1. MMGBSA

Table 1. MMGBSA Calculation

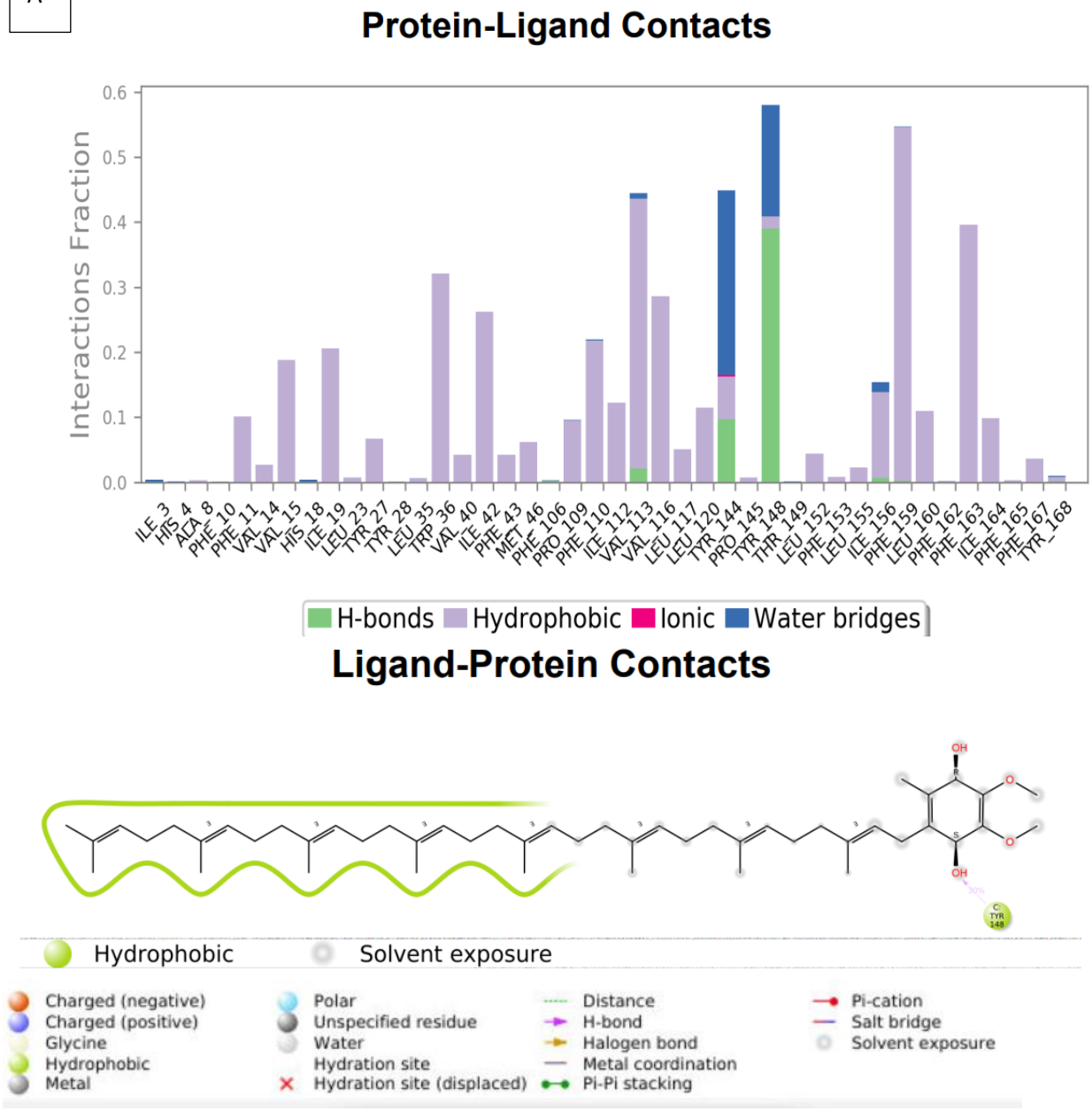
L124_G138	Binding Energy (kcal/mol)	Emodel_MD	F129_G143	Binding Energy (kcal/mol)	Emodel_MD
Ubiquinol	-112.8722	-73.747	Ubiquinol	-126.8153	-45.868
Fenamidone	-80.9935	-43.187	Fenamidone	-76.7195	-36.63
Mandestrobin	-61.7914	-41.374	Famoxadone	-54.0228	-48.809
Azoxystrobin	-68.6336	-57.91	Ametoctradin	-64.6404	-38.293
Captan	-36.3536	-25.133	Thiram	-52.4326	-31.147
Thiram	-25.1147	-32.646	Azoxytrobin	DNB	DNB
L124F_G138			F129L_G143		
Ubiquinol	-146.1665	-78.895	Ubiquinol	-139.0221	-53.13
Fenamidone	-74.0734	-45.443	Fenamidone	-72.1497	-43.909
Mandestrobin	-60.7936	-38.257	Famoxadone	-96.8131	-48.809
Azoxystrobin	-60.7889	-57.4	Ametoctradin	-36.6028	-23.568
Captan	-51.9781	-21.199	Folpet	-59.9621	-32.186
Thiram	-35.6427	-28.846	Thiram	-34.6025	-37.411
			Azoxystrobin	DNB	DNB
L124_G138A			F129_G143A		
Ubiquinol	-137.8718	-67.46	Ubiquinol	-92.6412	-61.849
Fenamidone	-77.8102	-45.726	Fenamidone	-56.6880	-37.063
Mandestrobin	-63.3806	-42.106	Famoxadone	-62.0463	-38.265
Azoxystrobin	-58.0502	-55.299	Ametoctradin	-21.4775	-26.039
Captan	-49.9644	-28.915	Azoxystrobin	-41.0854	-18.536
Thiram	-48.8370	-24.12	Folpet	-47.8349	-26.049
			Thiram	-51.8380	-26.899
			Azoxystrobin	DNB	DNB
L124F_G138A			F129L_G143A		
Ubiquinol	-158.1527	-73.468	Ubiquinol	-116.3582	-57.339
Fenamidone	-60.8398	-41.674	Fenamidone	-54.3397	-42.041
Mandestrobin	-62.4853	-42.106	Famoxadone	-74.9891	-46.843
Azoxystrobin	-43.0127	-55.299	Ametoctradin	-35.5459	-29.086
Captan	-48.0539	-24.12	Thiram	-36.3931	-32.139
Thiram	-42.5182	-28.915	Azoxystrobin	DNB	DNB

According to the average binding free energy and Emodel value, Ubiquinol as native ligand had very strong binding affinity toward Cytochrome b. When the target sites were L123 and G137 or their mutated version, high-risk fungicides like Fenamidoen and Mandestrobin showed lower binding free energy and Emodel value than low-risk fungicides (Thiram and Captan), which indicated that high-risk fungicides had more stronger binding affinity than low-risk fungicides.

When the active sites were L129 and G143 or their mutated version, high-risk fungicides still shower more tighter binding connection with Cytochrome b than low-risk fungicides.

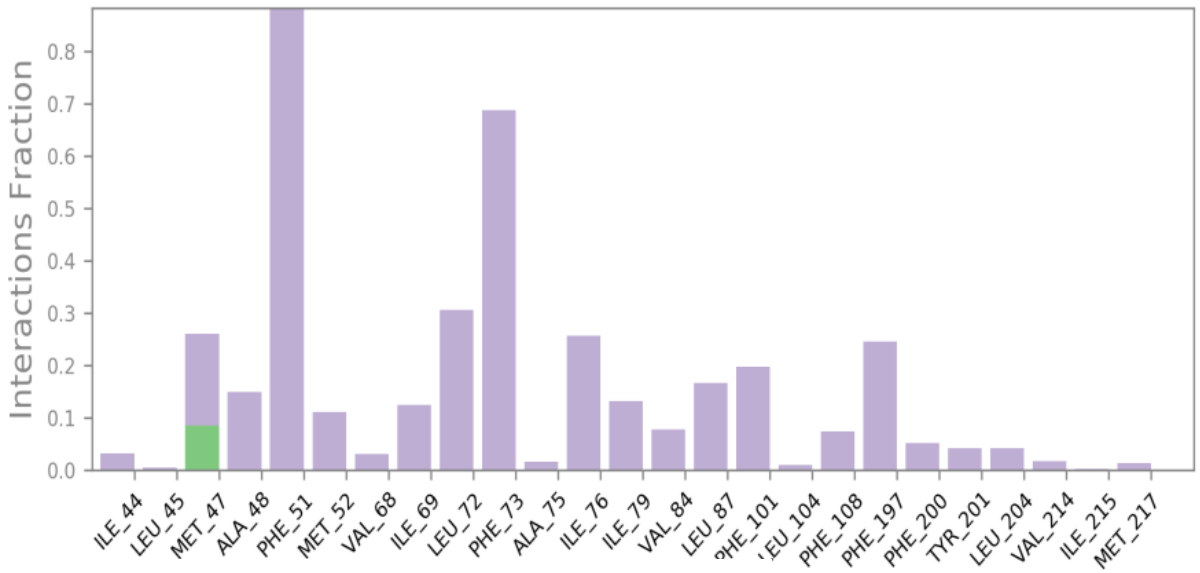
Interaction Diagram and Ligand Contact

A

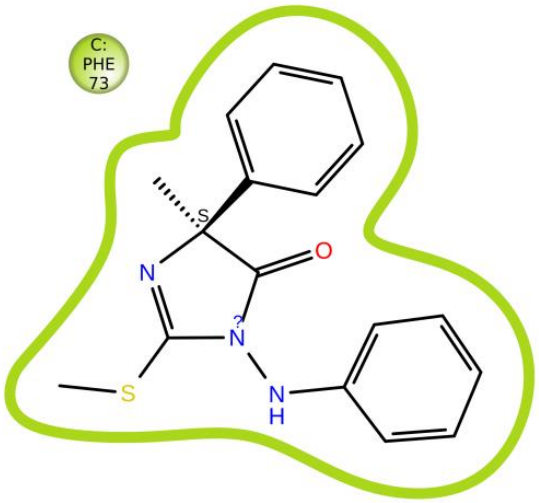


B

Protein-Ligand Contacts



Ligand-Protein Contacts



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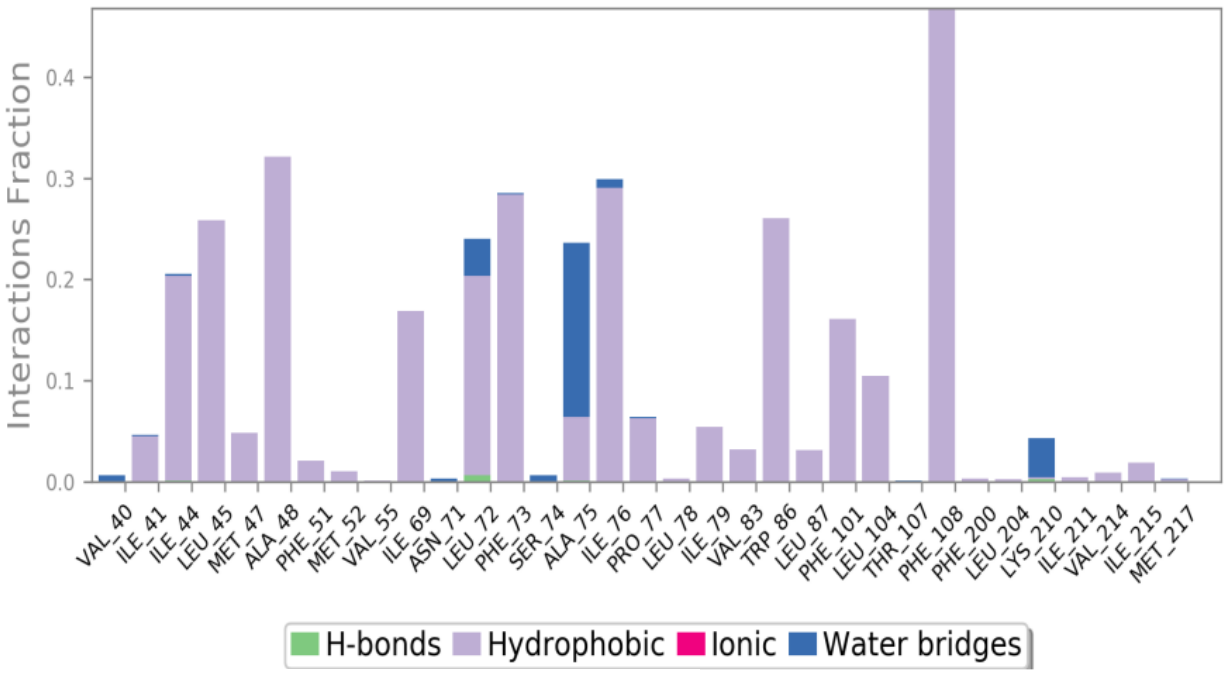
Protein-Ligand Contacts



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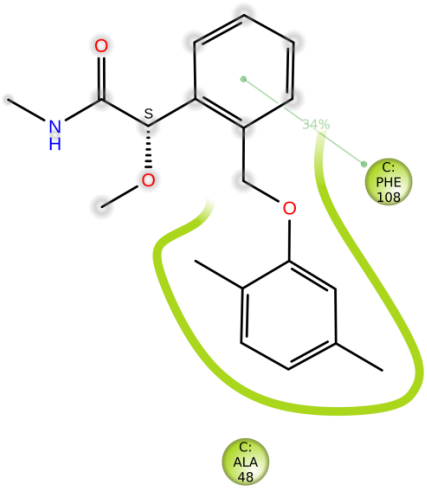

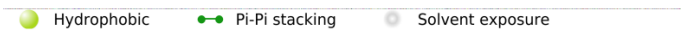

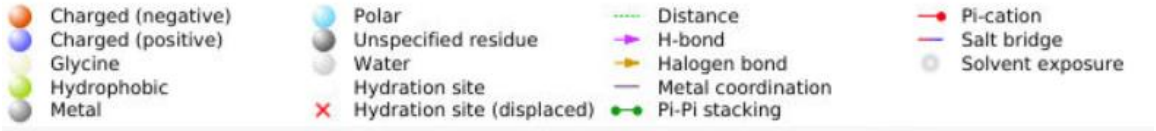

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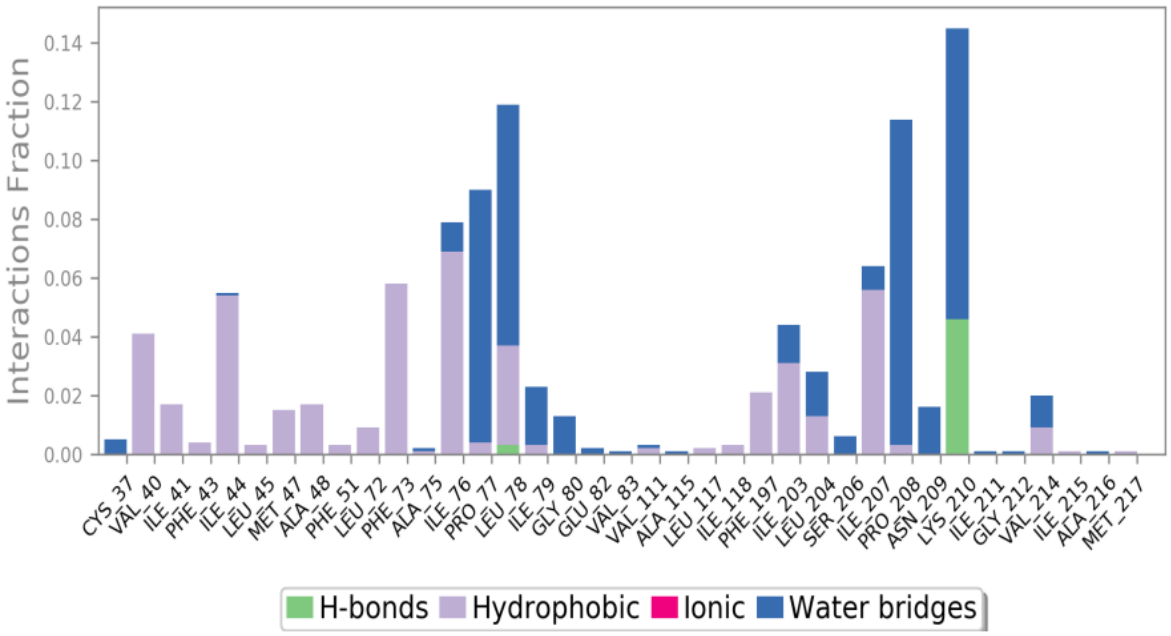

