SUPPROTING INFORMATION

Binding Conformation of 2-Oxoamide Inhibitors to Group IVA Cytosolic Phospholipase A_2 Determined by Molecular Docking Combined with Molecular Dynamics

Varnavas D. Mouchlis,[†] Vasiliki Michopoulou,[†] Violetta Constantinou-Kokotou, [#] Thomas Mavromoustakos,[†,*] Edward A. Dennis,[‡] George Kokotos[†,*]

[†]Laboratory of Organic Chemistry, Department of Chemistry, University of Athens, Panepistimiopolis, Athens 15771, Greece, [#]Chemical Laboratories, Agricultural University of Athens, Athens, Greece, [‡] Department of Chemistry and Biochemistry and Pharmacology, School of Medicine, University of California, San Diego, La Jolla, California 92093-0601, USA

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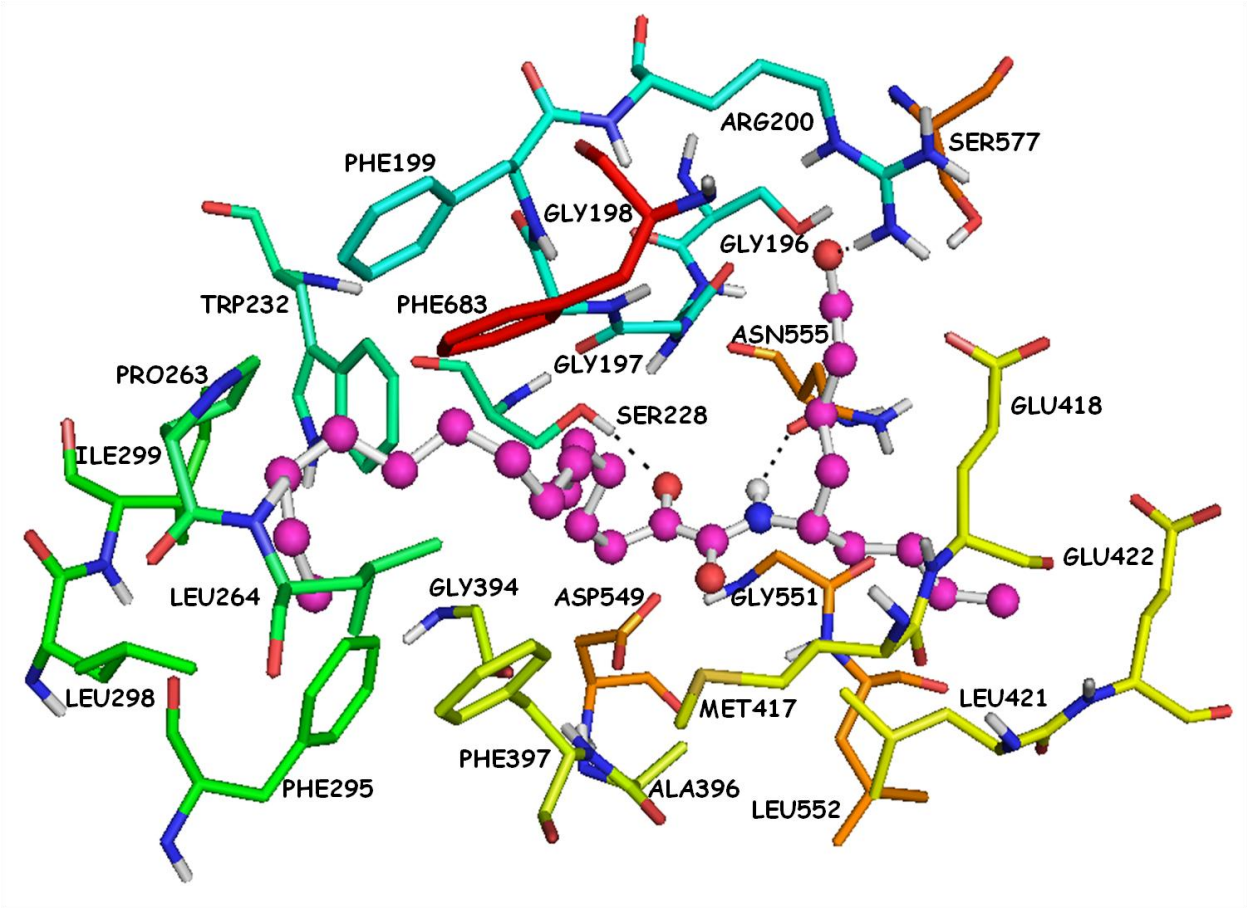
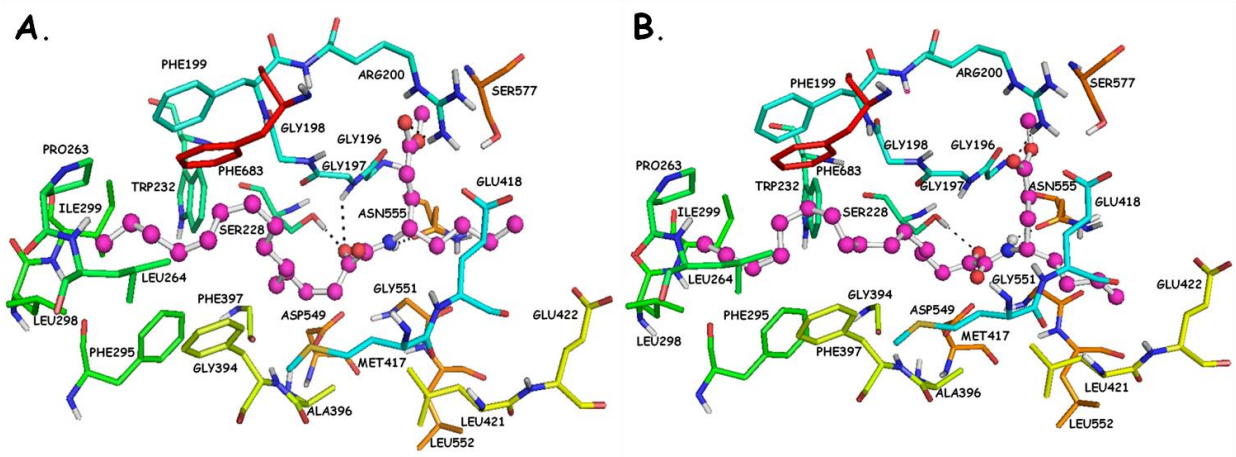
Data and discussion for the interactions of 2-oxoamide inhibitors

