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Supercomputer Modeling of Dual-Site Acetylcholinesterase (AChE) Inhibition

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Molecular docking is one of the most popular tools of molecular modeling. However, in certain cases, like development of inhibitors of cholinesterases as therapeutic agents for Alzheimer's disease, there are many aspects, which should be taken into account to achieve accurate docking results. For simple molecular docking with popular software and standard protocols, a personal computer is sufficient, however quite often the results are irrelevant. Due to the complex biochemistry and biophysics of cholinesterases, computational research should be supported with quantum mechanics (QM) and molecular dynamics (MD) calculations, what requires the use of supercomputers. Experimental studies of inhibition kinetics can discriminate between different types of inhibition—competitive, non-competitive or mixed type—that is quite helpful for assessment of the docking results. Here we consider inhibition of human acetylcholinesterase (AChE) by the conjugate of MB and 2,8-dimethyl-tetrahydro-γ-carboline, study its interactions with AChE in relation to the experimental data, and use it as an example to elucidate crucial points for reliable docking studies of bulky AChE inhibitors. Molecular docking results were found to be extremely sensitive to the choice of the X-ray AChE structure for the docking target and the scheme selected for the distribution of partial atomic charges. It was demonstrated that flexible docking should be used with an additional caution, because certain protein conformational changes might not correspond with available X-ray and MD data.

Keywords: acetylcholinesterase, Alzheimer's disease, molecular docking, atomic charges.

Introduction

Therapy of Alzheimer's disease (AD) involves inhibition of brain AChE to restore acetylcholine (ACh) levels. [9]. In addition to hydrolyzing ACh, AChE promotes aggregation of β-amyloid peptide through its interaction with the AChE peripheral anionic site (PAS). Thus, dual-site inhibitors of the active and PAS sites are expected to be disease-modifying agents [10].

To develop dual-site anti-AD drugs, we combined two known pharmacophores, methylene blue (MB) and carbolines into single conjugates (**MBC**) [14], see Fig. 1, and demonstrated that they were effective inhibitors of AChE capable of displacing propidium from the AChE PAS [1].

Docking and other computational methods have been used in drug design for decades. However biophysical constraints can hamper the predictive power of these approaches [2]. For example, AChE contains a gorge with a midpoint constriction ("bottleneck") that separates the PAS and active site regions [15]. Consequently, inhibition is determined not only by geometric and interaction energy factors, but also by binding dynamics [7]. In the present work, we analyzed the results of different molecular docking approaches for **MBC** into AChE and compared them to kinetic data, demonstrating mixed-type inhibition (Fig. 2). Thus, the compound should bind competetively to the active site and noncompetitively to the PAS, and docking should provide poses of the ligand both above and below the bottleneck.

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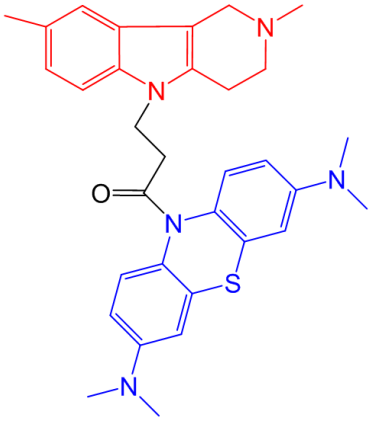


Figure 1. Structure of compound **MBC**, a conjugate of MB (colored blue) and 2,8-dimethyl-tetrahydro-γ-carboline (colored red)

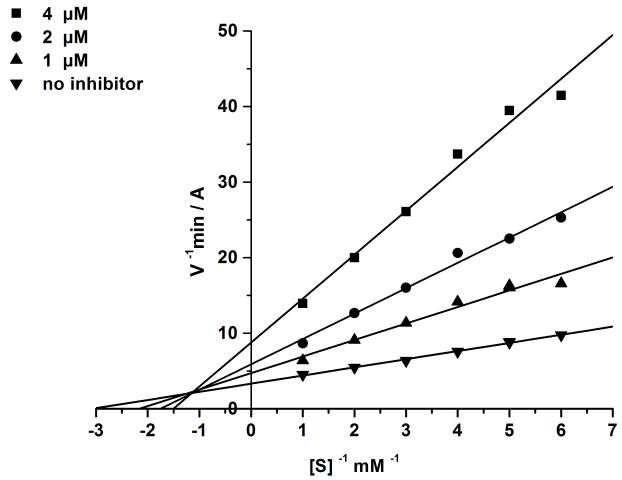
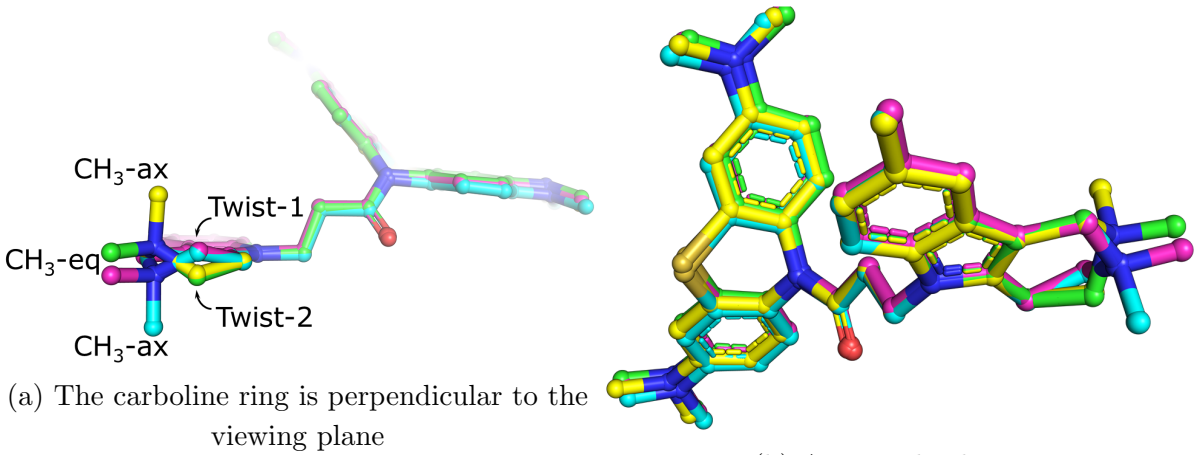


Figure 2. Steady state inhibition of AChE by this compound; Lineweaver-Burk double-reciprocal plots of initial velocity and substrate concentrations in the presence of the inhibitor, showing mixed-type inhibition

1. Methods

The carboline part of **MBC** contains a piperidine ring condensed with an aromatic system that implicates conformers and enantiomers. Using OpenEye OMEGA 2.5.1.4: OpenEye Scientific Software, Santa Fe, NM. `http://www.eyesopen.com` [5], 4 configurations of **MBC** were generated (Fig. 3).

pK_a values were calculated with Schrödinger Jaguar QM DFT pK_a module [16]. Geometries were optimized with Gamess-US [11] software (B3LYP/6-31G*). For docking, optimized ligand structures were used with Gasteiger partial atomic charges and those derived from QM results according to Mulliken and Löwdin schemes. Additionally, Schrödinger QM-Polarized Ligand Docking (PLD) [4] was used with extra precision docking and redocking; charges were calculated using the Jaguar accurate QM method. Five X-ray structures of human AChE (PDB IDs 4EY4-4EY8, [3]) were used for docking. Rigid docking was performed with AutoDock 4.2.6 [8] as described earlier [12]. For flexible docking, Schrödinger Glide Induced Fit [13] was used with AChE as a target. The docking volume included the entire gorge, and extra precision docking



(a) The carboline ring is perpendicular to the viewing plane

(b) A general side view

Figure 3. Overlaid configurations of the piperidine fragment of the γ-carboline ring of **MBC**

and scoring were employed. MD simulations were done in 0.15 M NaCl solution with previously published methods [6, 17].

2. Results

The PDB (`http://www.rcsb.org`) contains X-ray structures of *apo*-AChE (4EY4) and co-crystallized with different compounds: (-)-Huperzine A (4EY5); (-)-Galantamine (4EY6); Donepezil (4EY7); and fasciculin-2 (4EY8) [3]. The absence or presence of ligands affects the conformation of principal amino acids lining the gorge, e. g., Tyr337 and Tyr341 [15], see Fig. 4. We have previously reported significant differences in estimated binding energies for the same compounds with these targets [12]. Here, we show that the target X-ray structure determines whether or not ligand poses reflect mixed-type inhibition.