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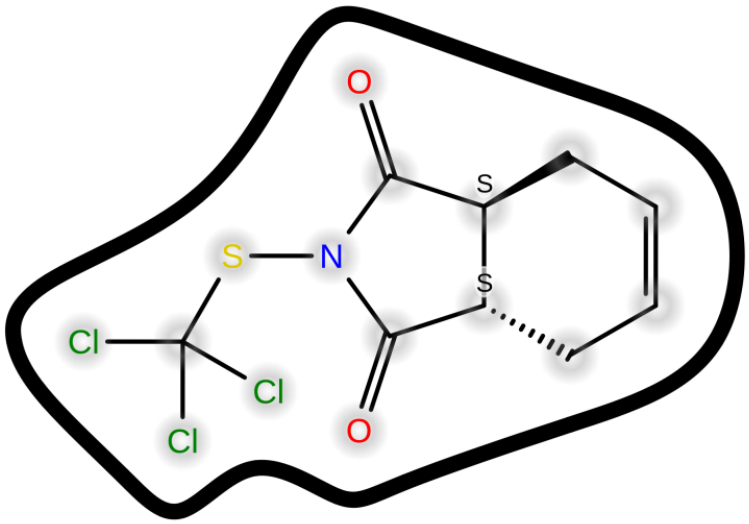

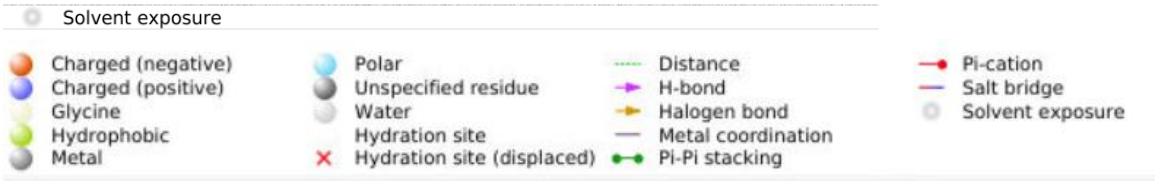

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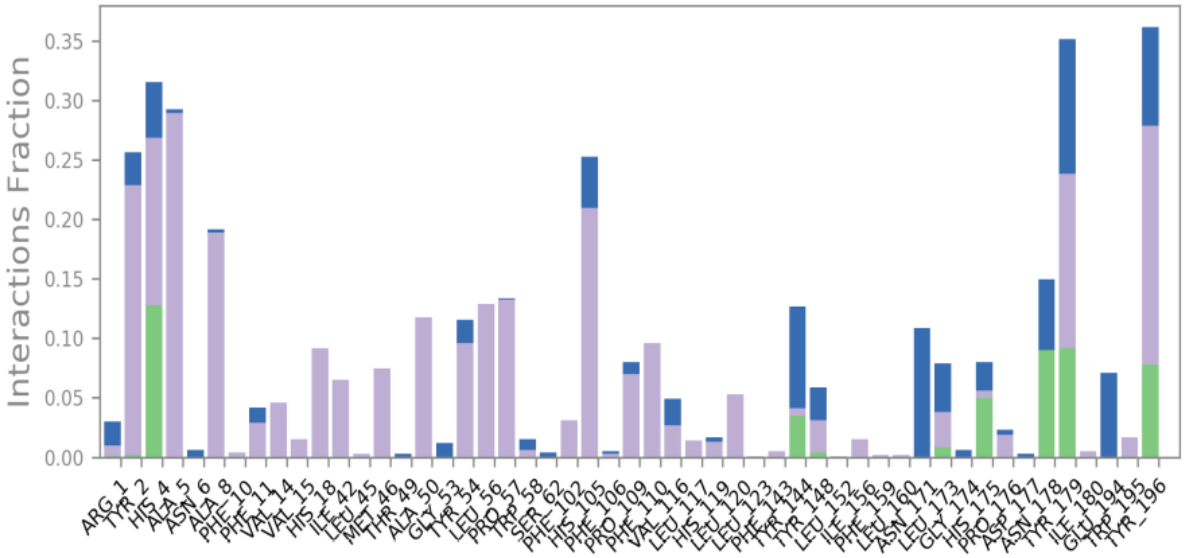
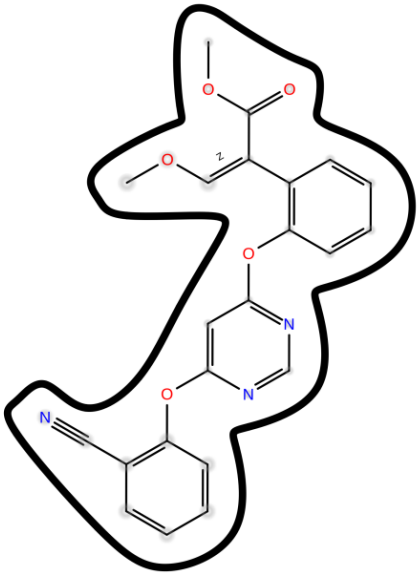
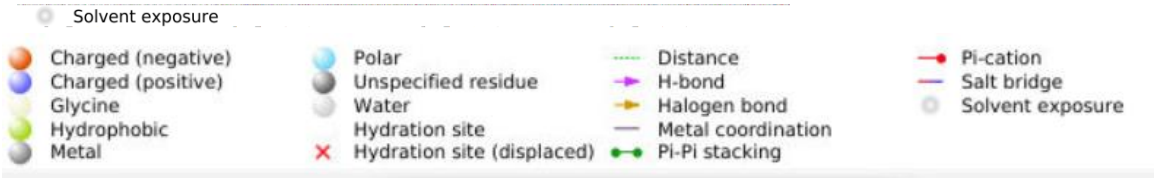
Protein-Ligand Contacts



Ligand-Protein Contacts





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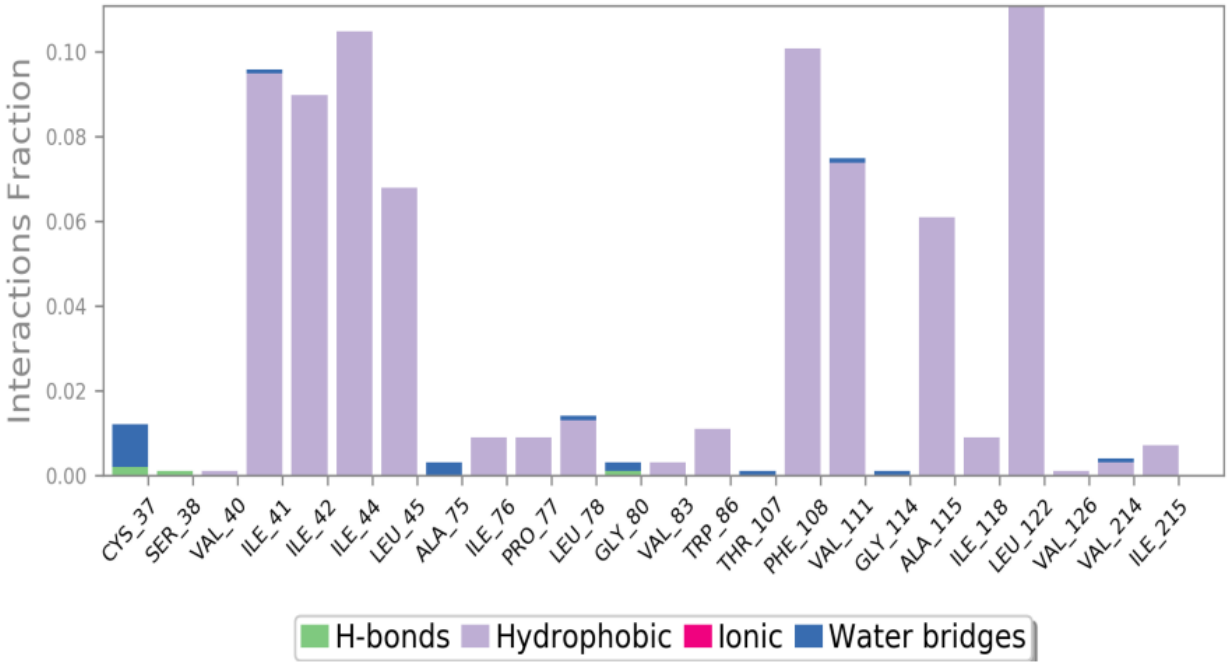
Protein-Ligand Contacts



Ligand-Protein Contacts

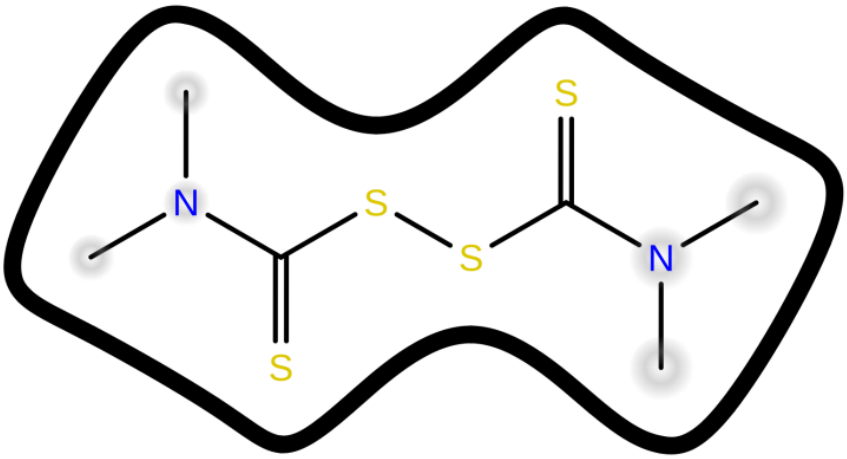
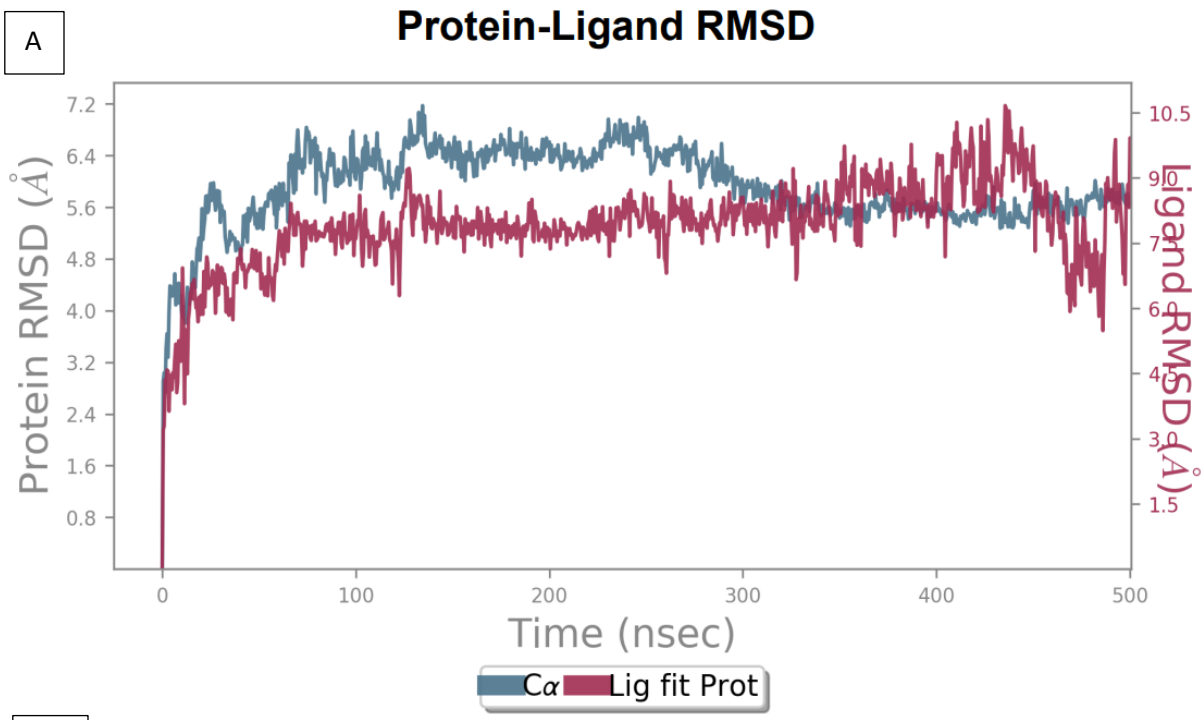


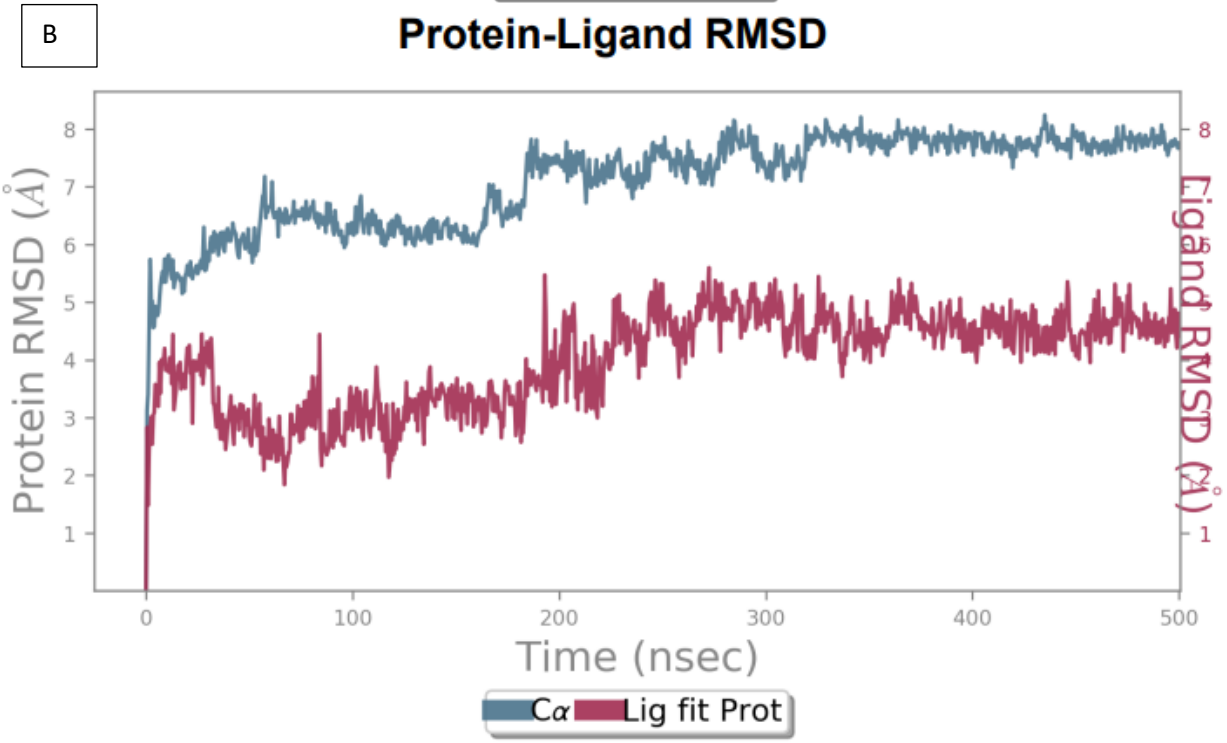
Figure 1. Protein-Ligand Interaction and contact toward active sites L123 and G137 (L45 and G60 on the figures). A) Ubiquinol with Cytochrome b; B) Fenamidone with Cytochrome b; C) Mandestrobin with Cytochrome b; D) Captan with Cytochrome b; E) Azoxystrobin with Cytochrome b; F) Thiram with Cytochrome b. The region started from 79 to 296 (0 to 217 on the figures).

Among the interaction between ligands and protein, hydrophobic binding interactions played a significant role. Since Ubiquinol was the original ligand bound to the Cytochrome b, it had strong multiple hydrophobic and Hydrogen bonding toward protein as expected. From Figure 1A, there was strong hydrophobic binding interaction at residus VAL190, PHE236, and PHE240 (VAL113, PHE159 and PHE163 on the figures) and it also had strong hydrogen binding at TRY226 (TYR148 on the figure). Fenamidone had stronger hydrophobic binding interaction at residue PHE129 (PHE51 on the figures) than Ubiquinol. Mandestrobin had hydrophobic contact at PHE186 (PHE108 on the figures). Azoxystrobin had multiply hydrophobic interaction, hydrogen binding and water bridge with the protein but they were weaker than Fenamidone and Mandestrobin. Captan had half water bridge and half hydrophobic binding interaction with protein. Thiram had mainly hydrophobic binding interaction with protein. Both Thiram and Captan showed much weaker binding interaction with protein due to smaller simulation time for the binding interaction.