The AX109 inhibitor is based on a *δ*-amino acid and exhibits a slightly lower experimental inhibitory activity than AX074 (Table 1, main article). The longer length of the linker between the 2-oxoamide functionality and the carboxylic acid moiety prevents the accommodation of the particular moiety near residue Ser577 (SF 1). Thus, the carboxylic acid participates only into one hydrogen bond with Arg200 (O...H 2.00 Å, O...N 2.90 Å). The 2-oxoamide functionality interacts with residues Ser228 (O...H 2.00 Å, O...O 2.90 Å) and Asn555 (H...O 2.40 Å, N...O 3.30 Å), but the hydrogen bond with residue Gly197 is not observed. The lack of the two hydrogen bonds with residues Gly197 and Ser577 may explain the reason that AX109 possesses lower polar score than that of AX074 (Table 1, main article). On the other hand, the hydrophobic score of the two inhibitors is almost the same because the long aliphatic 2-oxoacyl chain and the short aliphatic chain have the same number of carbon atoms and interact with the enzyme active site in a similar manner.



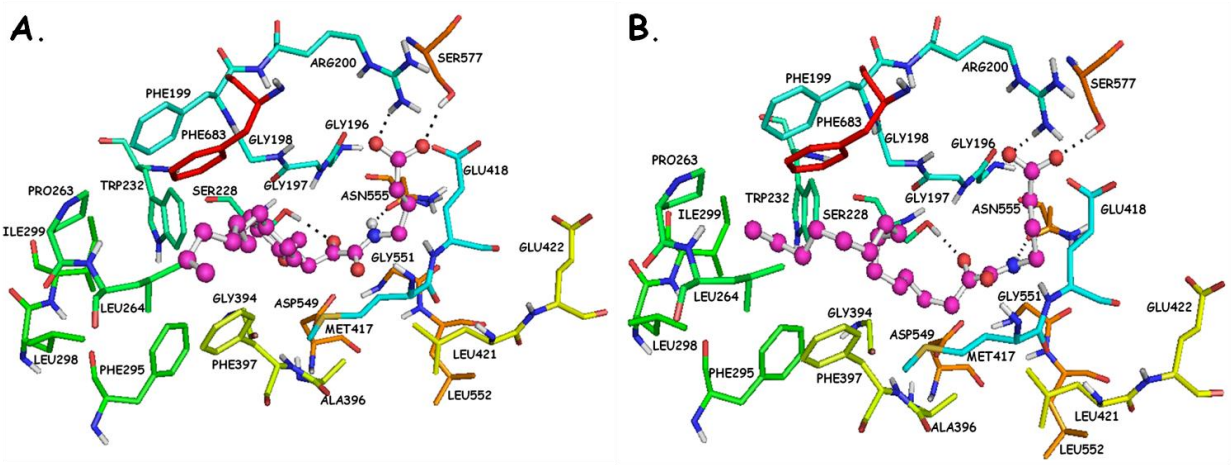
SF 1. AX109-GIVA $cPLA_2$ complex generated by the Surflex-Dock algorithm.

