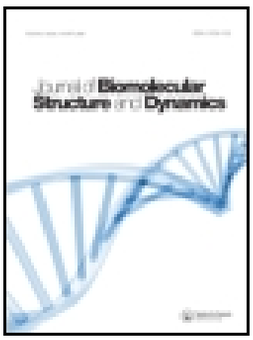

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Journal of Biomolecular Structure and Dynamics

ISSN: 0739-1102 (Print) 1538-0254 (Online) Journal homepage: http://www.tandfonline.com/loi/tbsd20

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To cite this article: Mohd. Shahbaaz, Suvardhan Kanchi, Myalowenkosi Sabela & Krishna Bisetty (2017): Structural basis of pesticide detection by enzymatic biosensing: A molecular docking and MD simulation study, Journal of Biomolecular Structure and Dynamics, DOI: 10.1080/07391102.2017.1323673

To link to this article: http://dx.doi.org/10.1080/07391102.2017.1323673

Publisher: Taylor & Francis
Journal: *Journal of Biomolecular Structure and Dynamics*
DOI: http://dx.doi.org/10.1080/07391102.2017.1323673

Structural basis of pesticide detection by enzymatic biosensing: A molecular docking and MD simulation study

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Abstract

Designing of rapid, facile, selective and cost-effective biosensor technology is a growing area for the detection of various classes of pesticides. The biosensor with these features can be achieved only through the various bio-components using different transducers. This study, therefore, focuses on the usage of molecular docking, specificity tendencies, and capabilities of proteins for the detection of pesticides. Accordingly, the four transducers, acetylcholinesterase (ACH), cytochromes P450 (CYP), glutathione S-transferase (GST) and protein kinase C (PKC) were selected based on their applications including neurotransmitter, metabolism, detoxification enzyme and protein phosphorylation. Then after molecular docking of the pesticides, fenobucarb, dichlorodiphenyltrichloroethane (DDT) and parathion onto each enzyme, the conformational behaviour of the most stable complexes was further analysed using 50 ns Molecular Dynamics (MD) simulations carried out under explicit water conditions. In the case of protein kinase C (PKC) and cytochrome P450 3A4 enzyme (CYP), the fenobucarb complex showed the most suitable combination of free energy of binding and inhibition constant -4.42 kcal/mol (573.73 μM) and -5.1 kcal/mol (183.49 μM) respectively. Parathion dominated for acetylcholinesterase (ACH) with -4.57 kcal/mol (448.09 μM) and lastly dichlorodiphenyltrichloroethane for glutathione S-transferase (GST), -5.43 kcal/mol (103.88 μM). The RMSD variations were critical for understanding the impact of pesticides as they distinctively influence the energetic attributes of the proteins. Overall, the outcomes from the extensive analysis provide an insight into the structural features of the proteins studied, thereby highlighting their potential use as a substrate in biorecognition sensing of pesticide compounds.

Keywords: Pesticides; Molecular Docking; Molecular Dynamics; Biosensors

1. Introduction

"The most intelligent species on earth poisons its food before eating it." These startling words by Rishi Miranhshah (Miranhshah 2011) reveal the stark reality of the destruction that we face today due to the relentless and injudicious use of pesticides. Pesticides are the chemical agents which prevent the proliferation of harmful pests and also interfere with the normal biological processes of other living organisms (Skládal et al. 1997; Amaral 2014). These are essential to the modern agriculture and extensively used to control the pests, reduce the growth of weeds, and boost the agricultural productivity, thus increases crop yields and reduce post-harvest losses (de Liñán Carral 2015; Fang et al. 2009; Odukkathil and Vasudevan 2013; Meng et al. 2013). A large amount of pesticides reaches the soil, air, water, food and other various systems through a drift that takes the pesticides to places downwind the point of application. This may prove to be detrimental to the various life forms due to their mutagenic, carcinogenic and tumorogenic (Caspers 1981; De Flora et al. 1993; Kuroda et al.1992; Rehana et al.1995).

The majority of the pesticides are neurotoxic compounds and irreversibly inhibits the enzyme acetylcholinesterase, an essential enzyme for the functioning of central nervous system in humans and insects. This inhibition leads to the accumulation of neurotransmitter acetylcholine in nerves which interferes with muscular activities and the functioning of vital organs, hence producing symptoms of serious infections which may even lead to death (Donarski et al. 1989; Chapalamadugu and Chaudhry 1992). Other target sites of various pesticides are: human erythrocytes (Suwalsky et al. 2005), reproduction and development system (Farag et al. 2010), endocrine system (Rawlings et al. 1998), liver, central nervous system and immune system (Gold et al. 1991; Fendick et al. 1990), etc. Thus, the analysis of pesticide residues is an important

concern due to their bioaccumulation effect, high toxicity and their long-term damage risk to both the environment and lives even though their concentration is very low.

Detection of pesticides at the levels established by the Environmental Protection Agency (EPA) remains a challenge. Several bio-recognition methods that have been reported for the detection of pesticides include enzymatic, whole cell, immunochemical, and DNA biosensors etc. The transducers selected for this study include acetylcholinesterase, cytochrome P450 3A4 enzyme, glutathione S-15 transferase and protein kinase C due to their established biological roles as neurotransmitter, metabolism, detoxification enzyme and protein phosphorylation (Sassolas, et al. 2012). Specifically, in the fabrication of biosensors for pesticides, mainly enzyme are used due to the pesticide inhibition capability on the enzyme by various neurotoxins (Mionetto, et al. 1994; Deo et al. 2005) and are very sensitive (able to detect 10^{-10} M), but they offer poor specificity (Simonian et al.1997).

The Acetylcholinesterase (ACH) is a neurotransmitter and easily undergoes hydrolysis to form choline and acetic acid. It is found at neuromuscular junctions and cholinergic synapses in the central nervous system, where its activity/concentration terminates synaptic transmission (Dhull et al. 2013). Cytochrome P450 3A4 enzyme (CYP) is an oxidase enzyme which enhances the different chemical reactions including heteroatom oxidation, epoxidation, and hydroxylation. CYP enzyme is mainly involved in the metabolism of several drugs and xenobiotics for bio-activation (Savary et al. 2014). Glutathione S-transferase (GST) is a detoxification enzyme, catalyzing glutathione (GSH) conjugation reactions. Protein phosphorylation process is the predominant molecular mechanism that can be achieved with protein kinase C (PKC) (Stanyon and Bernard 1999).

The scheming of simplistic, selective and economical biosensor technology is a budding area for the detection of various classes of pesticides. The biosensor with these features can be achieved only with the various bio-components with different transducers (Verma and Dhillon 2003). The chromatographic methods coupled to selective detectors have been traditionally used for the analysis of pesticides due to their sensitivity, reliability, and efficiency. Nevertheless, chromatographic techniques are time-consuming and laborious and require expensive equipments and highly-trained technicians (Jaffrezic-Renault 2001; Velasco-Garcia and Mottram 2003). Over the past decade, considerable attention has been given to the development of biosensors for the detection of pesticides as a promising alternative. Some electrochemical transducers have been used in biosensors for pesticides detection due to their high sensitivity (Andreescu and Marty 2006; Luque de Castro and Herrera 2003; Grieshaber et al. 2008). Additionally, their low cost, simple design and small size make them excellent candidates for the development of portable biosensors (Freire et al. 2003; Thévenot et al. 2001; Ronkainen et al. 2010; Yogeswaran and Chen 2008).

This study involves molecular docking in combination with molecular dynamics simulations to provide significant structural insights which can lead to the effective identification of potential biosensing enzymes for the electrochemical detection of pesticides. By keeping the future prospects in mind, the information generated through the in-silico analysis will be used to design the electrochemical biosensors for pesticides (Sabela et al. 2016; Bathinapatla et al. 2016). Therefore, the approach used in this work took advantage of the characteristic feature of ACE, CYP, GST and PKC molecules probing its ability as biosensors for the detection of a different class of pesticides. Literature studies revealed quite a few reports on the ACE performance as a biosensor for the detection of pesticides (Krasinski et al. 2005; Manetsch et al. 2004; Lin et al.

2005; Schulze et al. 2005; Montesinos et al. 2001). However, in the case of biomolecules such as CYP, GST, and PKC, there are no reports available regarding the structural basis for the detection of pesticide molecules in the available literature. Consequently, this study can provide an insight into the structural features of the proteins studied, thereby highlighting their potential use as a substrate in biosensing of pesticide compounds.

2. Material and Method

2.1. Molecular modelling

The structures of acetylcholinesterase (ACE, PDB ID – 5HF9), cytochrome P450 3A4 enzyme (CYP, PDB ID – 4D7D), glutathione S-transferase (GST, PDB ID – 4MPF), and protein kinase C (PKC, PDB ID – 5F9E) were remodelled to remove the discontinuities in the structural coordinates deposited in the biological database. The protein structures were predicted by satisfying the spatial restraints using MODELLER module of the Discovery Studio 2016 (DS, http://accelrys.com/products/collaborative-science/biovia-discovery-studio/). The generated 3-D models were minimized using the optimization and side chain refinement modules of DS. The structure of Fenobucarb (carbamate), DDT (organochlorine) and Parathion (organophosphate) pesticides were constructed by means of drawing utilities present in the DS and the geometries of the resulted structures were optimized by using the Density Functional Theory (DFT) method present in Dmol3 module of the DS.

2.2. Molecular docking and interaction assessment

The refined structures of proteins, as well as pesticides, were docked by utilizing the AutoDock 4 (Morris et al. 2009) package. AutoDock 4 performs the prediction of the bound conformation on the basis of free energy based on an empirical force field coupled with the Lamarckian Genetic Algorithm (Morris et al. 2009). The grid box of dimensions 50 x 70 x 60 Å along the

XYZ directions with grid spacing of 0.375 Å were created using the AutoGrid module. In order to increase the efficiency, the parameters associated with the Lamarckian genetic algorithm were set to the maximum efficiency value such as a number of individuals in population was set to 250 while the maximum number of energy evaluations was set to a "longer" interval. As a result, around 100 docked conformations were obtained which were grouped according to the RMSD tolerance of 2.0 Å. The efficiency of interactions was assessed in the form of free energy of binding which was calculated on the basis of the following equation (Morris et al. 2009):

$$\Delta G = \Delta G_{vdW} \sum_{i,j} \left(\frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^{6}} \right) + \Delta G_{Hbond} \sum_{i,j} E(t) \left(\frac{C_{ij}}{r_{ij}^{12}} - \frac{D_{ij}}{r_{ij}^{10}} + E_{Hbond} \right) + \Delta G_{elec} \sum_{i,j} \frac{q_i q_j}{(r_{ij}) r_{ij}} + \Delta G_{tor} N_{tor} + \Delta G_{sol} \sum_{i,j} S_i V_j e^{\left(-r_{ij}^2 / 2 \; ^2\right)}$$

Where, ΔG_{vdW} represents the van der Waals, ΔG_{Hbond} signifies Hydrogen Bonding while ΔG_{elec} and ΔG_{sol} denotes the free energy of Electrostatics and solvation respectively.