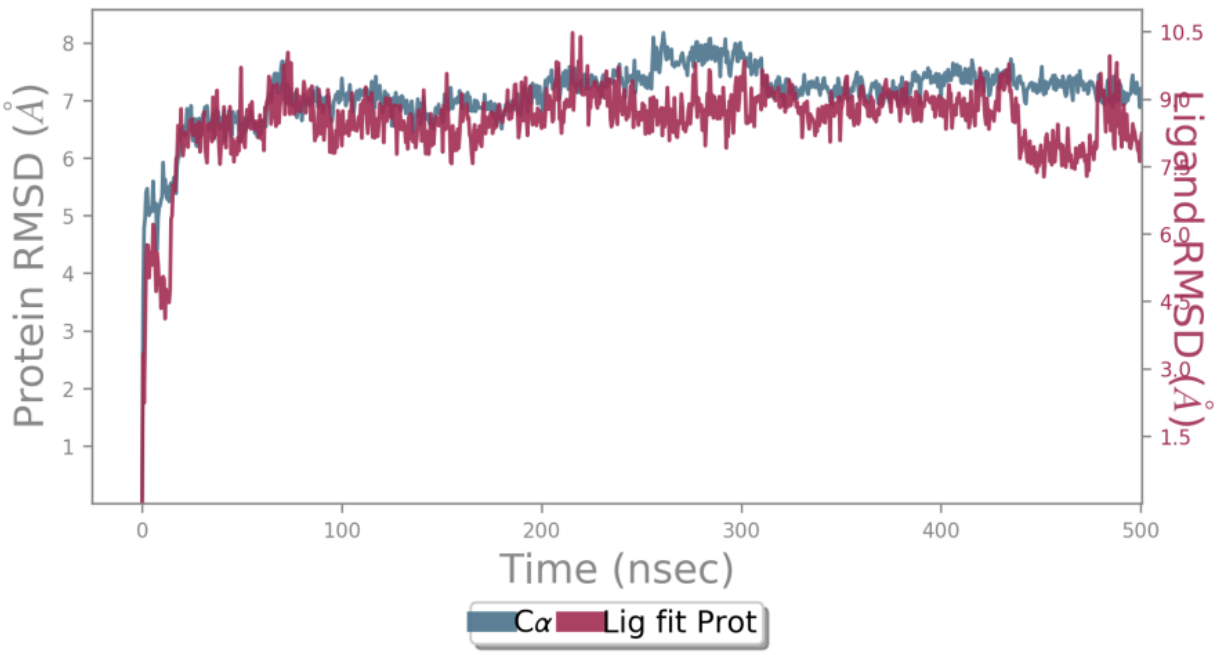
A


Protein-Ligand RMSD
Protein RMSD (Å)
Ligand RMSD (Å)
Time (nsec)
Cα
Lig fit Prot

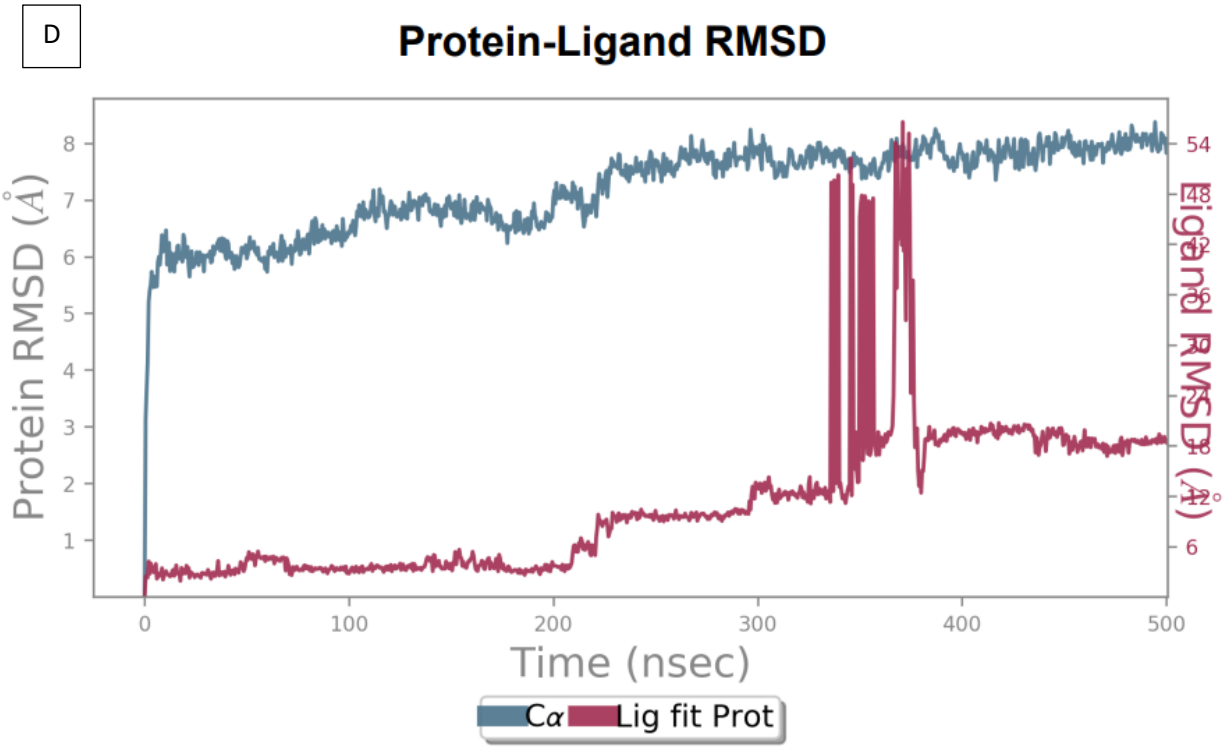
B


Protein-Ligand RMSD
Protein RMSD (Å)
Ligand RMSD (Å)
Time (nsec)
Cα
Lig fit Prot

C


Protein-Ligand RMSD
Protein RMSD (Å)
Ligand RMSD (Å)
Time (nsec)
Cα
Lig fit Prot

D


Protein-Ligand RMSD
Protein RMSD (Å)
Ligand RMSD (Å)
Time (nsec)
Cα
Lig fit Prot

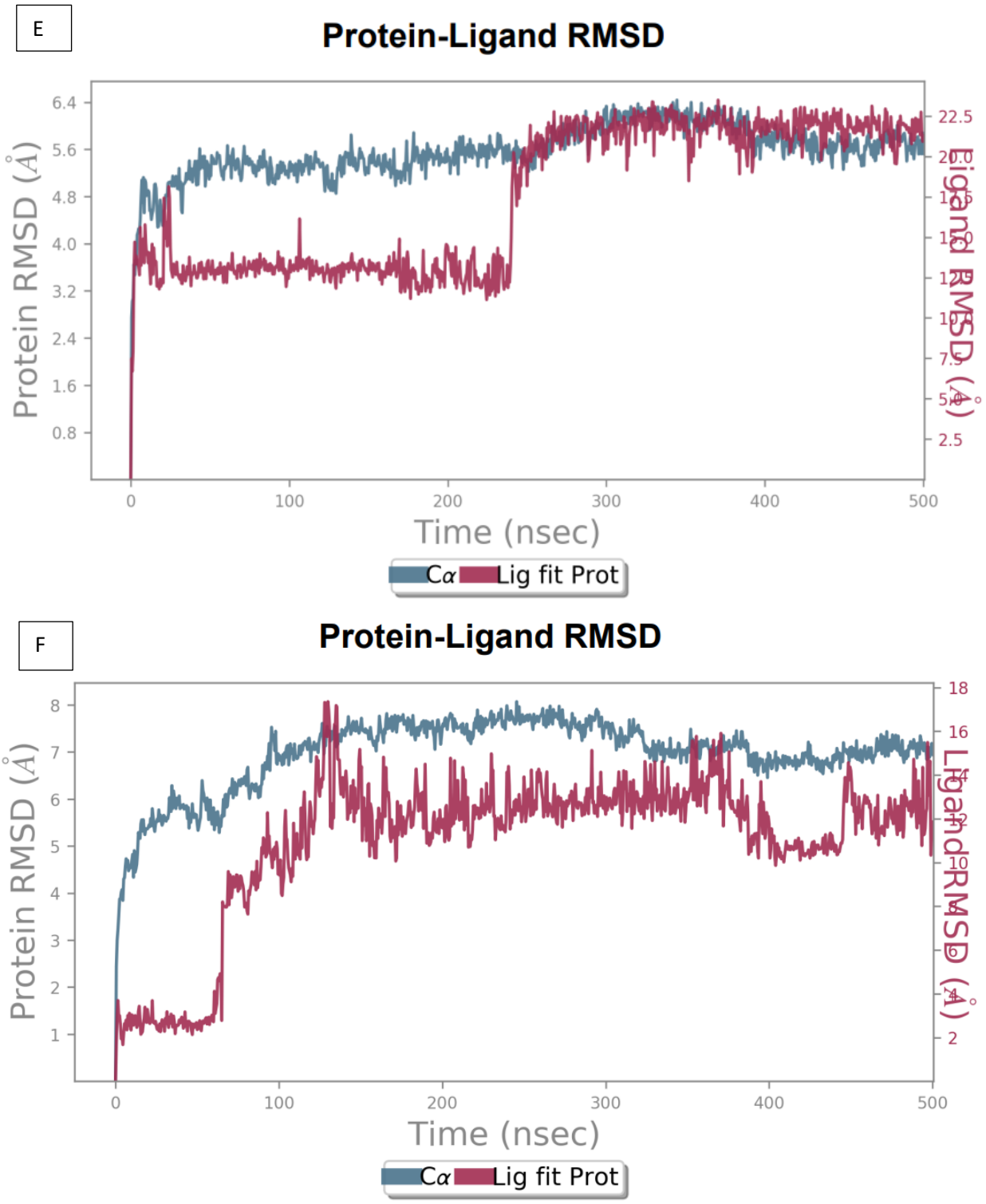


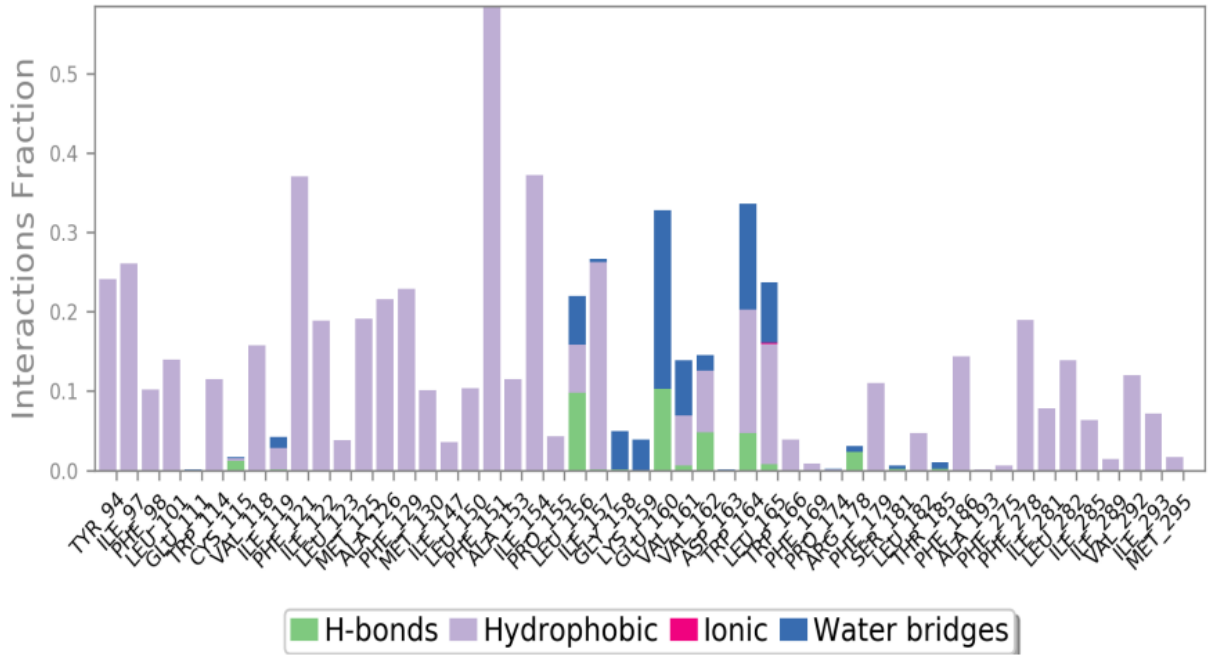
Figure 2. Protein and ligand RMSD (root mean square deviation) for the trajectory of each system toward active sites L124 and G138. Protein RMSD is shown in dark blue and Ligand RMSD is shown in red. A) Ubiquinol with Cytochrome b; B) Fenamidone with Cytochrome

b; C) Mandestrobin with Cytochrome b; D) Azoxystrobin with Cytochrome b; E) Thiram with Cytochrome b; F) Capatan with Cytochrome b.

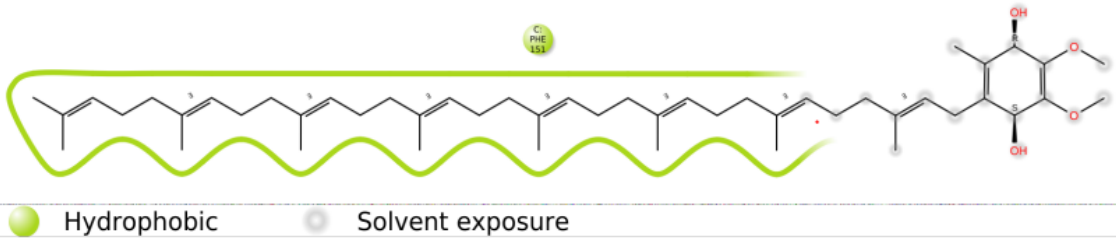
The simulation of Cytochrome b was equilibrated and the Ubiquinol bond tightly and stably during 500 ns Molecular Dynamics (MD) simulation. Protein simulation of Cytochrome b for Fenamidone and Mandestrobin was equilibrated. Mandestrobin bond more tightly than Fenamidone. Protein simulation for Azoxystrobin was equilibrated but Azoxystrobin only stayed closed to Cytochrome b before 200 ns and it diffused away from the protein. Both protein simulations for Thiram and Captan were equilibrated but Captan showed stronger binding affinity than Thiram. Fenamidone, Mandestrobin and Captan showed good performance on Ligand-contact and RMSD toward Cytochrome b among other high-risk and low-risk fungicides. They might be suitable option for fungicide combination.