Inhibitors AX073 and AX063 exhibit similar inhibitory activities and similar calculated binding affinities (Table 1, main article). These compounds possess a carboxymethyl ester group in place of the free carboxylic acid moiety. The experimental inhibitory activity of these compounds against the GIVA $cPLA_2$ is about 6-fold lower than that of AX074. The carboxylic acid moiety and the carboxymethyl ester group presumably act as a mimic of the phospholipid head group and it is expected to interact with the Arg200 residue. Although the hydrogen bond with Arg200 is present, the binding of these inhibitors shows that the methyl group prevents the placement of the carboxymethyl ester oxygen atom near residue Ser577, and the hydrogen bond with this residue is not observed (SF 2A and 2B). The orientation of the carboxymethyl ester group is similar for the two inhibitors and has to be denoted that it is positioned in a completely hydrophilic region near residue Arg200. With regard to the inhibitor AX063 (SF 2B), the hydrogen bond with Gly197 is also not observed. These inhibitors possess significantly lower polar and hydrophobic scores than those of AX074.



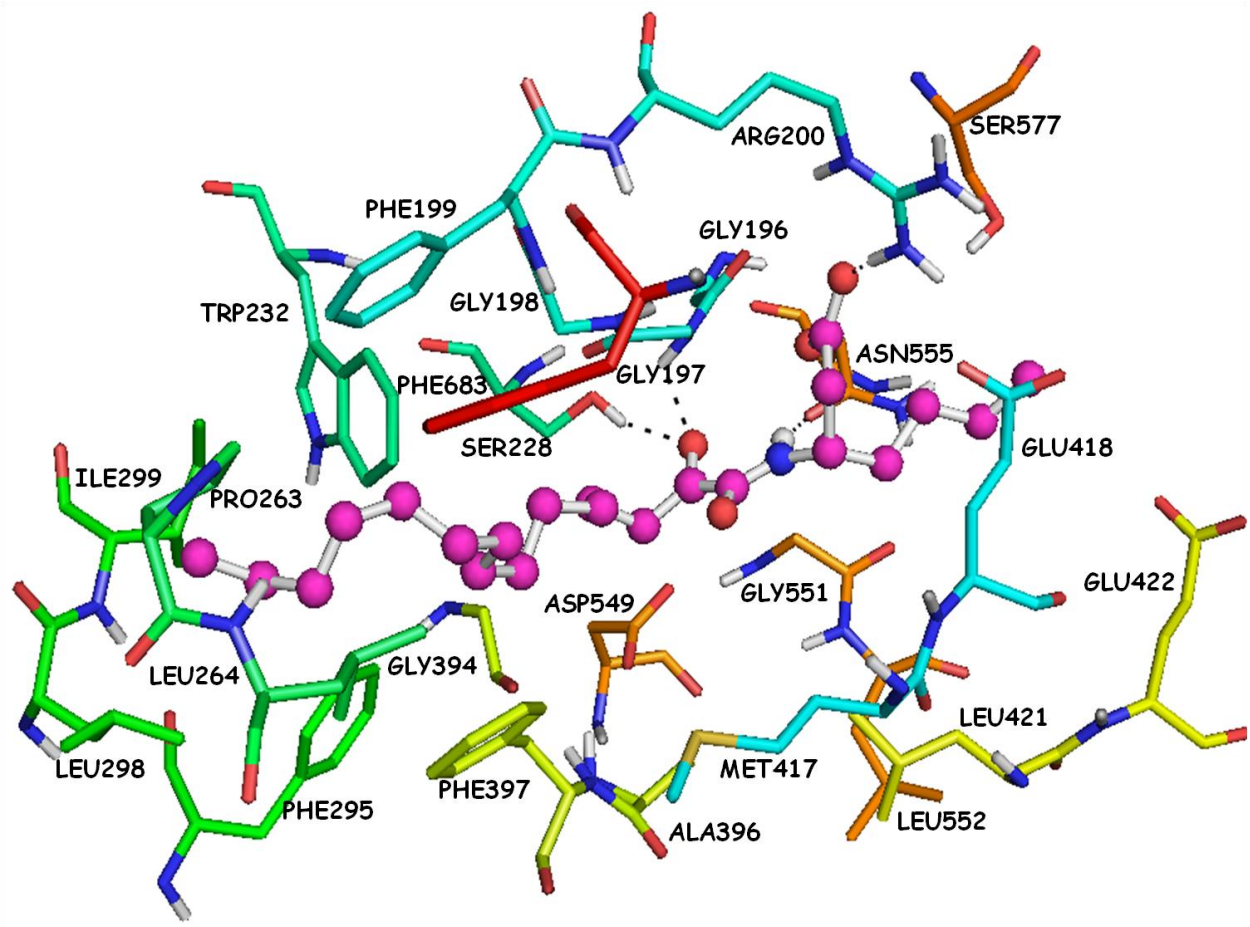
SF 2. (A) AX073-GIVA $cPLA_2$ complex generated by the Surflex-Dock algorithm; (B) AX063-GIVA $cPLA_2$ complex generated by the Surflex-Dock algorithm.