The QM-calculated pK_a value for the piperidine nitrogen was 7.84. Thus, under experimental conditions mimicking physiological pH 7.4, both protonated and non-protonated forms could be present. For this reason, both states were used for the docking study and analysis of results.

Partial atomic charges are crucial for docking results from the algorithms used in our study, as the charge distribution calculation scheme defines the estimated binding energies and geometries of complexes [4]. With respect to **MBC** docking into AChE, the influence of partial atomic charges was even more pronounced. In the case of *apo*-AChE as a target, poses below the bottleneck were obtained only for structures with partial charges derived from QM calculations according to the Löwdin scheme (Fig. 5). The results obtained with the Gasteiger scheme and derived from QM data according to the Mulliken scheme and Shrödinger QM PLD docking showed poorer occupation of the active site compartment for other targets (Fig. 5).

Only in the case of the AChE structure co-crystallized with Donepezil was **MBC** docked in full correspondence with experimental data (below and above the bottleneck) regardless of the partial atomic charges scheme (Fig. 5). This is ensured by the Tyr337 side chain, which forms the bottleneck, being rotated so that it does not block the gorge.

For AChE structures co-crystallized with Huperzine A and fasciculin-2 (Fig. 6), the **MBC** ligand could be found only in the PAS, which corresponds to non-competitive inhibition, and thus does not agree with experimental data.

