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

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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.

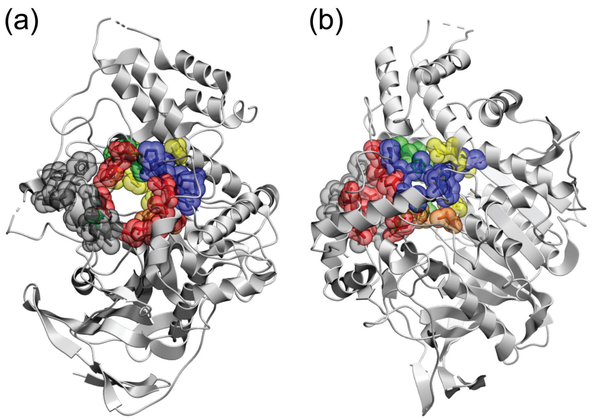
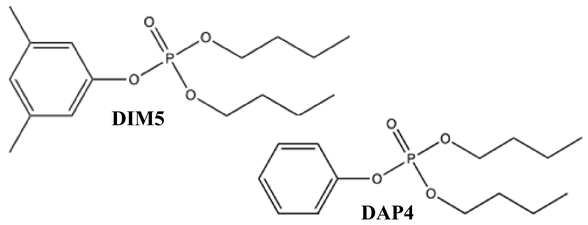


Figure 1. Visualization of 529-residue BChE in grayscale ribbon mode with active site residues shown as semi-transparent van der Waals surfaces (a) facing into the active site pocket from the gorge entrance and (b) rotated 90° about the vertical axis.



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]

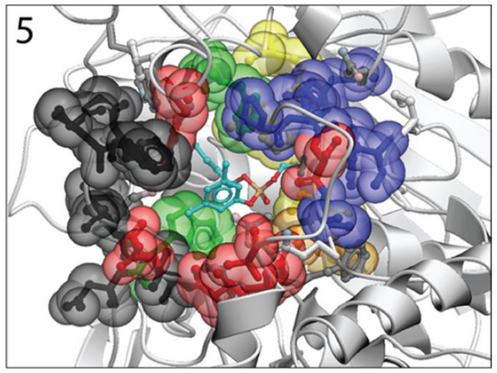


Figure 2. Medoid of the fifth binding mode of the BChE-DIM5 complex.

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.

Table 1. Contact table for DIM5: 1000 simulations.

1000 Sims DIM5	ASN68	ASP70	GLN119	ALA277	SER287	TYR332	SER198	GLU325	HIS438	GLY116	GLY117	ALA199	TRP82	ALA328	PHE329	TRP231	PRO285	LEU286	VAL288	PHE398	ILE69	GLN71	PHE73	PRO74	GLY75	PHE76	MET81	ASN83	SER79	TYR114	GLY115	PHE118	THR120	TYR128	GLU197	ASN397	TRP430	MET437	GLY439	TYR440	ILE442	Pop (%)
Mode	PAS						CAT			OAH			CBS			ABS					OML								Additional Protein 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			Ph		AK1	Ph	PO4		PO4	PO4	PO4	AK1	PO4	AK2	PO4	AK1	Ph	AK1	AK1	AK1	Ph								Ph		PO4	AK1	PO4		PO4		AK2		AK2			20.2
2			Ph		AK1	Ph	PO4		PO4	PO4	PO4	AK1	PO4	AK2	PO4	AK1	Ph	AK1	AK1	AK1	Ph					Ph	AK2		Ph		PO4		PO4				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		PO4		AK1	AK2	PO4		AK1	PO4	PO4	AK1	PO4	AK2	PO4	AK1	AK2	AK1	AK1	AK1	Ph												PO4									6.6
6		Ph	AK1		AK1	Ph	AK1		PO4	PO4	PO4	AK1	PO4	AK2	PO4	AK1	PO4	AK1	AK1	AK1						Ph	AK2						PO4				Ph					3.4

Electrostatic | Hydrogen Bonding | Charge-Dipole | π-stacking | van der Waals | Non-polar | Backbone

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	VAL288	PHE398	ILE69	GLN71	PHE73	PRO74	GLY75	PHE76	MET81	ASN83	SER79	TYR114	GLY115	PHE118	THR120	TYR128	GLU197	ASN397	TRP430	MET437	GLY439	TYR440	ILE442	Pop (%)
Mode	PAS						CAT			OAH			CBS			ABS					OML								Additional Protein Residues (APR)													
0			PO4			AK2	PO4		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			PO4	PO4	AK1	PO4		PO4	AK1	AK2	AK1	AK1	AK1			AK2									AK1	PO4									17.7
2			Ph		AK1	AK2	PO4		PO4	PO4	PO4	AK1	PO4	AK2	PO4	AK1	AK2	AK1	AK1	AK1	Ph										PO4	AK1	PO4		PO4				AK2			16.5
3	Ph		PO4			AK2	AK1		AK1	PO4	PO4	AK1	PO4	AK2	PO4	AK1	PO4	AK1	AK1	AK1												PO4									13.8
4			PO4		AK1	Ph	AK1			AK1	PO4	AK1	Ph		PO4	AK1	Ph	AK1	PO4	AK1	AK2											AK1	PO4									11.7
5						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											PO4	AK1	Ph		PO4				AK2			5.7
7			PO4			PO4				PO4	PO4		Ph		PO4		AK1																PO4									4

Electrostatic | Hydrogen Bonding | Charge-Dipole | π-stacking | van der Waals | Non-polar | Backbone

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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