Allosteric Regulation of GPX4 protein through Molecular Dynamics Energy Networks

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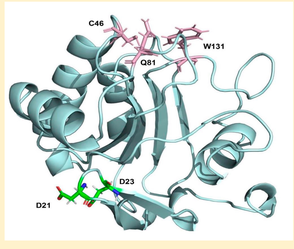
Abstract

GPX4 is an enzyme encoded by the human GPX4 gene which protects cells against membrane lipid peroxidation and preserves mitochondrial integrity of forebrain neurons. It opens the possibilities of potentially treating Alzheimer's Disease through elevating the GPX4 protein activity. Even though studies have identified that three kinds of mutations, including 2 point-mutations and one double mutation, are effective in enhancing GPX4 activity, the mechanism behind remains unknown. This research examines MD trajectories of GPX4 mutants from both conformational and energetics points of view. We seek to understand and explain the relationship between GPX4 mutations and their improved protein activity in the context of allosteric regulation.

Introduction

Single mutant D21A (mutant A), D23A (mutant B), and double mutant (mutant AB) all enhance the activity of GPX4 protein. Mutant AB have the strongest upregulation effect, followed by mutant A. Mutant B mildly improved the protein activity. Point mutations are treated as allosteric effectors here, as they affect and modulate GPX4 protein binding affinity from afar.



Molecular Dynamics Simulation

- 1000ns of molecular dynamic simulation for all 4 GPX4 systems.
- Systems all converged and stabilized to around 1Å.

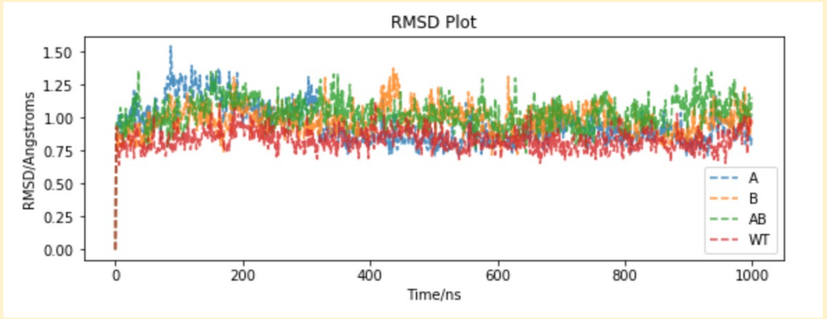


Fig 3. RMSD of 1000ns: *RMSD values show the similarity between the structures across the simulation.*

RMSF Analysis

- Mutant A has clear structural difference from the rest of the structures
- No obvious structural distinctions among the other systems.

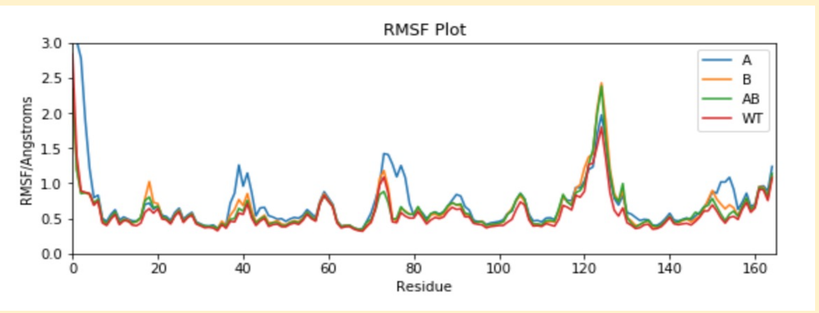
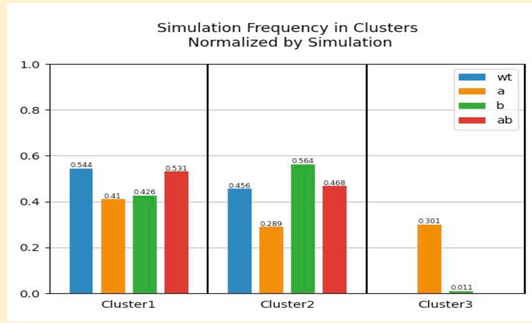


Fig 4. RMSF By Residue: RMSF plotted by each residue of the system.

Markov State Model (MSM) Analysis



- Mutant A having its own conformational cluster
- A improves the protein activity by adopting a favorable conformation
- However, B and AB have similar clustering to WT
- No meaningful about B and AB on the conformational level.