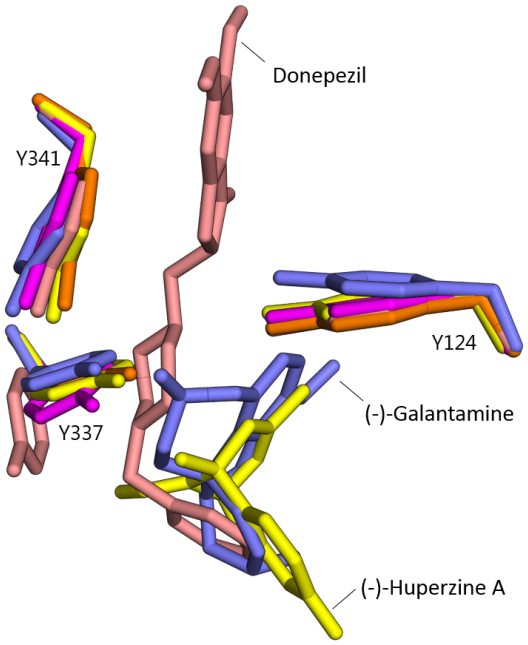


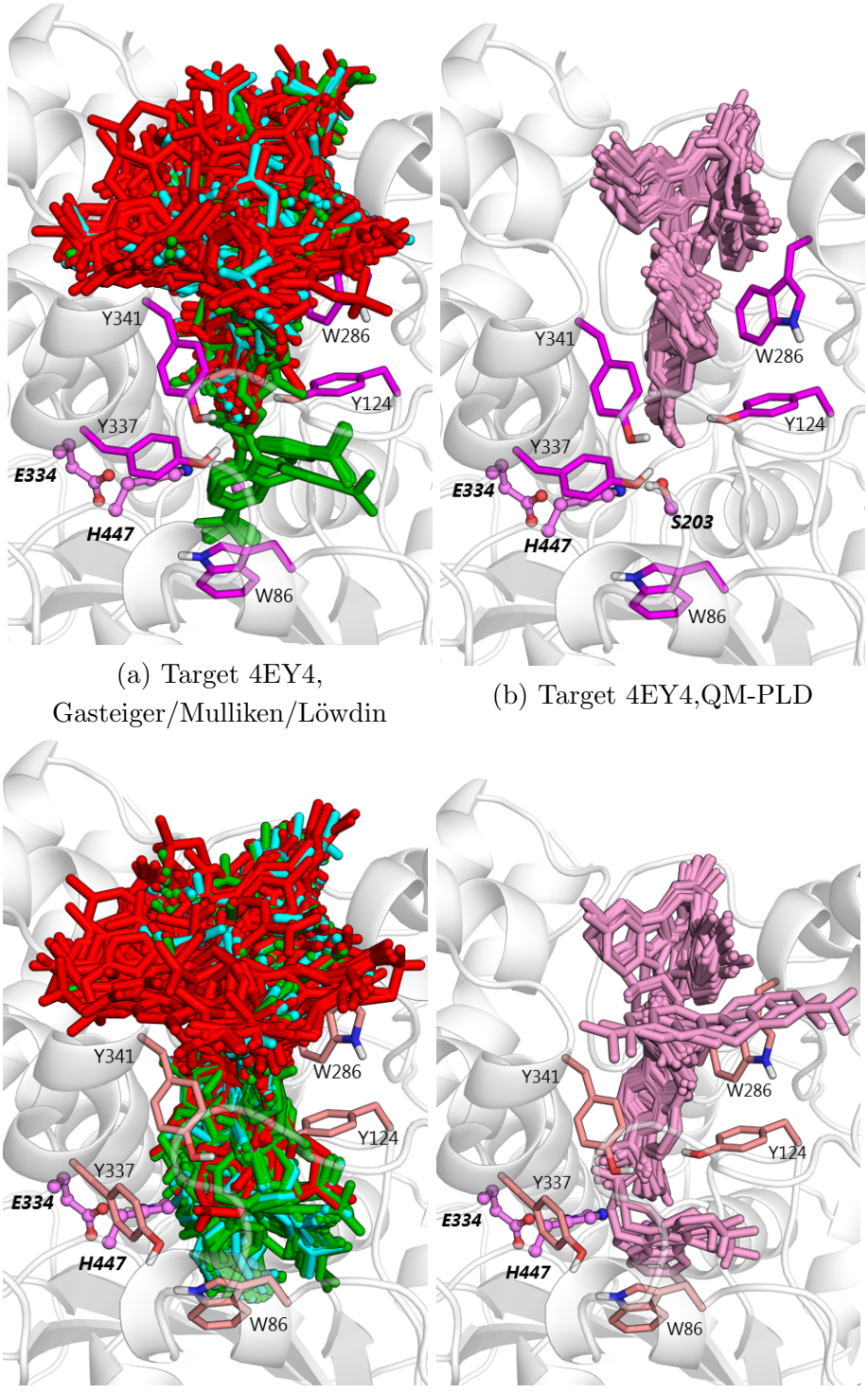
Figure 4. Overlay of X-ray structures of *apo*-state AChE (magenta); or AChE co-crystallised with (-)-Huperzine A (yellow), (-)-Galantamine (blue), Donepezil (salmon), and fasciculin-2 (orange). Principal amino acids of the gorge (Tyr341, Tyr337 and Tyr24) and ligands are shown

The Schrödinger Glide Induced Fit protocol for molecular docking of **MBC** provided positions similar to those obtained by rigid docking to 4EY7 as a target. The major difference in the compound's position was a flipped MB fragment, achieved through appreciable displacement of Phe297 and Tyr124, while conformational changes for other principal residues of the gorge were less significant (Fig. 7).

Conformations of Phe297 and Tyr124 side chains, namely, the χ_1 torsion angle for induced fit docking complexes, could be compared with conformations found in X-ray structures of human and mouse AChE and along MD trajectories for *apo*-AChE and AChE in complex with another bulky inhibitor [6]. We found side chain conformations from flexible docking different from those found in X-ray data or during MD simulations even with a bulky inhibitor in the gorge (Fig. 8). This suggests that results of the Induced Fit protocol of Glide should be compared with other available data and certain torsion angles should be fixed for redocking.

Conclusions

The results of kinetic and docking studies demonstrate the importance of choosing the right target structures. For bulky ligands, the structure of AChE co-crystallized with Donepezil (4EY7) gave the best agreement with experimental data. The use of different partial atomic charges also leads to markedly different docking results; the use of charges derived from QM calculations is advisable. Induced-fit docking should be used with caution; conformational changes of protein residues should be related to protein dynamics data (X-ray and MD) to avoid artifacts. Overall, to achieve reliable results, docking studies require the support of computationally demanding QM and MD calculations, as afforded by supercomputing facilities.