The generated conformations were re-scored on the basis of the scoring function present in DrugScoreX server (Neudert and Klebe 2011) and the conformation with the highest score was selected for the Molecular Dynamics (MD) simulations. In order to validate the generated outcomes the CDOCKER module was used, which is a CHARMM (Vanommeslaeghe et al. 2010) force field based docking algorithm implemented in DS.

Furthermore, the pharmacophore modelling was performed in order to identify the essential features between receptors and pesticides which are responsible for the inhibition activity of the studied compounds. The features were computed using the "Automatic Pharmacophore generation" as well as "Receptor-Ligand Pharmacophore generation" modules of DS. The pharmacophore features such as hydrophobic group (H), aromatic ring (R), hydrogen bond donor (D), hydrogen bond acceptor (A), negatively ionizable group (N), and positively ionizable group (P) were calculated. The generated pharmacophore models were analyzed and compared to the binding priorities of the ligands with the studied proteins. The pharmacophore based feature extraction was used to assess the moieties responsible for the inhibition of proteins, as it is considered as the significant tool to filter the significant therapeutic molecules in the process of drug design (Yao et al. 2017). The selectivity score generated by the DS during the pharmacophore modelling was to infer the binding affinity.

2.3. MD simulations

The generated docked complexes were subjected to MD simulations using GROMACS (Pronk et al. 2013) (version 5.1.2, installed on the Center for High Performance Computing (CHPC), Cape Town, South Africa). The topologies of the pesticide structures were generated by means of GROMOS96 53a6 force field (Oostenbrink et al. 2004). Due to the unavailability of suitable force field parameters for drug-like molecules in the GROMACS package, the PRODRG server (Schuttelkopf and van Aalten 2004) was used for the generation of piperine in topologies and coordinate files. The partial charges were corrected using DFT method of Gaussian which utilized the B3LYP 6-31G (d,p) basis set and the CHELPG program (Frisch et al. 2009). After the successful topology generation, all docked complexes were immersed in SPC/E water model (Zielkiewicz 2005) and the systems were neutralized by adding the counter ions. The neutralized

systems were energetically minimized by steepest descent and conjugate gradient algorithms with a convergence criterion of 0.005 kcal/mol. In order to increase the reliability of the MD simulations, the restraints were applied to the structure of the pesticides before the equilibration phase.

The equilibration phase was carried out separately in NVT (constant volume) as well as NPT (constant pressure) ensemble conditions, each with a 100 ps time scale. The temperature of the system was maintained at 300 K using Berendsen weak coupling method in both ensemble conditions along with pressure which was maintained at 1 bar by utilizing Parrinello-Rahman barostat in constant pressure ensemble. The final MD simulations were produced using LINCS algorithm for 50 ns time scale. The trajectory files were utilized for the analyses of each complex behaviour in the explicit water environment. The variations in the distances, H-bonds, RMSD (Root Mean Square Deviations), and Radius of Gyration (Rg) between the protein-pesticide complexes were analysed. Further analysis involved the calculation of the binding energy components using Molecular mechanics Poisson–Boltzmann surface area (MM-PBSA) protocols implemented in the *g_mmpbsa* package (Kumari, Kumar, and Lynn 2014) which provide deeper insights into the interaction mechanisms of the proteins and the pesticide molecules. The algorithm of the binding energy calculation *g_mmpbsa* package (Kumari, Kumar, and Lynn 2014) is based on the following equations:

$$\Delta G_{binding} = G_{complex} - (G_{protein} + G_{ligand})$$

Here, $G_{complex}$ signifies the total free energy of the binding complex and $G_{protein}$ and G_{ligand} are the measure of the total free energies of the isolated proteins as well as the ligands respectively. For

an individual entity, the general equation for the calculation of the free energy is given by the following equation:

$$G_x = \langle EMM \rangle - TS + \langle Gsolvation \rangle$$

Where, x represents either protein-ligand complex or individual components separately. ⟨ EMM ⟩ calculates the average molecular mechanic's potential energy in a vacuum and TS compute the entropic contribution to the free energy, in which T and S signify the temperature and entropy, respectively as well as the component $\langle G_{solvation} \rangle$ symbolize free energy of solvation.

3. Results

The pesticides toxicity in the untargeted organisms is considered as one of the biggest causes of the water pollution, arises mainly due to extensive agricultural and industrial activities (Verma and Bhardwaj 2015). The analytical methods used for the detection of the pesticides in the water resources are expensive, time-consuming and requires highly trained technicians (Verma and Bhardwaj 2015). Alternatively, the biosensors which involved the use of biomolecules as the key structural component to detect the neurotoxins and other pesticides with higher accuracies (Verma and Bhardwaj 2015). Therefore, the current study was undertaken to identify the possible enzymes which may function as a biosensor (Verma and Bhardwaj 2015).

3.1. Three-dimensional (3-D) structure refinements

In the primary stage, the concepts of the molecular modelling were used for the reconstruction of the protein 3-D structures. The Blastp and MODELLER modules present in the DS were used for the construction of homology models by satisfying the spatial restraints. The structures were further optimized and their stereochemical accuracy was assessed using Ramachandran plots. The modelled structure of acetylcholinesterase showed 99.5 % of the residues in the allowed

region of the Ramachandran plots, while cytochrome P450 showed 99.7 % in the allowed region. Similarly, the predicted models of glutathione S-transferase and protein kinase C showed 99.5% and 99.3% of their residues in the allowed region of the Ramachandran plots respectively. These observations indicated the reliability of the 3-D models.

3.2. Interaction studies

Subsequently, the molecular docking studies between the enzymes and the pesticide molecules were performed (Naz, Shahbaaz, Khan, et al. 2015; Naz, Shahbaaz, Bisetty, et al. 2015). All three categories of the pesticides were docked in the active site of the selected proteins using Autodock package. In the case of acetylcholinesterase, the parathion showed highest combined scores of inhibition constant of 448.09 μM and free energy binding which is calculated to be around -4.57 kcal/mol (**Table 1**). The interaction studies with fenobucarb showed higher values of inhibition constant as compared to the DDT, while higher binding energy was obtained with the latter pesticide (**Table 1**). Furthermore, the pharmacophore analysis showed the presence of 24 features, while only two were observed to be common with the receptor ACH protein (**Figure S2**).

Similarly, the fenobucarb showed the most suitable combination of generated parameters and selected for further analysis. The free energy of binding and inhibition constant for CYP-fenobucarb complex was calculated to be around -5.1 kcal/mol and 183.49 μM respectively (**Table 2**). In order to obtain a deeper insight, the pharmacophore analysis was performed, which showed that two features of fenobucarb may contribute to the inhibition, among nine predicted pharmacophore features (**Figure S5**). Likewise, the complex with DDT, the most widely used insecticide, was used for further analyses, which showed the highest inhibition constant as compared to the other studied proteins (**Table 3**) and three pharmacophore features involved in

the inhibition were also calculated (**Figure S8**). The parathion and fenobucarb complexes also showed the highest inhibition constant in contrast to the other proteins.

Moreover, the fenobucarb, DDT, and parathion were docked in the active site of PKC (**Figures 5** and **S10**). The highest score for the inhibition constant was obtained for PKC-Fenobucarb complex (**Table 4**), in addition to the four common pharmacophore features observed for studied receptor and pesticide (**Figure S11**). As the minimum features were detected in this case, the selectivity score of 5.6124 was predicted by DS indicating the specificity of PKC.

After selection of the protein- pesticide complexes with highest calculated affinities, they were subjected to 50 ns MD simulations in explicit water conditions and correlation of these findings with information available in the literature are discussed in the subsequent section.

4. Discussions

The recent developments in the detection of pesticides and other harmful chemicals using highly selective biomolecular based sensors form the basis of the current study, aimed at the classification of proteins, which can serve as potential biosensors (Guan et al. 2017; Jokar et al. 2017; Kumari et al. 2016; de Almeida et al. 2016; Mohammadi and Ghayeb 2016; Sharma, et al. 2016; Jokar et al. 2016). Biosensors can provide an alternative to the current techniques such as HPLC, GC-MC etc. for the detection of the pesticides mainly concern with food safety (Jokar et al. 2016). Previous studies showed that computational approaches can be used to screen and rank an array of the receptor structures and predict the interaction outcomes with a deeper understanding, a more cost effective alternative than laboratory experiments (Jokar et al. 2017). For this purpose computational techniques were used for the design of a potential biosensor and the outcomes of this study are discussed in this manuscript.

4.1. Caliber of Acetylcholinesterase (ACH) as a Future Biosensor: Interaction studies of ACH with Fenobucarb, DDT, and Parathion

The ACE was characterized to be a serine hydrolase and involved in the hydrolysis of acetylcholine, a neurotransmitter involved in the conducting of nerve impulses in a wide range of animals including humans (Fukuto 1990). The pesticide inhibition of the ACH takes place in an irreversible manner by the phosphorylation of serine hydroxyl, which increases the stability of the enzyme (Pundir and Chauhan 2012). The ACH has widely used in the formation of an inhibition based biosensor for the detection of harmful pesticides (Pundir and Chauhan 2012; de Almeida et al. 2016). Therefore, the ACH was selected in addition to other proteins as a possible candidate which can be used for designing an enzyme based biosensor (de Almeida et al. 2016; Mohammadi and Ghayeb 2016). All the three categories of the pesticides were docked in the active site of ACH using Autodock package. The parathion with highest binding scores was found to interact with Tyr121, Trp283, Ser290, Val291, and Tyr338 in the active site of ACH (**Figure 1A and B**). The inhibition of the ACH is two stepped process and took place by the phosphorylation of Serine hydroxyl group (Fukuto 1990), therefore, in the binding, the Ser290 was observed. Similar docked residues were observed in the case of fenobucarb and DDT (**Figure S1**).

The docked complex of ACH-parathion was subjected to 50 ns MD simulations. The partial charges were corrected by using the DFT based analysis (**Figure S3**). The energies associated with the binding of parathion with ACH were calculated using MM–PBSA method, which showed that the total free energy of binding was fluctuating in the range of -500 to -1000 kJ/mol (**Figure 1C)**. The vdW energies contributing towards the total energies to a greater extent as compared to electrostatic energies, therefore, the vdW interactions were observed in the

dominant position in the complex of ACH-parathion (Fang et al. 2014). The distance between the ACH and parathion was calculated using GROMACS utilities. The initial distance was observed to be around 0.4 nm and gradually decreases up to 10 ns time period, then continue fluctuating between the 0.25-0.3 nm but showed sudden increase around 50 ns (**Figure 2A**). The hydrogen bond estimation between the receptor and pesticide may reach up to 8 during the course of simulations (**Figure 2B**). The continuous fluctuations were observed in the RMSD and Rg plots which indicated that the ACH is energetically active (**Figure 2C and D**). The binding of parathion shifted the peaks of the curve indicated that the conformation of the ACH changed during the inhibition.