The docking complexes of GK165 and AX006 indicate the same hydrogen bonding with each other (SF 3A and 3B). The polar score is lower than that of AX074 because the hydrogen bond with Gly197 is not observed. The hydrophobic score is about 3.5 units lower than that of AX074. The significant decrease of the hydrophobic score is because of the lack of the hydrophobic interactions of the short linear aliphatic chain with the aforementioned small binding pocket.

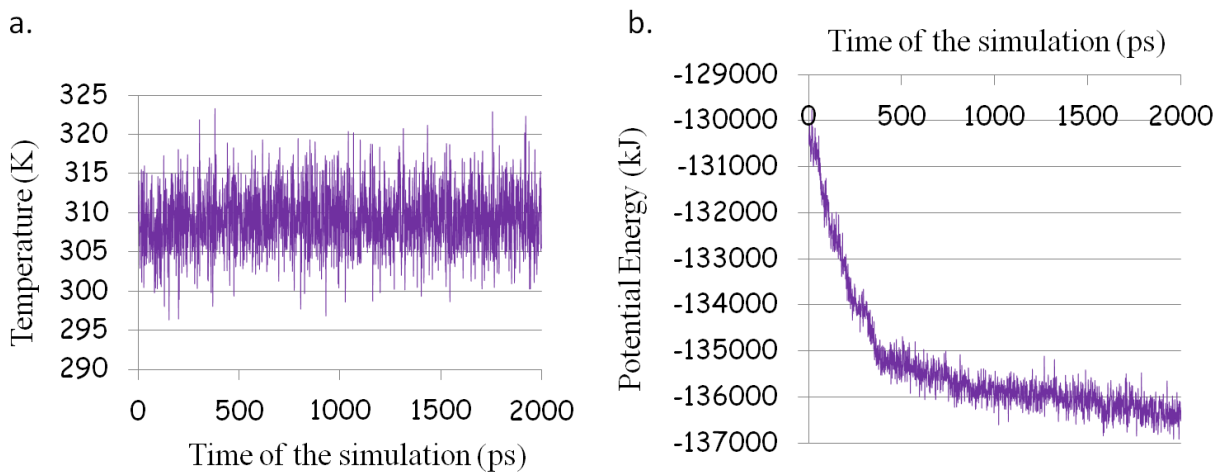


SF 3. (A) GK165-GIVA $cPLA_2$ complex generated by the Surflex-Dock algorithm; (B) AX006-GIVA $cPLA_2$ complex generated by the Surflex-Dock algorithm.

