

Thorough Identification and Characterization of Binding Modes via Molecular Modeling of BChE in complex with Di-n-alkyl Aryl Phosphate Derivatives CALIFORNIA STATE UNIVERSITY LONG BEACH

BACKGROUND

- Butyrylcholinesterase (BChE) is a nonspecific enzyme known to hydrolyze acetylcholine, a neurotransmitter associated with memory and learning functions,¹ making BChE associated with Alzheimer's Disease and dementia-like symptoms.
- One of our previous studies,² focused on simulations of thirteen organophosphate inhibitor-projects in complex with BChE, but failed to address and solve heuristic problems with the *k*-means clustering algorithm,³ which clusters BChE-inhibitor complexes into binding modes, or average conformations.
- Our last published study addressed the *k*-means' heuristic shortcomings using an intuitive statistical approach that will overcome the heuristic tendencies of k-means clustering and qualitatively validate clustering efficacy using internal metrics based on inter- and intra-cluster similarity.
- **Goal:** The study herein will revisit the thirteen organophosphate inhibitor projects and present reproducible and more accurate tabulations of contacts and interactions for each binding mode.



Figure 1. Visualization of 529-residue BChE in grayscale ribbon mode with active site residues shown as semi-transparent van der Waals surfaces (a) facing into the active site pocket from the gorge entrance and (b) rotated 90° about the vertical axis.



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METHODS

• Models:

- BChE model PDBID# 1P0I
- Inhibitors modeled & docked with ICM Pro
- Softwares & Parameters
 - Inhibitor partial charges calculated with Quacpac Tool Kit from OpenEye Scientific
 - General AMBER Force Field (GAFF)
 - BChE-inhibitor complex simulated using GROMACS 5.0.4 software (AMBER-03 FF for BChE)
 - Octahedral box using solvated with TIP3P water
 - 1.0 atm and 300 K (Berendsen barostat/thermostat)
 - 2.0 fs timestep, LINCS to constrain H atoms
 - Folding@Home: resulting structures sent to 1,000 computers around the world
 - 1000 simulations for each inhibitor, each simulation 100 – 110 ns with structures stored every 100 ps
 - Clustering Protocol from last publication ³

PRELIMINARY RESULTS

Contact tables efficiently display the various binding modes of enzyme-ligand complexes. The binding modes are organized in descending order by population. Each entry is the inhibitor functional group with the strongest interaction that is present at least 50% of the time. Displayed below are contact tables below for DIM5 and DAP4, the strongest and weakest inhibitors, respectively.