4.2. Caliber of Cytochrome P450 3A4 enzyme (CYP) as a Future Biosensor: Interaction studies of CYP with Fenobucarb, DDT, and Parathion

The human hepatic CYP catalyzes the bio-transformation of pesticides and resulted in the detoxification of these compounds (Abass et al. 2012). But the metabolism of organophosphorus and carbamate insecticides leads to the formation of higher toxic metabolites (Poet et al. 2003; Usmani, Hodgson, and Rose 2004). A variety of pesticides induces expression of the *CYP* gene (de Sousa et al. 1997), therefore, this protein can use as a candidate in the development of the pesticide-specific biosensor. Accordingly, the molecular docking studies were performed which validated the information available in the literature. The fenobucarb showed highest binding affinities with residues Phe57, Asp76, Arg106, Phe215, Met371, Arg372, and Glu374 of CYP (**Figure 3**). While for DDT and parathion, a lesser number of residues were detected in the interacting pocket of CYP (**Figure S4**).

Furthermore, the CYP-fenobucarb complex was subjected to 50 ns MD simulations in explicit solvent conditions with pesticide topology was generated using DFT-based methods

(**Figure S6**). On the basis of MM-PBSA analyses; the interaction energies, the total energy of binding was fluctuating around -200 kJ/mol, with the vdW and electrostatic energies, showed continuous fluctuation with the electrostatic energy was contributing towards the total energies to a greater extent as compared to around vdW (**Figure 3C**). Similarly, the dynamics of bonding between the CYP and fenobucarb was inferred from the calculated distance, which was fluctuating between 0.25 – 0.35 nm (**Figure 4A**). While the lesser number of hydrogen bonds were calculated in comparison with ACH-parathion complex (**Figure 4B**). The Rg plots signified the stable compactness of the complex as the variation in the values was observed around 2.25 nm (**Figure 4C**). But the RMSD variations were significantly affected by the binding of fenobucarb with CYP as the values started fluctuating at 0.6 nm from 0.4 nm, which indicated that the increased energetic attributes of the protein. These observations highlighted the potential of CYP in the biosensing of pesticide compounds.

4.3. Caliber of Glutathione S-transferase (GST) as a Future Biosensor: Interaction studies of GST with Fenobucarb, DDT, and Parathion

The GST is an enzyme involved in the formation of the defense against the insecticides such as decamethrin (Kostaropoulos et al. 2001) and usually considered as the target in designing novel insecticides (Wang et al. 2014). Therefore, the GST can be considered as the potential candidate for designing a biosensor for the detection of pesticide levels. The predicted 3-D structure of GST was used as a receptor and docked with all the categories of the pesticides discussed in the above sections. The DDT, with highest docking scores, was found to be interacted with Gly14, Arg15, Leu72, and Glu162 residues of GST (**Figure 5**), while similar interaction behavior was observed for other pesticides (**Figure S7**). Furthermore, the GST-DDT docked complex was centrally immersed in the solvation box containing water molecules and simulated for 50 ns

time scale. The energetic analysis of the interaction energies showed the presence of active interactions between the GST and DDT, with the total free energy of the binding was observed in the range of -200 – -300 kJ/mol (**Figure 5C**). The analyses of the trajectories showed that the distance between GST and DDT was fluctuated in the range of 0.25 – 0.3 nm (**Figure 6A**), while the hydrogen bond dynamics illustrated the presence of three bonds between the docked molecules (**Figure 6B**). Similar, the Rg and RMSD plots signified that binding of DDT resulted in little changes in the conformational preferences (**Figure 6C and D**). Therefore, GST can be used for the detection of both insecticide and pesticide compounds.

4.4. Caliber of Protein kinase C (PKC) as a Future Biosensor: Interaction studies of PKC with Fenobucarb, DDT, and Parathion

The PKC belongs to the family of serine/threonine kinases and catalyzed the phosphorylation of γ-aminobutyric acid receptors and regulates the effects of insecticides on neuronal channels and receptors present in insects (Murillo et al. 2011). But the effect of PKC in the modulation of the insecticide sensitivity still remains unclear (Murillo et al. 2011). Consequently, in order to understand the structural basis of PKC interaction with the pesticides, its structure was predicted and utilized for further analysis. The fenobucarb complex was selected on the basis of the generated parameters and was found to interact with Ser32, Leu53, Val58, Asp63, Cys66, and Glu70 (**Figure 7**).

The in-depth knowledge about the conformational behavior of PKC-fenobucarb complex was obtained through the principles of MD simulations and DFT studies (**Figure S12**). The MM-PBSA based analysis showed that the total free energy of binding and vdW energy was observed in the range of -150 – -200 kJ/mol and the negative values, indicating that the pesticide inhibition was energetically favorable (**Figure 7C**). The GROMACS utilities observed that the distance

between the PKC and fenobucarb was maintained in the range of 0.25-0.35 nm and hydrogen bonds also showed high fluctuations with maximum 3 bonds was present in the complex (**Figure 8A and B**). The Rg values showed a steady decrease up to 50 ns time scale indicated that the compactness of the structure was increased continuously throughout the MD simulations (**Figure 8C**). While the values in the RMSD curve showed a steady increase indicated that the PKC become energetically active with the advent of the MD simulations (**Figure 8D**). These observations indicated that the PKC is selectively inhibited by the pesticides and can be used for the specific detection.

5. Significance of the present study

The computational modelling is widely used instead of expensive physical experiments aiming at understanding the kinetic peculiarities/interaction studies of the target analyte with bio-recognizers (Dabulytė-Bagdonavičienė, Ivanauskas, and Razumas 2011; Botkin and Turova 2004). Initially, the main focus of this paper is to describe the molecular docking and dynamics studies on the interaction of pesticides (fenobucarb, dichlorodiphenyltrichloroethane (DDT) and parathion) with bio-recognizers (ACH, CYP, GST and PKC) to identify the potential candidate to design the effective biosensor for the detection of pesticides. Computational approaches were used to confirm some of the results already reported in the literature as a first step. However, to reduce the number of structures screened in the first pool of potential binding agents. In this way, we hope to both reduce the time taken to find appropriate bio-recognizers and to increase the chances of finding the best possible bio recognizers.

6. Conclusions

In this study, the structural basis of the possible candidates for the biosensing of the pesticides was analyzed and their interaction patterns were compared. The *in-silico* based studies provide new insights into the interacting residues of the proteins involved in the process of inhibition. Furthermore, MD simulation studies provided the understanding about the conformational behaviors of the studied protein-pesticide complexes. Moreover, the MM-PBSA analysis showed that the total binding energy for the PKC-fenobucarb complex was lower than the rest of the complexes indicating the highly specific sensing capabilities of PKC. In addition to ACH, which was validated to be the primary target for organophosphorus and carbamates, their role in the inhibition of other metabolically significant proteins has been established in this study. The generated outcomes can be used in other experimental studies for assessing the toxic effects of the pesticides. Specifically, GST showed the highest values of inhibition constants indicating its strong binding efficiency with the pesticides, but the PKC showed the highest specificity. Therefore, these proteins can further be utilized in designing a suitable biosensor for the detection of concentration level of the pesticides in water resources and food materials.

Conflict of interest

The authors have no substantial financial or commercial conflicts of interest.

Acknowledgements

The authors gratefully acknowledge the computational facility of Center for High Performance Computing (CHPC), Cape Town, South Africa and Durban University of Technology, Durban, South Africa.

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Figure legends

Figure 1. (A) The 3-D and 2D representation of stable docked conformation generated for ACH-parathion complex. **(B)** The plot showing the MM-PBSA results in the form of different free energies of binding.

Figure 2. (A) The graph depicting the variation of the distance between ACH and parathion. **(B)** The curve illustrating the variations in the hydrogen bonds observed during 50 ns MD simulations. **(C)** and **(D)** The graphical representation of the fluctuations observed in the Rg and RMSD values.

Figure 3. (A) The selected conformational pose of CYP-fenobucarb docked complex showing the interacting residues. **(B)** The MM-PBSA analysis based obtained curve showing the behavior of the interactions in the form of free energies of binding.

Figure 4. (A) and **(B)** The graphical view illustrating the dynamics observed in the bonding patterns between CYP and fenobucarb. **(C)** and **(D)** Rg and RMSD curves highlighting the changes in the conformational behavior observed during 50 ns MD simulations.

Figure 5. (A) The stable pose generated after molecular docking between the GST and DDT. **(B)** The MM-PBSA derived curve showing the fluctuations in the interacting free energies.

Figure 6. (A) and **(B)** The 2-D diagram describing the changes observed in the distance and hydrogen bonding patterns for GST and DDT complex. **(C)** and **(D)**The curves illustrating the changes in the Rg and RMSD values during 50 ns MD simulations.

Figure 7. (A) 3-D and 2-D representation of the interacting residues observed in the docked complex of PKC and fenobucarb. **(B)** The MM-PBSA based plots depicting the fluctuations in the interacting free energies.

Figure 8. (A) and **(B)** The illustration of the dynamics observed between PKC and fenobucarb in terms of distance and Hydrogen bonding. **(C)** and **(D)** The curves illustrating the changes in the Rg and RMSD values during 50 ns MD simulations.

