Induced-fit or Pre-existing equilibrium Dynamics? Lessons from Protein Crystallography and MD simulations on AchE and Implications for Structure-based Drug Design


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Outline

- Purpose
- Background
- Methods
- Results
- Conclusion
Purpose

- Compare acetylcholinesterase (AchE)-ligand complexes with side-chain conformations accessed by native AChE using molecular dynamics (MD) simulations
- Determine the binding mechanism that effects the conformational changes
- Understand conformational changes upon ligand binding to rationally design better Alzheimer's disease (AD) drugs
Acetylcholinesterase (AChE)

- An enzyme found in nerve and muscle cell synapses
- Termination of impulse transmission by rapid hydrolysis of Acetylcholine (AChE) into choline and acetate

Schematic diagram of a nerve synapse showing hydrolysis and synthesis cycle of acetylcholine. Also in the diagram is pre and post synaptic cleft as well as acetylcholinesterase activity.

http://www.cnsforum.com
AChE—Structure

- AChE monomer composed of 15 alpha helices and 11 beta strands.

- AChE acts rapidly (one molecule of ACh hydrolyzed in 40 μs), although the active site residues (Ser 200, Glu 327, His 440) are buried at the bottom of a deep & narrow gorge lined with aromatic residues.

G.Mustata: Secondary structure representation of the Torpedo californica AChE (Sussman et al., 1991); in green Trp84, Trp279, Phe330 (give the orientation of the gorge); in red Glu 199 (bottom of the gorge).

G. Mustata: Ribbon display of the Torpedo californica AChE (Sussman et al., 1991); in green, the catalytic triad of the active site (Ser200, Glu327, His440); in red Trp84, Glu199, Trp279, Phe330.
Structure—Active sites

- PAS
  - Trp 279
- CAS
  - Trp 84
- Lining of aromatic residues

Xu et al., *Protein Science* (2008), 17:601-605

3D structure of native TcAChE (PDB access code 1ea5). Catalytic-triad residues are in red, the active-site gorge in green, and PAS Trp279 and CAS Trp84 in blue.
AChE Inhibitors

- Irreversible
- Pseudo-irreversible
- Transition state analogues
- Reversible
  - Unifunctional: interacts with either the PAS or CAS
  - Bifunctional: interacts with both PAS and CAS
Binding Models

A. Lock and Key
B. Induced Fit
C. Preexisting Equilibrium

Lock and key model

Induced fit model

Differential ligand positioning together with Preexisting equilibrium model
## Methods—Structures

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METHODS—Experiment

MD SIMULATION
- Crystal structures were put in a 10.6 cubic nm box and solvated in water
- Conditions: NPT (constant number moles, pressure and temperature)
  - Pressure 1 bar
  - Temperature 300K
- Software
  - GROMACS package using NPT and periodic boundary conditions
- Simulation on each structure run for 20 ns
- $\chi_1$ and $\chi_2$ defining Trp 279's side chain conformation angles were recorded at 1 ps intervals
Results

- 5 islands of distinct conformations were accessed in the simulation.
- Trp 279 conformation in the 89 crystal structures studied, native or complexed, lay within the 5 islands accessed in the simulation.
- There were seven conformation groups observed experimentally.

Time evolution graph showing side chain angles. Colors represent MD islands as well as conformation groups: Khaki: crystallographic groups a and b; blue: group c; cyan: group d; gray: group f; mauve: group g.

Xu et al., *Protein Science* (2008), 17:601-605
Ramachandran plot showing side chain conformation graph from a 20 ns MD simulation (gray dots) and 89 crystals (black triangles). There are 5 islands and seven conformation groups. The white pentacle indicate conformation of TcAChE (1EA5) which the simulation experiment is based on.

Xu et al., Protein Science (2008), 17:601-605
Results—MD Simulations

- **Group d,f**
  - Conformations at the margins of MD islands
  - Bind to preexisting model and also cause small induced fit

- **Group g**
  - Preexisting model

- **Group e**
  - Contain PAS Trp279 that was not accessed in the simulation of Native Ache
  - Induced model likely

Ramachandran plot showing side chain conformation graph from a 20 ns MD simulation (gray dots) and 89 crystals (black triangles). There are 5 islands and seven conformation groups. The white pentacle indicate conformation of TcAChE (1EA5) which the simulation experiment is based on.

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Conclusion

- MD simulations showed conformational heterogeneity that might profit rational drug design – i.e. design new inhibitors using several AchE-ligand complexes taking into account the conformational change of the amino acids involved in ligand binding.

- Ligands that contact several sites simultaneously, bifunctional, may involve aspects of all binding modes. Thus, rational drug design should be based upon both structure and structural dynamics.

- Protein conformational diversity is essential for a wide range of biological functions. X-ray crystallography combined with other biophysical techniques will not only add a dynamical dimension to structural insight but will also validate conformational sampling from MD simulations.
Acknowledgements

- Dr Gabriela Mustata. PhD
References


- [www.biomedcentral.com/.../1472-6807-7-31-1.jpg](http://www.biomedcentral.com/.../1472-6807-7-31-1.jpg)