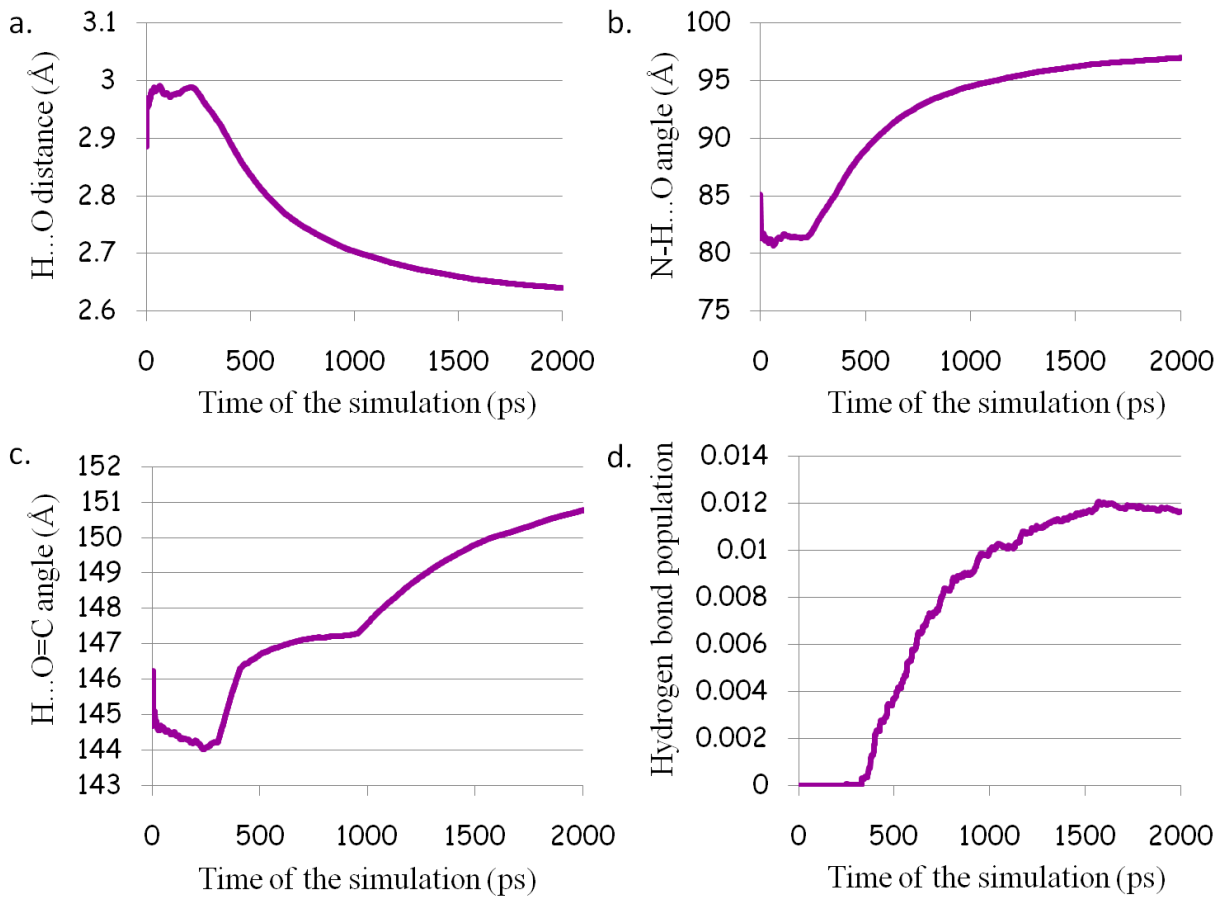
Our previous structure-activity relationship studies have indicated that decreasing the distance between the 2-oxoamide functionality and the carboxyl group results in a decrease of the inhibitory activity. Inhibitor AX021 is based on a β-amino acid and is about 9-fold less potent than AX074 (Table 1, main article). The presence of a polar group (carboxylic acid moiety or carboxymethyl ester group) in all the 2-oxoamide inhibitors constitutes a replacement of the phospholipid substrate head group and it is expected to interact with Arg200. The γ- or δ-spacing between the polar group and the 2-oxoamide functionality (three or four carbon atoms, respectively) is present in all of the potent inhibitors. Based on the docking complex of AX021, it is believed that the β-spacing (two carbon atoms) between the polar group and the 2-oxoamide functionality prevents the accommodation of the carboxylic moiety near residues Arg200 and Ser577 (SF4). Thus, the carboxylic acid moiety participates into a hydrogen bond with Arg200 (O...H 2.00 Å, O...N 2.90 Å) and the polar score of the AX021 is significantly lower than that of AX074 (Table 1, main article). The hydrophobic score is also significantly lower because β-spacing is short and the short aliphatic chain is not accommodated inside the small binding pocket and lacks interactions with it.



