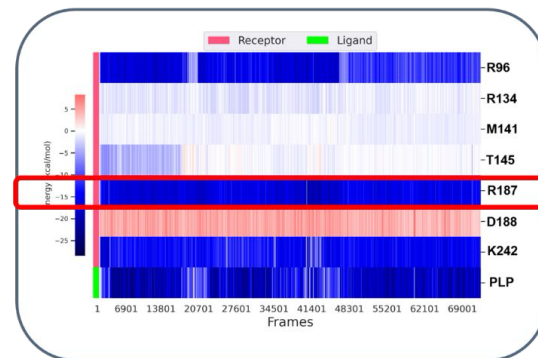
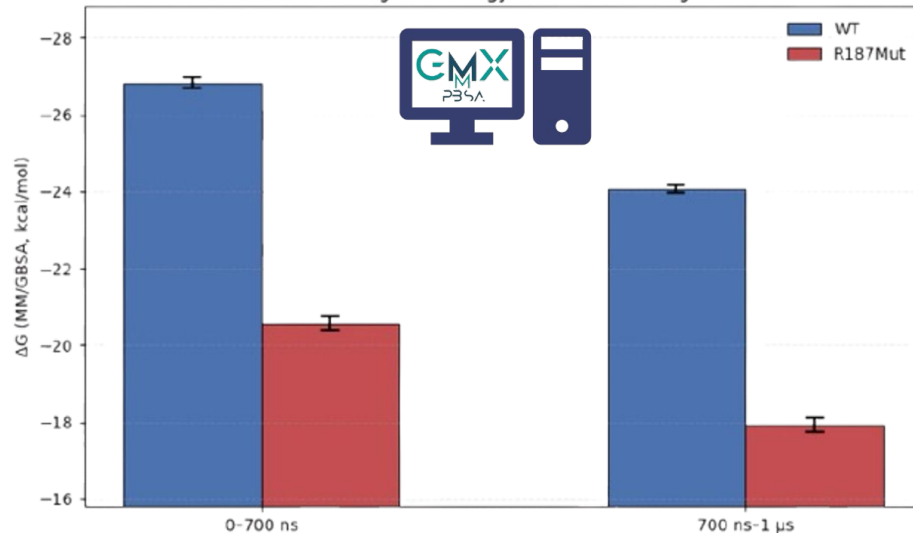
i



VAR_058095 → **R187P**



Binding free energy: Alanine Scanning



- ❑ **R187P** may impair **electrostatic stabilization** of PLP, eliminating the positively charged side chain
- ❑ **Pro** may induce local **structural distortion** in helix **H4**



AF3 predicts a consistent SLC25 carrier → **SLC25A38**



Docking, MD and MM/PBSA support **preferential binding** of **PLP** over glycine



PLP remained stably bound throughout the simulations, whereas **glycine dissociated** within ~10 ns

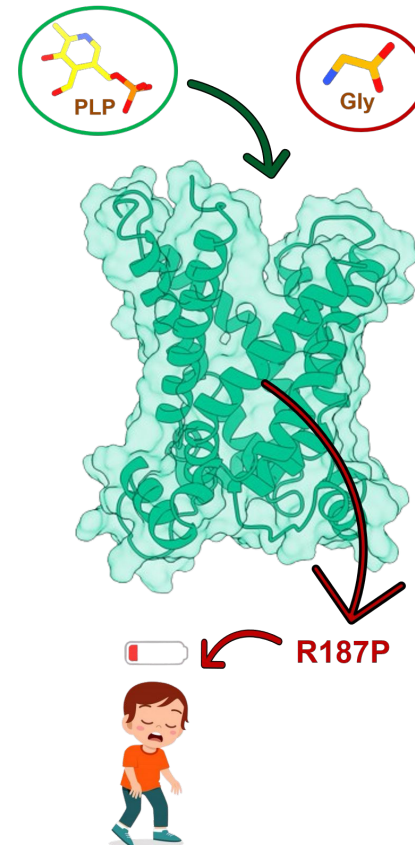


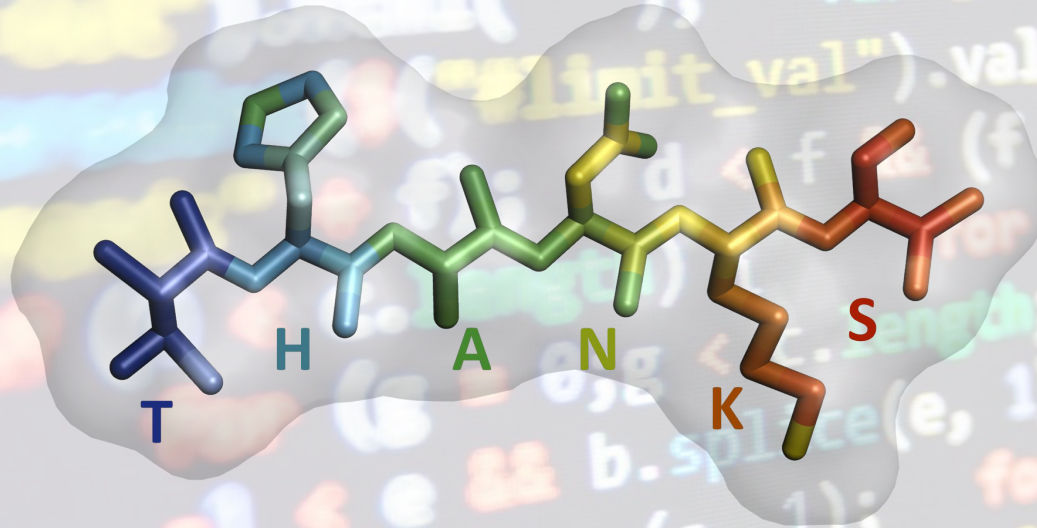
Arg187 is one of the major contributors to PLP stabilization through **electrostatic interactions** with the phosphate group



