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ACCELERATED COMMUNICATION

Induced-fit or preexisting equilibrium dynamics? Lessons from protein crystallography and MD simulations on acetylcholinesterase and implications for structure-based drug design

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Abstract

Crystal structures of acetylcholinesterase complexed with ligands are compared with side-chain conformations accessed by native acetylcholinesterase in molecular dynamics (MD) simulations. Several crystallographic conformations of a key residue in a specific binding site are accessed in a simulation of native acetylcholinesterase, although not seen in rotamer plots. Conformational changes upon ligand binding thus involve preexisting equilibrium dynamics. Consequently, rational drug design could benefit significantly from conformations monitored by MD simulations of native targets.

Keywords: X-ray crystallography; protein dynamics; conformational energy landscape; ligand binding; structure-based drug design; molecular dynamics simulation; acetylcholinesterase

X-ray crystallography provides a time and ensemble-averaged picture of a protein's three-dimensional structure. An individual molecule in solution, however, constantly fluctuates among many conformational substates in a complex energy landscape (Frauenfelder et al. 1991). That the resulting "structural dynamics" is essen-

tial for biological activity is evident in the process of ligand binding. Indeed, in many cases only a conformational change permits accommodation of a ligand, in contrast to the classical lock-and-key mechanism, in which the two rigid binding partners are of complementary shape. Two mechanisms have been offered to rationalize conformational changes upon ligand binding, i.e., induced-fit (Koshland 1958) and preexisting equilibrium dynamics (Monod et al. 1965; Tsai et al. 1999). In the former, binding of a ligand induces, and thus precedes, the conformational change, whereas in the latter it binds to a specific preexisting conformation already present in the set of conformational substates accessed by equilibrium dynamics. The difference is subtle but might be a crucial issue in structure-based drug design (Steuber et al. 2007). Here, we

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compare the repertoire of crystallographic structures of the enzyme acetylcholinesterase (AChE), complexed with various ligands, with conformations accessed by native AChE in molecular dynamics (MD) simulations in order to explore the origin of conformational changes involved in ligand binding.

The main role of AChE is rapid hydrolysis of the neurotransmitter acetylcholine (ACh) (Silman and Sussman 2005). The crystal structure of *Torpedo californica* (*Tc*) AChE (Sussman et al. 1991) showed its active site to be near the bottom of a narrow, 20 Å deep gorge, containing a catalytic triad and a conserved Trp, Trp84, which is the main residue of the catalytic anionic site (CAS) (Fig. 1). A second Trp, Trp279, is the major residue in the “peripheral” anionic site (PAS), at the gorge mouth, which may serve as a relay station for incoming substrate and interact allosterically with the active site (Rosenberry et al. 2005). A variety of ligands bind to AChE, including first-generation anti-Alzheimer drugs, organophosphorous (OP) nerve agents, and OP and carbamate insecticides (Silman and Sussman 2005). In most cases, no conformational change is observed in Trp279 upon binding. However, certain bifunctional inhibitors, bridging the CAS and PAS, induce drastic conformational changes in Trp279 and in the equivalent residue of AChEs from other species (Bourne et al. 2004; Colletier et al. 2006; Ekstrom et al. 2006; Rydberg et al. 2006). Such inhibitors are being developed as potential second-generation anti-Alzheimer drugs (Du and Carlier 2004) and as reactivators after OP poisoning (Ekstrom et al. 2006).