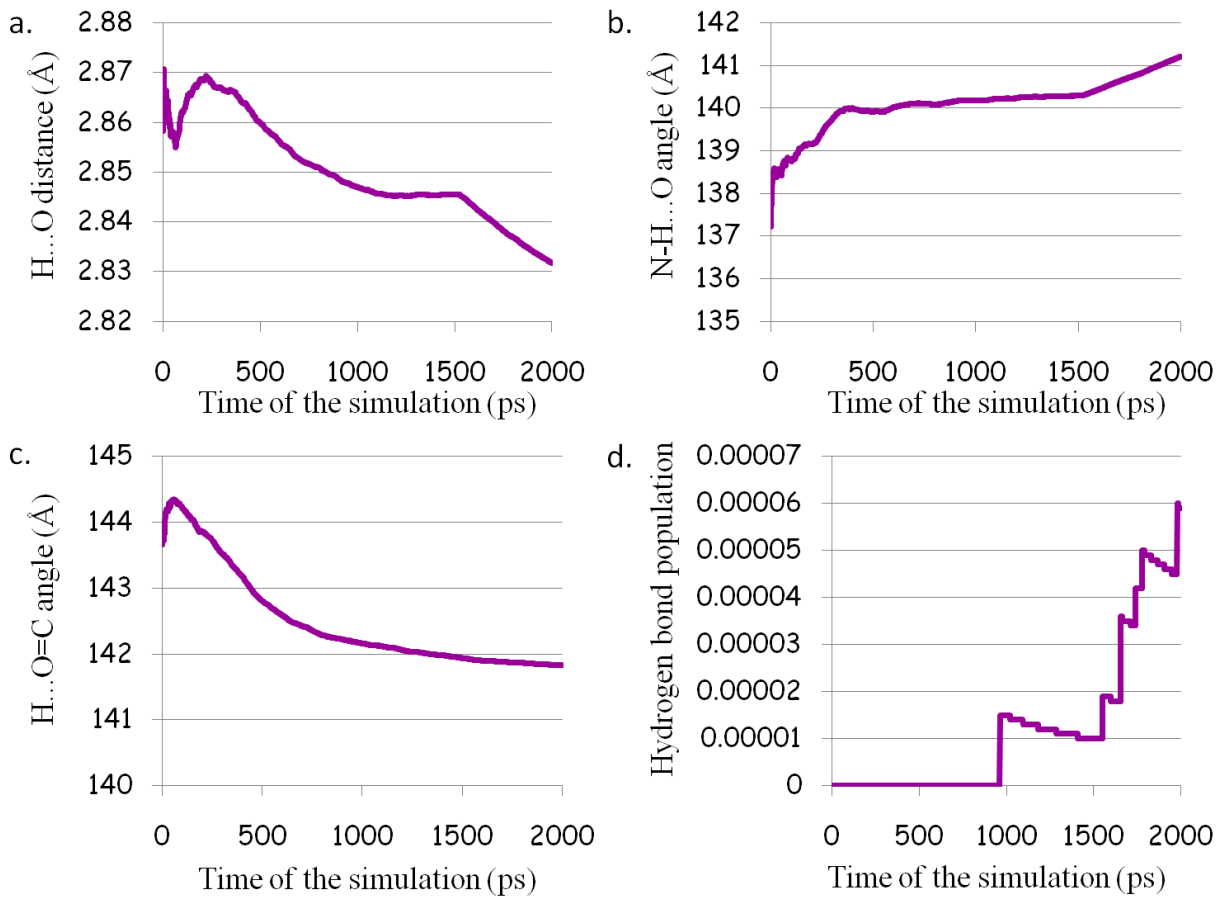
SF4. AX021-GIVA cPLA$_2$ complex generated by the Surflex-Dock algorithm.