A

Protein-Ligand Contacts

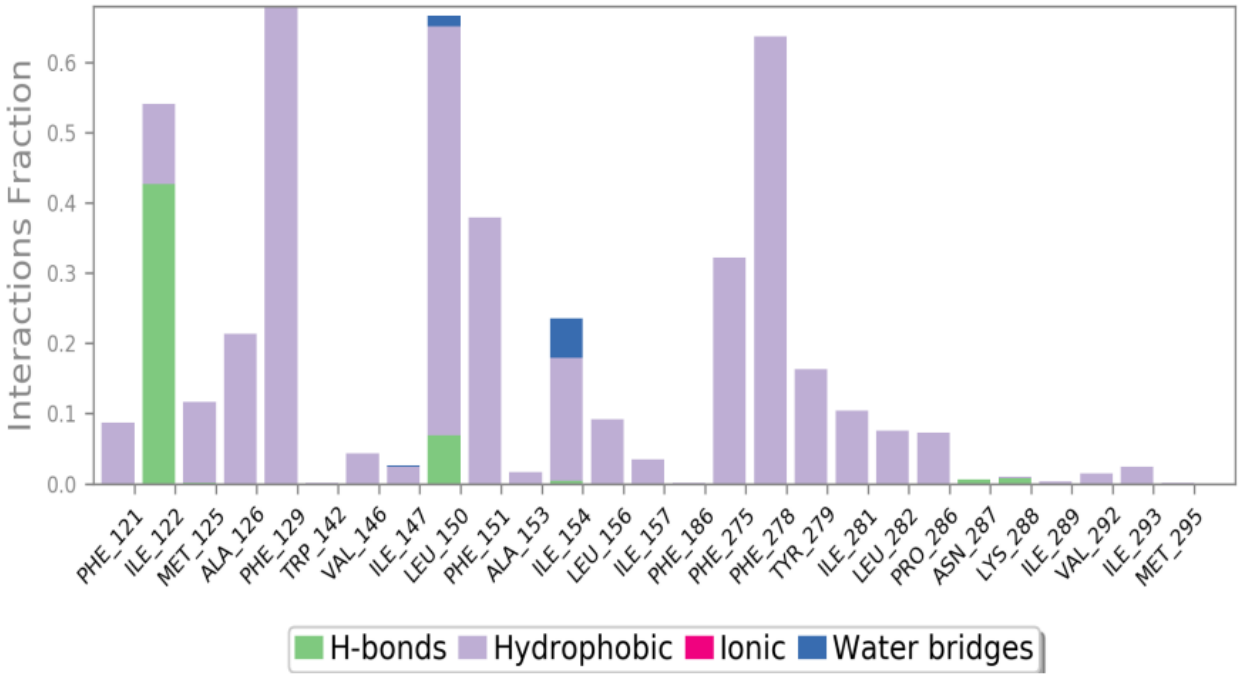


Ligand-Protein Contacts

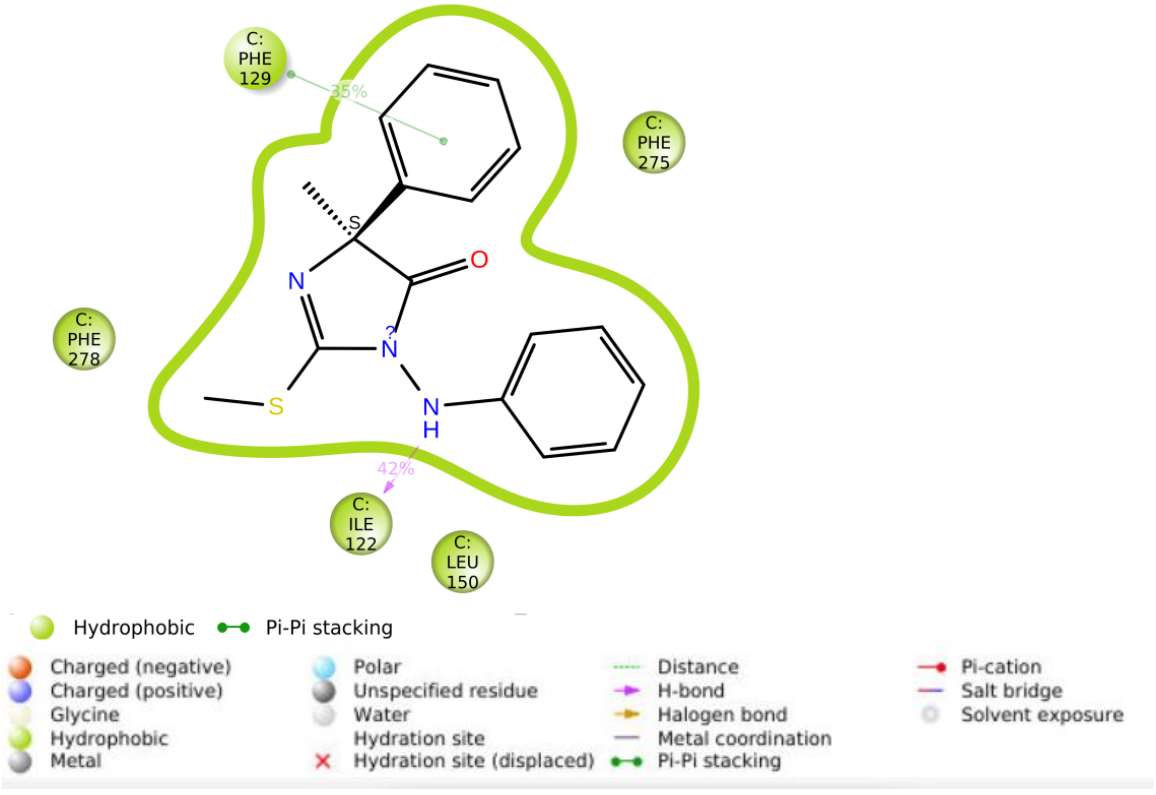


B

Protein-Ligand Contacts

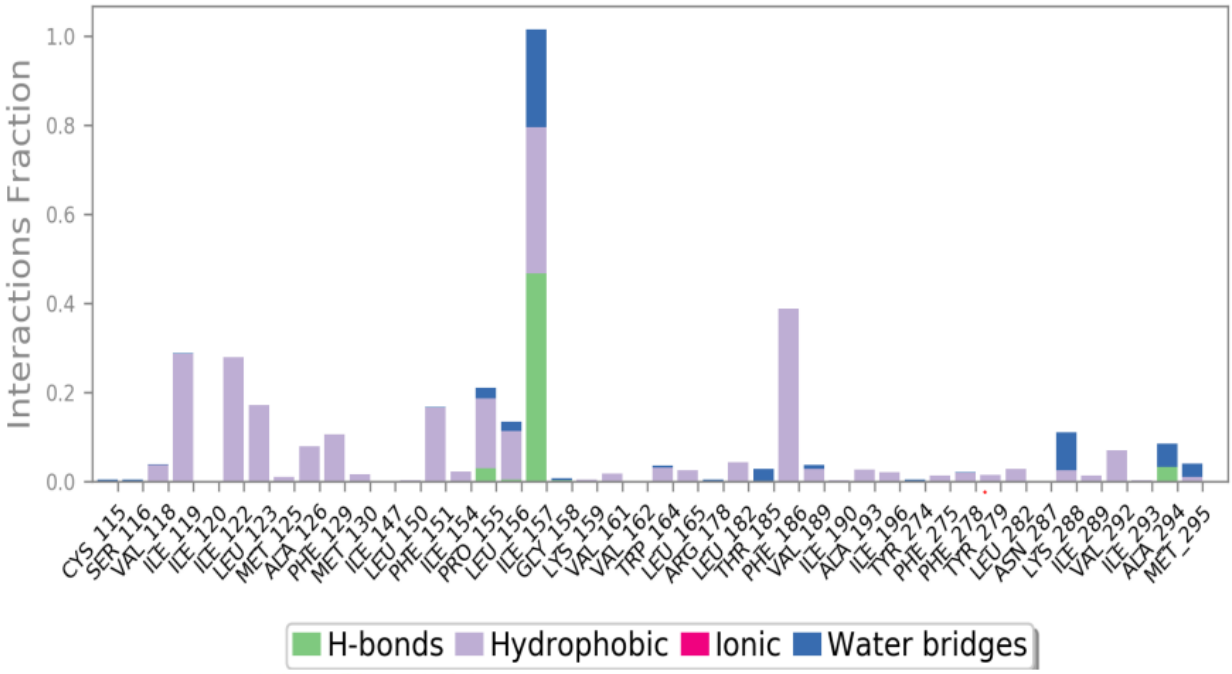


Ligand-Protein Contacts



C

Protein-Ligand Contacts



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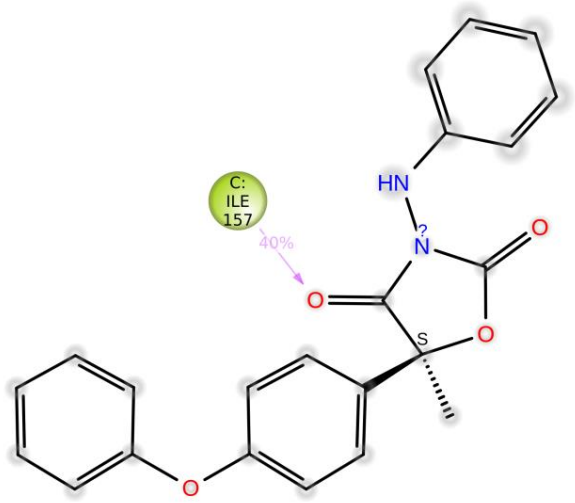
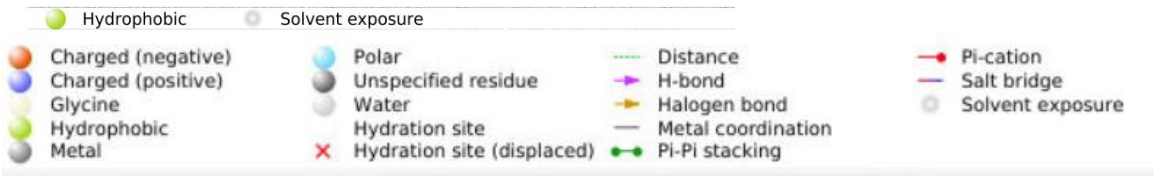
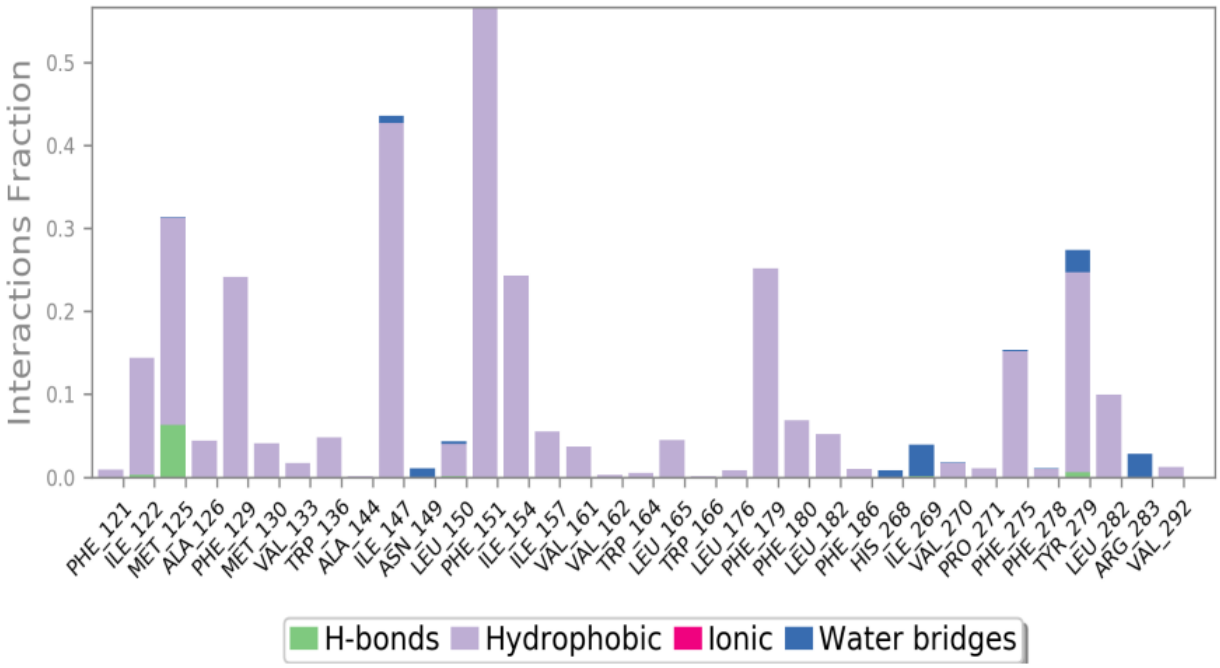


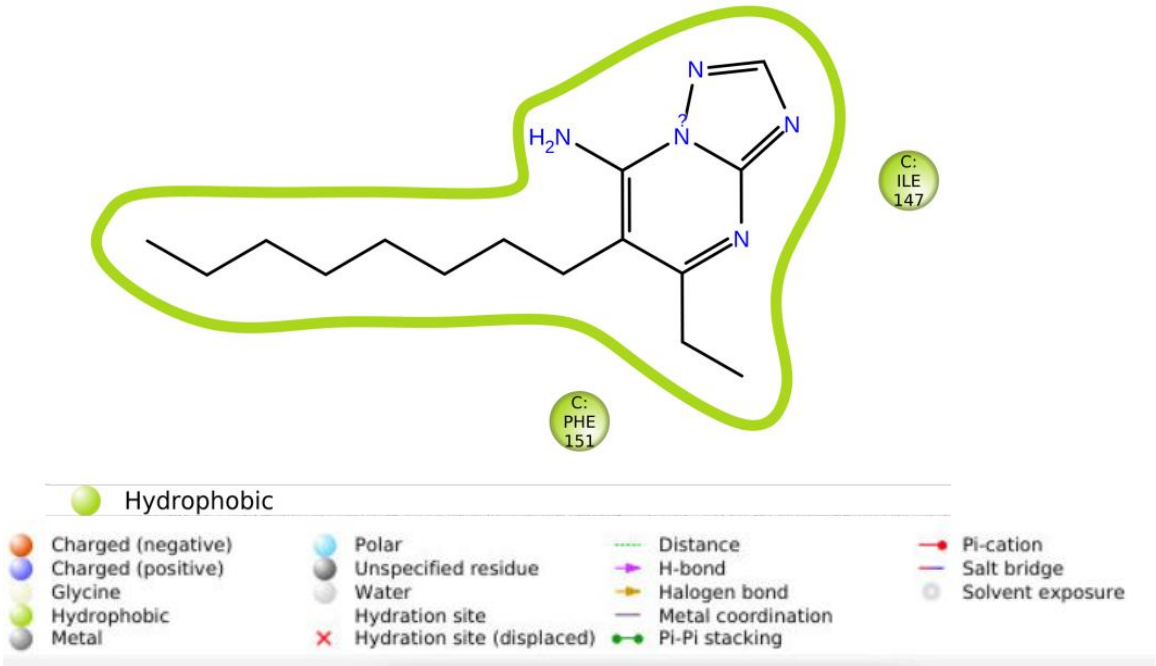


D

Protein-Ligand Contacts



Ligand-Protein Contacts



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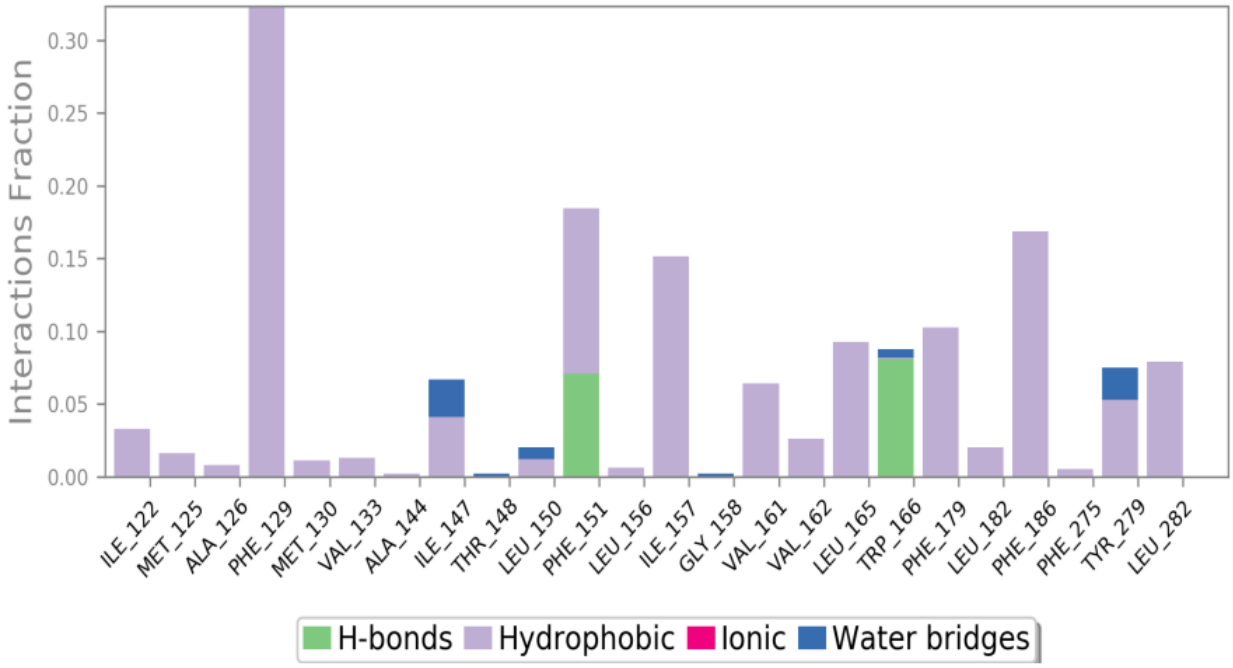
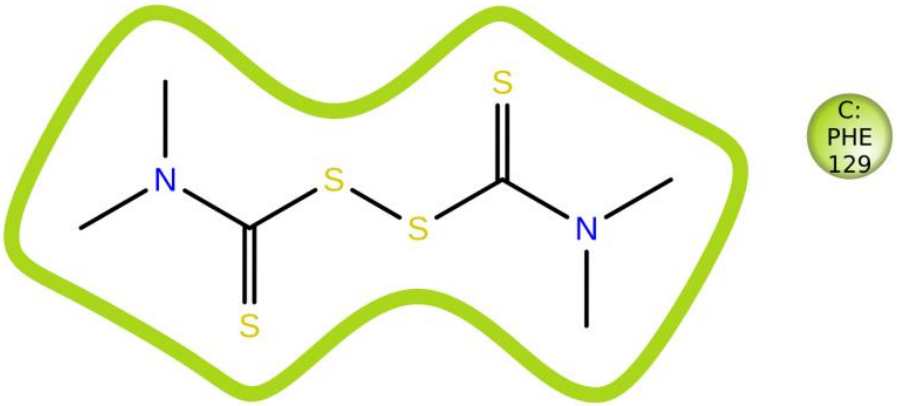
Protein-Ligand Contacts