Appendix A. Supplementary data

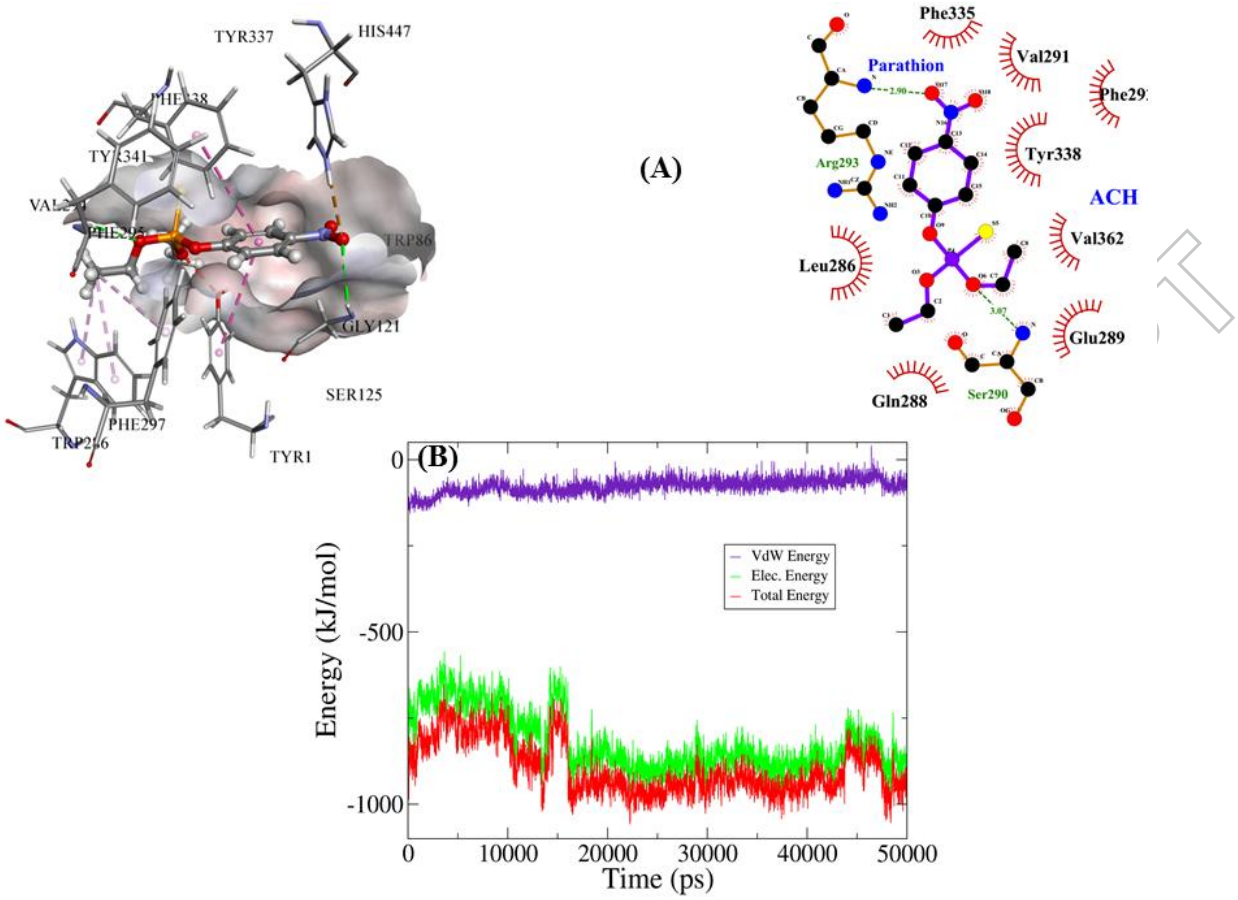
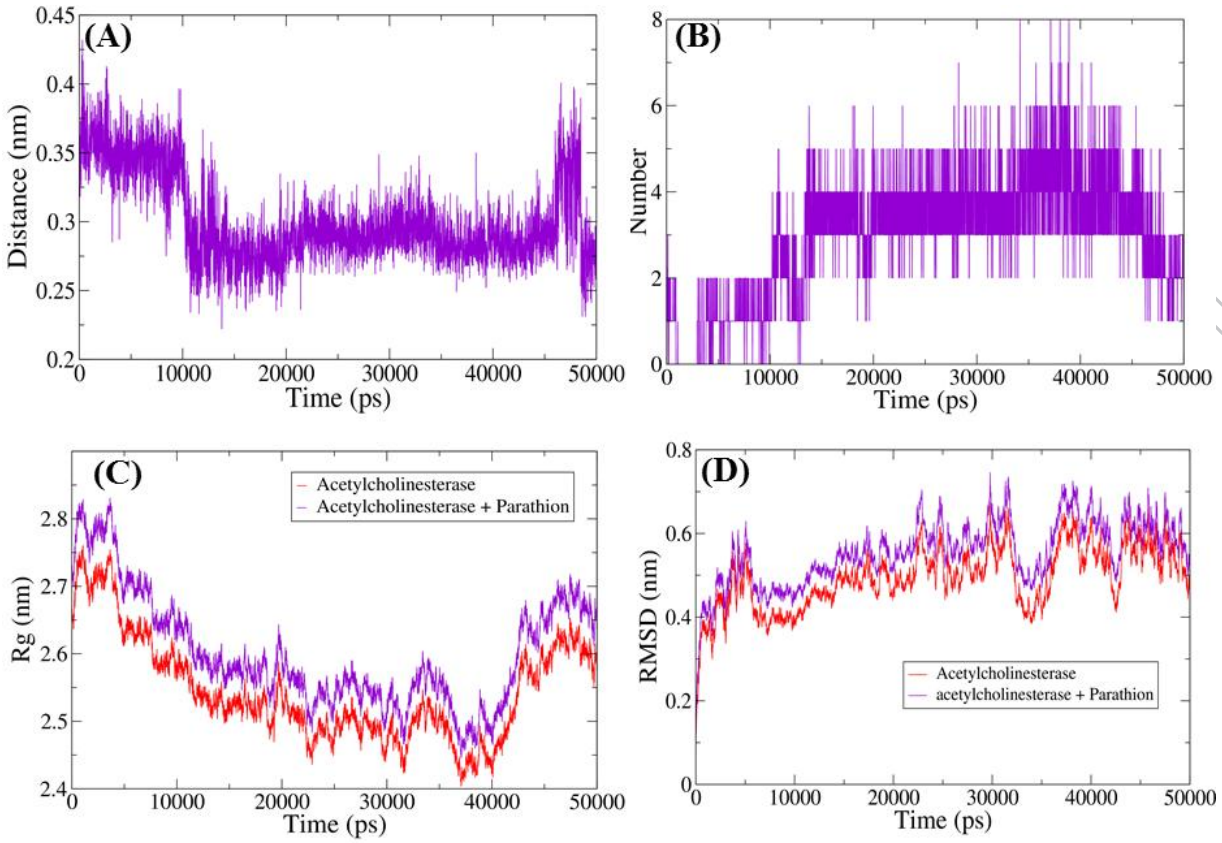


Figure 1.



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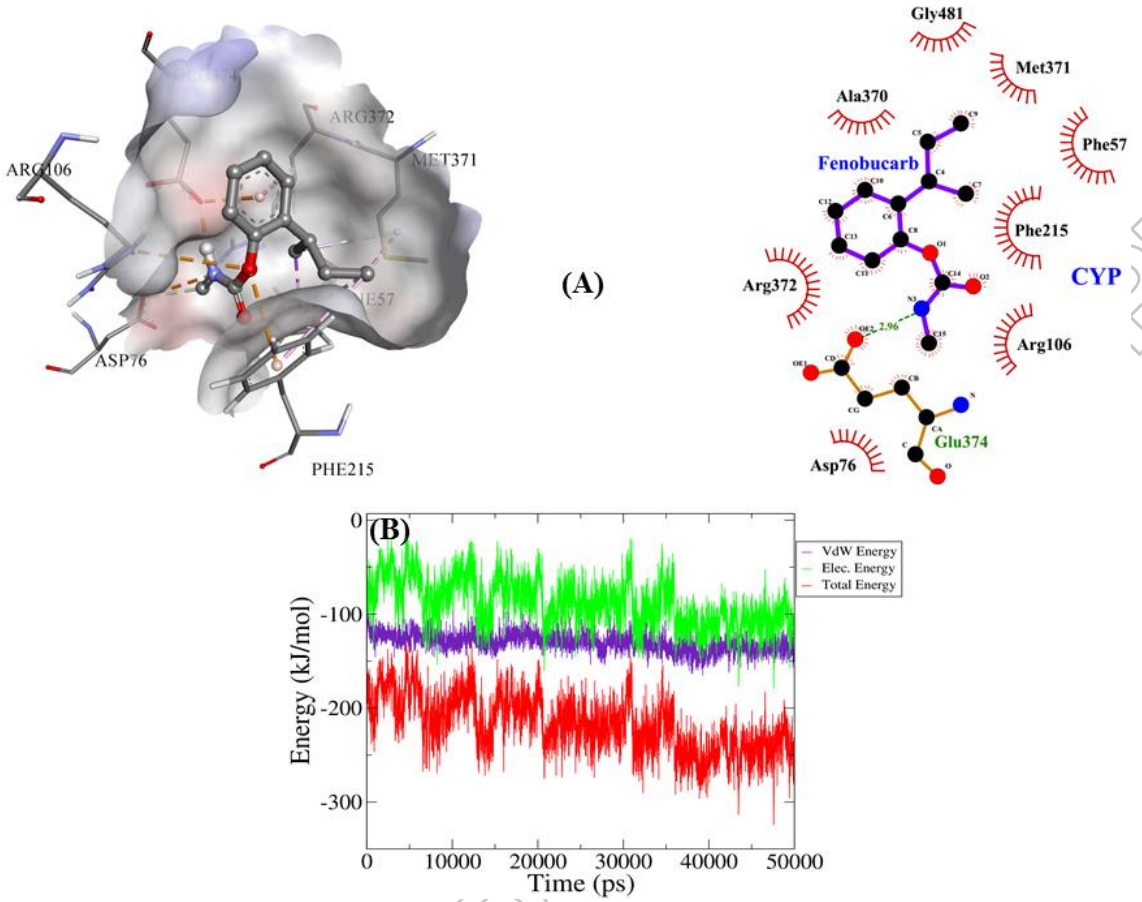