Fig 4. MSM Result of Conformations: *Histogram showing the contributions of the four GPX4 constructs in each of the three clusters of the MD-MSM. Each of the three clusters (denoted 1-3) are composed of different percentages from each of the four systems*

Energy Networks Analysis

- Allosteric effect is about force-based signal propagates through the network
- Observe chains of residues interactions taking place at various scales of distances / electrostatic communication channel

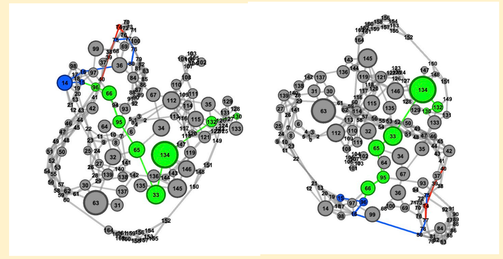


Fig 5. 3% of edges Mutant AB ***Fig 6. 3% of edges Mutant WT***

- Sampled 10% of the total frames to create energetic networks
- Nodes: the protein residues
- Edges: the degree of pair wise electrostatic interaction
- Network visualized using Gephi

- Important edges are filtered with average node degree.
- Shortest electrostatic pathways (SEPs): shortest path from mutation site residues to active site residues.
- SEPs are considered as important for transmitting allosteric signals.

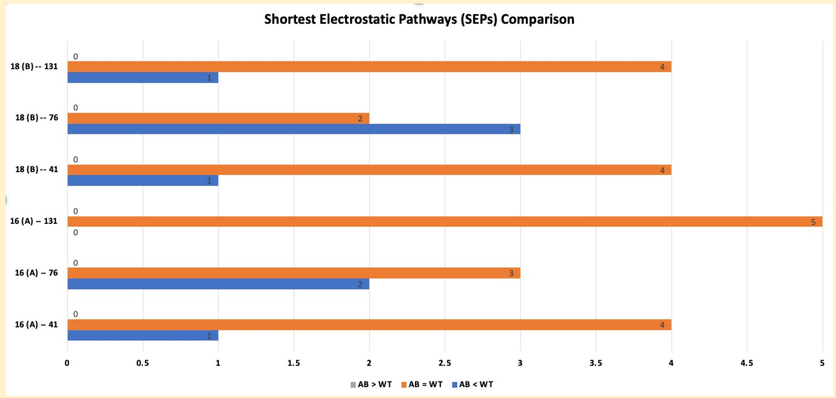


Fig 7. SEPs comparison chart: *comparison of 30 pairs of SEPs of mutant AB comparing to mutant WT*

- WT never has a better SEPs than AB.
- 30% of the SEPs are shorter in the mutant AB.
- SEPs from mutant AB use more well-connected residues on the SEPs.
- Better and more efficient channels for AB to transmit the allosteric signals.

Heat Kernel Analysis

- Heat kernel simulate heat propagating through the system.
- Heat preferentially flows through the proteins along the allosteric pathways.
- Standard PCA reduces the dimensions to R3 for visualization.
- The embedding results captures the structural changes of A.

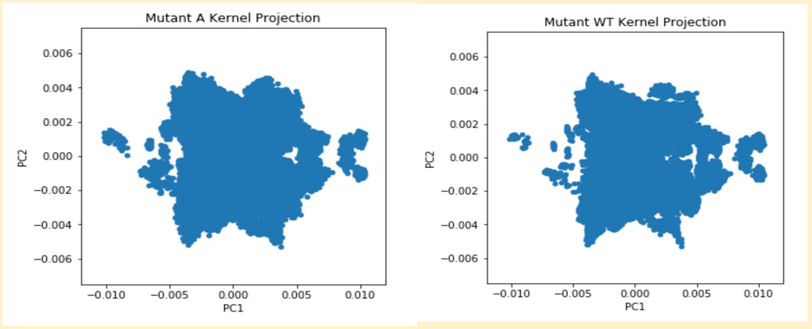


Fig 8. Residues projection for PC1 and PC2

- Comparison of energetic changes by residues through variances
- Mutation site residues did not show conformational changes
- However, they are significantly different from WT on the energetics

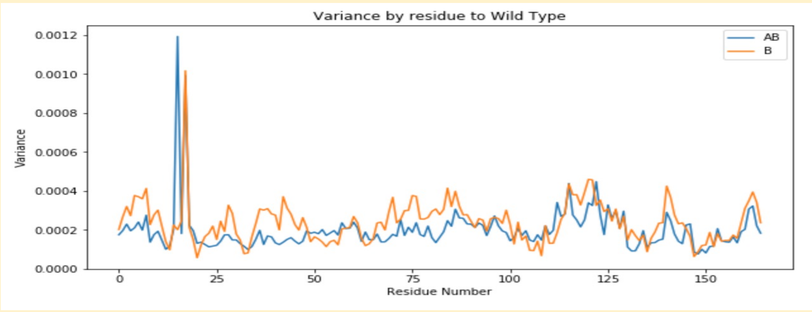


Fig 9. Variance by Residues compared to WT based on the heat kernel projections

Future Directions

We are currently mapping the observations made on the electrostatic networks back on to the structures of the molecules to provide more links to the biochemical viewpoint of this project.

Acknowledgements

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