The key contribution of Arg187 to PLP binding offers a **mechanistic explanation** for the **R187P SIDBA2** variant

Together, these data support the hypothesis that SLC25A38 contributes to mitochondrial PLP homeostasis through PLP transport



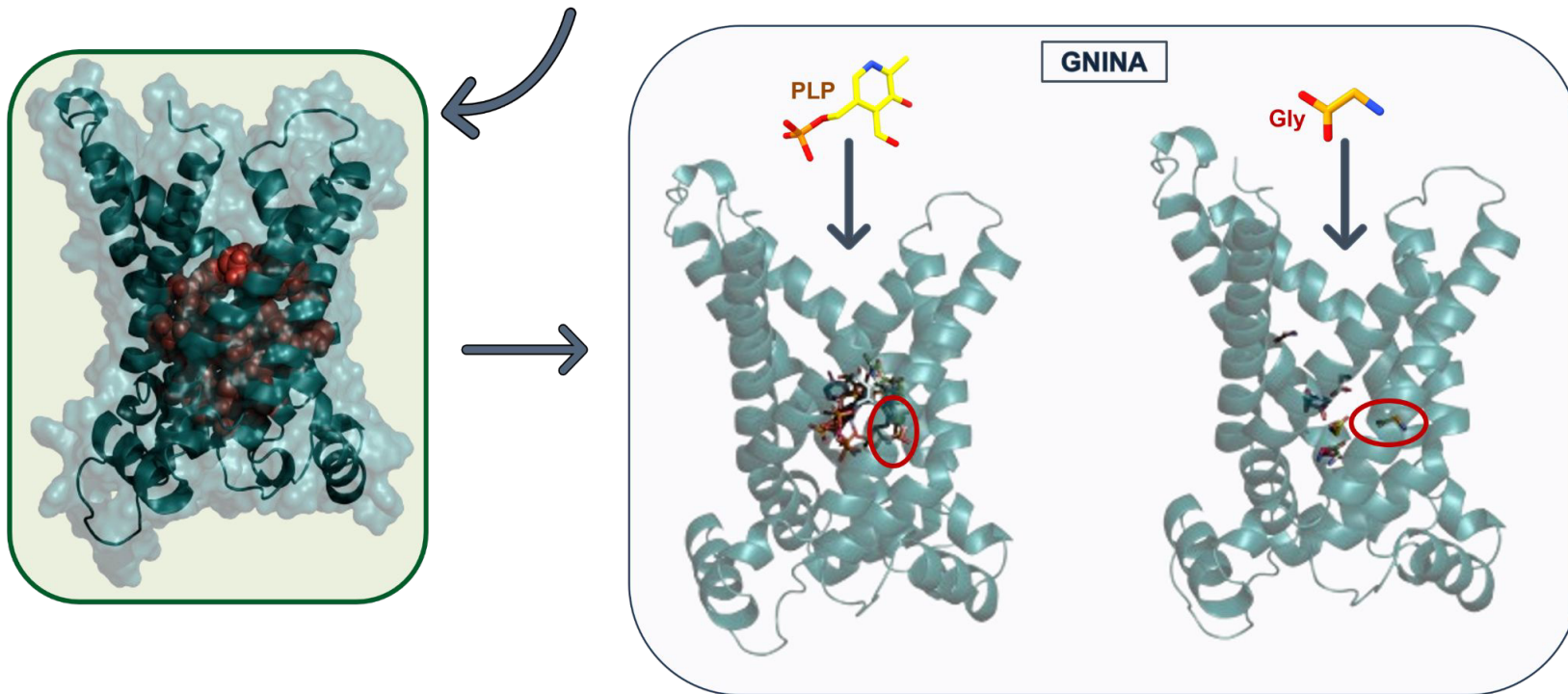


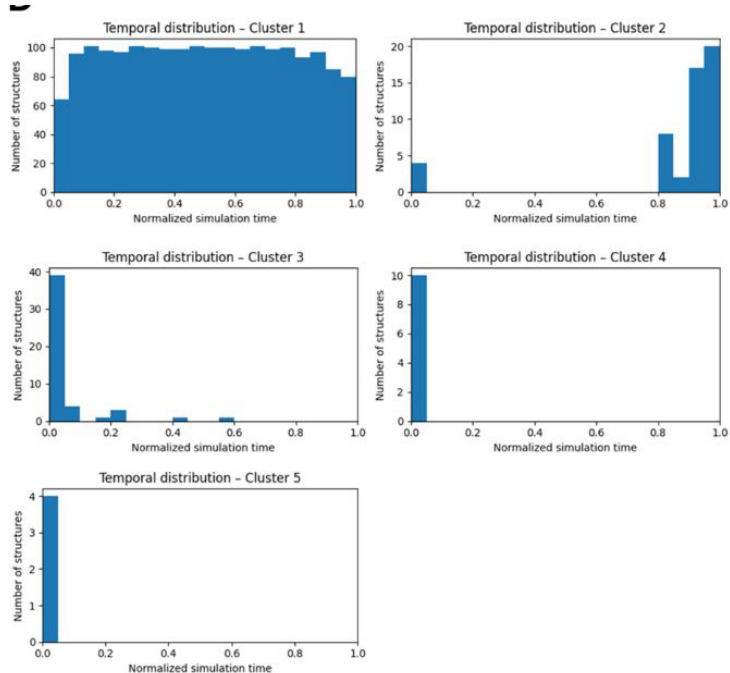
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THANKS

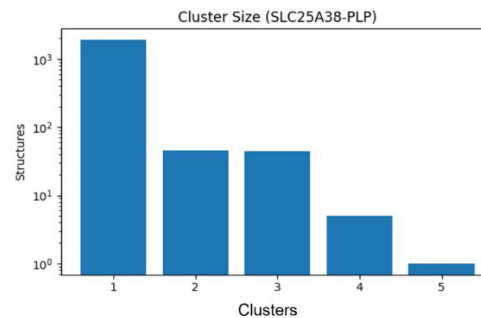
for your attention

- **P2Rank** identified a primary binding pocket to guide **GNINA** Molecular Docking

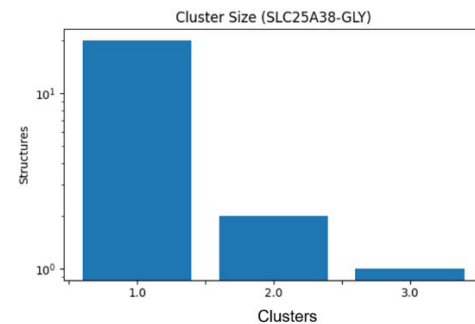




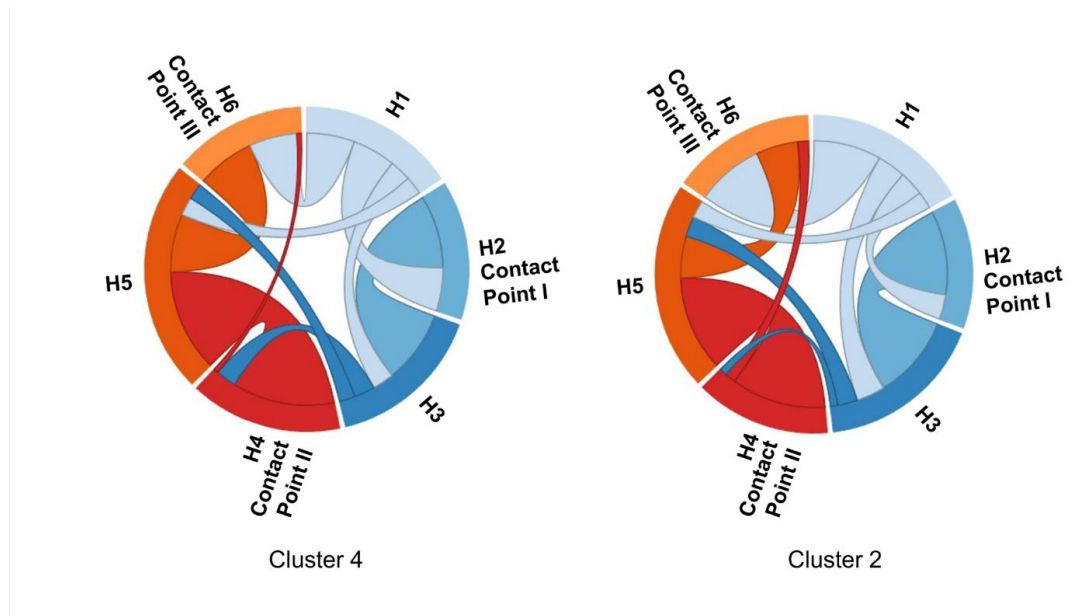
A



B



Distribution of individual SLC25A38 conformational clusters over the entire simulation



Transmembrane helices contacts representation. Residue-residue interaction networks among the SLC25A38 helices during the MD simulation with PLP. On the left is the centroid of cluster 4, taken as the initial conformation at the start of the simulation; on the right is the centroid of cluster 2, taken as the final conformation at the end of the simulation.