| 1000 Sims DIM5 | ASN68 | ASP70 | GLN119 | ALA277 | SER287 | TYR332 | SER 198 | GLU325 | HIS438 | GLY116 | GLY117 | ALA 199 | TRP82 | ALA328 | PHE329 | TRP231 | PRO285 | LEU286 | VAL288 | PHE398 | ILE69 | GLN71 | PHE73 | PR074 | GLY75 | PHE76 | MET81 | ASN83 | SER79 | TYR114 | GLY115 | PHE118 | THR120 | TYR128 | GLU197 | A SN397 | TRP430 | MET437 | GLY439 | TYR440 | ILE442 | Pop (%) |
|---|-------------|-------|--------|--------|--------|--------|---------|--------|------------------|--------|--------|----------------------------|----------------|-------------|--------|---------|-----------------|-------------------|-------------|----------|--------|-------|--|-------|-------|----------------|-------|-------|---|---------------|--------|-------------------|------------------|---------|--|---------|--------|---------------|--------|--------|-----------|------------|
| Mode | ode PAS CAT | | | | | | | | OAH | | CBS | | | | ABS | 5 | | | | | ON | AL | | | | | | | Additional Prote | | | | n Residues (APR) | | | | | | | | | |
| 0 | | | Ph | | AK1 | Ph | PO4 | | PO4 | PO4 | PO4 | AK1 | PO4 | AK2 | PO4 | AK1 | AK2 | AK1 | AK1 | AK1 | Ph | | | | | | | | | | PO4 | AK1 | PO4 | | PO4 | | AK2 | | | | | 28.7 |
| 1 | 2 | - | Ph | | AK1 | Ph | PO4 | | PO4 | PO4 | PO4 | AK1 | PO4 | AK2 | PO4 | AK1 | Ph | AK1 | AK1 | AK1 | Ph | | | | - 32 | | | 2 - D | Ph | | PO4 | AK1 | PO4 | (0) | PO4 | | AK2 | | AK2 | | | 20.2 |
| 2 | | | Ph | | AK1 | Ph | PO4 | | PO4 | PO4 | PO4 | AK1 | PO4 | AK2 | PO4 | AK1 | Ph | AK1 | AK1 | AK1 | Ph | | Ĵ. | Ĵ | 1 îl | Ph | AK2 | | Ph | | PO4 | | PO4 | 20 - 11 | | | AK2 | AK2 | AK2 | | | 18.7 |
| 3 | | | Ph | | | Ph | PO4 | | PO4 | PO4 | PO4 | AK1 | PO4 | AK2 | PO4 | AK1 | PO4 | AK1 | AK1 | AK1 | Ph | | | | | | | | | | PO4 | AK1 | PO4 | | PO4 | | AK2 | | AK2 | | | 13.2 |
| 4 | | | PO4 | | AK1 | Ph | AK1 | | | AK1 | PO4 | AK1 | Ph | Ph | PO4 | AK1 | Ph | AK1 | PO4 | AK1 | AK2 | | | | | | | | | | | AK1 | PO4 | | | | | | | | | 9.2 |
| 5 | Ph | 1 | PO4 | | AK1 | AK2 | PO4 | 1 | AK1 | PO4 | PO4 | AK1 | PO4 | AK2 | PO4 | AK1 | AK2 | AK1 | AK1 | AK1 | Ph | | | | | - 32 | | 0 Q | 3 - 32 | | 0 30 | | PO4 | Q (- | | | | | 6 | 2 | 10 - 10 P | 6.6 |
| 6 | | Ph | AK1 | | AK1 | Ph | AK1 | 20 | PO4 | PO4 | PO4 | AK1 | PO4 | AK2 | PO4 | AK1 | PO4 | AK1 | AK1 | AK1 | | | 1 | i i | 1 ii | Ph | AK2 | | | | | | PO4 | | | | Ph | | 2 | (| | 3.4 |
| Table 2. Contact table for DAP4: 1000 simulations. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1000 Sims DAP4 | ASN68 | ASP70 | GLN119 | ALA277 | SER287 | TYR332 | SER198 | GLU325 | HIS438 | GLY116 | GLY117 | ALA199 | TRP82 | ALA328 | PHE329 | TRP231 | PRO285 | LEU286 | VAL 288 | PHE398 | ILE69 | GLN71 | PHE73 | PRO74 | GLY75 | PHE76 | MET81 | ASN83 | SER79 | TYR114 | GLY115 | PHE118 | THR120 | TYR128 | GLU197 | ASN397 | TRP430 | MET437 | GLY439 | TYR440 | ILE442 | Pop (%) |
| Mode | | | PA | S | | | (| CAT | | | OAH | | - | ABS | | | | | | OM | IL. | | | | | Additional Pro | | | | | | in Residues (APR) | | | | | | | | | | |
| 0 | | | PO4 | | | AK2 | P04 | | PO4 | PO4 | PO4 | AK1 | PO4 | AK2 | PO4 | AK1 | PO4 | AK1 | AK1 | AK1 | Ph | | | | | | | | | | | AK1 | PO4 | | PO4 | | AK2 | | AK2 | AK2 | | 24 |
| 1 | Ph | | PO4 | | AK1 | AK2 | AK1 | | 004 | PO4 | PO4 | AK1 | PO4 | ALCO. | P04 | AK1 | AK2 | AK1 | AK1 | AK1 | Dh | | AK2 | | | - | _ | | | | DOA | AK1 | PO4 | | DOA | | | | 11/0 | _ | - | 17.7 |
| 2 | Dh | | POA | | AK1 | AK2 | AK1 | - | AK1 | PO4 | PO4 | AK1 | P04 | AK2 | P04 | AK1 | PO4 | AK1 | AK1 | AK1 | FII | 2 | | | - | - | - | | | _ | P04 | ANT | PO4 | _ | P04 | | | | AK2 | - | | 10.5 |
| 3 | Fn | | P04 | - | AK1 | Ph | AK1 | | ANT | AK1 | PO4 | AK1 | Ph | AR2 | P04 | AK1 | Ph | AK1 | PO4 | AK1 | AK2 | | | | - | + | - | | | - | | AK1 | PO4 | - | - | | | | | + | - | 13.8 |
| 5 | | | 1 | | ANT | AK2 | PO4 | | PO4 | PO4 | AK1 | AK1 | PO4 | AK2 | PO4 | AK1 | PO4 | AK1 | AK1 | AK1 | | - | | | | - | | | | | AK1 | | PO4 | | PO4 | | AK2 | | AK2 | AK2 | - | 6.6 |
| 6 | | | Ph | | AK1 | Ph | PO4 | | PO4 | PO4 | PO4 | PO4 | Ph | AK2 | PO4 | AK1 | Ph | AK1 | AK1 | PO4 | | 6 | | | | | | | | | PO4 | AK1 | Ph | | PO4 | | | | AK2 | | | 5.7 |
| 7 | 3 | | PO4 | | | PO4 | | | | PO4 | PO4 | 8 | Ph | | PO4 | | AK1 | 4 8 | | | | 5 | | | S | | | | 338 | | | | PO4 | | | | ×— | 9.—39 | 1 | | | 4 |
| Electrostatic Hydrogen Bonding onal Institutes of Health under Award Numbers; UL1GM118979; TL4GM | | | | | | | | | GM1 ² | 18980 |) Ch | arge-l GM1 ⁻ | Dipol 18978 | e 8. The | e cont | cent is |] π-s solely | tackir y the i | ng respo | onsibili | ity of | the a | van der Waals authors and does not nece | | | | | | Non-polar Ssarily represent the official vie | | | | | | Backbone s of the National Institutes c | | | | | | | |