Ligand-Protein Contacts



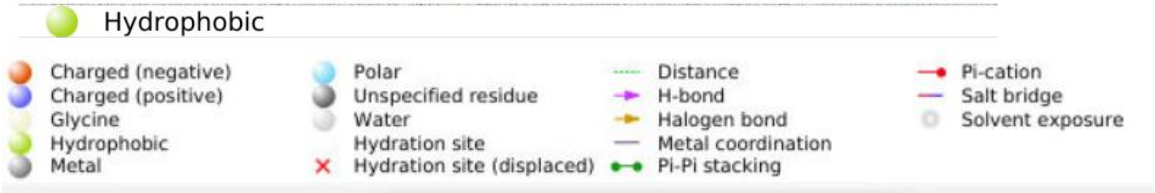
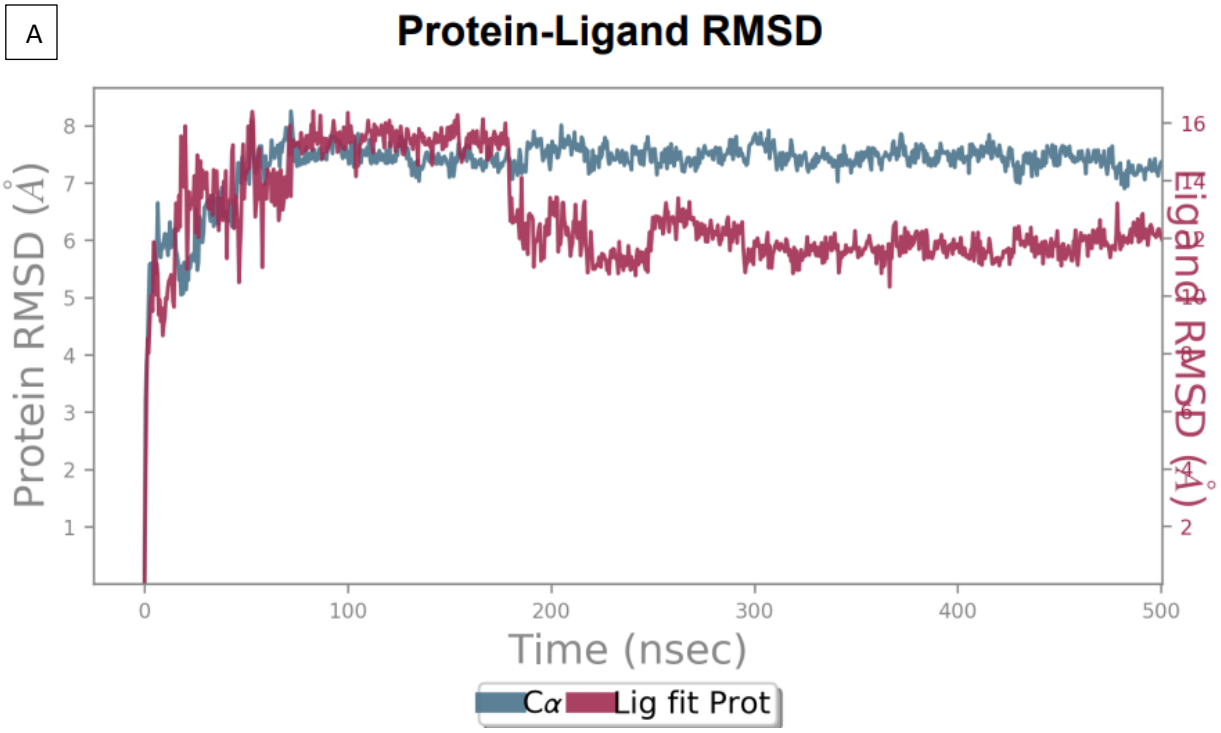


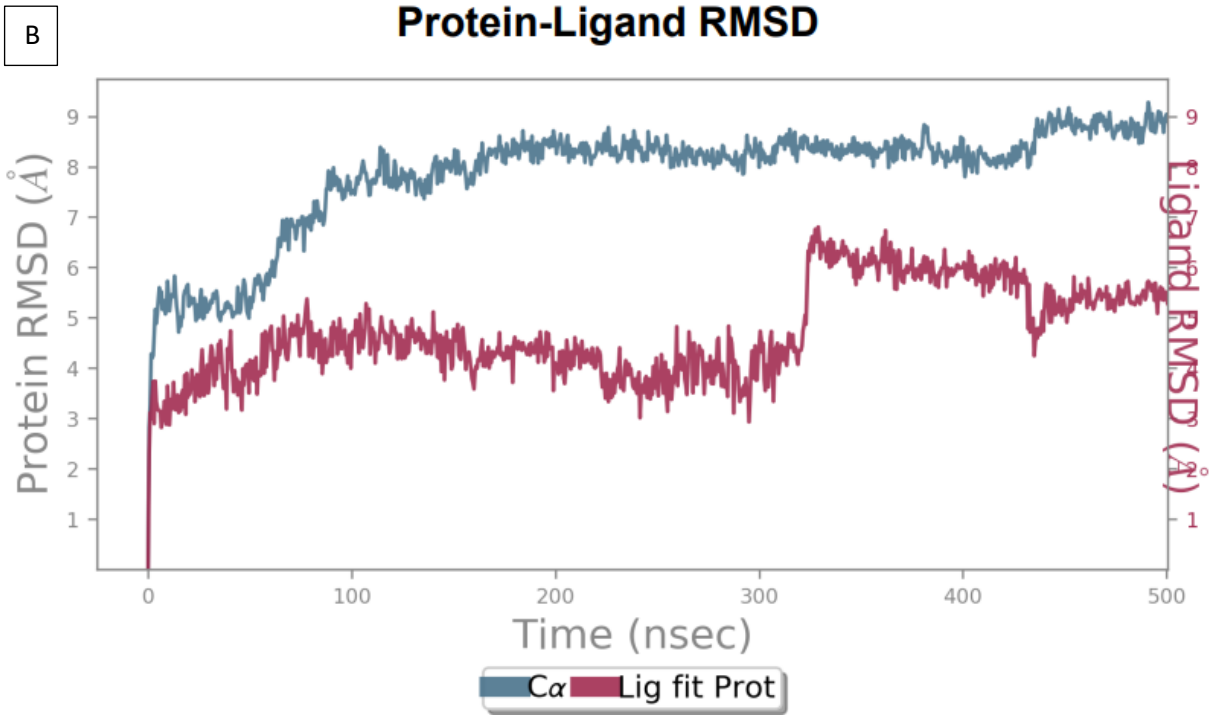
Figure 3. Protein-Ligand Interaction and contact toward active sites L129 and G143. A) Ubiquinol with Cytochrome b; B) Fenamidone with Cytochrome b; C) Famoxadone with Cytochrome b; D) Ametoctradin with Cytochrome b; E) Thiram with Cytochrome b. The region started from 79 to 296.

Hydrophobic binding interaction was the main type of binding relationship between ligands and Cytochrome b. Ubiquinol showed multiple strong hydrophobic binding affinity with Cytochrome b at PHE121, PHE151 and ILE154. Fenamidone had strong hydrophobic binding at PHE129 and LEU150 and PHE278; it also had strong hydrogen binding at ILE122. Famoxadone only had strong binding affinity at ILE157, including hydrogen bond, hydrophobic binding and water bridge. Amectoctradin had strong hydrophobic binding affinity at ILE147 and PHE151. Thiram showed strong hydrophobic binding affinity at PHE129.

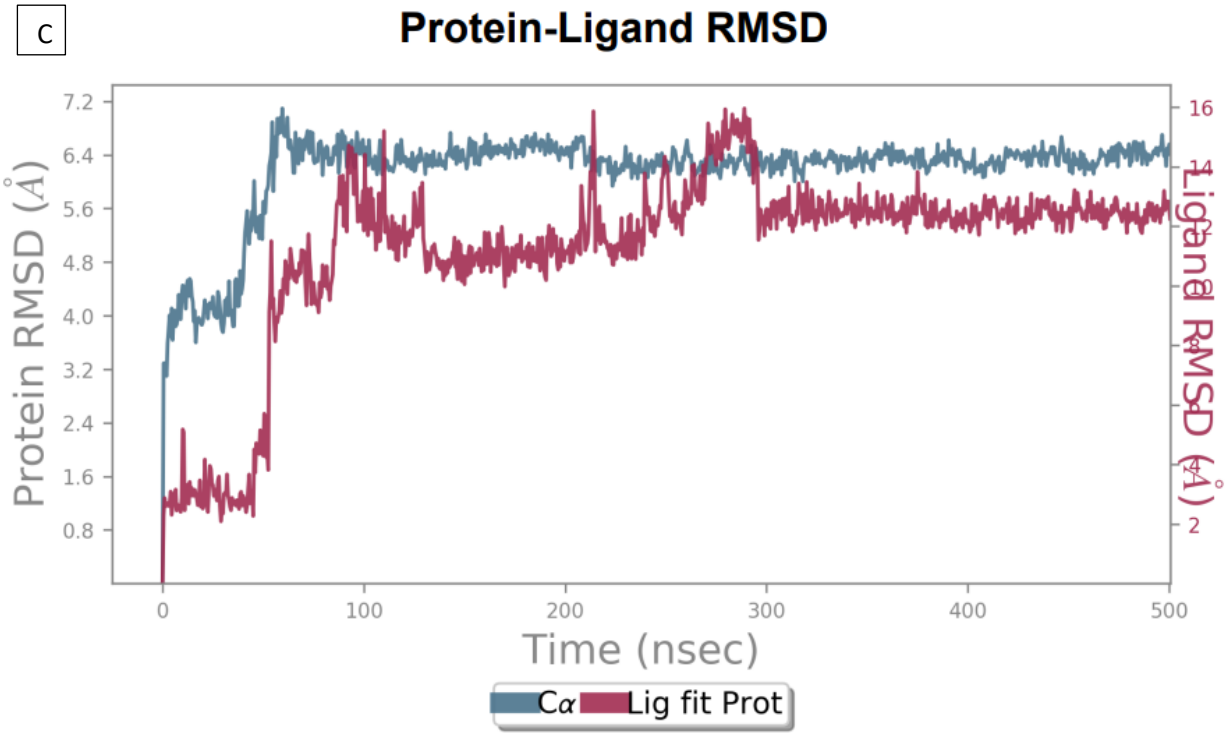
A


Protein-Ligand RMSD
Protein RMSD (Å)
Ligand RMSD (Å)
Time (nsec)
Cα
Lig fit Prot

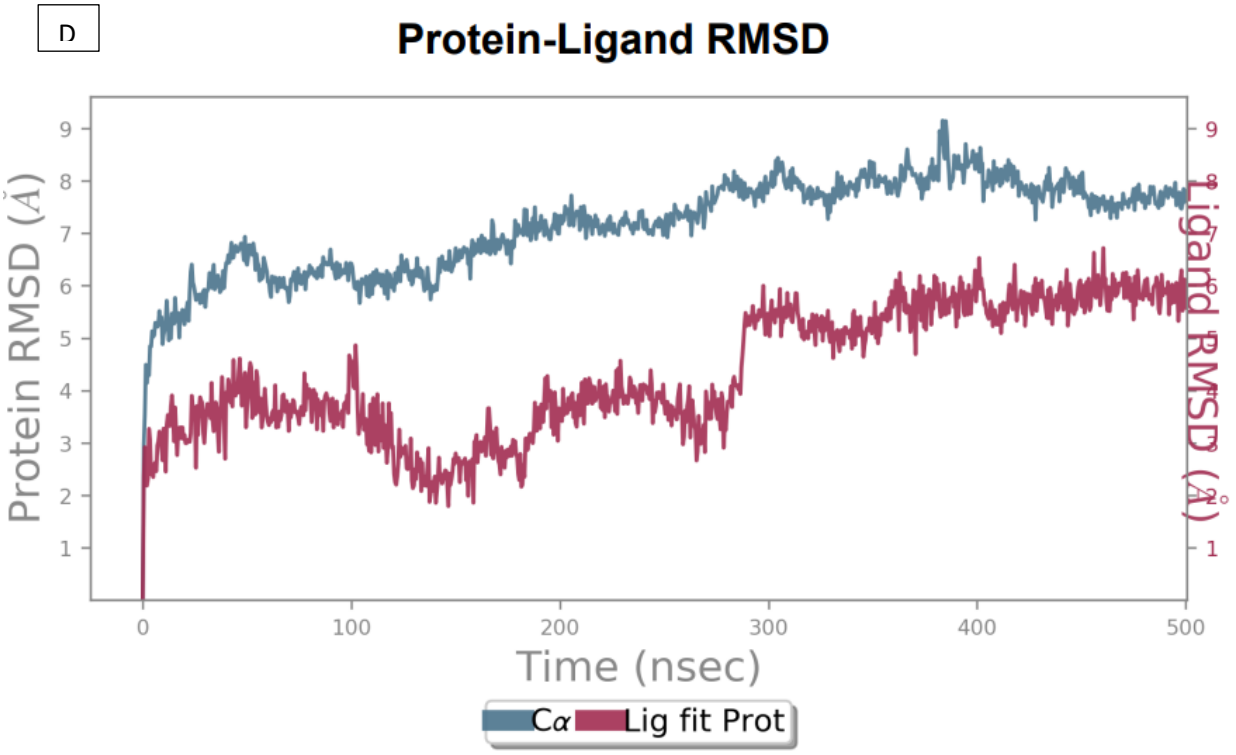
B


Protein-Ligand RMSD
Protein RMSD (Å)
Ligand RMSD (Å)
Time (nsec)
Cα
Lig fit Prot

C


Protein-Ligand RMSD
Protein RMSD (Å)
Ligand RMSD (Å)
Time (nsec)
Cα
Lig fit Prot

D


Protein-Ligand RMSD
Protein RMSD (Å)
Ligand RMSD (Å)
Time (nsec)
Cα
Lig fit Prot

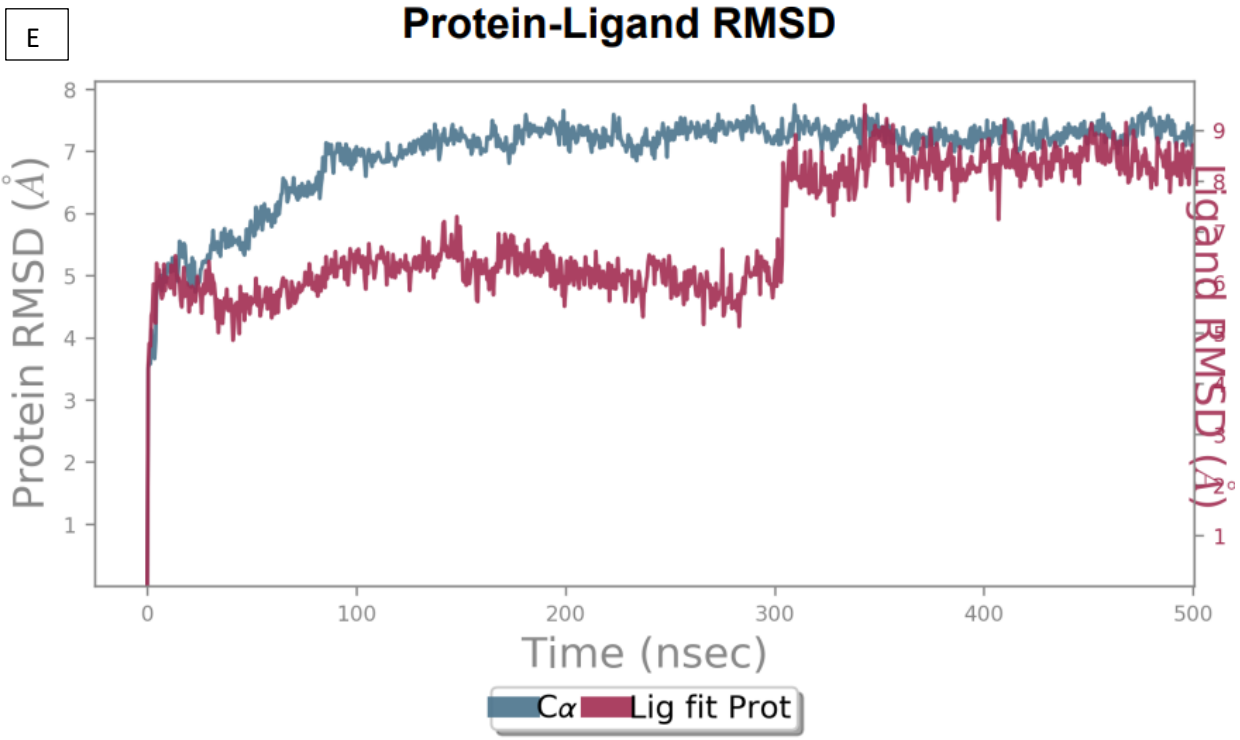
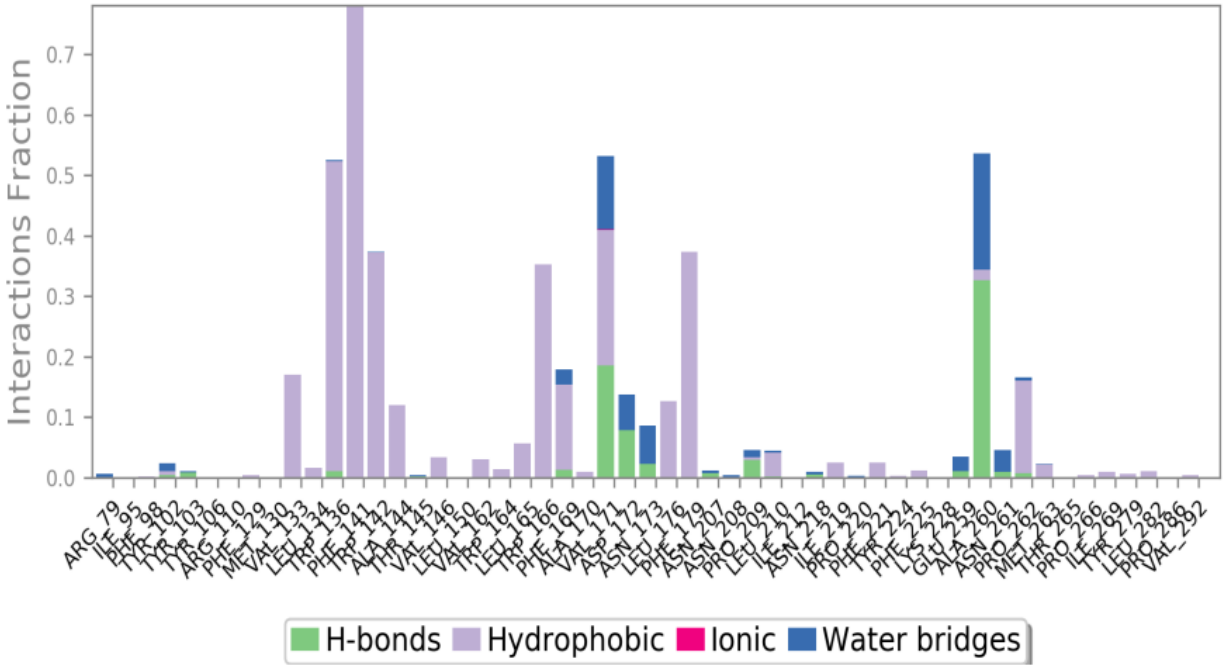


Figure 4. Protein and ligand RMSD for the trajectory of each system active sites L129 and G143. Protein RMSD is shown in dark blue and Ligand RMSD is shown in red. A) Ubiquinol with Cytochrome b; B) Fenamidone with Cytochrome b; C) Famoxadone with Cytochrome b; D) Ametoctradin with Cytochrome b; E) Thiram with Cytochrome b. The region started from 79 to 296.