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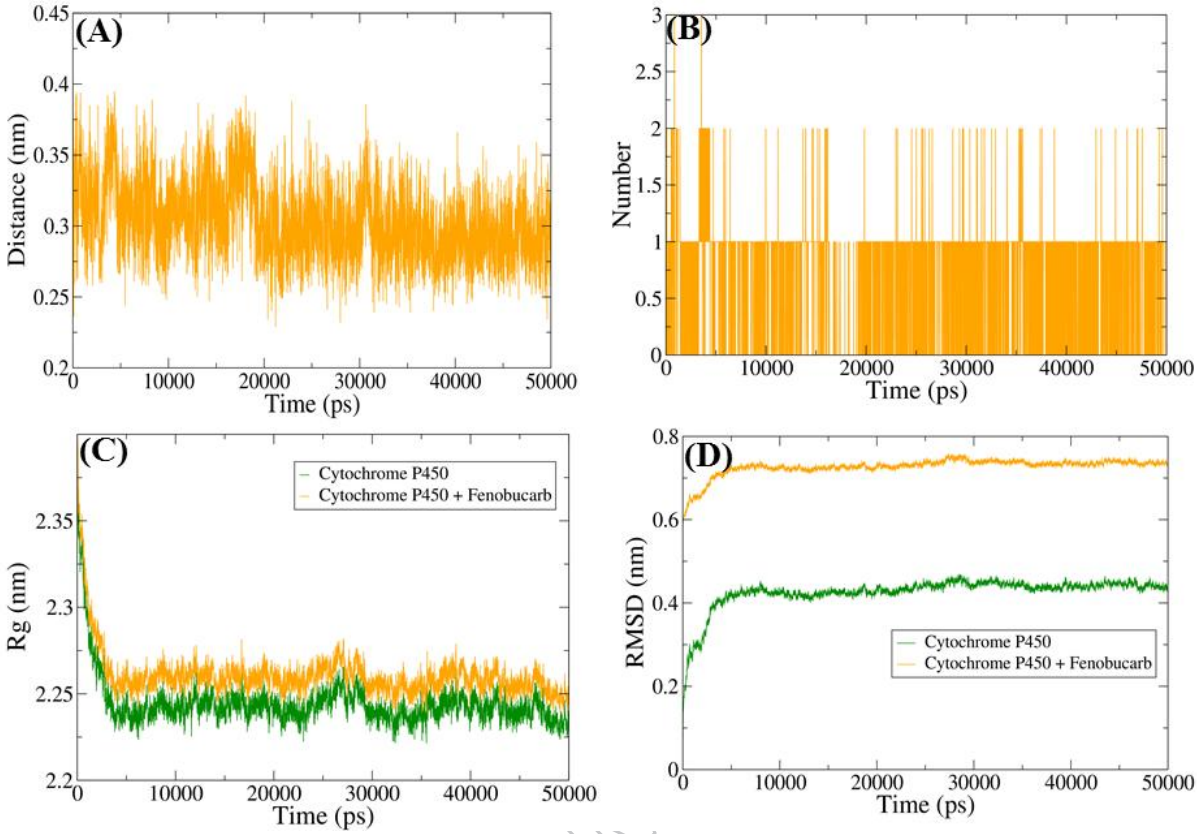

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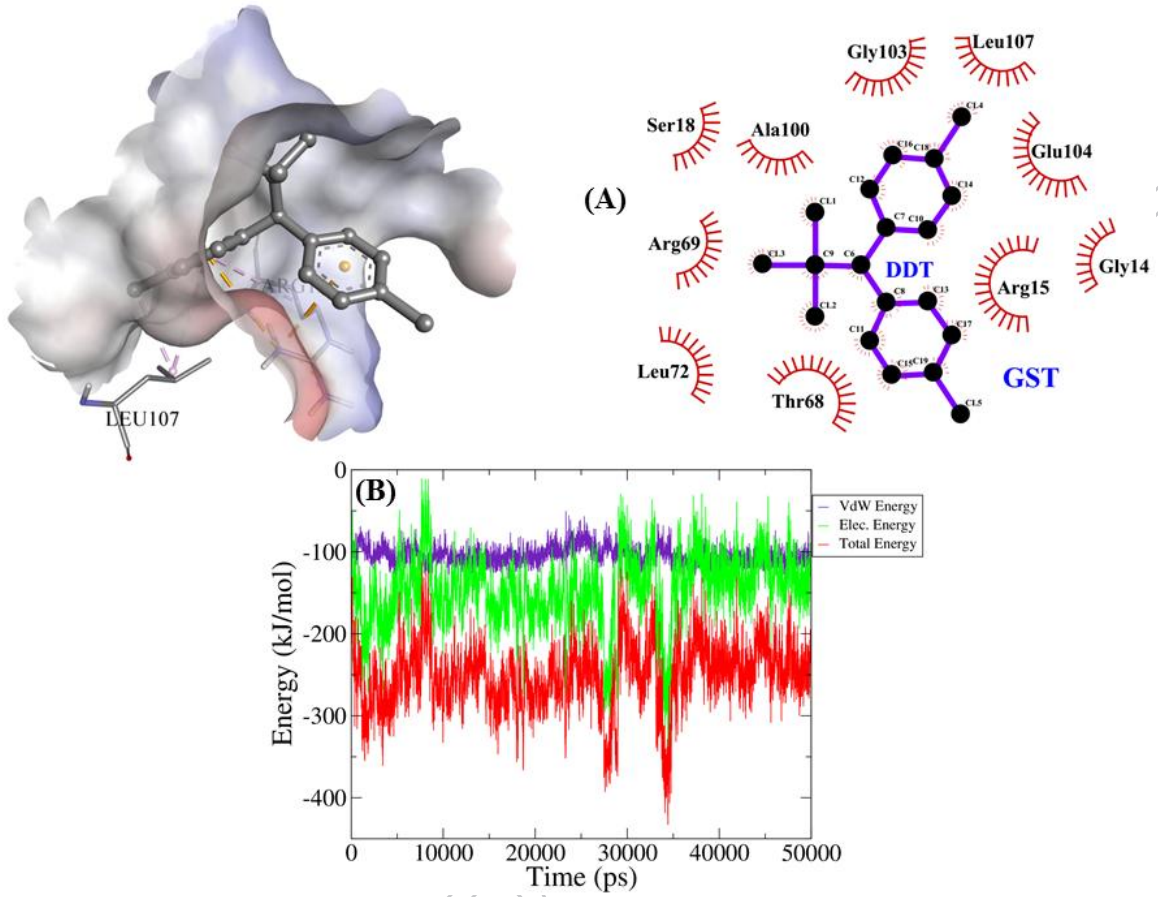
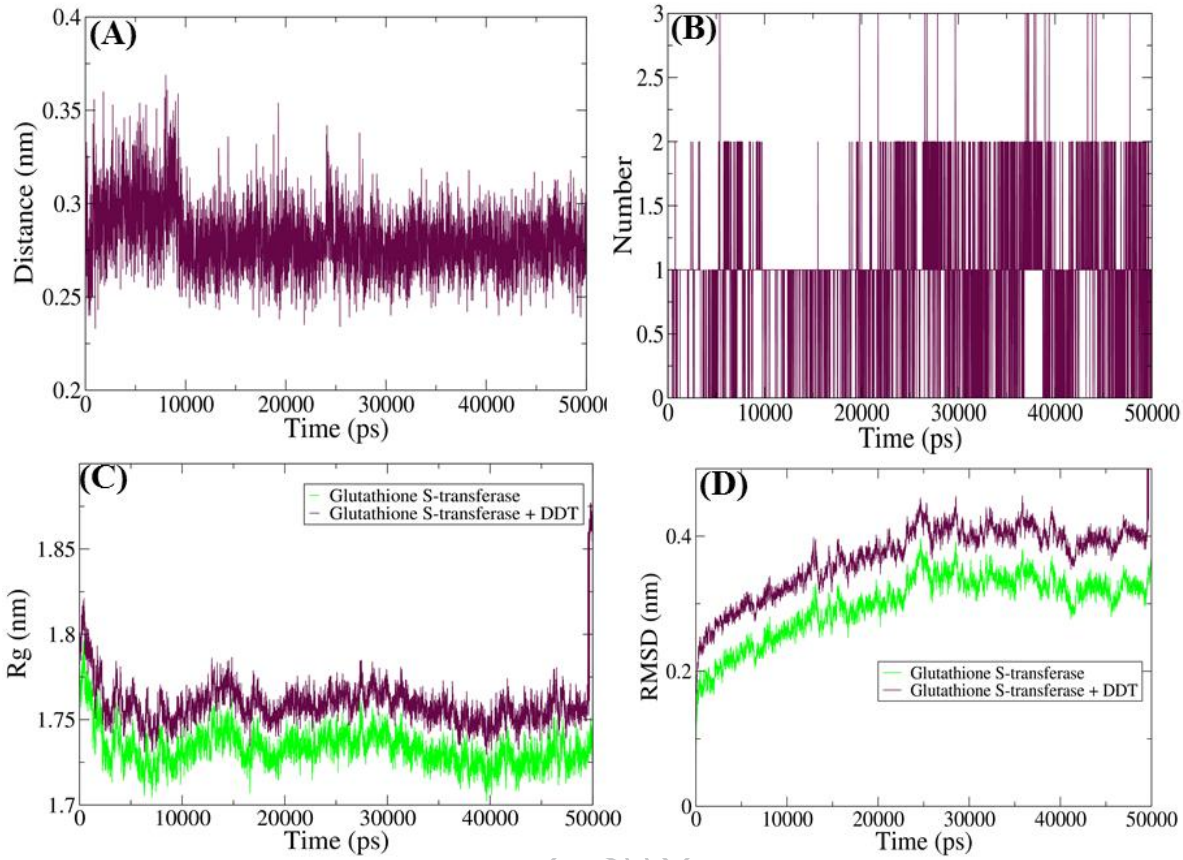


Figure 5.



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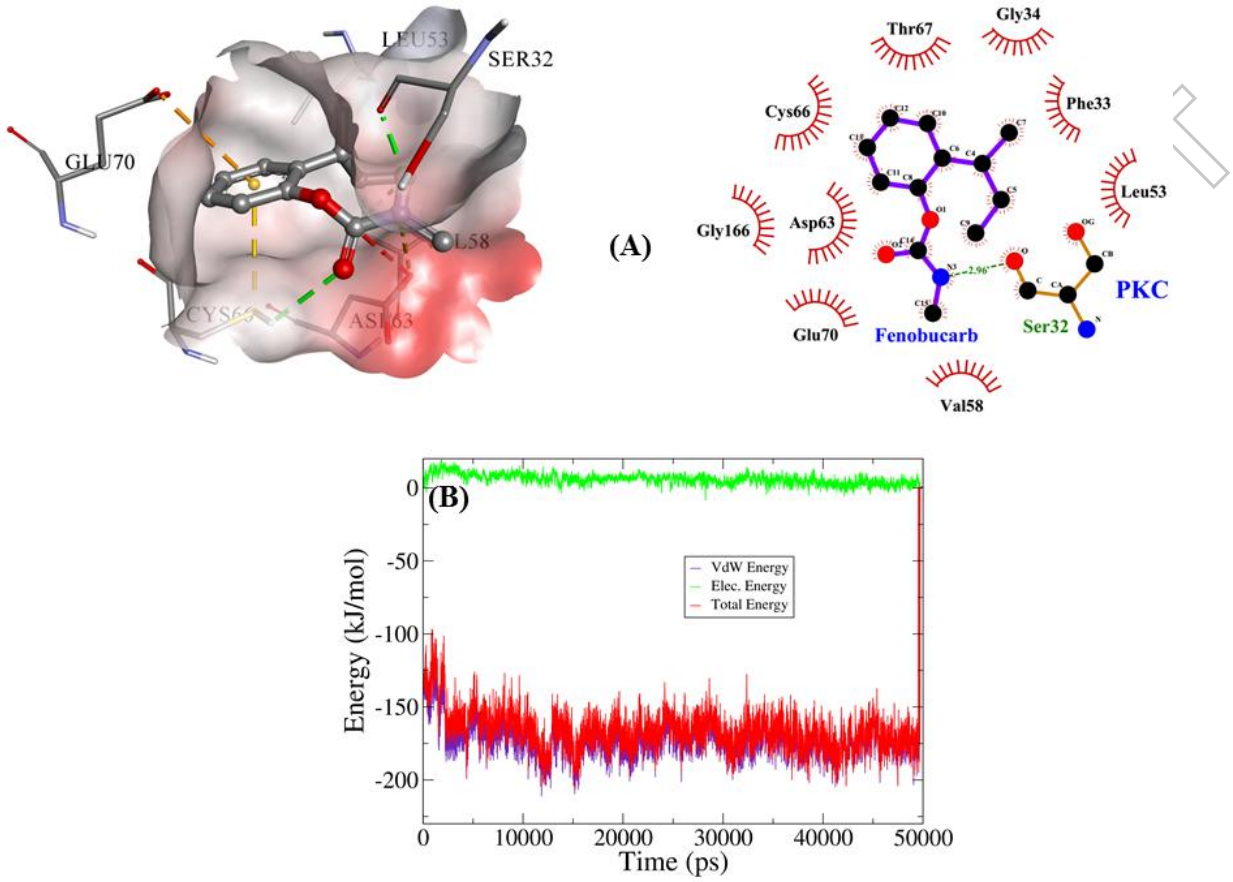

Figure 7.