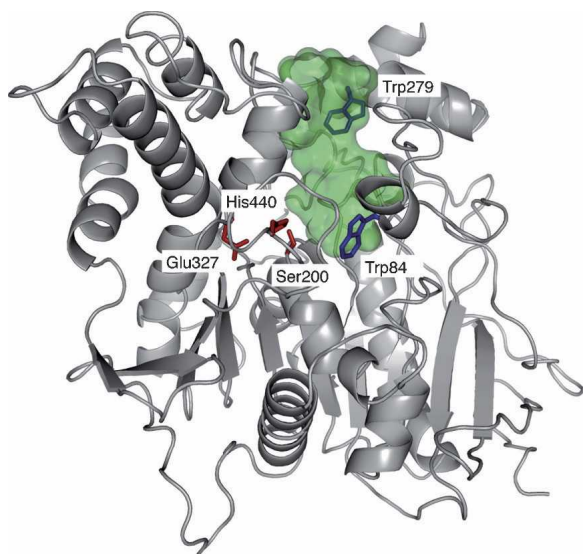


Figure 1. 3D structure of native *Tc*AChE (PDB access code 1ea5). Catalytic-triad residues are in red, the active-site gorge in green, and PAS Trp279 and CAS Trp84 in blue.

Here we addressed the issue of whether conformational changes in the PAS Trp are due to induced fit or to selection of a preexisting conformation by comparing conformations accessed in MD simulations with those seen in crystal structures. Some of the side-chain conformations of this residue in crystallographic AChE–ligand complexes, which differ from that in native AChE, are already accessed in a MD simulation of native AChE. Certain conformational changes upon ligand binding thus involve preexisting equilibrium dynamics. Others are suggested to involve induced fit only, or both preexisting equilibrium dynamics and induced fit. Structure-based drug design might thus benefit from the equilibrium conformations in MD simulations of the native enzyme since they are shown to be validated by those seen in crystallographic complexes.

Results and Discussion

Hydrated MD studies were carried out over 20 ns, starting from the native *Tc*AChE structure (PDB ID code 1ea5). The χ_1 and χ_2 angles defining Trp279’s side-chain conformation were recorded at 1-ps intervals (Fig. 2A). A broad conformational space is sampled in this simulation, consisting of five islands of distinct conformations. Comparison with the Trp279 conformations of 89 published crystal structures of AChEs from various species (Table 1), either native or complexed with ligands, reveals that several experimentally determined conformations are within the five islands accessed in the simulation (Fig. 2A). The largest number (groups a–c in Fig. 2A) are close to those in native AChEs. Ligands binding to the conformations in groups a–c either do not interact with Trp279 directly or involve a lock-and-key mechanism. Those that differ markedly from the native conformation (groups d–g) are mostly of bifunctional inhibitor complexes. Only some mAChE complexes with oxime reactivators (Ekstrom et al. 2006) (group e) contain a PAS Trp conformation not accessed in the simulation trajectory, and conformational changes are thus most likely generated by an induced-fit mechanism. The PAS Trp conformations observed in the crystal structures of groups d, f, and g are present in the preexisting equilibrium dynamics of AChE and are selected, and thus accumulate, during ligand binding. If, in contrario, they had been created primarily by induced fit, we would not expect them to be accessed in the MD simulation of native AChE, as is indeed the case for those in group e. Conformations in groups d and f are at the margins of MD islands and may contain both preexisting equilibrium dynamics and a small induced-fit component during ligand binding. We note that a single 20-ns MD simulation might not sample the conformational space of a side chain fully. It is striking, however, that a standard χ_1, χ_2 rotamer plot of tryptophan (Laskowski et al. 1993)