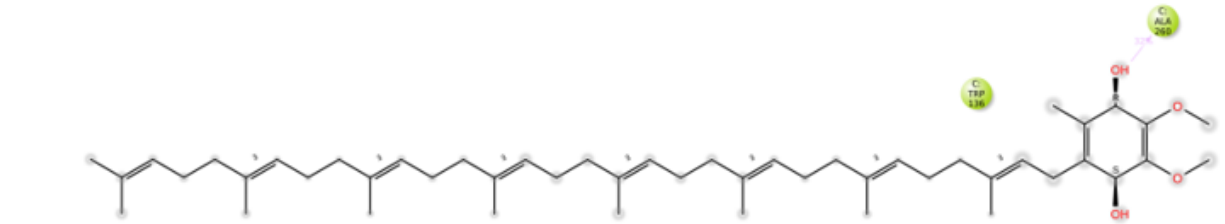
Protein simulation of Cytochrome b was equilibrated with Ubiquinol and Ubiquinol stably bound to the protein. Protein simulations of Cytochrome b for Fenamidone and Famoxadone were equilibrated after 100 ns and Fenamidone bounded more closer to the protein than Famoxadone. Protein simulations of Cytochrome b for Ametoctradin and Thiram was equilibrated and Ametoctradin bound stably to the protein. According to Protein-Ligand contact and RMSD valur, Fenamidone, Famoxadone, Ametoctradin and Thiram showed stable binding interactions with Cytochrome b for wild version.

