Supporting Information for

Engineering Dynamic Surface Peptide Networks on Butyrylcholinesterase$_{G117H}$ for Enhanced Organophosphosphorus Anticholinesterase Catalysis

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METHODS

Computational modeling of BChE conformational flexibility

Ideally conformations collected along the entire reaction pathway are required for accurate identification of the functionally important enzyme flexibility. For the reaction catalyzed by BChE, this would require the use of quantum mechanical modeling or hybrid quantum-classical (QM/MM) modeling. These types of calculations are very computationally expensive. Fortunately, we have shown previously that the functionally important dynamical regions can be identified by using MD simulations of a few selected states along the reaction pathway (*J. Phys. Chem. B*, 113, 16669-16680). There are several important concerns regarding the modeling of intermediate states with classical MD simulations, including how to model the different electronic states (corresponding to different states and/or intermediates) and how to sample conformations associated with the higher energy states during the reaction pathway. In the past, researchers have used the approach of using multiple MD simulations for modeling different electronic states and applying restraints on key distances (or angles) between atoms involved in the reaction mechanism. This approach of modeling intermediate states (such as transition state(s)) with classical force-fields has been successfully used for computational modeling of BChE for cocaine hydrolysis and anticocaine medication development (Refs. 38 and 39 in main manuscript).

Based on previously proposed acylation and hydrolysis mechanism (Ref. 37 in main manuscript), MD simulations were performed for 4 states: reactant (R), intermediate state (IN1), intermediate state (IN2) and product (P) states for the hydrolysis reaction (shown in Figure S1). For modeling

we used butyrylcholine (BCh), the native substrate of the enzyme, as the goal was to identify the intrinsic dynamics/flexibility of the enzyme associated with the mechanism. Standard AMBER parameters were used for serine and histidine (single or double protonation). Substrate, products and intermediate(s), colored in light blue in Figure S1, were parameterized following the protocol described in the AMBER manual. The tetrahedral intermediate structure in IN1 was created by adding a bond between hydroxyl oxygen of Ser198 and the carbon from the carbonyl group of BCh intermediate. This bond is indicated in purple color in Figure S1.

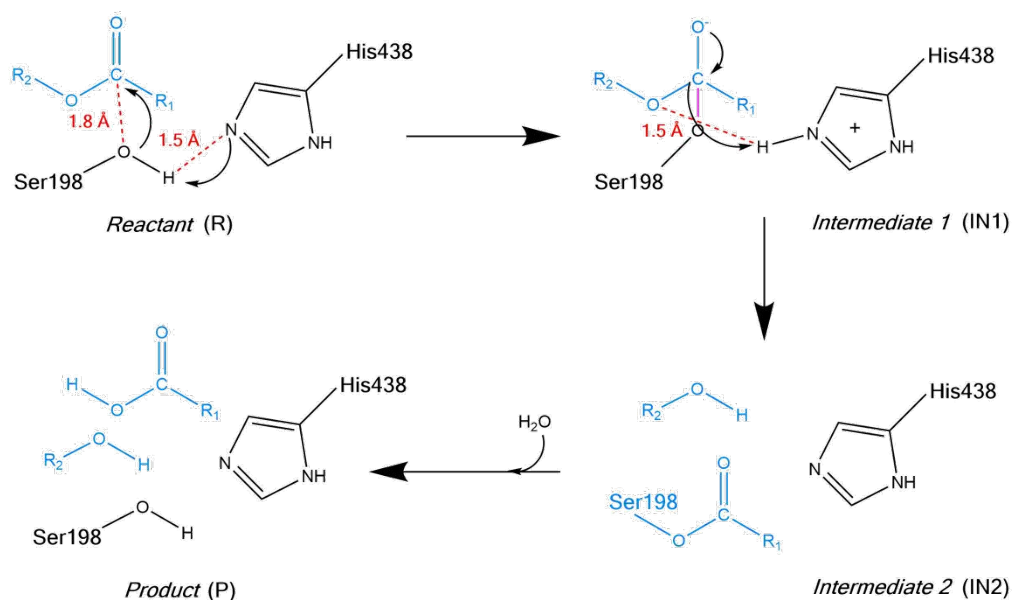

Figure S1: States during the mechanism of BChE used for simulation. The native substrate BCh was used for modeling. The distance restraints applied are shown by dashed red lines and equilibrium distances are listed in red (in Angstroms). Force constant of 2.5 kcal/mol·Å^2 were used for all restraints.

