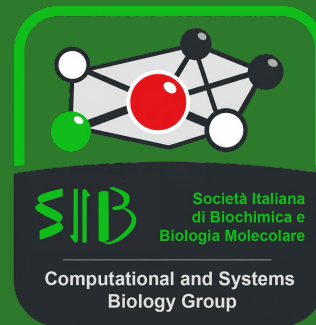


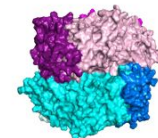
S2 · 11:40–11:55

Architecture and Dynamics of a Supramolecular Oxygen Transport System in Human Homogentisate 1,2-Dioxygenase

Alfonso Trezza

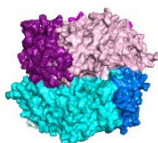
University of Siena





Architecture and dynamics of a supramolecular oxygen transport system in Human Homogentisate 1,2-Dioxygenase

(Alfonso Trezza et al., 2026. *Journal of Structural Biology (Elsevier)*, 218;3. <https://doi.org/10.1016/j.jsb.2026.108341>.)



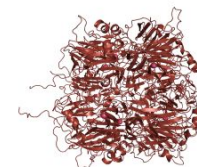
Alfonso Trezza, Ph.D.

University of Siena

Department of Biotechnology, Chemistry, and Pharmacy

One Health Lab (CB-AI hub)

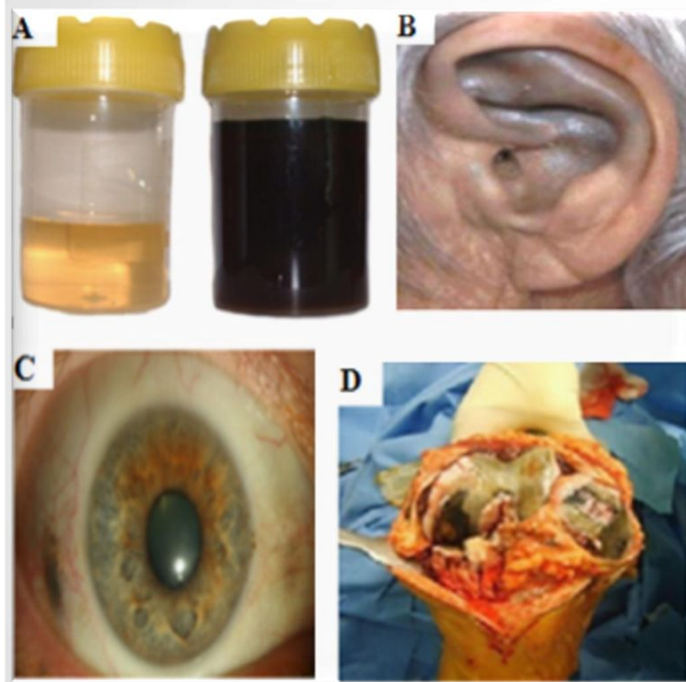
(Computational Biochemistry and Artificial Intelligence Lab)



Computational and Systems Biology Group · SIB · June 16, 2026



Background: HGD & Alcaptonuria



- Ultra-rare disease (950 patients in the world)
 - Autosomal recessive disease
- Defect in Homogentisate 1,2 dioxygenase (HGD) that produces Homogentisic Acid (HGA) accumulation
 - Multisystemic disorder

State of art: HGD Mutation



DEPARTMENT
OF MOLECULAR
BIOLOGY



INSTITUTE FOR CLINICAL
AND TRANSLATIONAL
RESEARCH

HGD mutation database

homogentisate 1,2-dioxygenase (HGD) 

Curator: Dr. Andrea Zatkova (andrea.zatkova@savba.sk)

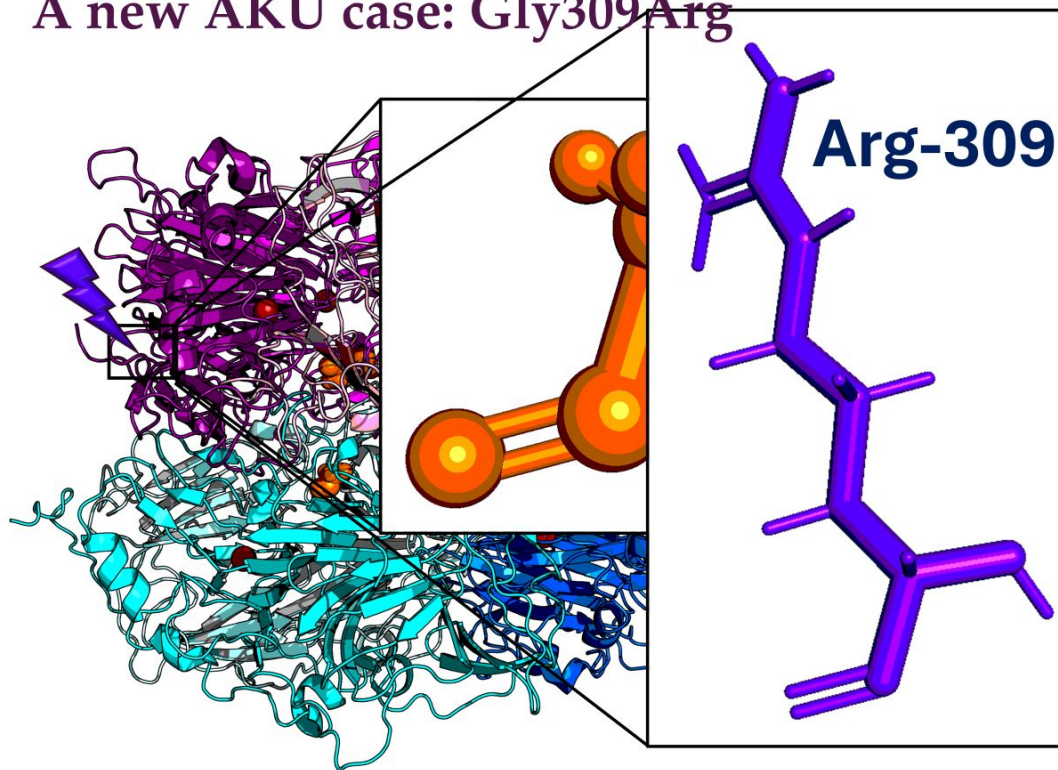
Total number of unique DNA variants reported	264
Total number of individuals with variant(s)	766
Total number of variants reported	1497

Predicted mutation effect*:

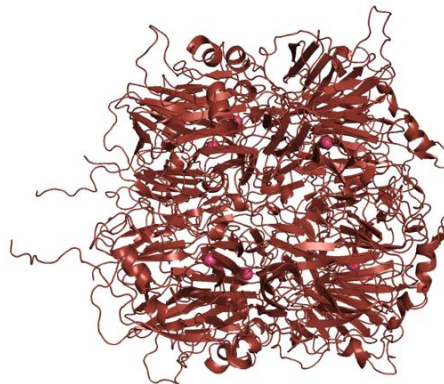
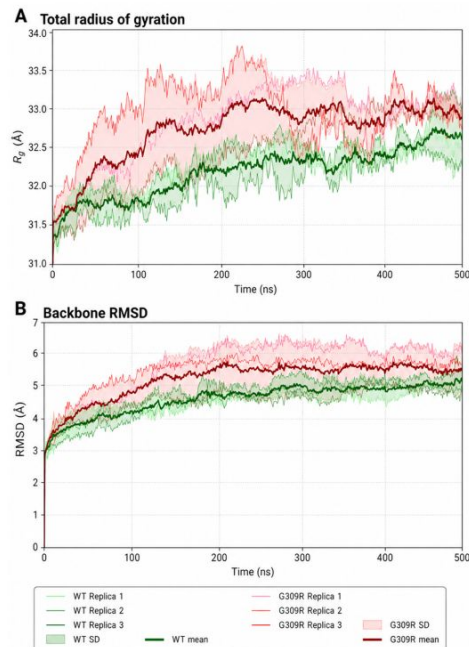
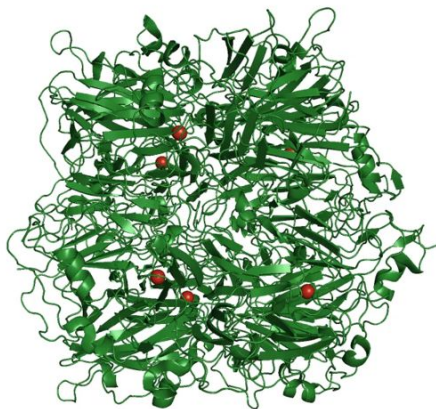
1. Protomer destabilisation
2. Hexamer distruption
3. Active Site distruption

Predicted Mutation Effect*: The effects of the novel missense variants on stability and protein-protein affinity was assessed in the context of the molecular interactions of the wild-type residue using **mCSM** Pires et al. (2014a) and **DUET** Pires et al. (2014b), University of Cambridge and Melbourne.

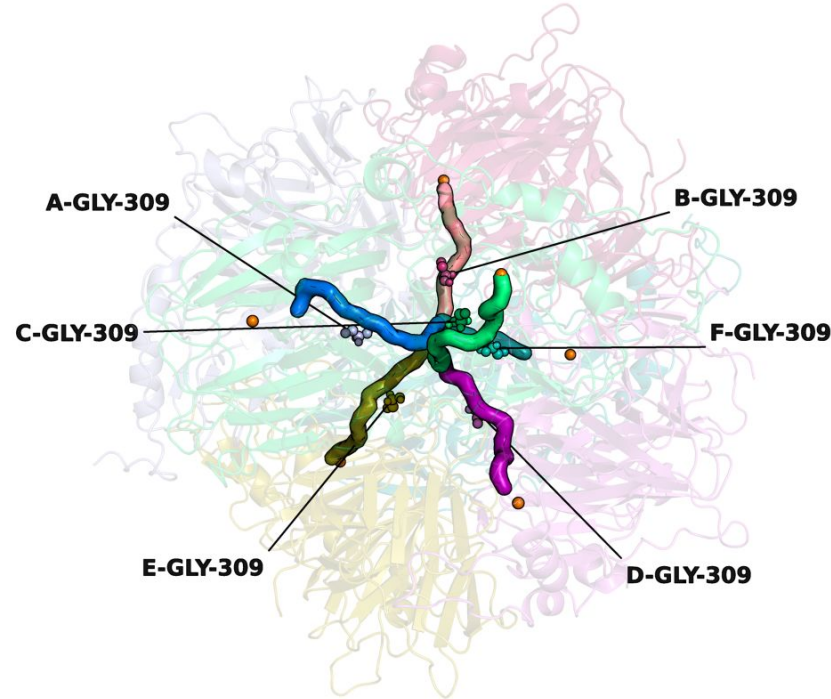
A new AKU case: Gly309Arg



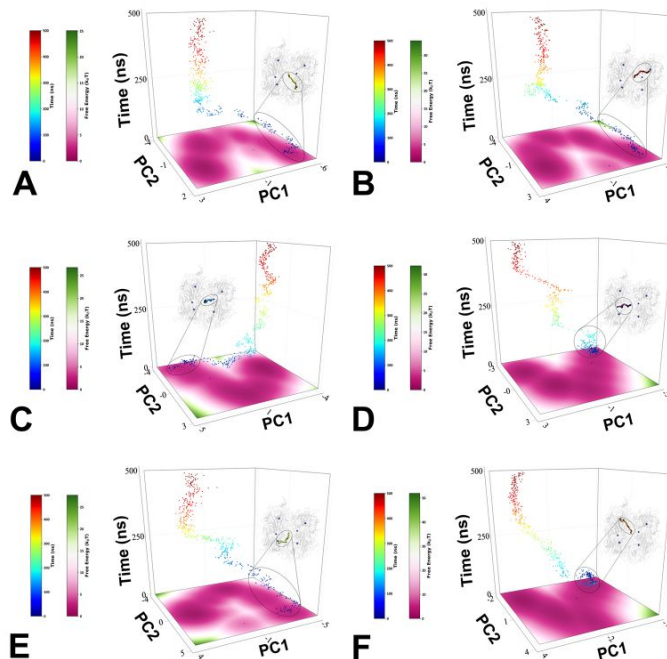
Results: Structural stability

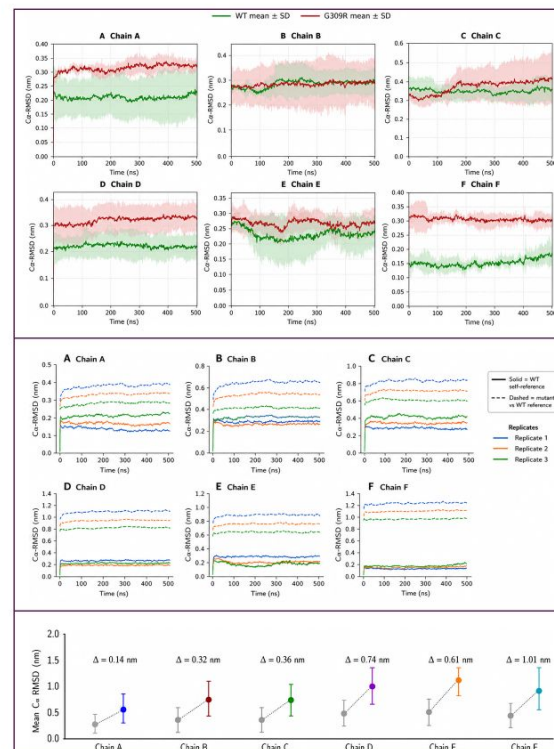
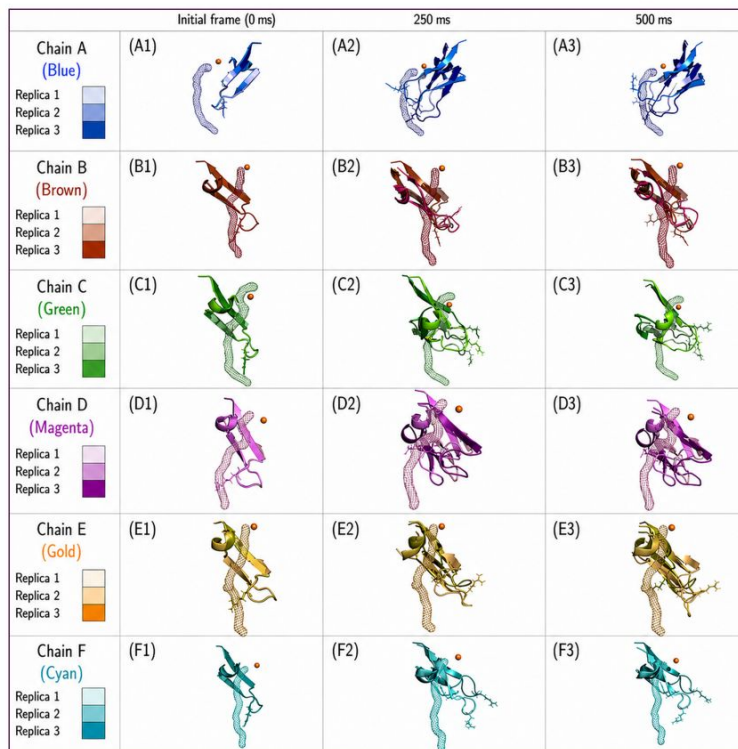


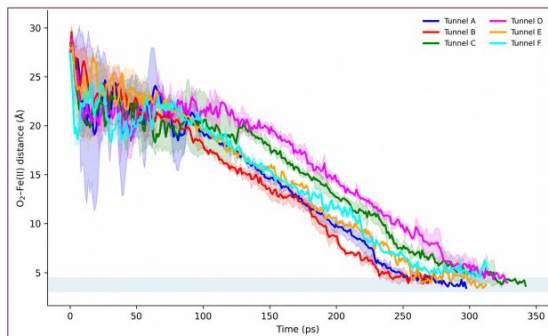
Results: Gly309 Localization and tunnel network in the WT



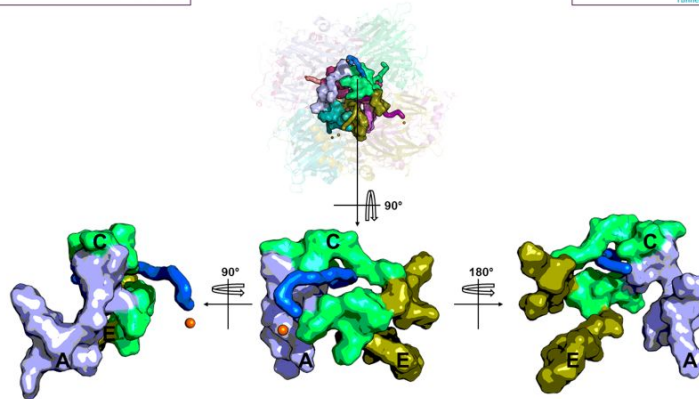
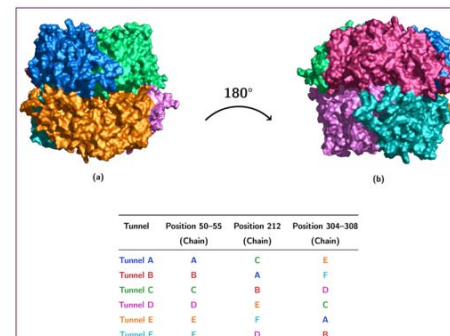
Results: PCA/FEL characterization of WT tunnel-associate conformational states

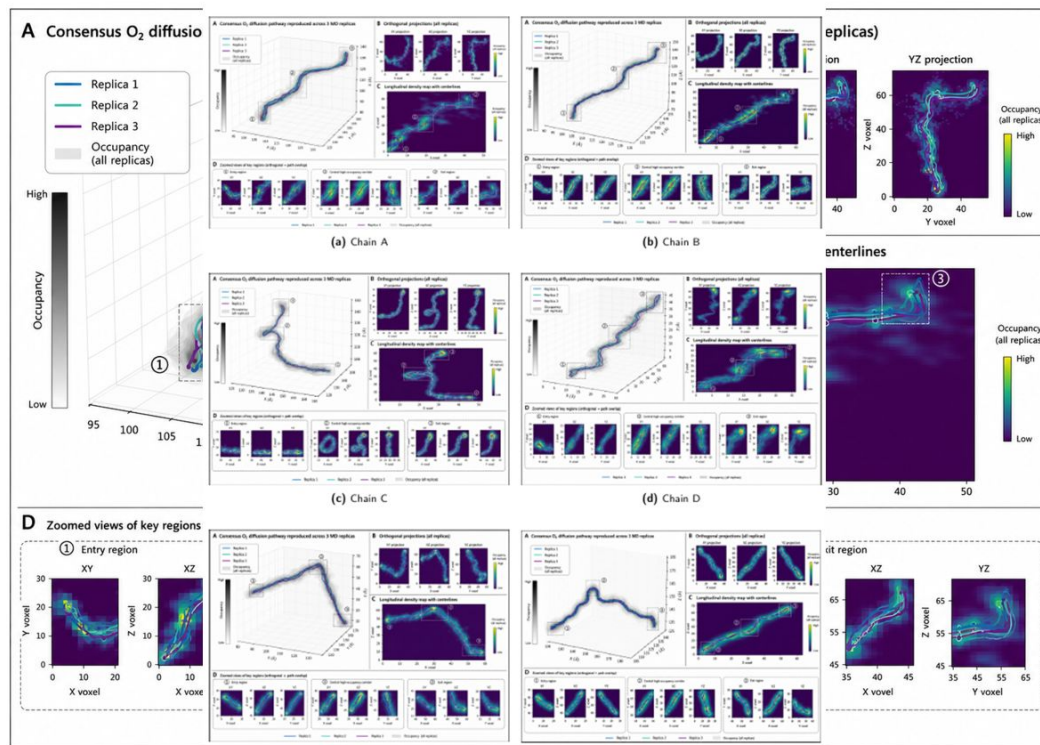


Results: G309R-induced β -hairpin displacement in HGD

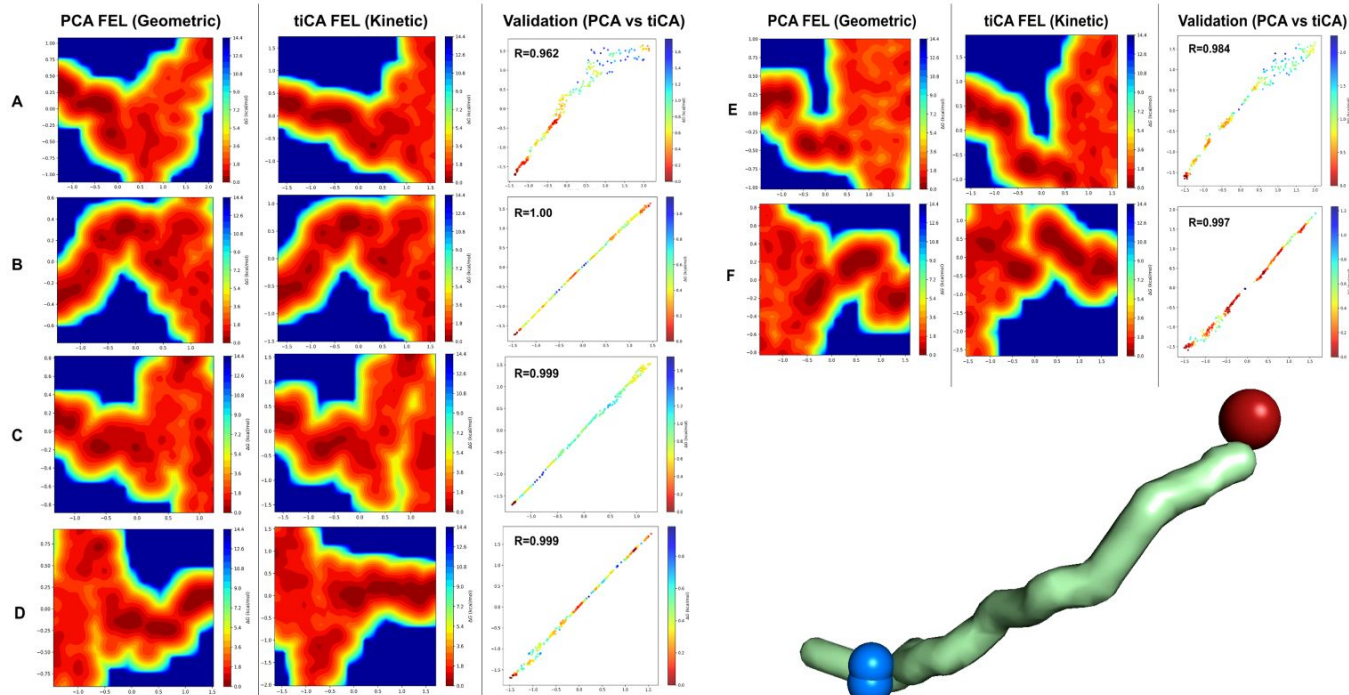
Results: O₂ translocation through WT tunnel toward catalytic Fe(II) center by SMD

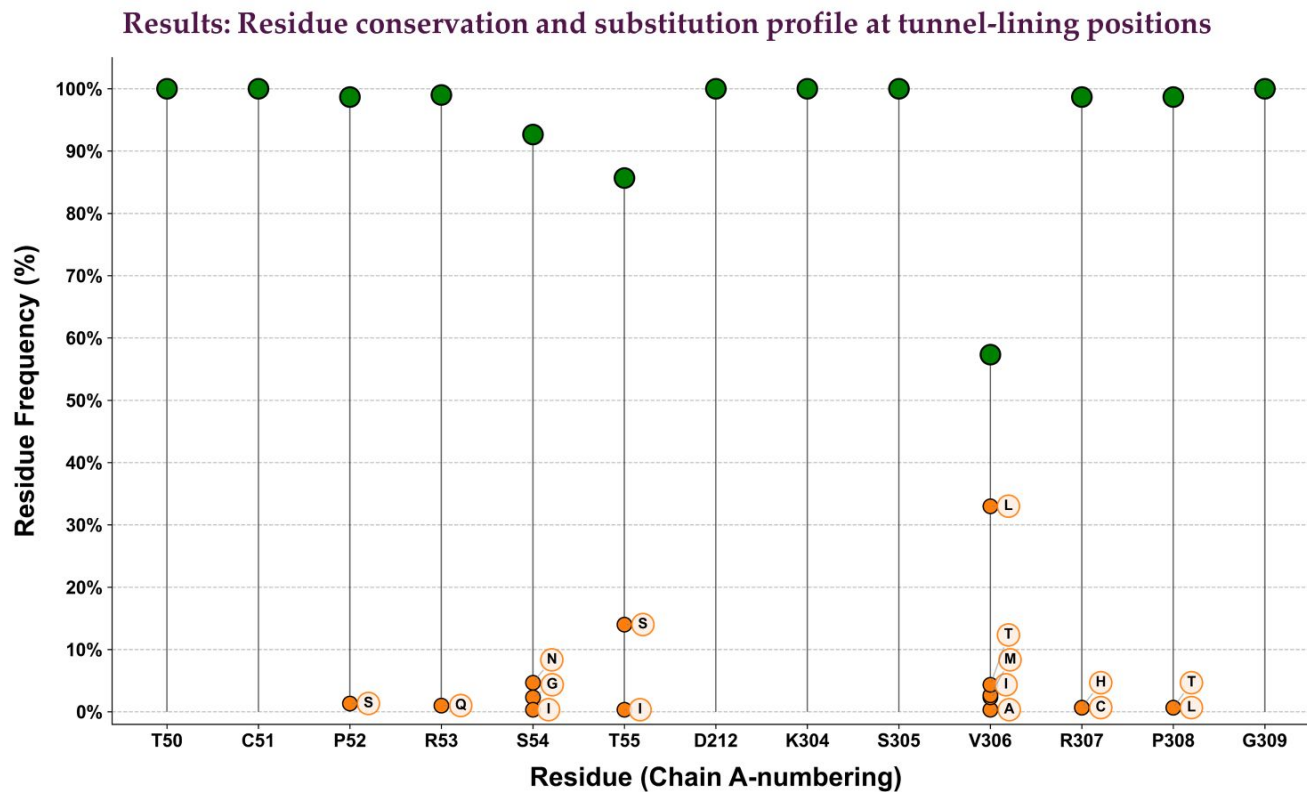
in-house Python script
Md Analyses



Results: Consensus O_2 diffusion pathways

Results: PCA/tICA free-energy analysis





Strengths

- I. The study proposes a new mechanistic interpretation of HGD dysfunction, showing that loss of activity may arise from impaired oxygen access rather than only from active-site damage or global destabilization.
- II. It integrates multiple computational layers, including replicated molecular dynamics, tunnel mapping, residue-level pathway analysis, spatial occupancy, PCA/tICA-based validation, free-energy landscapes, and evolutionary profiling.
- III. The WT/G309R comparison is mechanistically strong because it uses a real AKU-associated variant to link a single-residue substitution with disruption of O₂ trafficking.
- IV. The identification of six symmetry-related and dynamically supported O₂ pathways provides a clear structural framework for interpreting oxygen delivery in HGD.

Targeted future developments

- I. Points to improve: The mechanism remains computational and should ideally be supported by experimental validation, for example site-directed mutagenesis or activity assays on tunnel-lining residues.
- II. O₂ diffusion could be further strengthened by enhanced-sampling approaches or explicit ligand-migration simulations.
- III. Additional AKU-associated variants should be analyzed to determine whether impaired oxygen trafficking is a general mechanism or mainly specific to G309R.
- IV. The therapeutic implications are promising but still indirect and should be presented as future perspectives rather than immediate translational conclusions.

Conclusion



This study identifies oxygen trafficking as a previously unrecognized structural determinant of HGD catalytic competence. The G309R AKU-associated mutation selectively disrupts O₂-access pathways by altering a tunnel-associated β -hairpin, impairing catalytic function without major global destabilization.



These findings redefine HGD dysfunction as a consequence not only of active-site or oligomeric defects, but also of impaired oxygen delivery through mutation-sensitive internal pathways.



To our knowledge, this work provides the first residue-level dynamic description of oxygen diffusion in human HGD and establishes a mechanistic framework that may be relevant to oxygen trafficking in other dioxygenases.



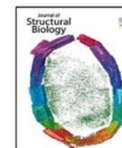
Overall, O₂-access pathways emerge as functional structural elements with potential relevance for AKU variant interpretation and future mechanism-informed therapeutic strategies.

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Research Article

Architecture and dynamics of a supramolecular oxygen transport system in human homogentisate 1,2-Dioxygenase

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Francesca Furlan^b, Annalisa Santucci^{a,c,d}^a Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Via Aldo Moro, 2, Siena, 53100, Italy^b Centro clinico di riferimento regionale per lo screening metabolico allargato, SC Pediatria Pneumoinfettivologia, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Via Commenda 9, Milano, 20122, Italy^c Industry 4.0 Competence Center ARTES 4.0, Viale Rinaldo Piaggio, Pontedera, 56025, Italy^d MetabERN, Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Via Aldo Moro, 2, Siena, 53100, Italy

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Alkaptonuria
Multiple sequence alignment
Molecular modeling

ABSTRACT

Alkaptonuria (AKU) is an ultra-rare inherited metabolic disorder caused by impaired activity of homogentisate 1,2-dioxygenase (HGD), a Fe(II)-dependent enzyme that catalyzes the oxidative cleavage of homogentisic acid in the tyrosine degradation pathway. Although high-resolution structures of human HGD have been solved, a fundamental mechanistic question has remained unresolved: how molecular oxygen reaches the deeply buried catalytic iron required for catalysis. Here, we identify a previously unreported AKU-associated HGD variant, c.925G>A (p.G309R), and use it as a mechanistic perturbation reference to dissect the structural determinants of oxygen access. By integrating replicated classical and steered molecular dynamics simulations with transient pocket detection, tunnel mapping, O₂ spatial-occupancy analysis, residue-level tunnel composition, and PCA/tICA-based dynamic validation, we identify a structurally accessible and dynamically supported O₂-translocation architecture connecting the central pore of the hexameric enzyme to the non-heme Fe(II) active sites. This pathway is not intrinsic to a single subunit but emerges from a cooperative arrangement of residues contributed by three protomers, generating six symmetry-related O₂-access routes per hexamer. The G309R



ONE-HEALTH LAB

<https://one-health.unisi.it/>



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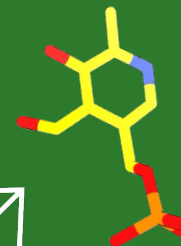
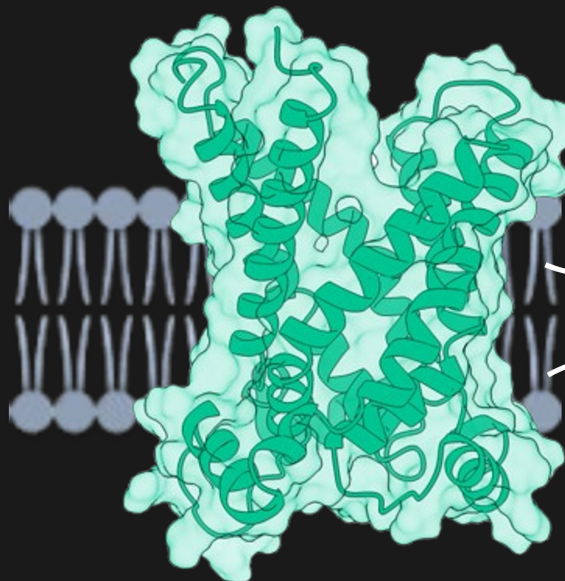


SLC25A38 binding of Pyridoxal 5'-Phosphate: A computational Study



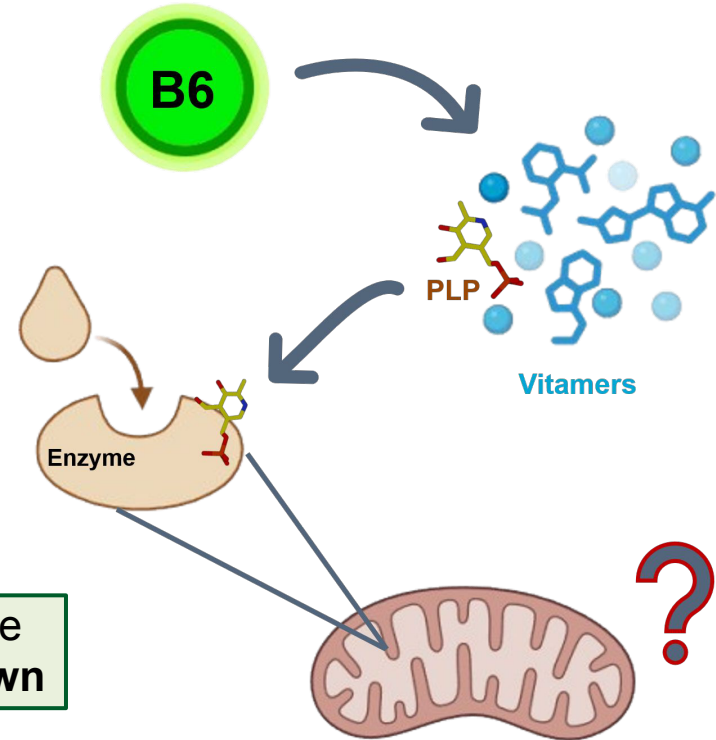
Miriana Quaranta

Sapienza University of Rome

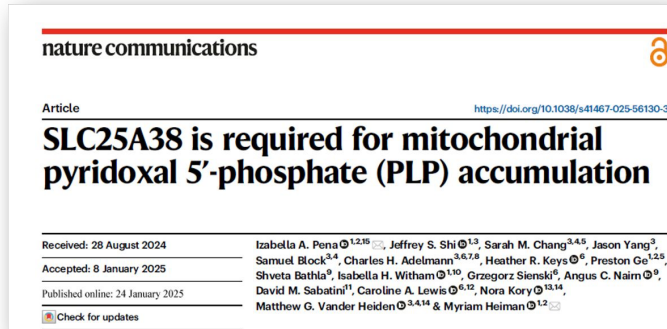
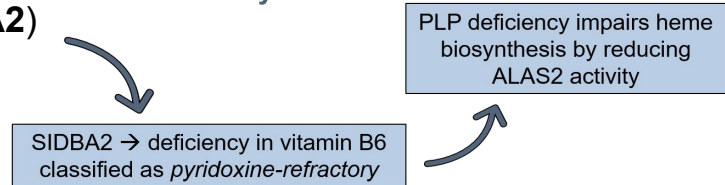
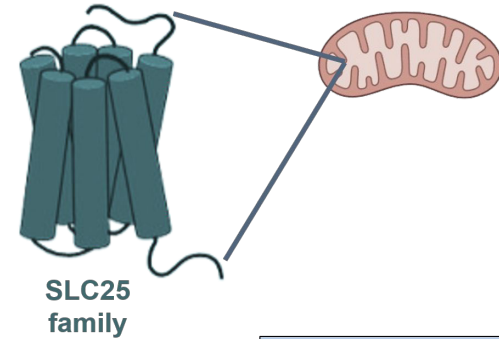


- Vitamin B6 includes biologically active **vitamers**
- Phosphorylated forms □ **Pyridoxal 5'-Phosphate (PLP)**
- Key enzymatic cofactor
- ~60 PLP-dependent enzymes
- One third of PLP-dependent enzymes localized in **mitochondria**

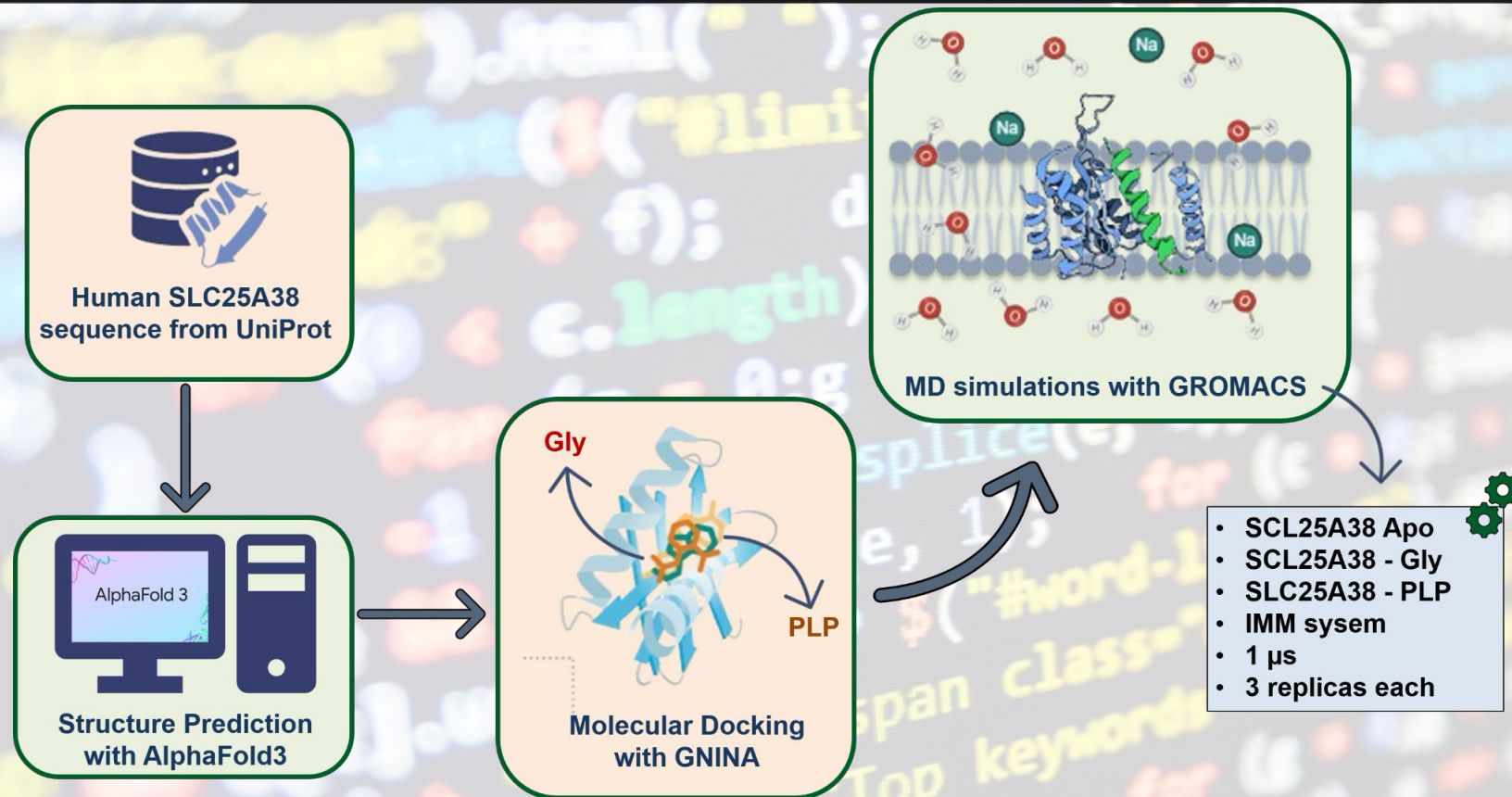
The **mechanisms** of PLP transport across the mitochondrial inner membrane remain **unknown**



- 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**)



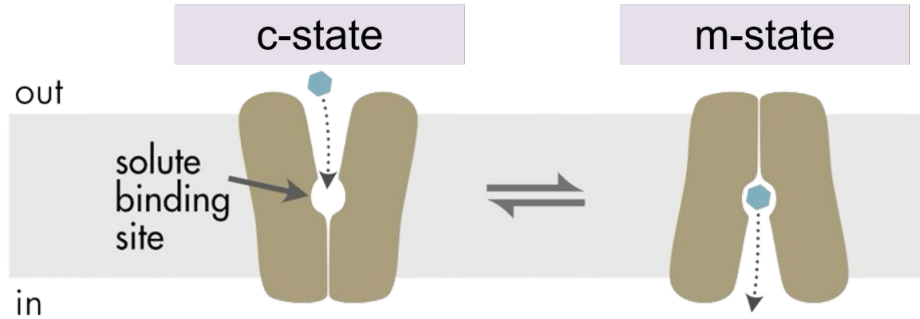
SLC25A38 mediates mitochondrial **PLP** transport; its loss reduces **SHMT2** activity and consequently mitochondrial **glycine synthesis**



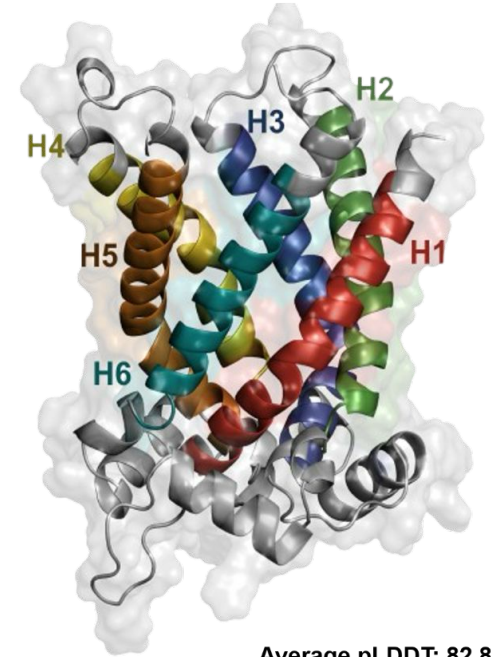
Results: Structural Modelling of SLC25A38

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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



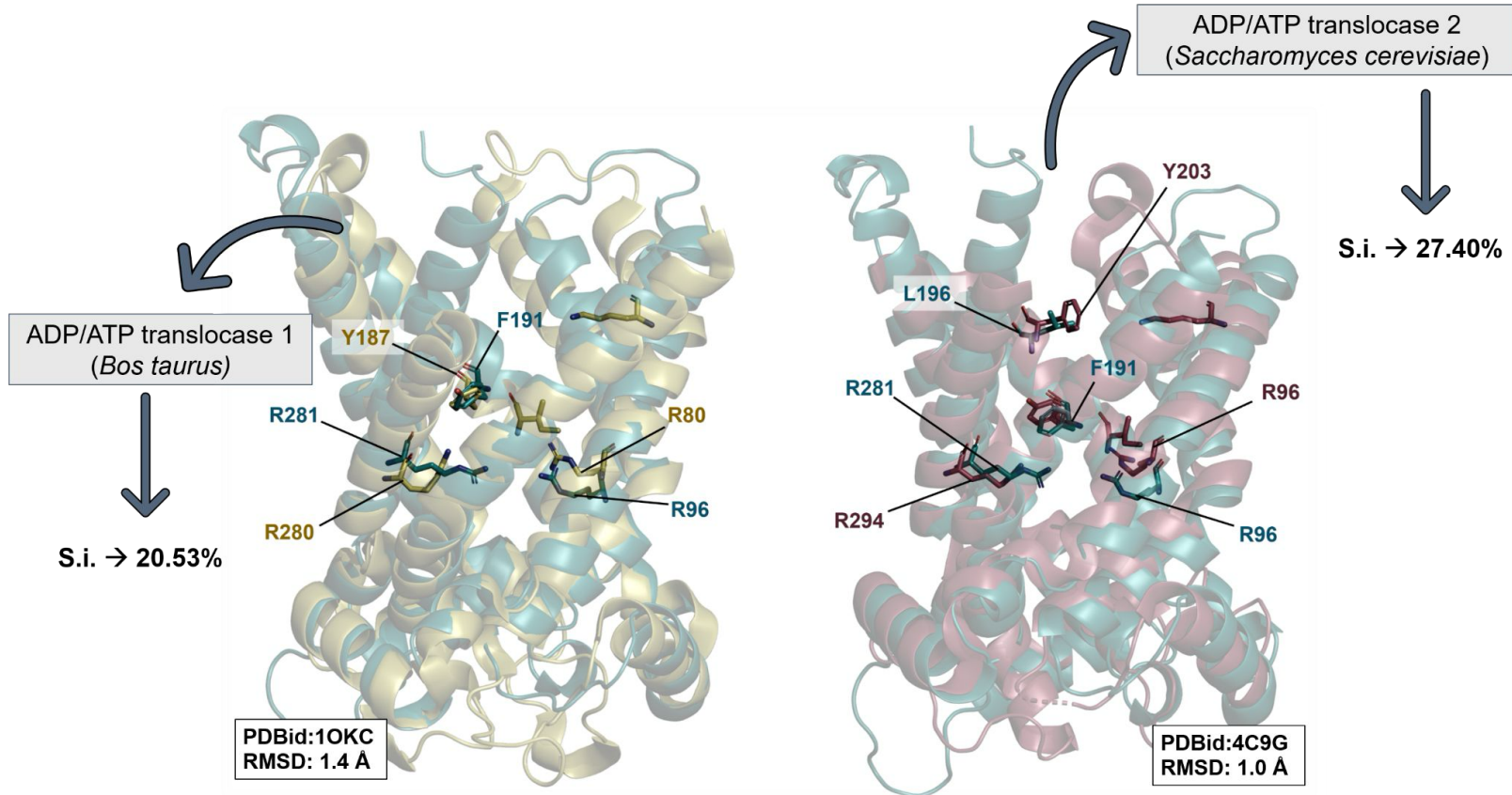
**Predicted Human SLC25A38
(c-state)**

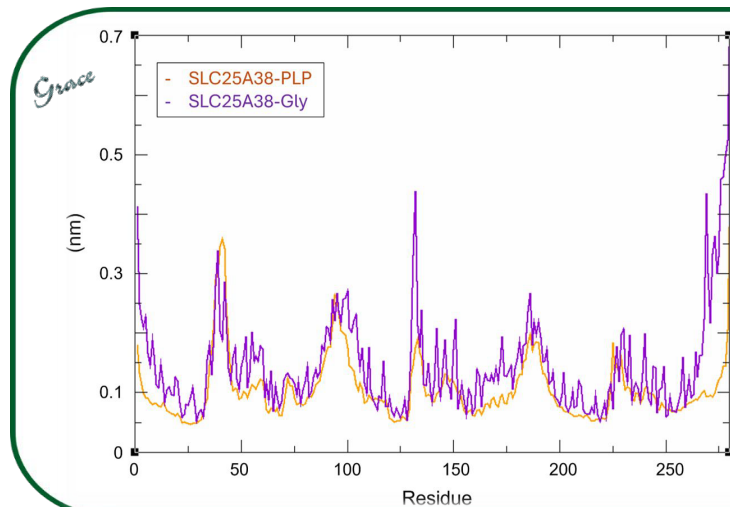


Average pLDDT: 82.88

Results: Structural Modelling of SLC25A38

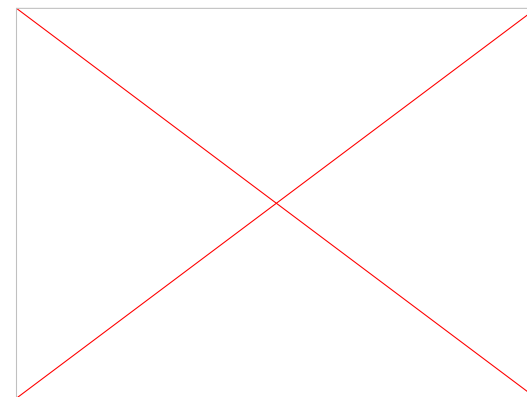
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SLC25A38-PLP

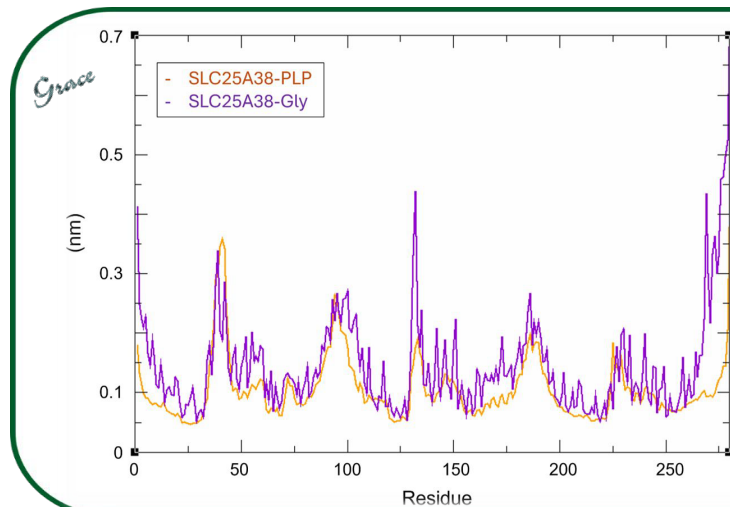
SLC25A38-Gly



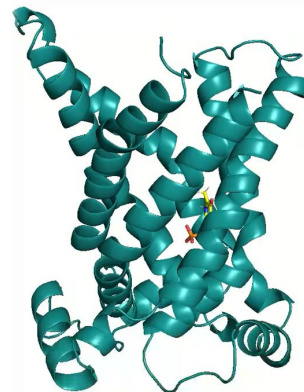
- **Lower residue flexibility** in presence of **PLP**
- **Glycine** remains bound only for the first **~10 ns**
- The **PLP-bound** system shows **reduced energy** fluctuations

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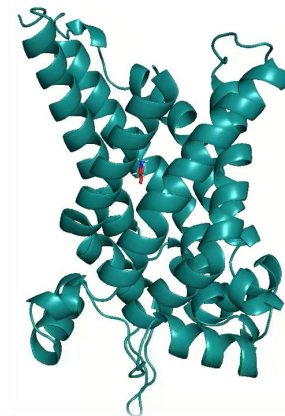
- Force field → **CHARMM General Force Field (CGenFF)**
- System → **Mitochondrial internal Membrane**
- Water model → **TIP3P**
- Length → **1 μ s** (triplicate)



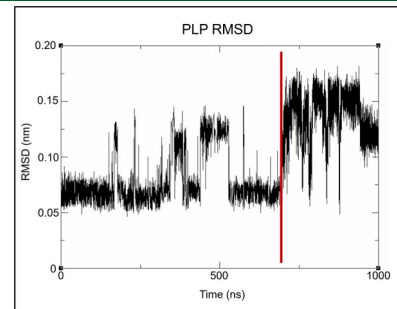
SLC25A38-PLP



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**



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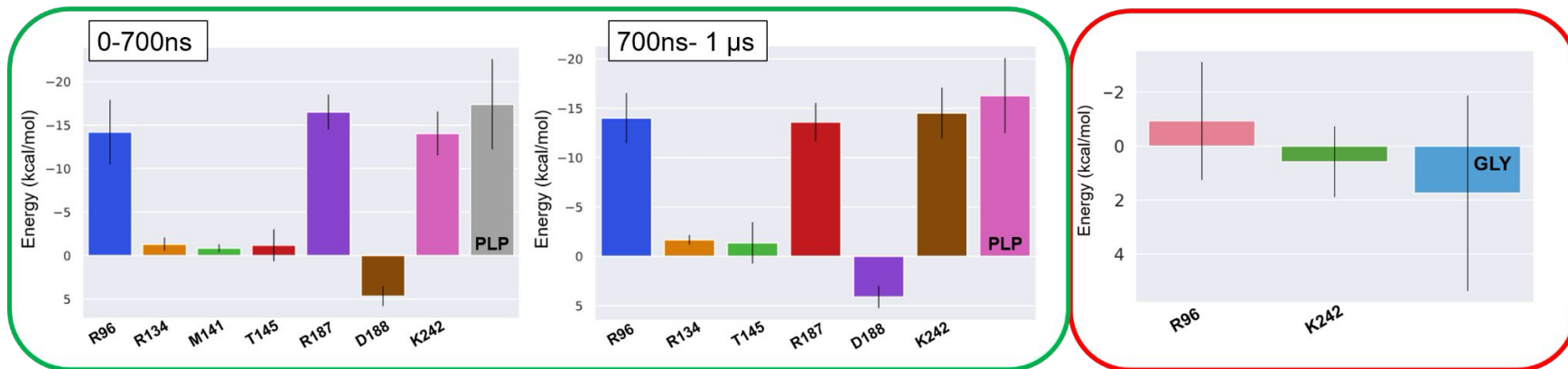
- Force field → CHARMM General Force Field (CGenFF)
- System → Mitochondrial internal Membrane
- Water model → TIP3P
- Length → 1 μ s (triplicate)

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



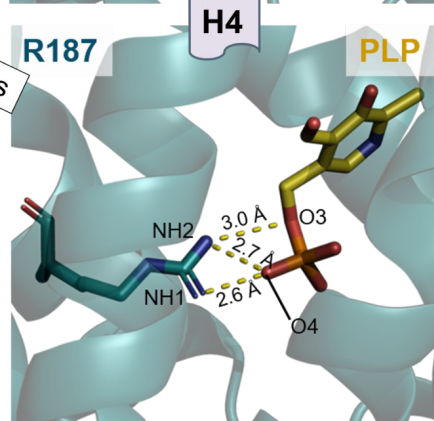
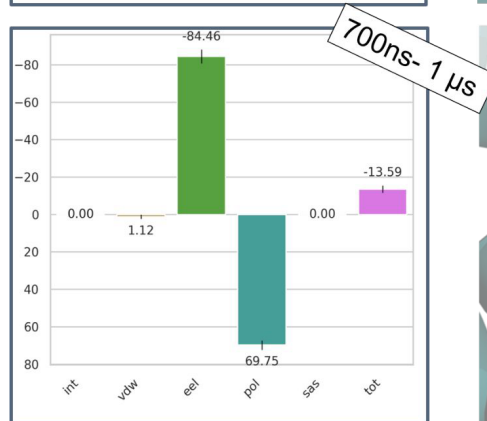
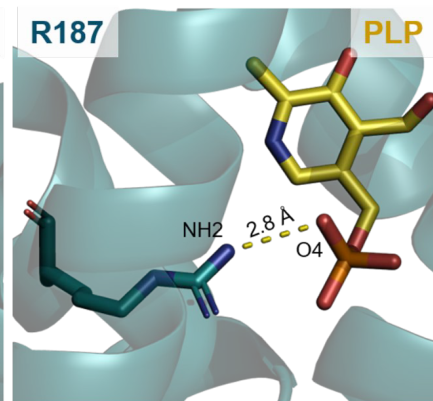
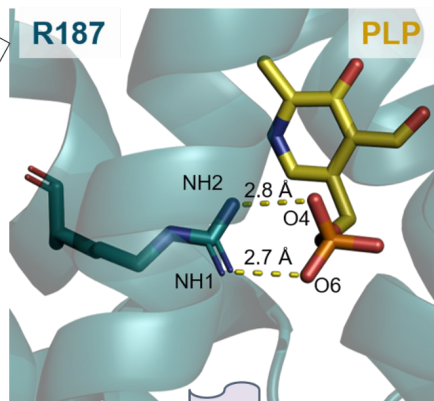
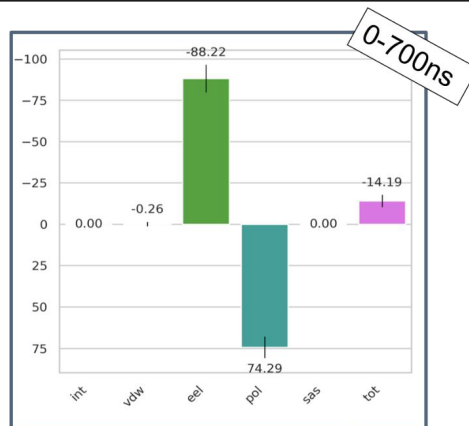
PLP binding is energetically **favorable**, driven by conserved charged residues

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

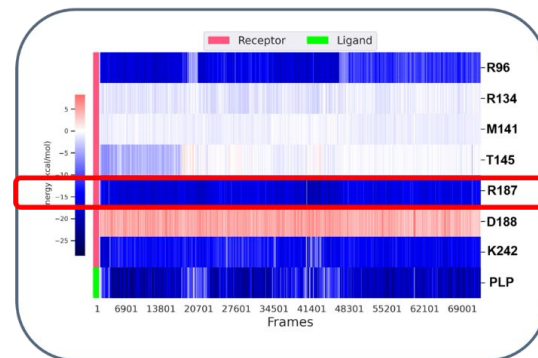
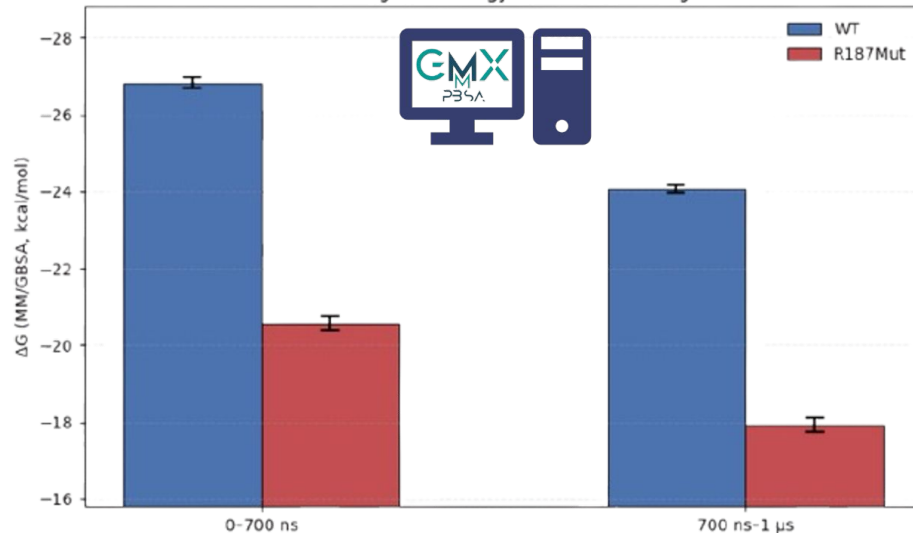
i



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

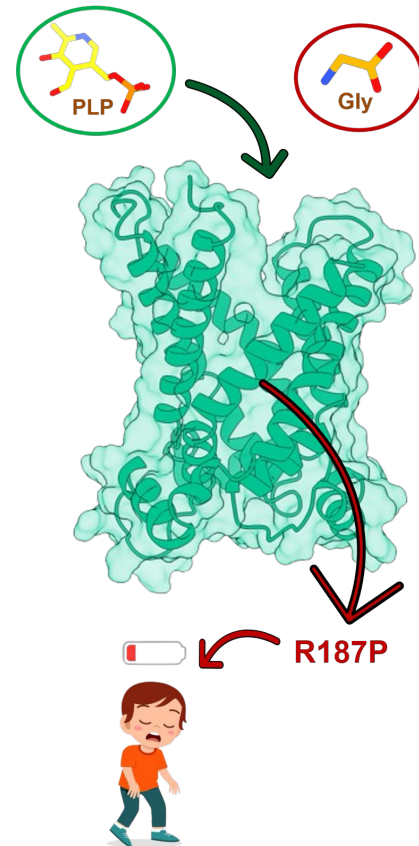


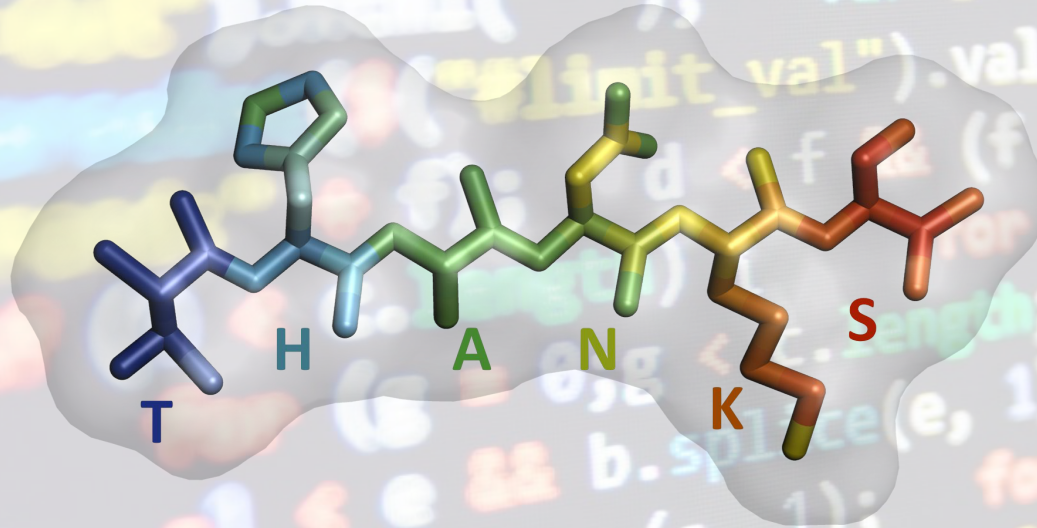
Arg187 is one of the major contributors to PLP stabilization through **electrostatic interactions** with the phosphate group



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



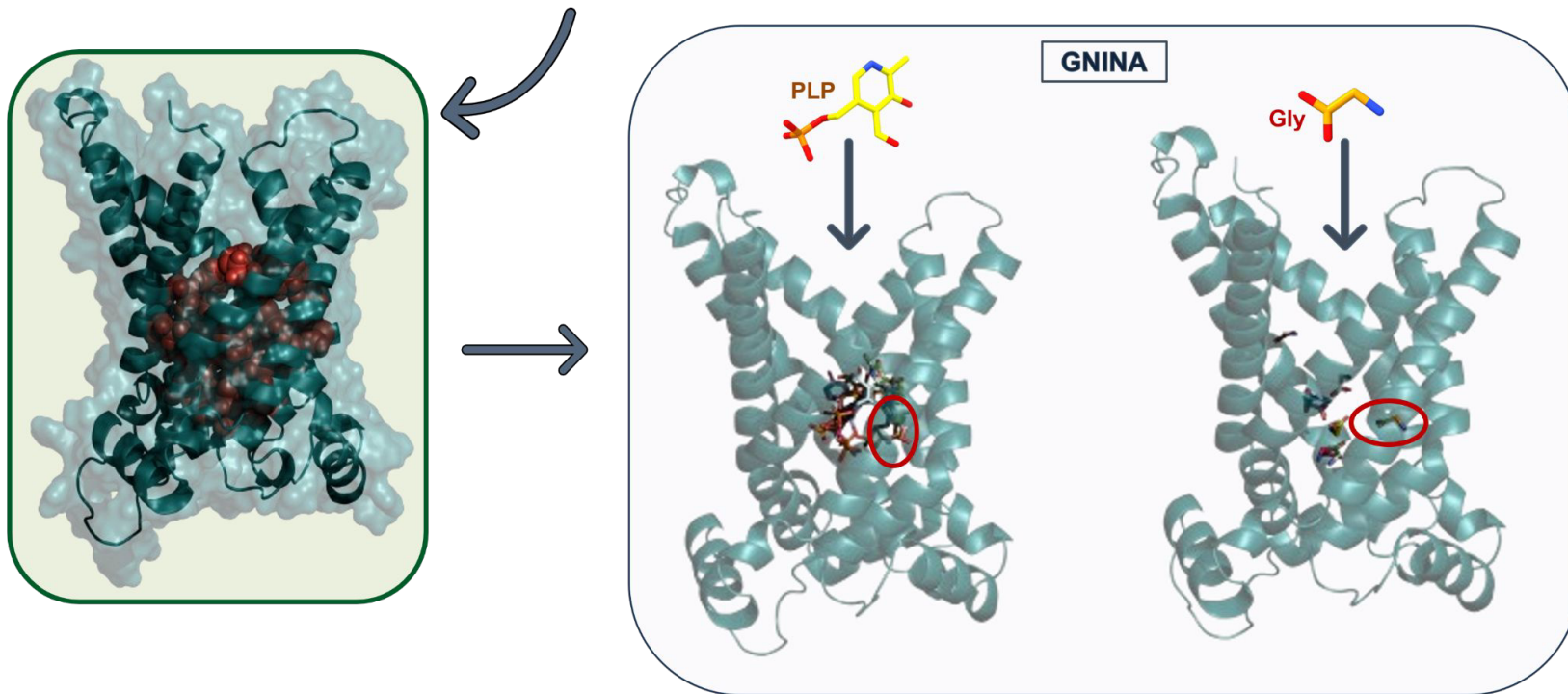


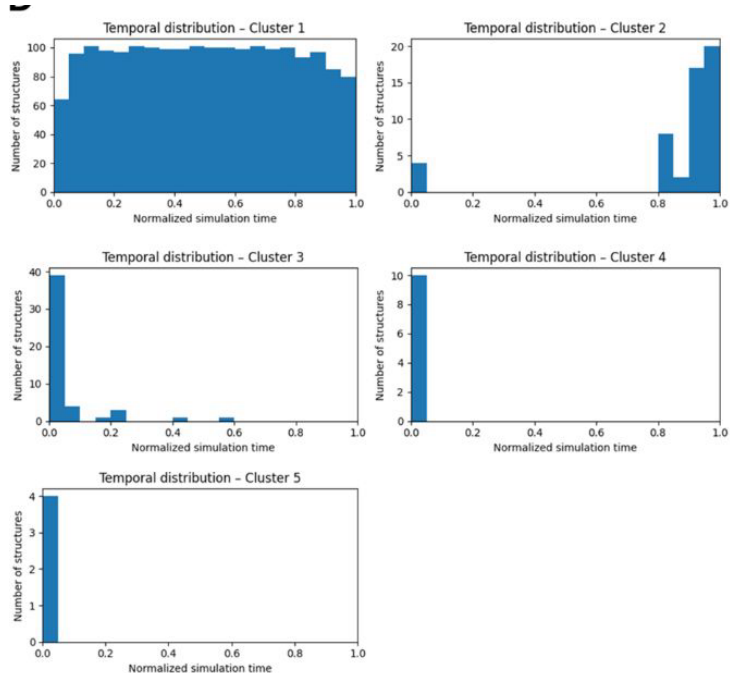
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THANKS

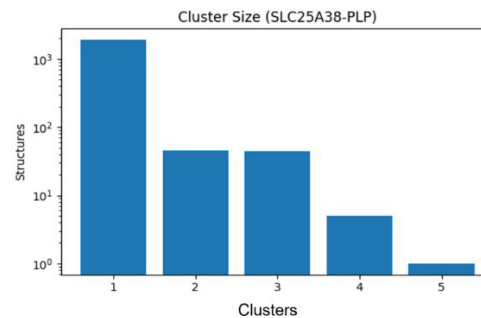
for your attention

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

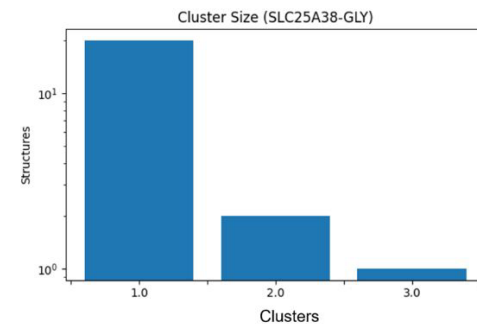




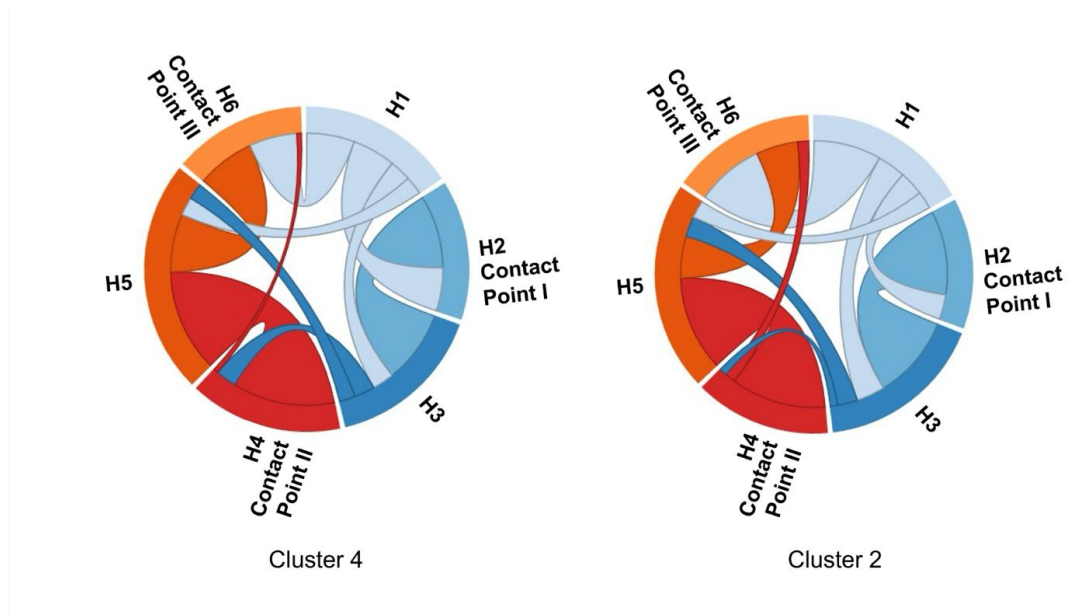
A



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.