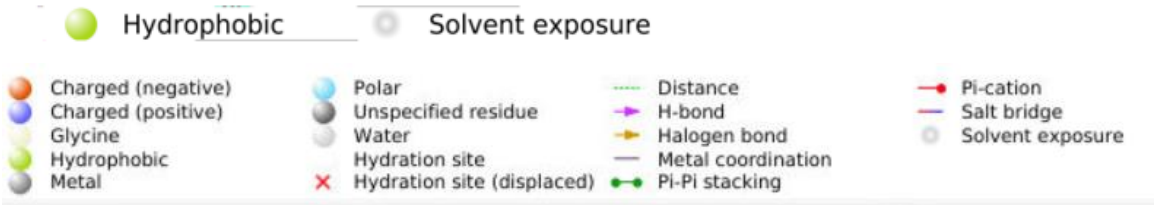
A

Protein-Ligand Contacts



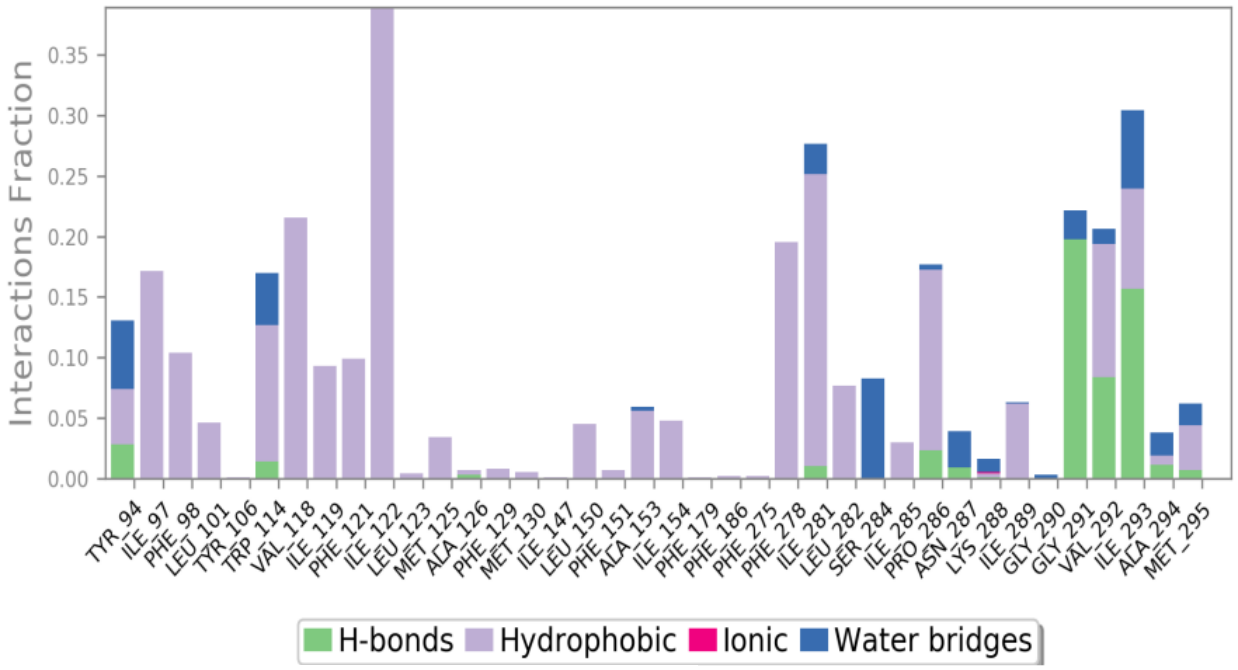
Ligand-Protein Contacts



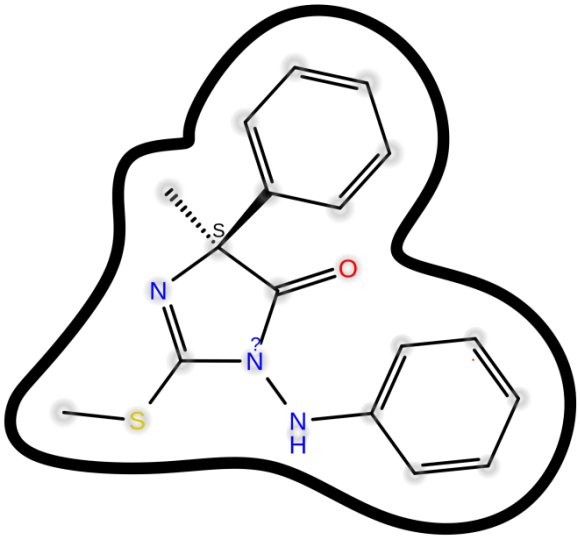


B

Protein-Ligand Contacts

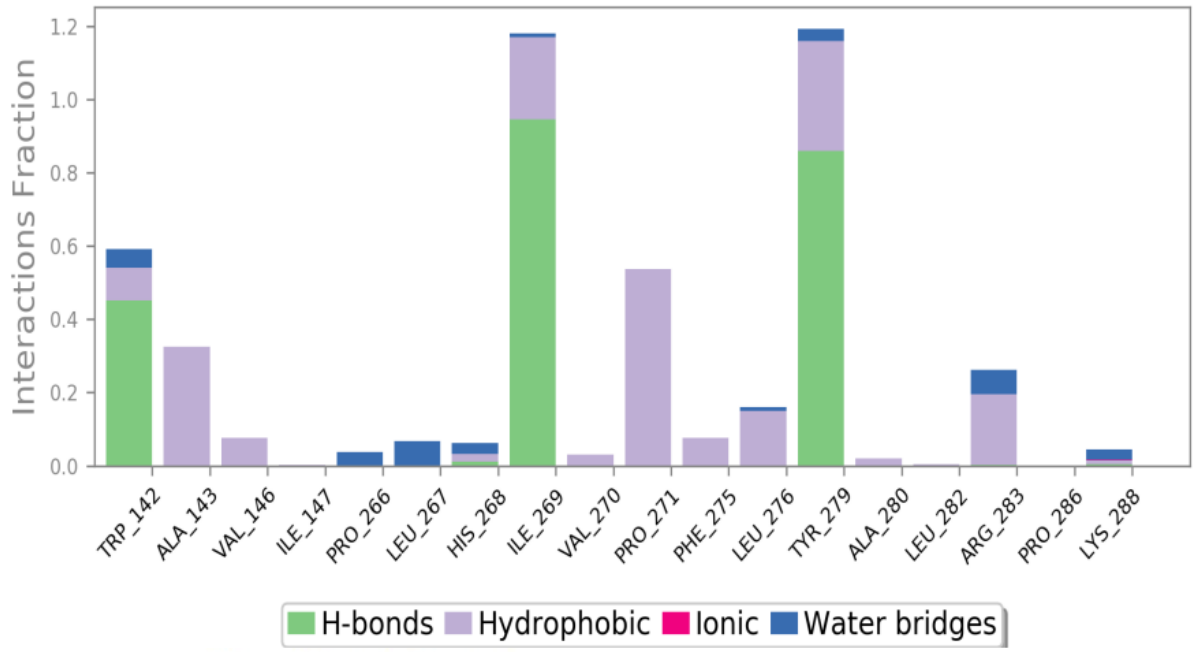


Ligand-Protein Contacts

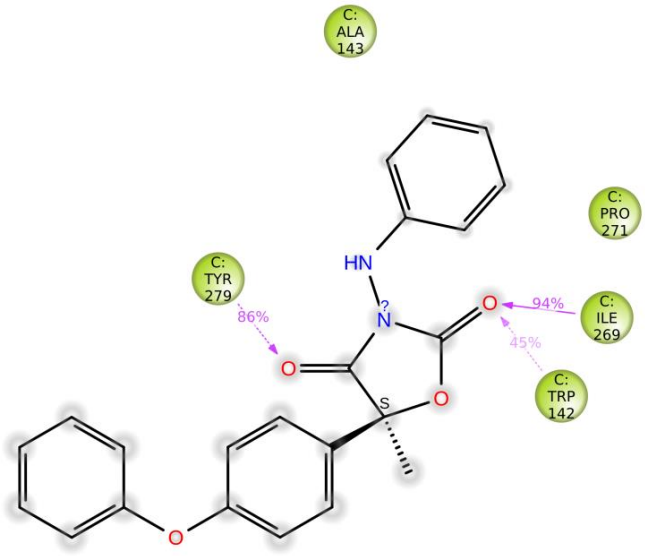


C

Protein-Ligand Contacts

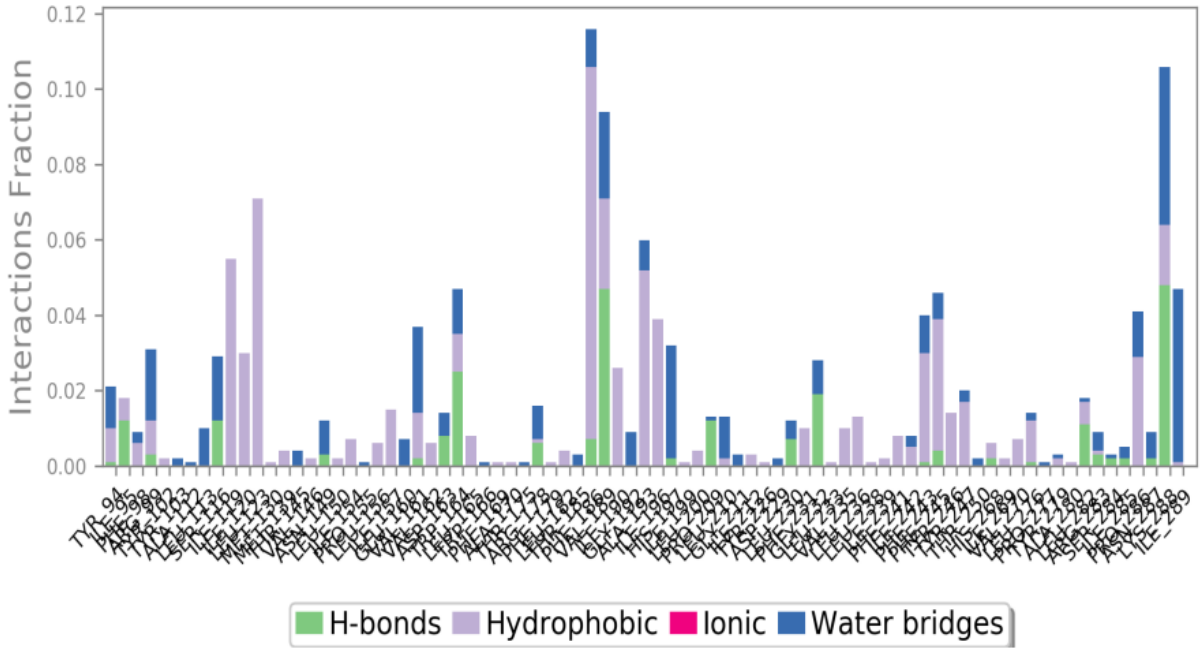


Ligand-Protein Contacts

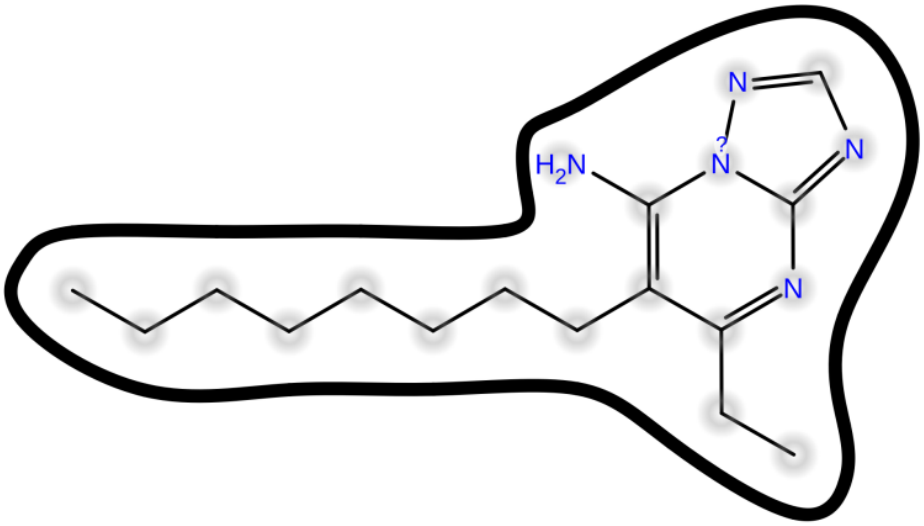


D

Protein-Ligand Contacts

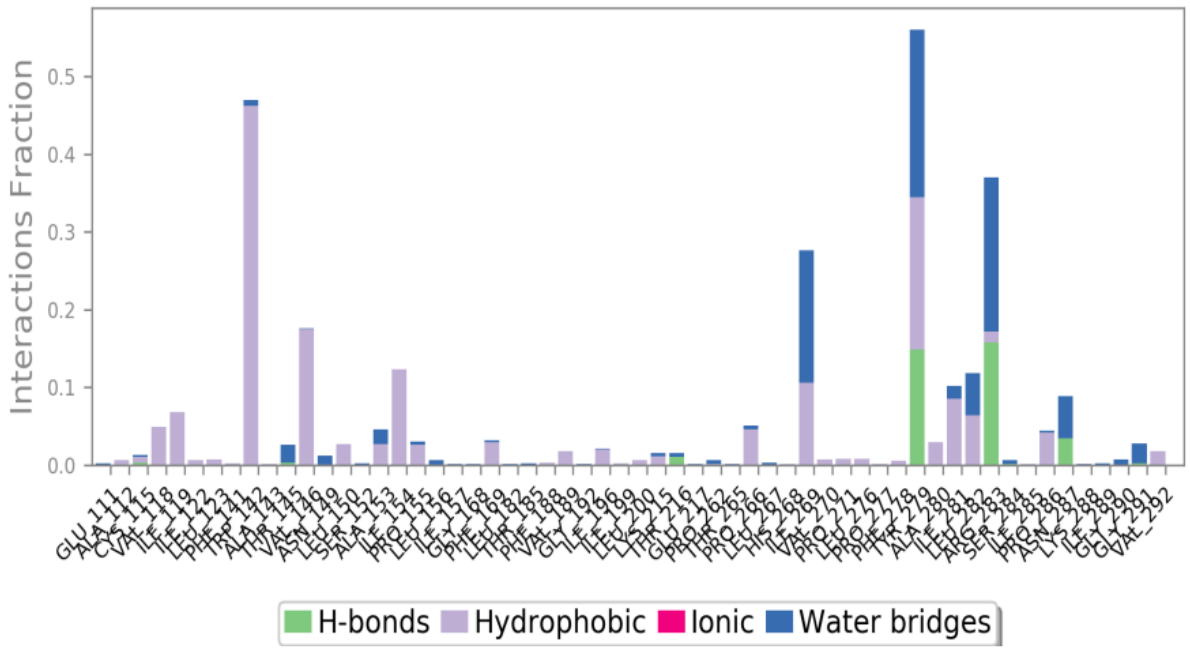


Ligand-Protein Contacts

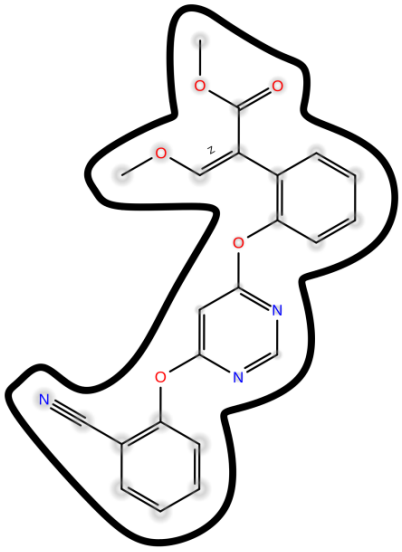


E

Protein-Ligand Contacts

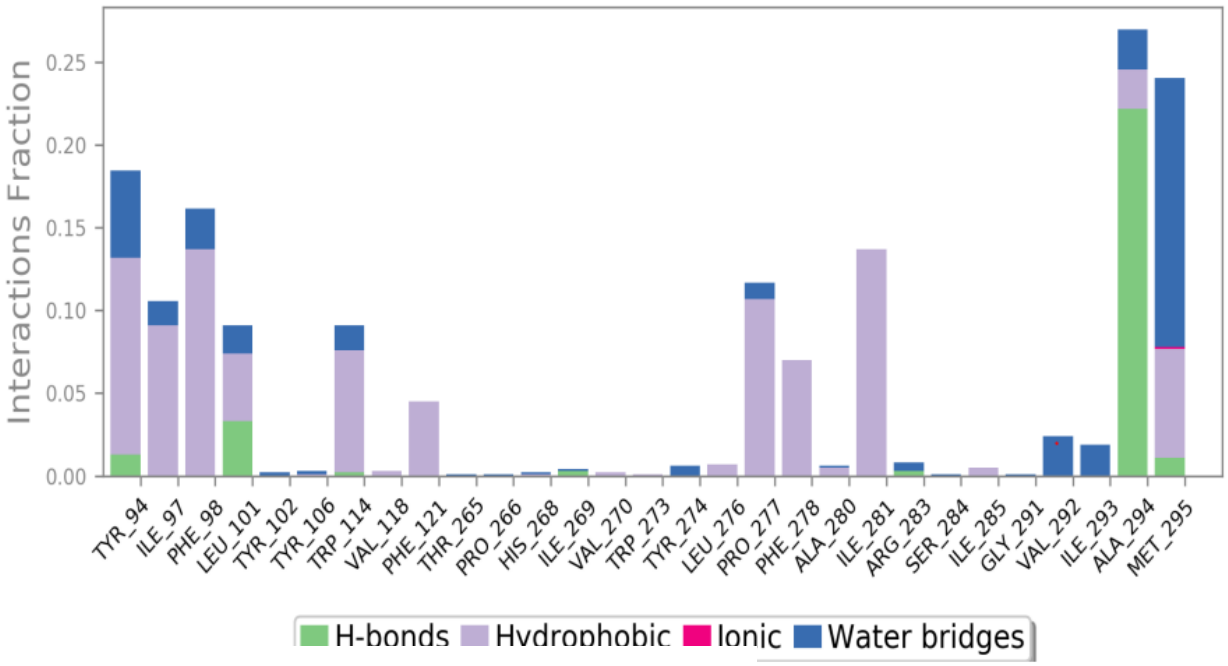


Ligand-Protein Contacts

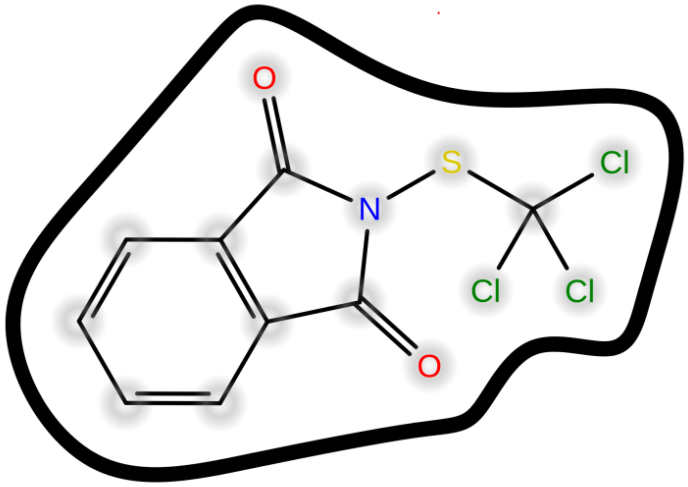


F

Protein-Ligand Contacts



Ligand-Protein Contacts



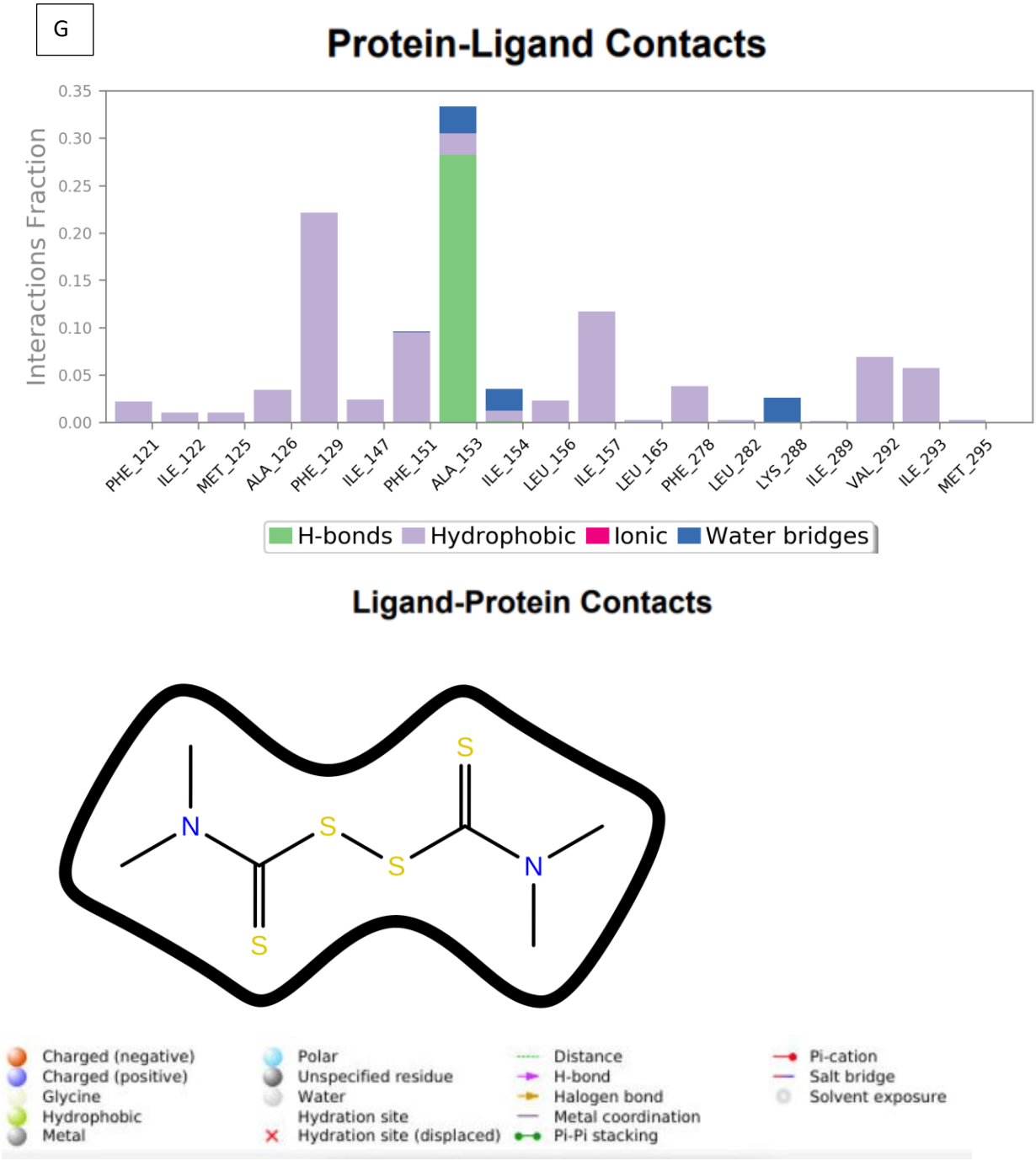
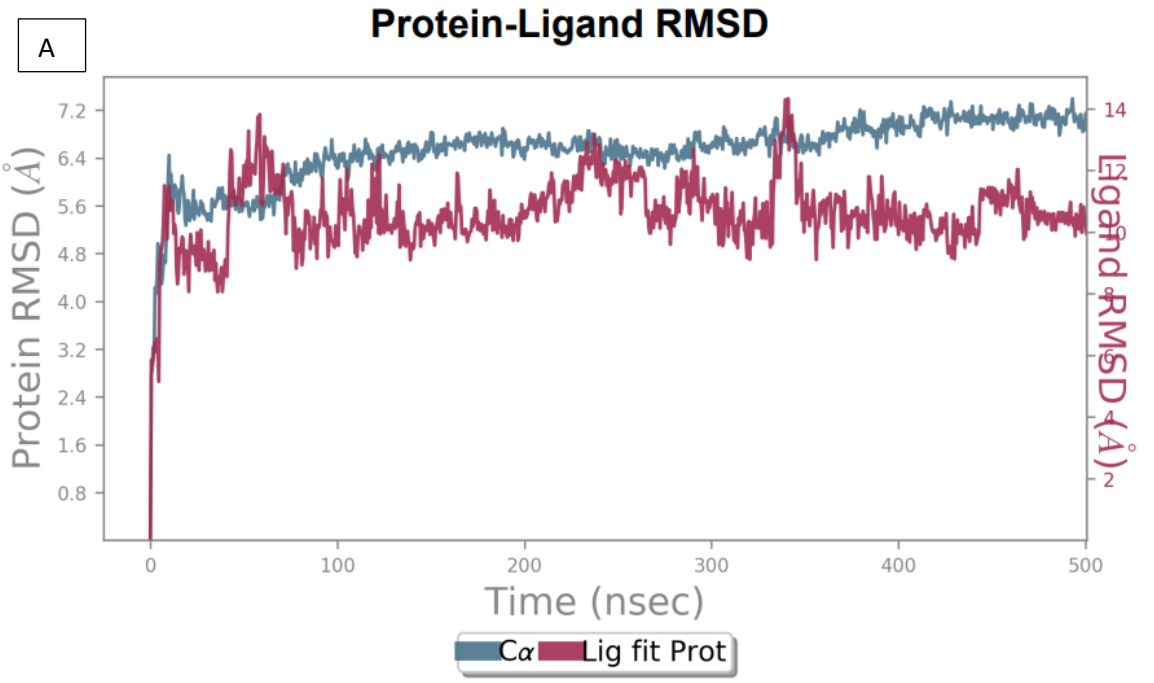
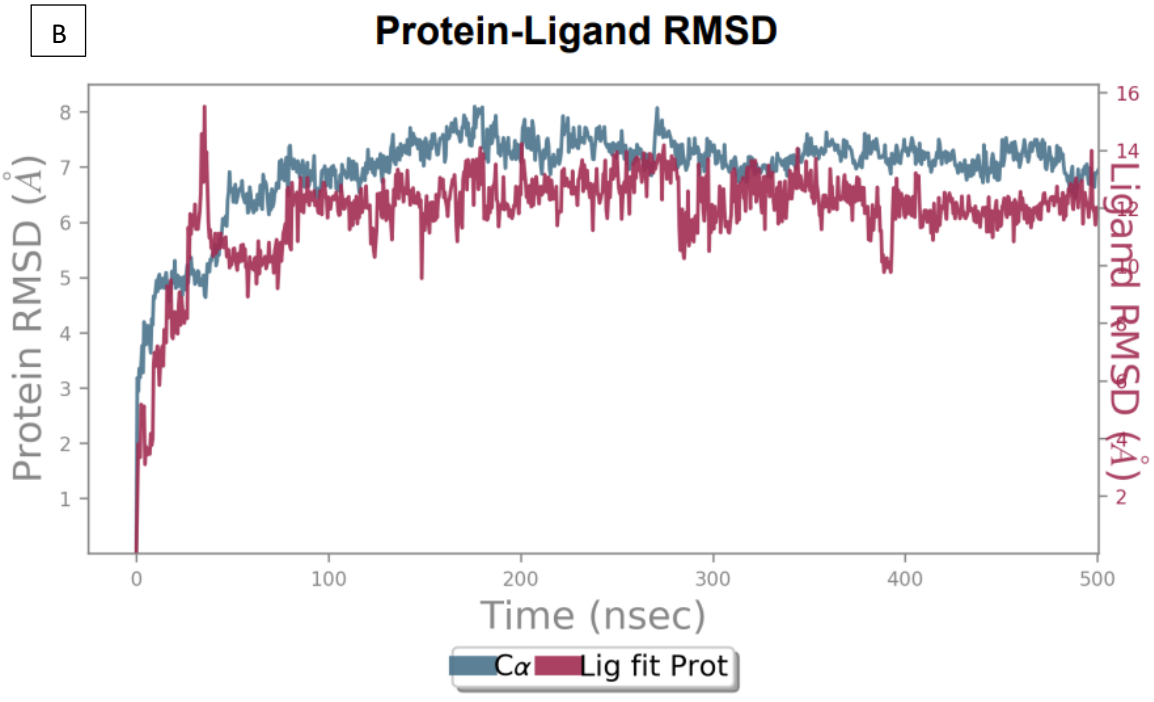


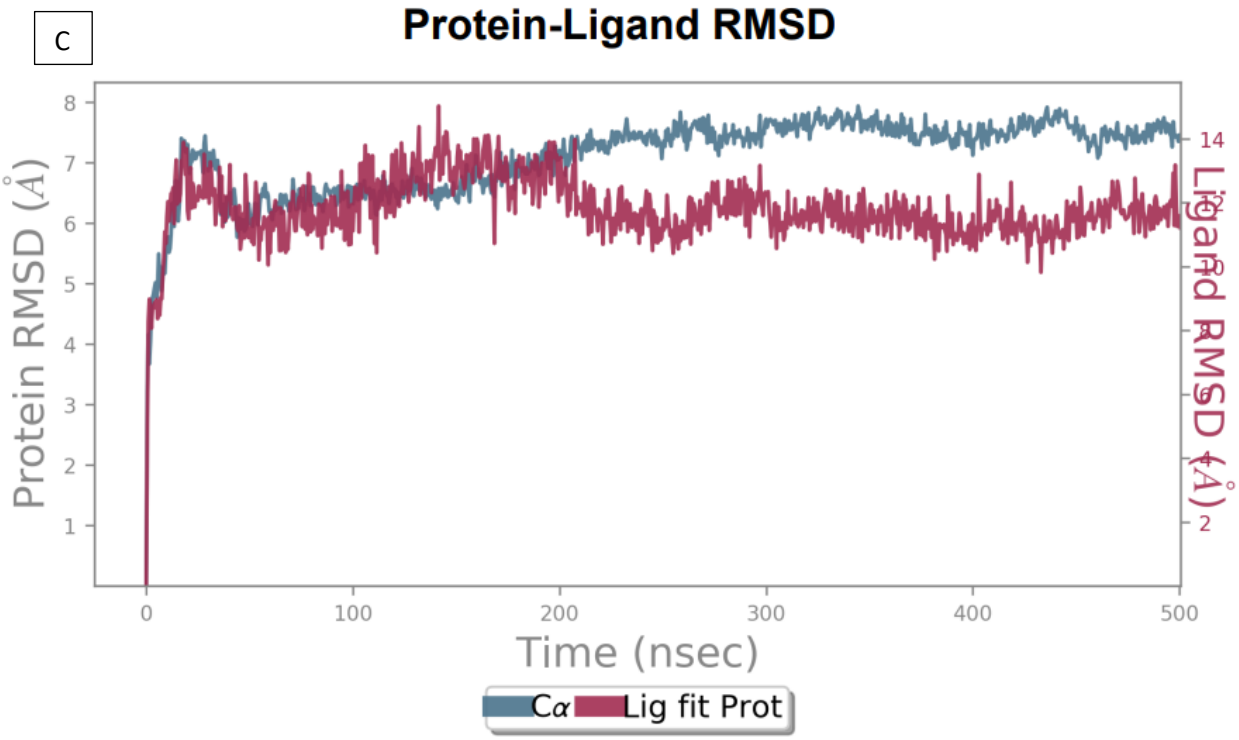
Figure 5. Protein-Ligand Interaction and contact toward active sites L129 and G143A mutated version. A) Ubiquinol with Cytochrome b; B) Fenamidone with Cytochrome b; C) Famoxadone with Cytochrome b; D) Ametoctradin with Cytochrome b; E) Azoxystrobin with Cytochrome b F) Folpet with Cytochrome b; G) Thiram with Cytochrome b. The region started from 79 to 296.