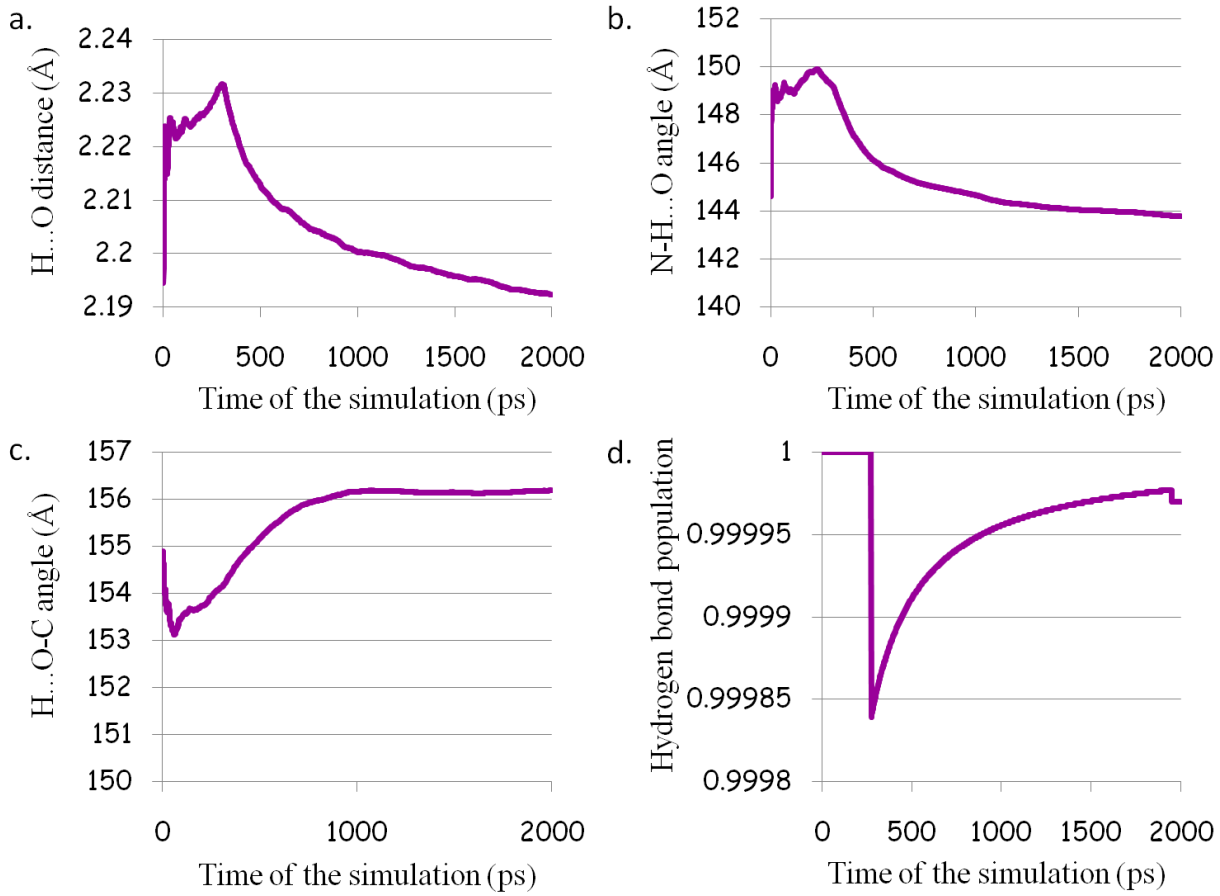
SF 5. Plots for: a. the temperature; and b. the potential energy of the system during the MD simulation.