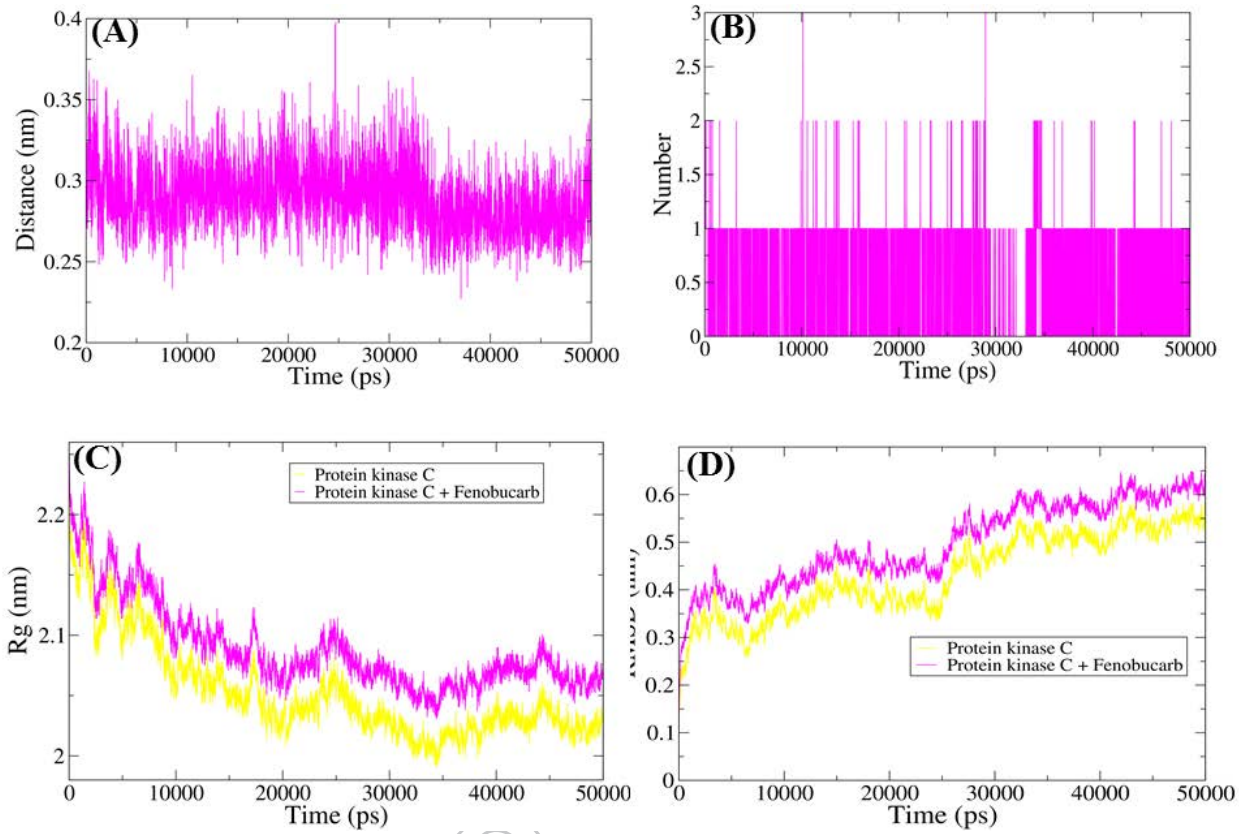

Figure 8.

Table 1: List interaction parameters generated after molecular docking of acetylcholinesterase and pesticides

S. No	Generated parameters	Pesticide		
		Fenobucarb	**DDT**	**Parathion**
1.	Selected pose	21	27	8
2.	Free energy of Binding (kcal/mol)	-5.27	-6.37	-4.57
3.	Inhibition Constant (μM)	138.15	21.41	448.09
4.	vdW + Hbond + desolv Energy (kcal/mol)	-6.46	-7.25	-7.02
5.	Intermolecular energy (kcal/mol)	-6.46	-7.27	-6.66
6.	Total internal (kcal/mol)	-0.73	-0.98	-0.92
7.	Torsional energy (kcal/mol)	1.19	0.89	2.09

Table 2: List interaction parameters generated after molecular docking of cytochrome and pesticides

S. No	Generated parameters	Pesticide		
		Fenobucarb	**DDT**	**Parathion**
1.	Selected pose	6	5	12
2.	Free energy of Binding (kcal/mol)	-5.1	-6.54	-5.03
3.	Inhibition Constant (μM)	183.49	16.15	206.93
4.	vdW + Hbond + desolv Energy (kcal/mol)	-6.11	-7.42	-6.24
5.	Intermolecular energy (kcal/mol)	-6.29	-7.43	-7.11
6.	Total internal (kcal/mol)	-0.72	-1.04	-0.77
7.	Torsional energy (kcal/mol)	1.19	0.89	2.09

Table 3: List interaction parameters generated after molecular docking of glutathione-S-transferase and pesticides

S. No	Generated parameters	Pesticide		
		Fenobucarb	**DDT**	**Parathion**
1.	Selected pose	26	13	4
2.	Free energy of Binding (kcal/mol)	-4.5	-5.43	-4.11
3.	Inhibition Constant (μM)	501.29	103.88	968.91
4.	vdW + Hbond + desolv Energy (kcal/mol)	-5.81	-6.3	-5.79
5.	Intermolecular energy (kcal/mol)	-5.7	-6.33	-6.2
6.	Total internal (kcal/mol)	-0.72	-0.99	-0.88
7.	Torsional energy (kcal/mol)	1.19	0.89	2.09

Table 4: List interaction parameters generated after molecular docking of protein kinase C and pesticides

S. No	Generated parameters	Pesticide		
		Fenobucarb	**DDT**	**Parathion**
1.	Selected pose	26	21	29
2.	Free energy of Binding (kcal/mol)	-4.42	-6.11	-5.32
3.	Inhibition Constant (μM)	575.53	33.22	125.1
4.	vdW + Hbond + desolv Energy (kcal/mol)	-5.63	-7.04	-5.12
5.	Intermolecular energy (kcal/mol)	-5.61	-7.0	-7.41
6.	Total internal (kcal/mol)	-0.73	-0.94	-0.96
7.	Torsional energy (kcal/mol)	1.19	0.89	2.09

Structural basis of pesticide detection by enzymatic biosensing: A molecular docking and MD simulation study

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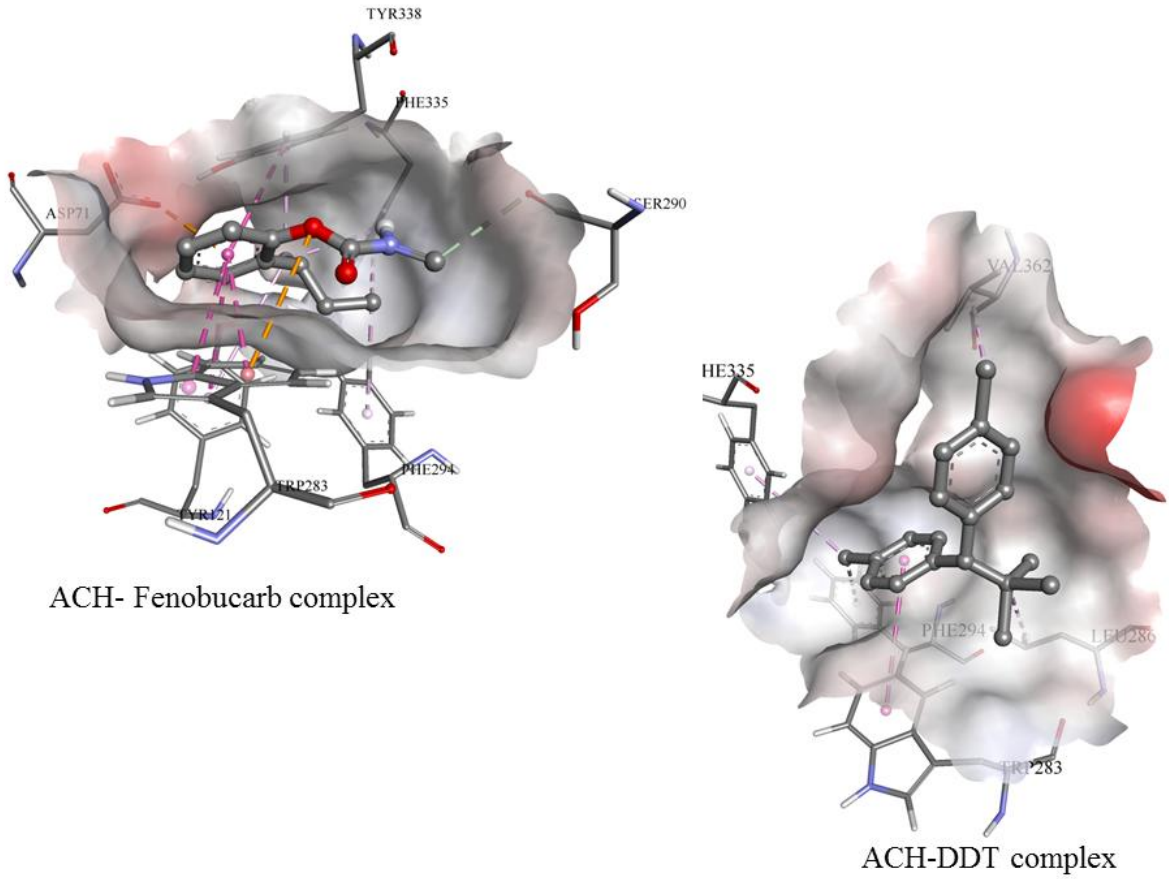