There were strong hydrophobic binding interaction and hydrogen bond at PHE141 and ALA260 for Ubiquinol. Fenamidone showed strong hydrophobic binding interaction toward ILE122 and ILE281; it also had hydrogen bonding at GLY291. Famoxadone showed very strong hydrogen bonding at ILE269 and TRY279. Ametoctradin had multiply weak interaction toward the protein. Azoxystrobin had strong hydrophobic binding interaction at TRP142 and multiply interaction TRY279. Folpet had hydrogen bonding at ALA294 and water bridge at MET295. Thiram showed hydrogen bonding at ALA153 and hydrophobic binding interaction at PHE129.

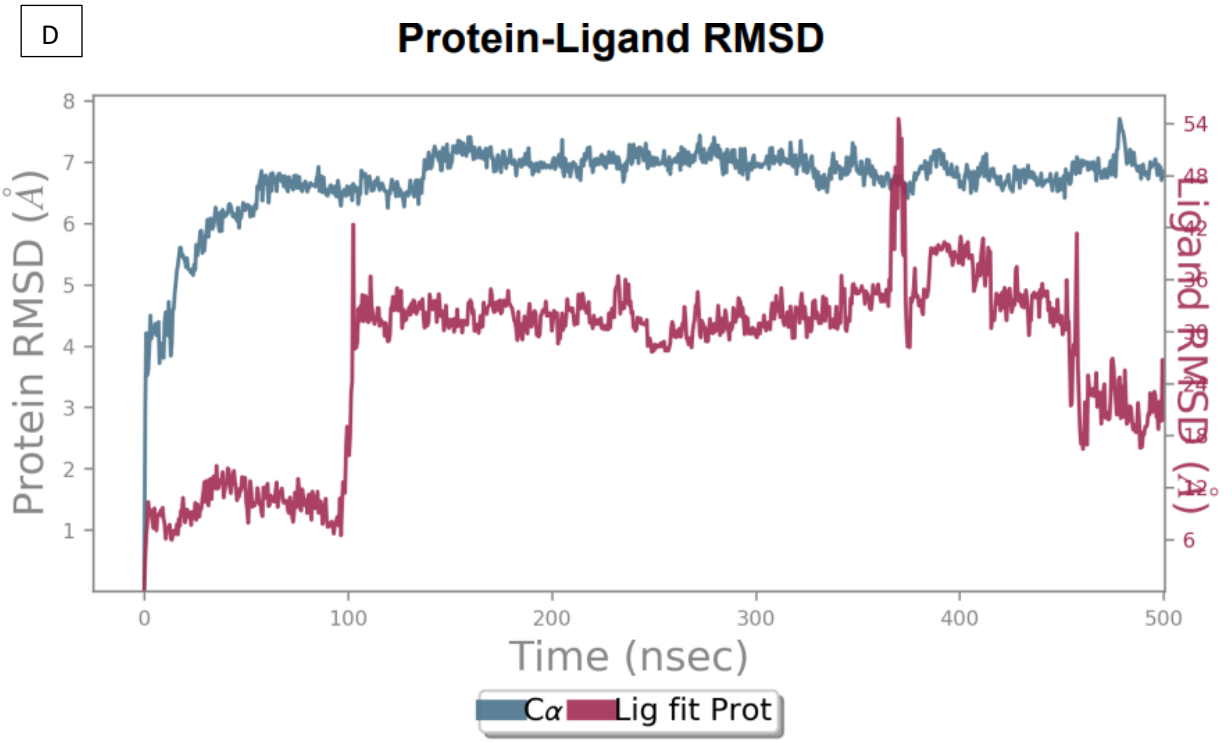




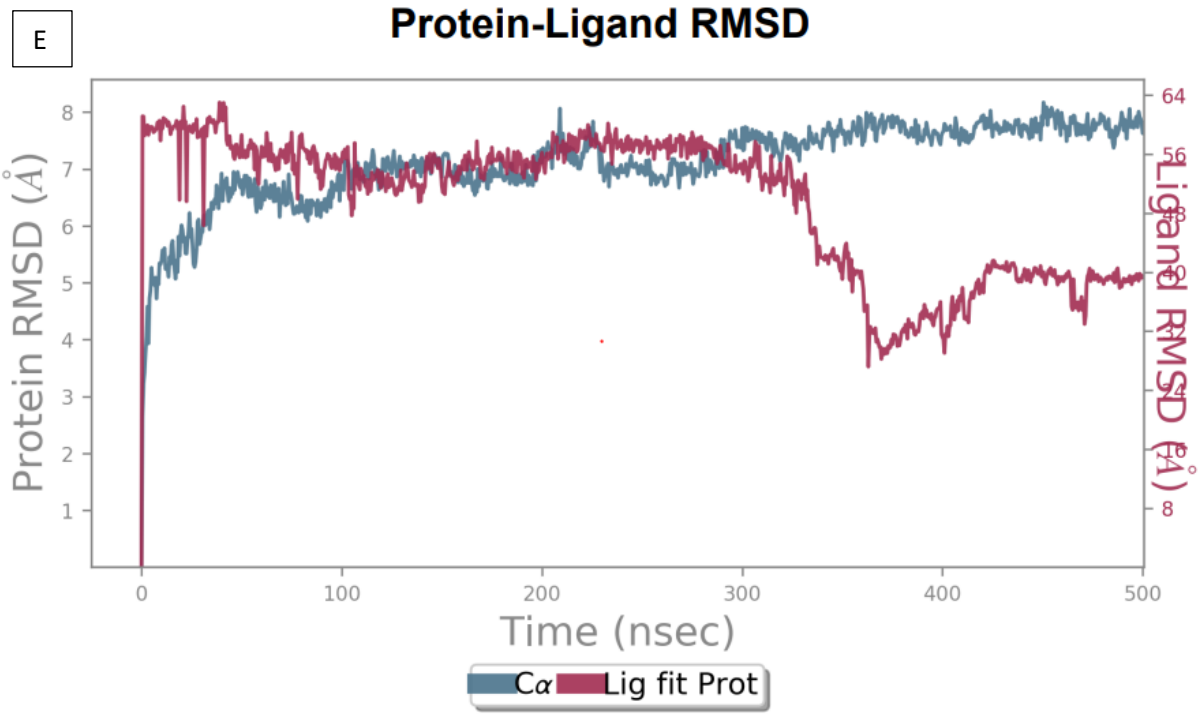
C


Protein-Ligand RMSD
Protein RMSD (Å)
Ligand RMSD (Å)
Time (nsec)
Cα
Lig fit Prot

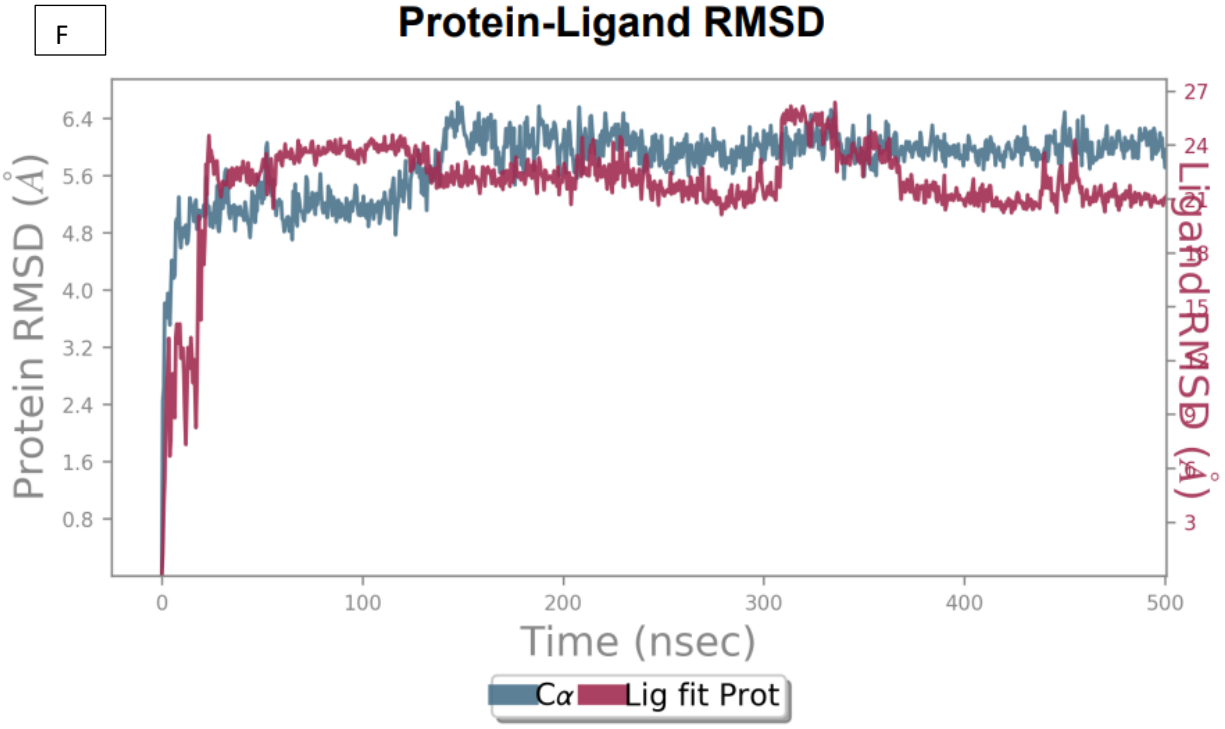
D


Protein-Ligand RMSD
Protein RMSD (Å)
Ligand RMSD (Å)
Time (nsec)
Cα
Lig fit Prot

E


Protein-Ligand RMSD
Protein RMSD (Å)
Ligand RMSD (Å)
Time (nsec)
Cα
Lig fit Prot

F


Protein-Ligand RMSD
Protein RMSD (Å)
Ligand RMSD (Å)
Time (nsec)
Cα
Lig fit Prot

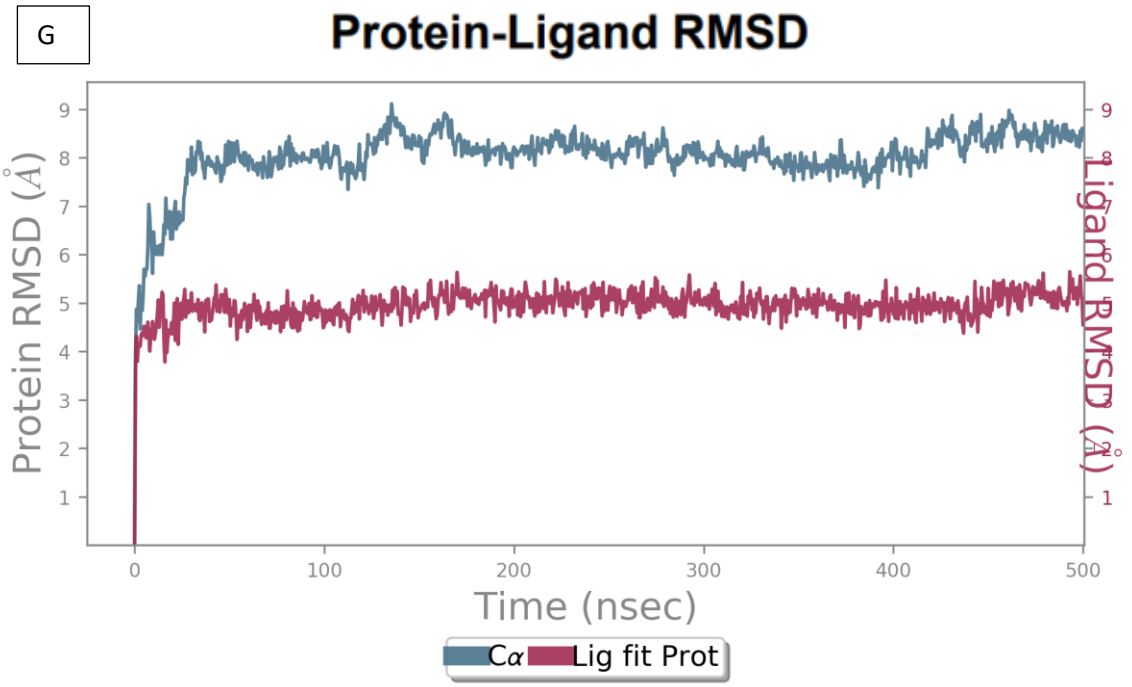
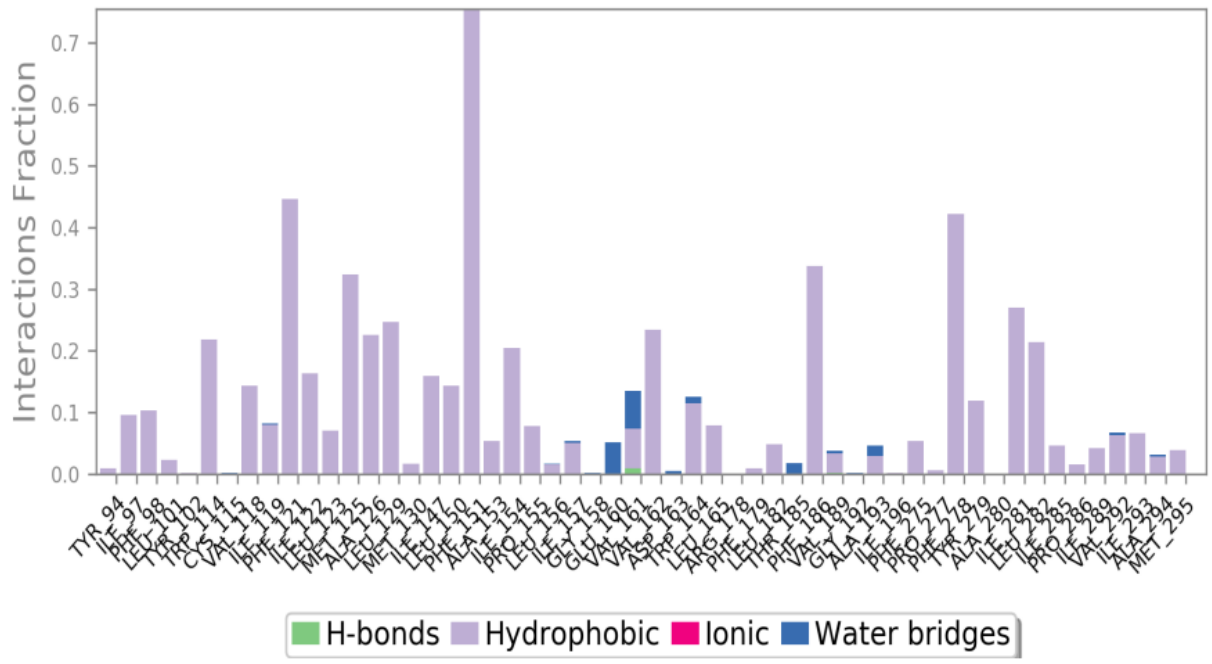


Figure 6. Protein and ligand RMSD (root mean square deviation) for the trajectory of each system toward F129 and G143A mutated version. Protein RMSD is shown in dark blue and Ligand RMSD is shown in red. A) Ubiquinol with Cytochrome b; B) Fenamidone with Cytochrome b; C) Famoxadone with Cytochrome b; D) