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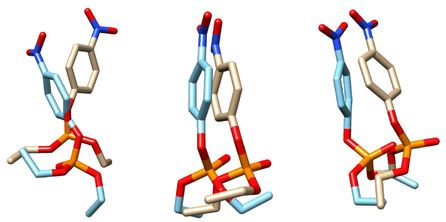
Identification of the binding mode of diethyl p-nitrophenyl phosphate to phosphotriesterase

Léa El Khoury[1], David L. Mobley[1], Dongmei Ye[2], Susan B. Rempe[2]
[1] Department of Pharmaceutical Sciences, University of California, Irvine, California 92697, United States
[2] Center for Biological and Engineering Sciences, Sandia National Laboratories, Albuquerque, New Mexico 87123, United States

Background

- Organophosphorus (OP) compounds are among the most toxic chemical substances and are widely used as insecticides, pesticides and chemical warfare agents.
- The most important enzyme inhibited by OP compounds is acetylcholinesterase (AChe). Inactivation of AChe function results in the accumulation of acetylcholine, leading to death due to serious respiratory disorders.
- Organophosphorus hydrolase (OPH), also called phophotriesterase, is a metalloenzyme that can hydrolyze various OP agents in the circulatory system.
- The best OPH substrate found to date is the insecticide diethyl p-nitrophenyl phosphate (paraoxon).
- Most structural and kinetic studies assume that the binding orientation of paraoxon is identical to that of diethyl 4-methylbenzylphosphonate, which is the only substrate analog co-crystallized with OPH.

Three paraoxon docked poses were selected



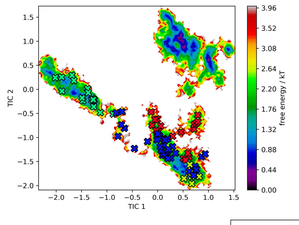
Pose A; RMSD: 2.7 Å
Pose B; RMSD: 2.7 Å
Pose C; RMSD: 2.5 Å

Superposition of the paraoxon docked poses before MD (in tan) and their corresponding binding poses after MD (in blue).

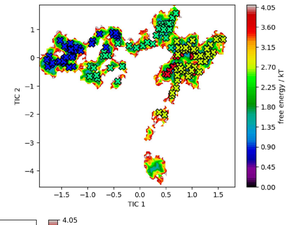
- HYBRID (OpenEye Inc.) was used to dock paraoxon using the substrate analog diethyl 4-methylbenzylphosphonate as a reference ligand.
- After short MD simulations on 13 predicted docked poses, 3 different poses were selected based on their stability in the active site.

MD simulations on the selected poses suggest different potential binding modes of paraoxon

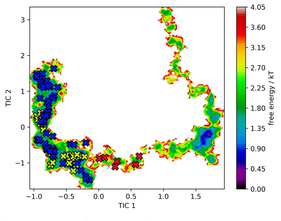
Pose A



Pose B

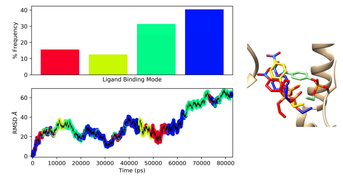


Pose C

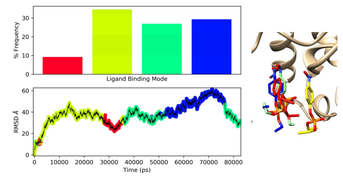


We constructed Markov State Models and applied a clustering approach to identify the different binding modes of paraoxon.

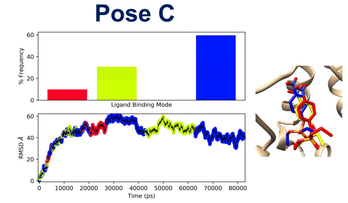
Pose A



Pose B



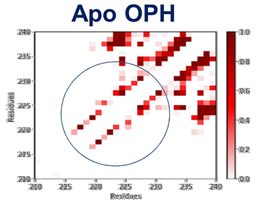
Pose C



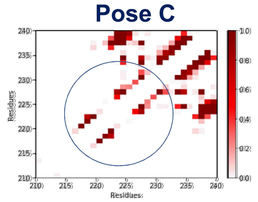
- **Starting from three different docked poses, we have identified several potential paraoxon binding modes.**
- **We found that these binding modes are the results of the transition of paraoxon rotatable bond between multiple states.**

Structural rearrangements occur in the binding site upon substrate binding

Apo OPH

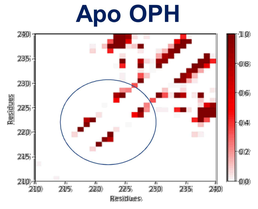


Pose C

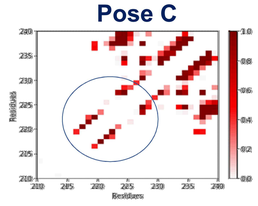


Paraoxon binding changes the protein-protein interaction profile, thus inducing a secondary structure shift in the OPH binding site.

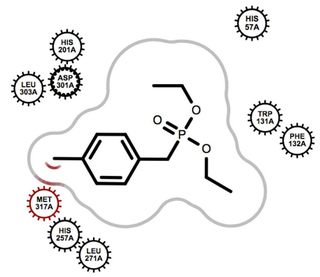
Apo OPH



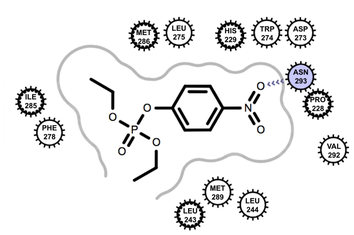
Pose C



The binding orientation of paraoxon is different than that of the substrate analog



The interactions of the substrate analog diethyl 4-methylbenzylphosphonate with the OPH binding site residues.



The interactions of one of paraoxon binding modes with the OPH binding site residues.

References

[1] Kuo, J.M., Chae, M.Y. and Raushel, F.M., 1997. Perturbations to the active site of phosphotriesterase. Biochemistry, 36(8), pp.1982-1988.
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Acknowledgments

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