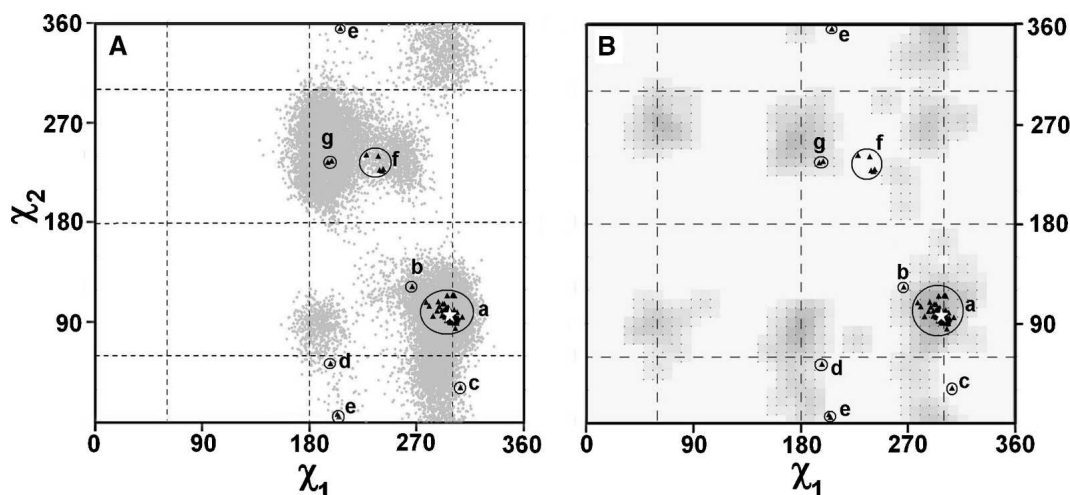


Figure 2. χ_1 and χ_2 angles of Trp279 side-chain conformations from simulation, X-ray crystallography, and a rotamer library. (A) Conformations from a 20-ns MD simulation (gray dots) and 89 crystallographic AChE structures deposited in the PDB (black triangles). Simulated conformations form five islands. The white pentacle indicates the conformation in the crystal structure (1ea5) that the simulation is based on. Experimental conformations fall in seven groups: a, most native and complex structures; b, native *Dm*AChE (Harel et al. 2000) (1QO9); c, *Tc*AChE/tacrine (Harel et al. 1993) (1ACJ); d, mAChE/TZ2PA6syn (Bourne et al. 2004) (1Q83); e, mAChE/HI-6 (Ekstrom et al. 2006) (2GYU), mAChE/HLO-7 (2JEY), and mAChE/HLO-7/tabun (monomer A, 2JEZ) complexes; f, *Tc*AChE/A7 (Rydberg et al. 2006) (2CKM), *Tc*AChE/A8 (Rydberg et al. 2006) (1ODC), *Tc*AChE/NF595 (Colletier et al. 2006) (2CEK), mAChE/obidoxime (Ekstrom et al. 2006) (2GYW), and mAChE/HLO-7/tabun (monomer B, 2JEZ) complexes; g, mAChE/ortho-7 (Ekstrom et al. 2006) (2GYV) and mAChE/ortho-7/tabun (2JF0) complexes. (B) Most favorable conformations predicted by PROCHECK (Laskowski et al. 1993) based on an experimental library of rotomers (gray areas). The same experimental conformations as in A are presented (black triangles and white pentacle).

would miss several of these regions, in particular group f (Fig. 2B).

More than one of the seven conformational groups (a–g) are accessed in crystalline structures of a given space group (Table 1). Thus, complexes of a trigonal crystal form ($P3_121$) of *Tc*AChE display Trp279 conformations in groups a, c, and f. Consequently, differences in

crystal packing in the different space groups are not at the origin of the differences in conformations of groups a–g. However, the number of accessible Trp279 conformations for a particular space group might be restricted.

AChE is well suited to addressing the origin of conformational changes upon ligand binding, since a large number of crystallographic complexes are available for