Table 1. Contact table for DIM5: 1000 simulations.

| 1000 Sims DIM5 | A SN68 | ASP70 | GLN119 | ALA277 | SER287 | TYR332 | SER 198 | GLU325 | HIS438 | GLY116 | GLY117 | ALA 199 | TRP82 | ALA328 | PHE329 | TRP231 | PR0285 | LEU286 | VAL288 | PHE398 | ILE69 | GLN71 | PHE73 | PR074 | GLY75 | PHE76 | MET81 | A SN83 | SER79 | TYR114 | GLY115 | PHE118 | THR120 | TYR128 | GLU197 | ASN397 | TRP430 | MET437 | GLY439 | TYR440 | ILE442 | Pop (%) |
|--|---------|-------|--------|--------|--------|--------|---------|---------|--------|--------|-----------|---------------------------|----------------|-------------|--------|---------------|--------------|-------------------|-------------|---------|--------|---------------|-------|---------------|-------|-------|-------|--|----------------|---------------|--------|--------|--------|---|--------|------------------|--------------|--------|--------|--------|--------------|------------|
| Mode | ode PAS | | | | | | | CAT OAH | | | | | CBS ABS | | | | | | S | | | | | ON | OML | | | | | | | Add | lition | al Pro | otein | Resid | sidues (APR) | | | | | |
| 0 | | | Ph | | AK1 | Ph | P04 | | PO4 | PO4 | PO4 | AK1 | PO4 | AK2 | PO4 | AK1 | AK2 | AK | 1 AK | I AK1 | Ph | | | | | | | | | | PO4 | AK1 | PO4 | | PO4 | | AK2 | | | | | 28.7 |
| 1 | 2 | 2 | Ph | | AK1 | Ph | P04 | | PO4 | PO4 | PO4 | AK1 | PO4 | AK2 | PO4 | AK1 | Ph | AK | 1 AK | I AK1 | Ph | | | | 2 8 | | | 2 - 32 2 | Ph | | PO4 | AK1 | P04 | 60 | PO4 | | AK2 | 1 | AK2 | | 90 - A | 20.2 |
| 2 | | | Ph | | AK1 | Ph | PO4 | | PO4 | PO4 | PO4 | AK1 | PO4 | AK2 | PO4 | AK1 | Ph | AK | 1 AK | I AK1 | Ph | | | 8 86 23 00 | 03 | Ph | AK2 | . 03 | Ph | | PO4 | | PO4 | 20 X | | | AK2 | AK2 | AK2 | | 20 2 10 2 | 18.7 |
| 3 | | | Ph | | | Ph | PO4 | | PO4 | PO4 | PO4 | AK1 | PO4 | AK2 | PO4 | AK1 | PO4 | AK' | 1 AK | I AK1 | Ph | | e - 1 | e - 17 | 18 | | | | | | PO4 | AK1 | PO4 | 3 | PO4 | | AK2 | | AK2 | | | 13.2 |
| 4 | | | PO4 | | AK1 | Ph | AK1 | | | AK1 | PO4 | AK1 | Ph | Ph | PO4 | AK1 | Ph | AK | 1 PO | 4 AK1 | AK2 | | | | | | | | | | - | AK1 | PO4 | | | | | | | | | 9.2 |
| 5 | Ph | ÷ | PO4 | | AK1 | AK2 | PO4 | 1 | AK1 | PO4 | PO4 | AK1 | PO4 | AK2 | PO4 | AK1 | AK2 | AK | 1 AK | AK1 | Ph | | | -1 | - 32 | - 22 | | 1 (R | | | o - 33 | | PO4 | Q - 13 | | - | | 1 2 | ÷ | ş | 90 - A | 6.6 |
| 6 | | Ph | AK1 | | AK1 | Ph | AK1 | | PO4 | PO4 | PO4 | AK1 | PO4 | AK2 | PO4 | AK1 | PO4 | AK' | 1 AK | AK1 | | | | | 1 | Ph | AK2 | | | | s 25 | | PO4 | 1 | | | Ph | | 2 | ĺ. | | 3.4 |