Figure S1. The obtained docked complexes for ACH

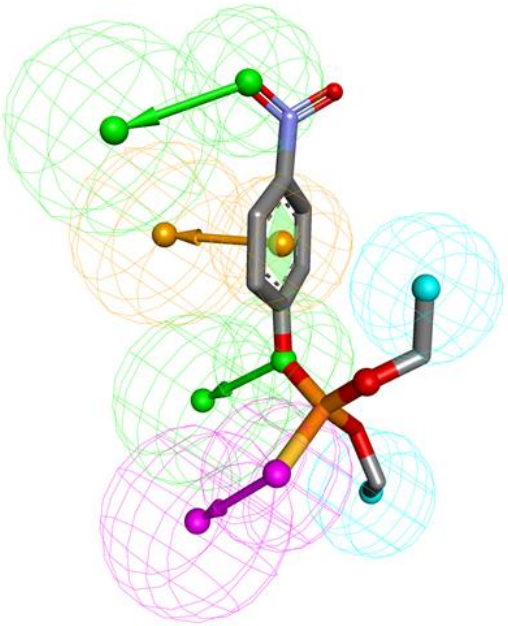
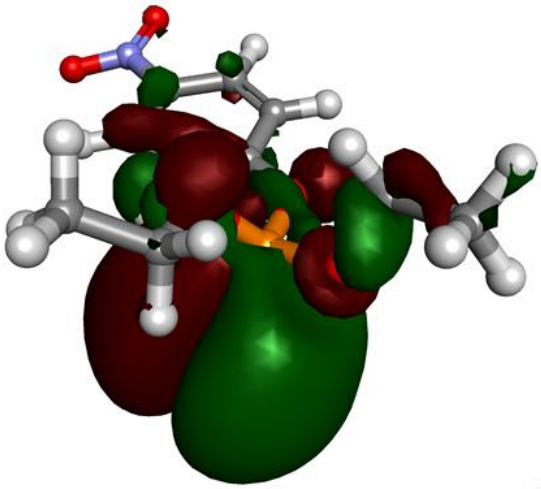
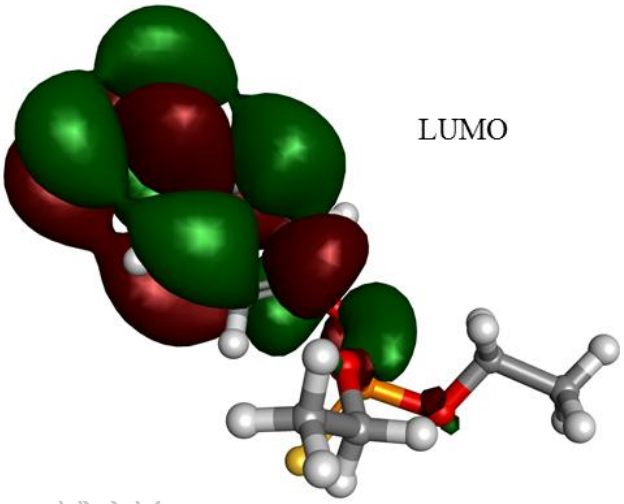


Figure S2. The generated pharmacophore features for docked complex



Acetycholine-parathion complex



HOMO

LUMO

HOMO energy = -0.21 Ha
LUMO energy = -0.10 Ha
Band gap energy = 0.11 Ha

Total energy = -1633.96 Ha

Figure S3. The DFT generated parameters

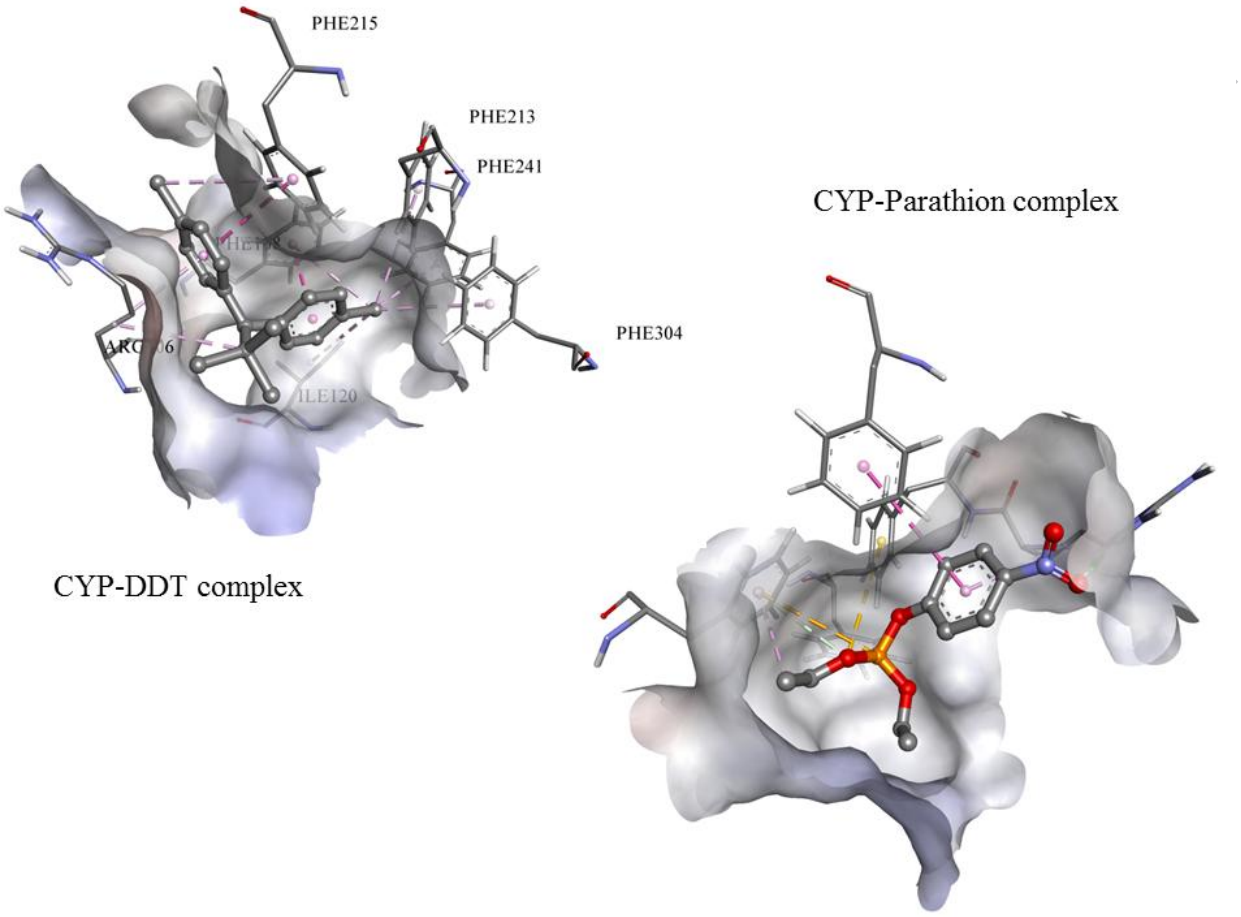


Figure S4. The obtained docked complexes for CYP

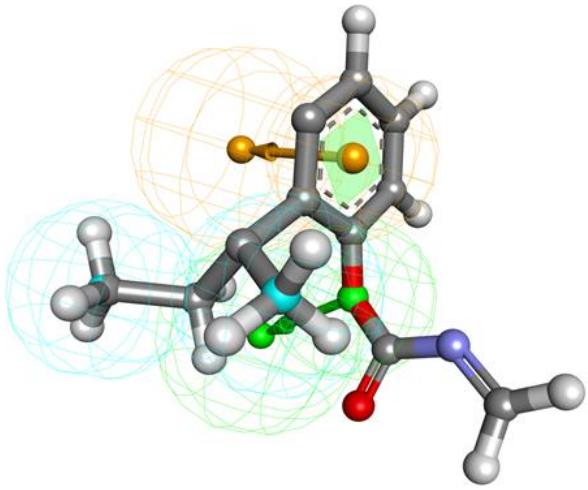
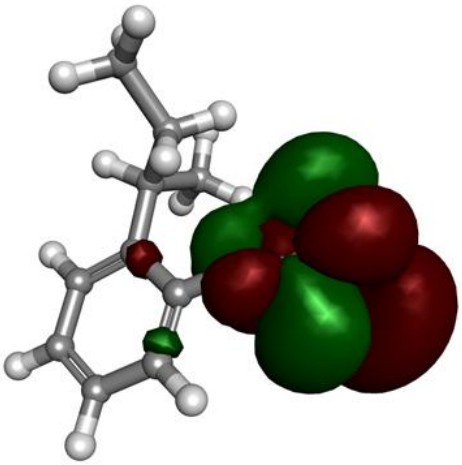


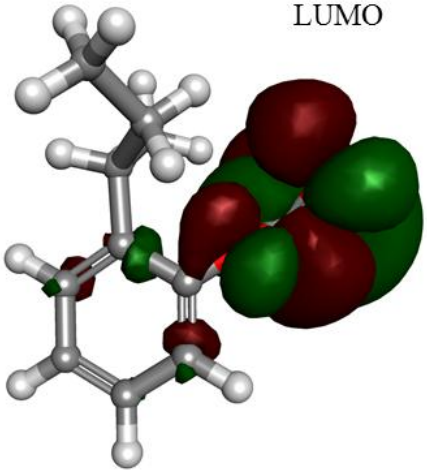
Figure S5. The generated pharmacophore features for docked complex