Table 1. The 89 crystal structures of AChE from five different species

Species	PDB code and space group	Residue number
<i>Tc</i> AChE	^c 1acj ($P3_121$), ^a 1acl ($P3_121$), ^a 1amn ($P3_121$), ^a 1ax9 ($P3_121$), ^a 1cfj ($P3_121$), ^a 1dx6 ($P3_121$), ^a 1e3q ($P3_121$), ^a 1e66 ($P3_121$), ^a1ea5 ($P3_121$), ^a 1eve ($P3_121$), ^a 1fss ($P2_12_12$), ^a 1gpk ($P3_121$), ^a 1gpn ($P3_121$), ^a 1gqr ($P3_121$), ^a 1gqs ($P3_121$), ^a 1h22 ($P3_121$), ^a 1h23 ($P3_121$), ^a 1hbj ($P3_121$), ^a 1jjb ($P3_121$), ^a 1oce ($P3_121$), ^f 1ode ($P3_121$), ^a 1qid ($P3_121$), ^a 1qti ($P3_121$), ^a 1som ($P3_121$), ^a 1u65 ($P3_22_1$), ^a 1ut6 ($P3_121$), ^a 1vot ($P3_121$), ^a 1vxo ($P3_121$), ^a 1vxr ($P3_121$), ^a 1w4l ($P3_121$), ^a 1w6r ($P3_121$), ^a1w75 ($P2_12_12_1$), ^a 1w76 ($P2_12_12_1$), ^a 1zgb ($P3_121$), ^a 1zgc ($P2_12_12_1$), ^a 2ace ($P3_121$), ^a 2ack ($P3_121$), ^a 2bag ($P3_121$), ^a 2c4h ($P3_121$), ^a 2c5f ($P3_121$), ^a 2c5g ($P3_121$), ^a 2c58 ($P3_121$), ^f 2cek ($P3_121$), ^f 2ckm ($P3_121$), ^a 2cmf ($P3_121$), ^a 2dfp ($P3_121$), ^a 2j4f ($P3_121$)	W279
<i>Ee</i> AChE	^a 1eea (F222), ^a 1c2b (F222), ^a 1c2o (C2)	W283
<i>Dm</i> AChE	^a 1dx4 ($P4_32_12$), ^a1qo9 ($P4_32_12$), ^a 1qon ($P4_32_12$)	W321
mAChE	^a1j06 ($P2_12_12_1$), ^a 1j07 ($P2_12_12_1$), ^a 1ku6 ($P6_522$), ^a 1maa ($P2_12_12_1$), ^a 1mah ($P6_522$), ^a 1n5m ($P2_12_12_1$), ^a 1n5r ($P2_12_12_1$), ^a 1q83 ($P2_12_12_1$), ^a 1q84 ($P2_12_12_1$), ^a 2c0p ($P2_12_12_1$), ^a 2c0q ($P2_12_12_1$), ^c 2gyu ($P2_12_12_1$), ^c 2gyv ($P2_12_12_1$), ^c 2gyw ($P2_12_12_1$), ^a 2h9y ($P2_12_12_1$), ^a 2ha0 ($P2_12_12_1$), ^a 2ha2 ($P2_12_12_1$), ^a 2ha3 ($P2_12_12_1$), ^a 2ha4 ($P2_12_12_1$), ^a 2ha5 ($P2_12_12_1$), ^a 2ha6 ($P2_12_12_1$), ^a 2ha7 ($P2_12_12_1$), ^a 2jey ($P2_12_12_1$), ^c 2jez ($P2_12_12_1$), ^c 2jfo ($P2_12_12_1$), ^a 2jge ($P2_12_12_1$), ^a 2jgf ($P2_12_12_1$), ^a 2jgg ($P2_12_12_1$), ^a 2jgh ($P2_12_12_1$), ^a 2jgi ($P2_12_12_1$), ^a 2jgj ($P2_12_12_1$), ^a 2jgk ($P2_12_12_1$), ^a 2jgl ($P2_12_12_1$), ^a 2jgm ($P2_12_12_1$)	W286
hAChE	^a 1b41 (H32), ^a 1f8u (H32)	W286

The space group is given in brackets. The PDB codes of the native AChE data sets are highlighted in bold italics. The corresponding residue number of W279 in each species is shown. The superscripts indicate the group (a–g) to which each conformation belongs in Figure 2.

analysis. Our results for AChE suggest that the equilibrium dynamics of native proteins, as monitored by MD simulations, indeed encompass preexisting side-chain conformations selected by ligands. Rational drug design, based on structural information, might profit by taking into account conformational heterogeneity observed in MD simulations. In particular, we suggest that docking assays be performed starting from several protein structures in which amino acids involved in ligand binding are in the various conformations displayed in the MD simulations. Ligands that might not bind to the (average) native protein structure, but rather to a minor conformation in the preexisting equilibrium dynamics, could thus be identified.

Binding of ligands contacting several sites simultaneously may involve aspects of preexisting equilibrium dynamics, induced-fit, and lock-and-key mechanisms. Thus, some bifunctional AChE inhibitors (Bourne et al. 2004; Colletier et al. 2006; Ekstrom et al. 2006; Rydberg et al. 2006) select a preexisting conformation at the PAS but bind with only minor concomitant conformational changes at the CAS, where they fit in a lock-and-key manner. Rational drug design should thus be based upon both structure and structural dynamics. In this context, structural information from NMR ensembles can also benefit structure-based drug design (Damm and Carlson 2007).

Preexisting equilibrium dynamics is also involved in protein–protein interactions (Goh et al. 2004), being capable of conferring multi-specificity upon antibodies in which different preexisting conformations recognize different antigens (James et al. 2003). Evidence is thus growing that a protein's conformational diversity is essential for a wide range of biological functions. X-ray crystallography, together with other biophysical techniques, can thus add a dynamical dimension to the structural insight it provides, and validate conformational sampling from MD simulations.

Materials and Methods

MD simulation

A monomer of native *TcAChE*, determined at 1.8 Å resolution (Sussman et al. 1991), served as the starting structure for simulation (PDB code: 1ea5). The protein, together with crystal water molecules, was inserted into a box of dimensions $10.6 \times 10.6 \times 10.6 \text{ nm}^3$, with the minimal distance of the protein from its walls being 2.0 nm. This box was solvated by use of a simple point charge (SPC) water model (Berendsen et al. 1981). The solvated box was then submitted to energy minimization. Subsequently, counter ions were added, so as to provide a neutral simulation system. Energy minimization was then repeated on the whole system. After convergence had been reached, the solvent, the counter ions, and the protein were

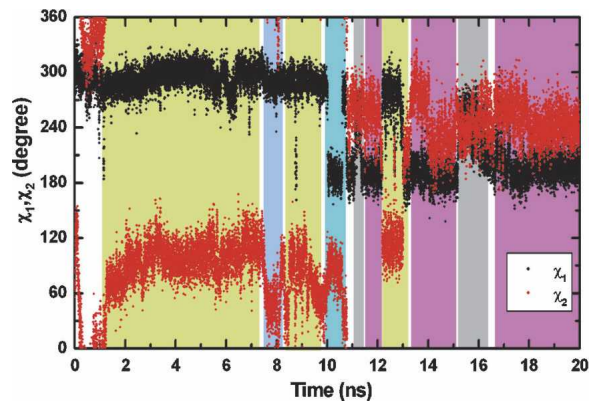


Figure 3. Time dependence of χ_1 and χ_2 along the 20-ns trajectory. Colored bars indicate the five islands shown in Figure 2A. Khaki: island containing crystallographic groups a and b; blue: island containing group c; cyan: island containing group d; gray: island containing group f; mauve: island containing group g.

coupled separately to a temperature bath at 300 K. The whole system was then equilibrated for 20 ns. The final root mean square deviations (RMSDs) of $C\alpha$ atoms and of all protein atoms are 2.0 and 2.6 Å, respectively, relative to the crystallographic starting structure. The Ramachandran plot of phi/psi angles, however, shows no increase in “outliers” relative to the native structure. The time evolution of the Trp279 conformation during the 20-ns simulation is shown in Figure 3.

MD simulations were carried out with a GROMACS package (Berendsen et al. 1995; Lindahl et al. 2001), using NPT and periodic boundary conditions. The GROMOS96 force field (van Gunsteren et al. 1996) was applied to the protein. The pressure was kept constant at 1 bar by coupling to a Berendsen barostat with $\tau_p = 1.0 \text{ ps}$ and a compressibility of $4.5 \times 10^{-5} \text{ bar}$ (Berendsen et al. 1984). The temperature was maintained at 300 K by coupling to a Berendsen thermostat with a coupling time of $\tau_T = 0.1 \text{ ps}$ (Berendsen et al. 1984). The LINCS method (Hess et al. 1997) was used to restrain bond lengths, allowing an integration step of 2 fs. The coordinates of the whole system were saved every 500 steps. Electrostatic interactions were calculated using the Particle-Mesh Ewald (PME) algorithm (Darden et al. 1993; Essmann et al. 1995).