We performed 4 separate MD simulations, 100 ns in duration, for each of the R, IN1, IN2, P states. The restraints were applied are indicated by red dashed lines (equilibrium distances listed) in Figure S1, and the force constants were 2.5 kcal/mol·Å^2 in both directions (when the inter-atomic distance is less and greater than the equilibrium distances). The equilibrium distances and

force constants were selected to allow the atoms to move, thereby, allowing other enzyme residues to sample different conformations associated the reaction pathway.

Note that previously QM/MM modeling has used an intermediate and two transition states involving a water molecule, however, as our focus was on identifying the dynamics of distal residues we used a simplified mechanism with a single transition state previously described in reference 36 of the main manuscript.

In addition, a single MD simulation of 100 ns duration was also performed with paraoxon in substrate conformation. The results confirmed that flexibility of BChE was similar to the 4 state reaction pathway modeling with BCh substrate described above.

RESULTS

Movies of the molecules dynamics (MD) simulations

24 movies (in Quicktime .mov format) of the following MD simulations are provided. (Note due to the large size of these files have been deposited in the DANS large data set facility and can be accessed at: https://doi.org/10.17026/dans-xym-nu36)

1. $BChE_{G117H}$ in complex with native substrate BCh
2. $BChE_{G117H}$ in complex with substrate paraoxon
3. $BChE_{G117H}$ in complex with substrate DFP
4. $BChE_{G117H}$ in complex with substrate EthP
5. Mutant $BChE_{G117H}$ with engineered loop ENG in complex with native substrate BCh
6. Mutant $BChE_{G117H}$ with engineered loop ENG in complex with substrate paraoxon
7. Mutant $BChE_{G117H}$ with engineered loop ENG in complex with substrate DFP

8. Mutant $BChE_{G117H}$ with engineered loop ENG in complex with substrate EthP
9. Mutant $BChE_{G117H}$ with engineered loop ENA in complex with native substrate BCh
10. Mutant $BChE_{G117H}$ with engineered loop ENA in complex with substrate paraoxon
11. Mutant $BChE_{G117H}$ with engineered loop ENA in complex with substrate DFP
12. Mutant $BChE_{G117H}$ with engineered loop ENA in complex with substrate EthP
13. Mutant $BChE_{G117H}$ with engineered loop ENI in complex with native substrate BCh
14. Mutant $BChE_{G117H}$ with engineered loop ENI in complex with substrate paraoxon
15. Mutant $BChE_{G117H}$ with engineered loop ENI in complex with substrate DFP
16. Mutant $BChE_{G117H}$ with engineered loop ENI in complex with substrate EthP
17. Mutant $BChE_{G117H}$ with engineered loop ENT in complex with native substrate BCh
18. Mutant $BChE_{G117H}$ with engineered loop ENT in complex with substrate paraoxon
19. Mutant $BChE_{G117H}$ with engineered loop ENT in complex with substrate DFP
20. Mutant $BChE_{G117H}$ with engineered loop ENT in complex with substrate EthP
21. Mutant $BChE_{G117H}$ with engineered loop ENR in complex with native substrate BCh
22. Mutant $BChE_{G117H}$ with engineered loop ENR in complex with substrate paraoxon
23. Mutant $BChE_{G117H}$ with engineered loop ENR in complex with substrate DFP
24. Mutant $BChE_{G117H}$ with engineered loop ENR in complex with substrate EthP

Legend for the movies: The enzyme structure is shown as gray cartoon, the engineered loop in red, with substrate and key residues displayed as sticks. The substrate is shown green sticks, catalytic triad as cyan carbon sticks, and H117 as magenta sticks. The duration of simulations is 200 nanoseconds (ns).