HOMO

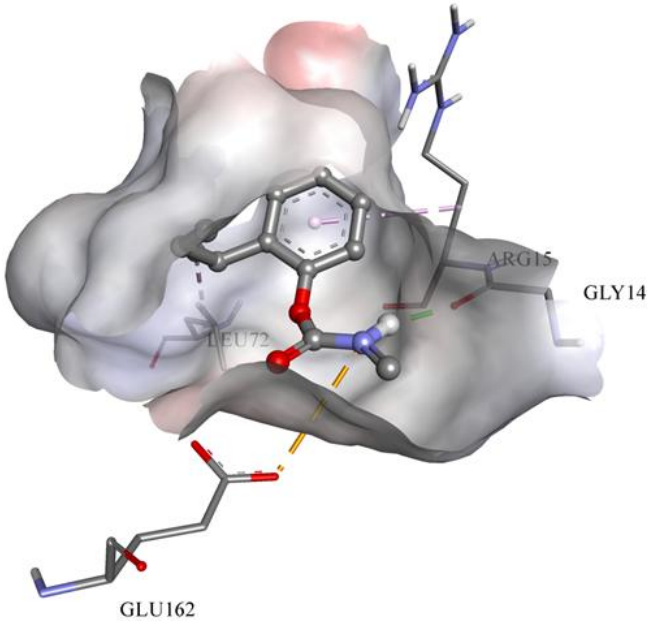
Cytochrome P450-Fenobucarb complex

LUMO

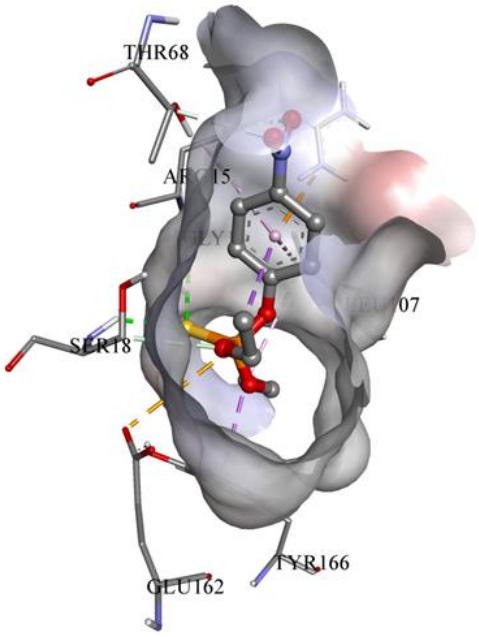


HOMO energy = -0.17 Ha
LUMO energy = -0.04 Ha
Band gap energy = 0.13 Ha
Total energy = -712.09 Ha

Figure S6. The DFT generated parameters



GST-fenobucarb complex



GST-parathion complex

Figure S7. The obtained docked complexes for GST

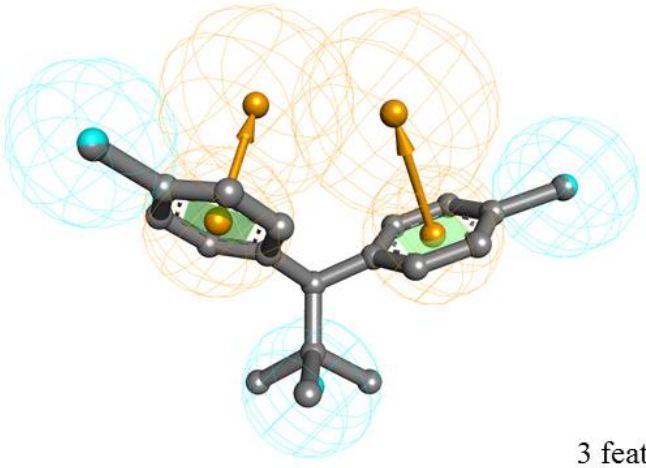
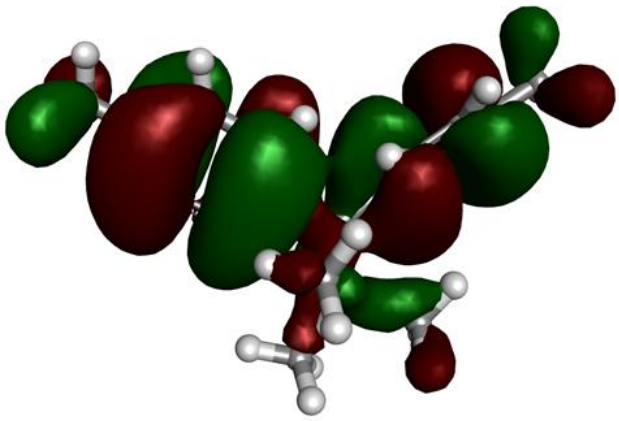


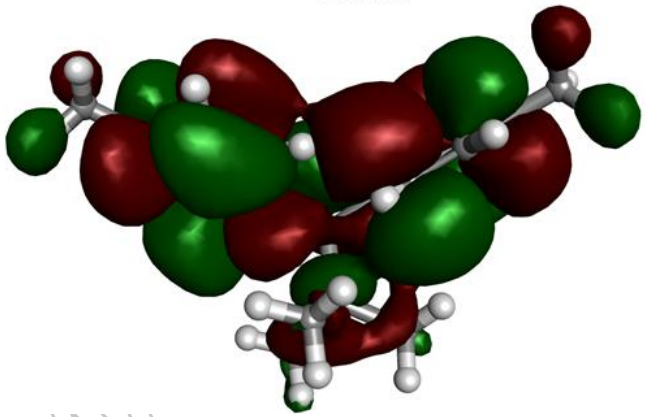
Figure S8. The generated pharmacophore features for docked complex



Glutathione S-transferase-DDT complex

HOMO

LUMO



HOMO energy = -0.21Ha
LUMO energy = 0.0058 Ha
Band gap energy = 0.224 Ha
Total energy = -784.52 Ha

Figure S9. The DFT generated parameters

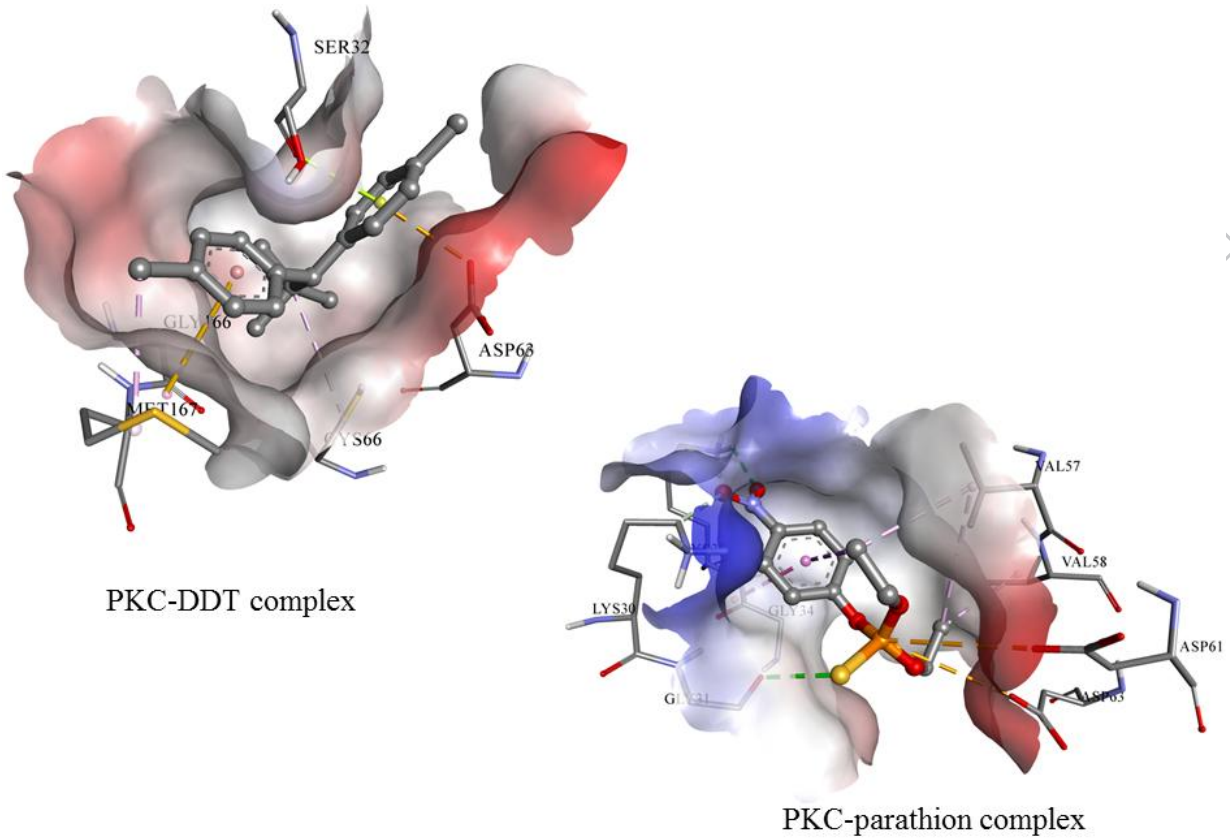
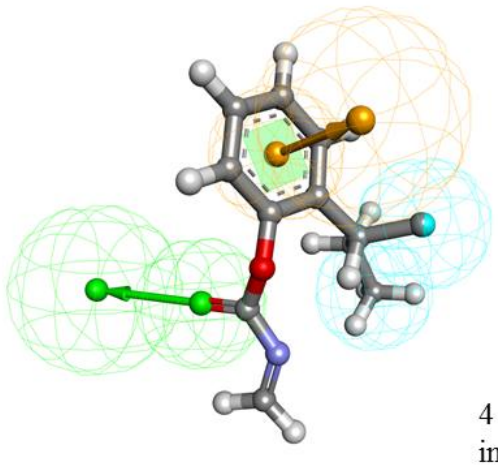


Figure S10. The obtained docked complexes for PKC

Protein kinase C-Fenobucarb complex



HB_ACCEPTOR: 4

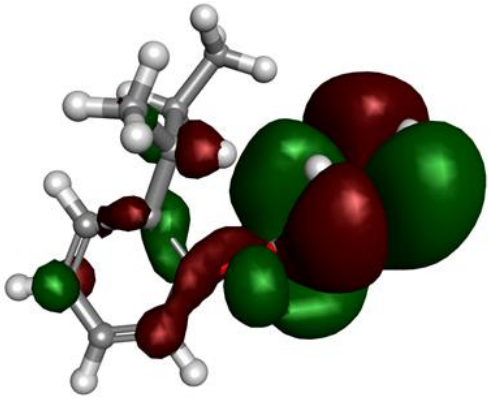
HYDROPHOBIC: 3

RING_AROMATIC: 2

4 features match the receptor-ligand interactions: AHHH

Selectivity Score: 5.6124

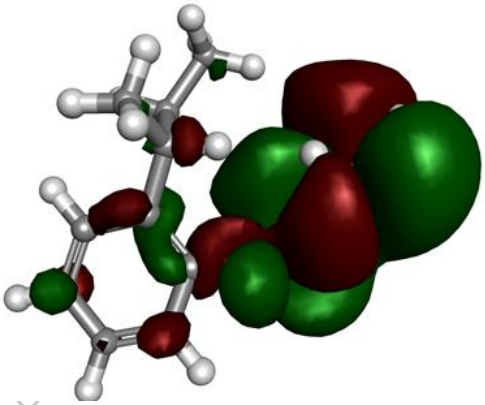
Figure S11. The generated pharmacophore features for docked complex



Protein kinase C-Fenobucarb complex

HOMO

LUMO



HOMO energy = -0.17 Ha
LUMO energy = -0.049 Ha
Band gap energy = 0.12 Ha
Total energy = -712.09 Ha

Figure S12. The DFT generated parameters