Crystallographic data

Currently, 97 crystal structures of AChE are available from the PDB. The majority are for *TcAChE* (55 structures) and *mAChE* (34 structures), as well as two, three, and three structures, respectively, for human, *Electrophorus*, and *Drosophila* AChE. Of the nine radiation-damaged native *TcAChE* structures (Weik et al. 2000), only one, i.e., 1qid, was selected. Thus, 89 crystal structures are used for the χ_1 and χ_2 angles analysis of W279. The PDB codes of the 89 structures are listed in Table 1 and the space group of the corresponding crystal form is given.

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References

- Berendsen, H.J.C., Postma, J.P.M., van Gunsteren, W.F., and Hermans, J. 1981. Interaction models for water in relation to proteins hydration. In *Intermolecular forces* (ed. B. Pullman), pp. 331–342. Reidel, Dordrecht.
- Berendsen, H.J.C., Postma, J.P.M., van Gunsteren, W.F., DiNola, A., and Haak, J.R. 1984. Molecular dynamics with coupling to an external bath. *J. Chem. Phys.* **81**: 3684–3690.
- Berendsen, H.J.C., van der Spoel, D., and van Drunen, R. 1995. GROMACS: A message-passing parallel molecular dynamics implementation. *Comput. Phys. Commun.* **91**: 43–56.
- Bourne, Y., Kolb, H.C., Radic, Z., Sharpless, K.B., Taylor, P., and Marchot, P. 2004. Freeze-frame inhibitor captures acetylcholinesterase in a unique conformation. *Proc. Natl. Acad. Sci.* **101**: 1449–1454.
- Colletier, J.P., Sanson, B., Nachon, F., Gabellieri, E., Fattorusso, C., Campiani, G., and Weik, M. 2006. Conformational flexibility in the peripheral site of *Torpedo californica* acetylcholinesterase revealed by the complex structure with a bifunctional inhibitor. *J. Am. Chem. Soc.* **128**: 4526–4527.
- Damm, K.L. and Carlson, H.A. 2007. Exploring experimental sources of multiple protein conformations in structure-based drug design. *J. Am. Chem. Soc.* **129**: 8225–8235.
- Darden, T., York, D., and Pedersen, L. 1993. Particle mesh Ewald: An N -log(N) method for Ewald sums in large systems. *J. Chem. Phys.* **98**: 10089–10092.
- Du, D.M. and Carlier, P.F. 2004. Development of bivalent acetylcholinesterase inhibitors as potential therapeutic drugs for Alzheimer's disease. *Curr. Pharm. Des.* **10**: 3141–3156.
- Ekström, F., Pang, Y.P., Boman, M., Artursson, E., Akfur, C., and Börjegen, S. 2006. Crystal structures of acetylcholinesterase in complex with HI-6, Ortho-7 and obidoxime: Structural basis for differences in the ability to reactivate tabun conjugates. *Biochem. Pharmacol.* **72**: 597–607.
- Essmann, U., Perera, L., Berkowitz, M.L., Darden, T., Lee, H., and Pedersen, L.G. 1995. A smooth particle mesh Ewald potential. *J. Chem. Phys.* **103**: 8577–8592.
- Frauenfelder, H., Sligar, S.G., and Wolynes, P.G. 1991. The energy landscapes and motions of proteins. *Science* **254**: 1598–1603.
- Goh, C.S., Milburn, D., and Gerstein, M. 2004. Conformational changes associated with protein–protein interactions. *Curr. Opin. Struct. Biol.* **14**: 104–109.
- Harel, M., Schalk, I., Ehret-Sabatier, L., Bouet, F., Goeldner, M., Hirth, C., Axelsen, P.H., Silman, I., and Sussman, J.L. 1993. Quaternary ligand binding to aromatic residues in the active-site gorge of acetylcholinesterase. *Proc. Natl. Acad. Sci.* **90**: 9031–9035.
- Harel, M., Kryger, G., Rosenberry, T.L., Mallender, W.D., Lewis, T., Fletcher, R.J., Guss, J.M., Silman, I., and Sussman, J.L. 2000. Three-dimensional structures of *Drosophila melanogaster* acetylcholinesterase and of its complexes with two potent inhibitors. *Protein Sci.* **9**: 1063–1072.
- Hess, B., Bekker, B., Berendsen, H.J.C., and Fraaije, J.G.E.M. 1997. LINCS: A linear constraint solver for molecular simulations. *J. Comput. Chem.* **18**: 1463–1472.
- James, L.C., Roversi, P., and Tawfik, D.S. 2003. Antibody multispecificity mediated by conformational diversity. *Science* **299**: 1362–1367.
- Koshland, D.E. 1958. Application of a theory of enzyme specificity to protein synthesis. *Proc. Natl. Acad. Sci.* **44**: 98–104.
- Laskowski, R.A., MacArthur, M.W., Moss, D.S., and Thornton, J.M. 1993. PROCHECK: A program to check the stereochemical quality of protein structures. *J. Appl. Crystallogr.* **26**: 283–291.
- Lindahl, E., Hess, B., and van der Spoel, D. 2001. Gromacs 3.0: A package for molecular simulation and trajectory analysis. *J. Mol. Model.* **7**: 306–317.
- Monod, J., Wyman, J., and Changeux, J.P. 1965. On the nature of allosteric transitions: A plausible model. *J. Mol. Biol.* **12**: 88–118.
- Rosenberry, T.L., Johnson, J.L., Cusack, B., Thomas, J.L., Emani, S., and Venkatasubban, K.S. 2005. Interactions between the peripheral site and the acylation site in acetylcholinesterase. *Chem. Biol. Interact.* **157–158**: 181–189.
- Rydberg, E.H., Brumshtein, B., Greenblatt, H.M., Wong, D.M., Shaya, D., Williams, L.D., Carlier, P.F., Pang, Y.P., Silman, I., and Sussman, J.L. 2006. Complexes of alkylene-linked tacrine dimers with *Torpedo californica* acetylcholinesterase: Binding of Bis5-tacrine produces a dramatic rearrangement in the active-site gorge. *J. Med. Chem.* **49**: 5491–5500.
- Silman, I. and Sussman, J.L. 2005. Acetylcholinesterase: 'Classical' and 'non-classical' functions and pharmacology. *Curr. Opin. Pharmacol.* **5**: 293–302.
- Steuber, H., Zentgraf, M., La Motta, C., Sartini, S., Heine, A., and Klebe, G. 2007. Evidence for a novel binding site conformer of aldose reductase in ligand-bound state. *J. Mol. Biol.* **369**: 186–197.
- Sussman, J.L., Harel, M., Frolow, F., Oefner, C., Goldman, A., Toker, L., and Silman, I. 1991. Atomic structure of acetylcholinesterase from *Torpedo californica*: A prototypic acetylcholine-binding protein. *Science* **253**: 872–879.
- Tsai, C.J., Kumar, S., Ma, B., and Nussinov, R. 1999. Folding funnels, binding funnels, and protein function. *Protein Sci.* **8**: 1181–1190.
- van Gunsteren, W.F., Billeter, S.R., Eising, A.A., Hunenberger, P.H., Krüger, P., Mark, A.E., Scott, W.R.P., and Tironi, I.G. 1996. *Biomolecular simulations: The GROMOS96 manual and user guide*. Vdf Hochschulverlag AG an der ETH Zuerich, Zurich, Switzerland.
- Weik, M., Ravelli, R.B., Kryger, G., McSweeney, S., Raves, M.L., Harel, M., Gros, P., Silman, I., Kroon, J., and Sussman, J.L. 2000. Specific chemical and structural damage to proteins produced by synchrotron radiation. *Proc. Natl. Acad. Sci.* **97**: 623–628.