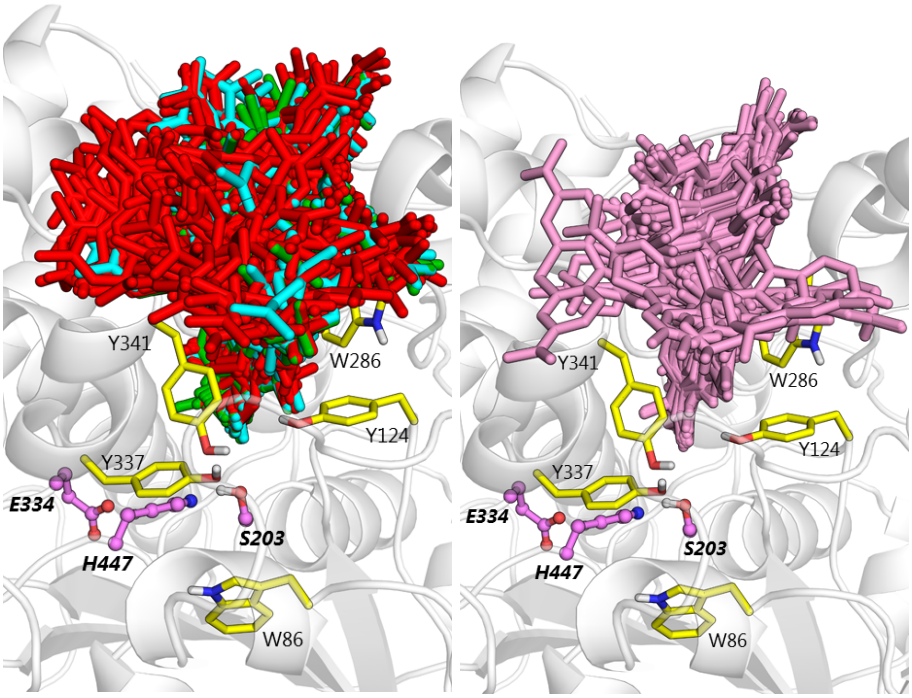
(a) Target 4EY4, Gasteiger/Mulliken/Löwdin

(b) Target 4EY4,QM-PLD

(e) Target 4EY7, Gasteiger/Mulliken/Löwdin

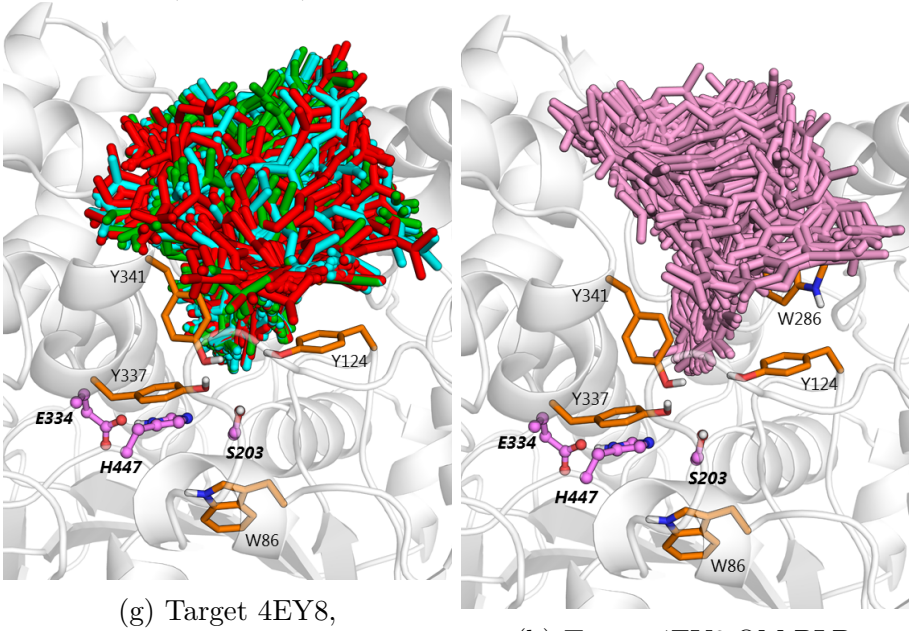
(f) Target 4EY7,QM-PLD

Figure 5. Molecular docking results of conjugate **MBC** into AChE, corresponding to experimental data. Carbon atoms of the target AChE amino acids are colored according to Fig. 4; catalytic residues are colored violet. In the left columns, cyan color shows poses obtained with partial atomic charges derived from the Gasteiger scheme, red—derived from QM calculations according to the Mulliken scheme, and green—derived from QM calculations according to the Löwdin scheme. Results of Schrödinger QM-PLD for each X-ray AChE structure are shown separately in the right column—ligand poses are colored pink