| Table 2. Contact table for DAP4: 1000 simulations. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Sims DAP4 | ASN68 | ASP70 | GLN11 | ALA27 | SER28 | TYR33 | SER19 | GLU32 | HIS438 | GLY116 | GLY117 | ALA19 | TRP82 | ALA32 | PHE32 | TRP23 | PRO28 | LEU28(| VAL 28 | PHE39 | ILE69 | GLN71 | PHE73 | PRO74 | GLY75 | PHE76 | MET81 | ASN83 | SER79 | TYR11 | GLY11 | PHE118 | THR12 | TYR128 | GLU19 | ASN39 | TRP43 | MET43 | GLY43 | TYR44(| ILE442 | Pop (%) |
| Mode | | | PA | \S | | | 1 | CAT | | | OAH | | - | CBS | | | aleste. | ABS | 5 | | | | | OM | L | | | | Additional Pro | | | | | | | n Residues (APR) | | | | | | |
| 0 | - | | P04 | | | AK2 | PO4 | | PO4 | P04 | P04 | AK1 | P04 | AK2 | P04 | AK1 | P04 | AK1 | AK1 | AK1 | Ph | | | | _ | | _ | | | | | AK1 | P04 | | P04 | | AK2 | | AK2 | AK2 | | 24 |
| 1 | Ph | | PO4 | | AK1 | AK2 | AK1 | | 004 | P04 | P04 | AK1 | PO4 | AIZO. | P04 | AK1 | AK2 | AK1 | AK1 | AK1 | Dh | | AK2 | | | | _ | | | | DOA | AK1 | PO4 | | 004 | | | | 11/0 | | _ | 17.7 |
| 2 | - | | PI | | AK1 | AK2 | PU4 | - | P04 | PO4 | PO4 | AK I | P04 | AKZ | P04 | AK I | PO4 | AKI | AKI | AKI | Pff | | - | _ | - | - | - | _ | 2 2 | | P04 | AKI | P04 | | P04 | | | | AK2 | | | 16.5 |
| 3 | Ph | - | P04 | | A1/4 | Ph | AK1 | | ANT | 1/14 | PO4 | AK1 | Ph | AKZ | P04 | AK1 | Ph | AK1 | PO4 | AK1 | AK2 | | | - | - | - | - | | | | | AK1 | P04 | - | | | | | | - | - | 13.8 |
| 5 | - | | 101 | | ANT | AK2 | PO4 | | PO4 | PO4 | AK1 | AK1 | PO4 | AK2 | PO4 | AK1 | PO4 | AK1 | AK1 | AK1 | ru ve | - | - | - | - | | - | - | | _ | AK1 | 11111 | PO4 | | PO4 | | AK2 | | AK2 | AK2 | | 6.6 |
| 6 | | | Ph | | AK1 | Ph | PO4 | | PO4 | PO4 | PO4 | PO4 | Ph | AK2 | PO4 | AK1 | Ph | AK1 | AK1 | PO4 | | - | | - | - | | | | | | PO4 | AK1 | Ph | | PO4 | - | | | AK2 | | - | 5.7 |
| 7 | - | | PO4 | | | PO4 | 1 | 9 - 0 | | PO4 | PO4 | - | Ph | | PO4 | | AK1 | 1 | | | - | 6 | - | | | | | | 8 - 8 | ÷ | | 9 - P | PO4 | | | | | a | | | | 4 |
| Electrostatic Hydrogen Bonding ional Institutes of Health under Award Numbers; UL1GM118979; TL4GN | | | | | | | | | GM11 | 8980 | Ch | arge- GM1 ⁻ | Dipol 18978 | e 3. The | e con | tent is | π-s solel | tackir y the i | ng respo | nsibili | ity of | van der Waals | | | | | | Non-polar necessarily represent the offic | | | | | | Backbone views of the National Institutes of | | | | | | | | |



Figure 2. Medoid of the fifth binding mode of the BChE-DIM5 complex.

CONCLUSION & FUTURE WORK

Interaction motifs between BChE and thirteen separate inhibitor projects will be studied and compared. Future studies will improve on the generation of contact tables by introducing a weighted cut-off for specific interactions, thereby changing the entries to reflect more physically relevant contacts.

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