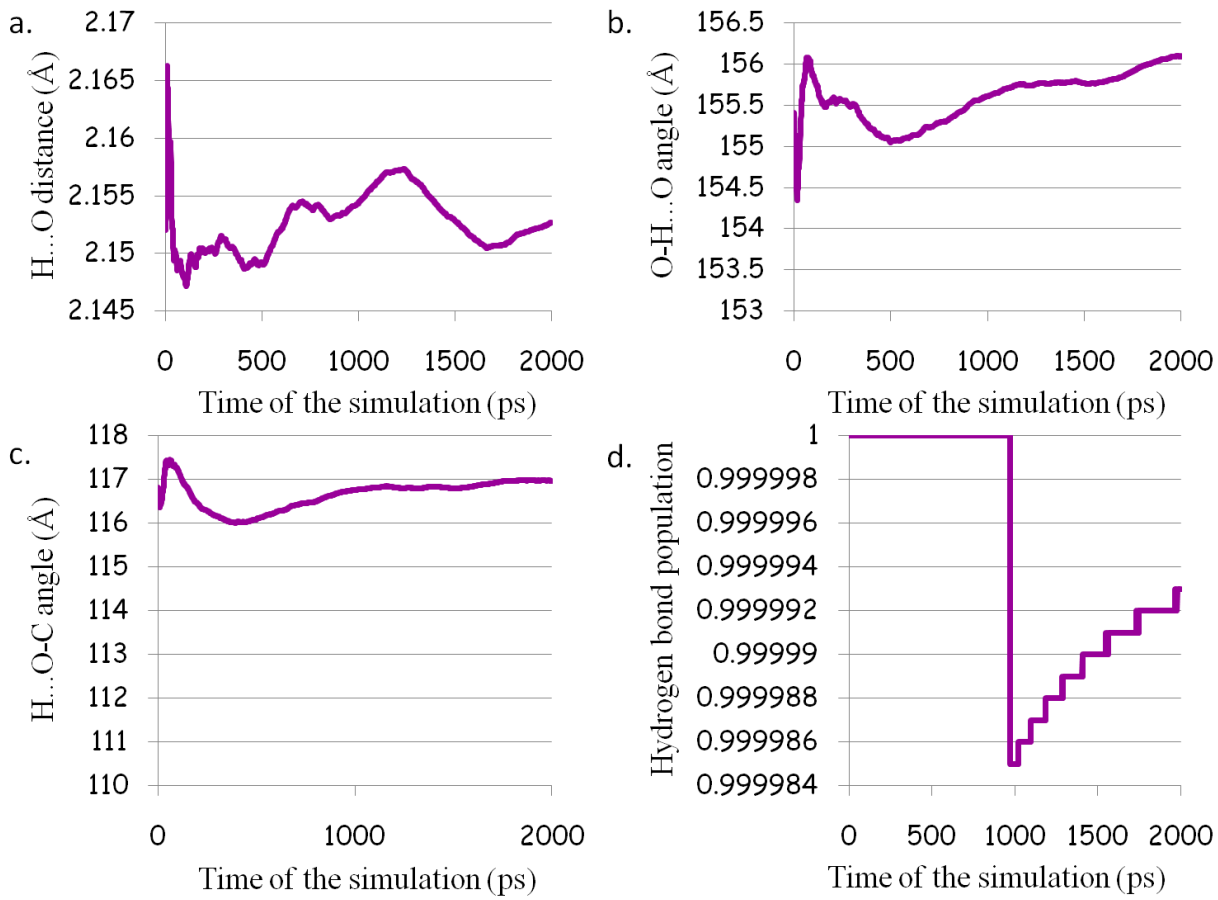
SF 6. Plots for the hydrogen bond between the 2-oxoamide functionality and Gly197: a. the H...O distance; b. the N-H...O angle; c. the H...O=C angle; and d. the population of the hydrogen bond.



SF 7. Plots for the hydrogen bond between the 2-oxoamide functionality and Asn555: a. the H...O distance; b. the N-H...O angle; c. the H...O=C angle; and d. the population of the hydrogen bond.



SF 8. Plots for the hydrogen bond between the carboxylic acid moiety and Arg200: a. the H...O distance; b. the N-H...O angle; c. the H...O-C angle; and d. the population of the hydrogen bond.



SF 9. Plots for the hydrogen bond between the carboxylic acid moiety and Ser577: a. the H...O distance; b. the O-H...O angle; c. the H...O-C angle; and d. the population of the hydrogen bond.