(c) Target 4EY5, Gasteiger/Mulliken/Löwdin

(d) Target 4EY5,QM-PLD



(g) Target 4EY8, Gasteiger/Mulliken/Löwdin

(h) Target 4EY8,QM-PLD

Figure 6. Molecular docking results of conjugate **MBC** into AChE, not reflecting experimental data. Coloring according to Fig. 5

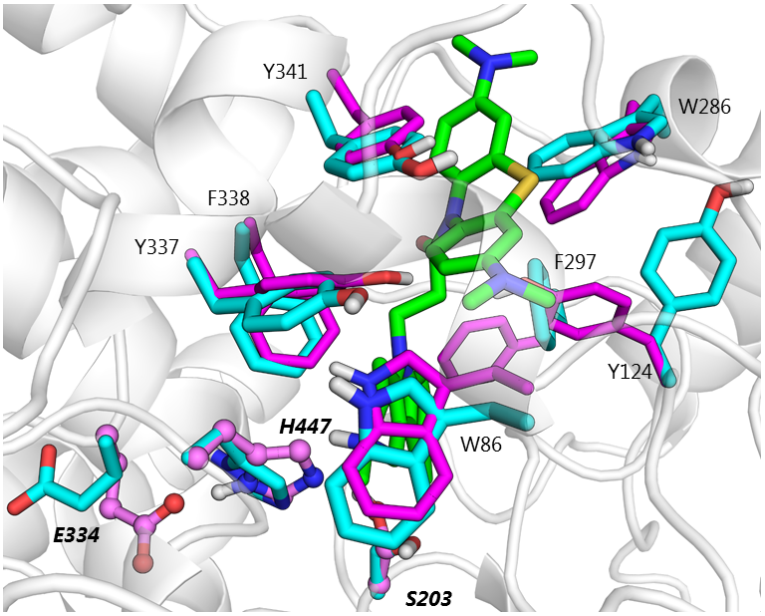
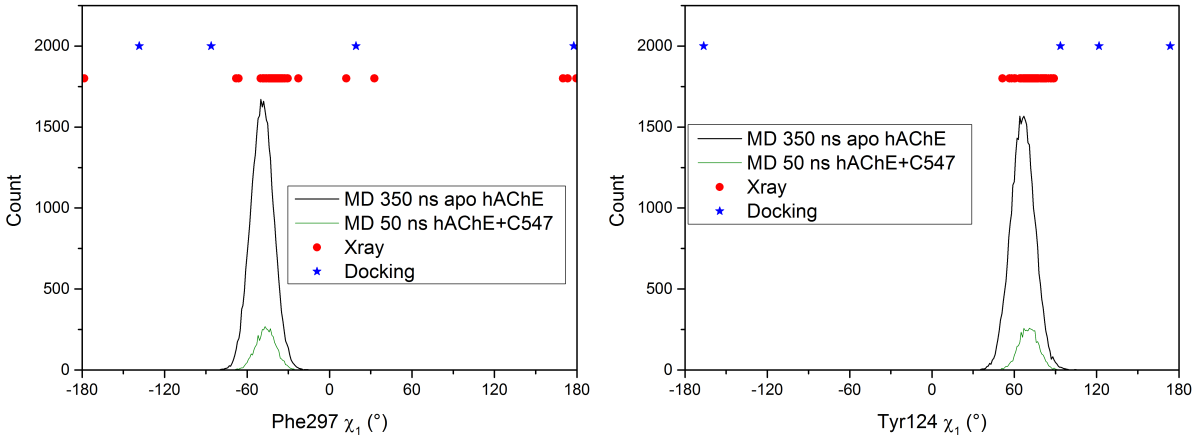


Figure 7. Protein-inhibitor complex obtained as a result of Induced Fit procedure (Schrödinger/Glide). The **MBC** ligand carbon atoms are green and protein carbon atoms are cyan. The docked complex is overlaid with the *apo*-AChE X-ray structure (carbon atoms are magenta)



(a) Phe297 (b) Tyr124

Figure 8. Distribution of values of χ_1 torsion angle over MD trajectories for *apo*-AChE (black line), total length 350 ns and 50 ns with the bulky inhibitor C-547 [6] (green line). Corresponding values for X-ray structures of human and mouse AChE available in the PDB are overlaid on the distribution plot with red points, and Induced Fit docking results are overlaid with blue stars

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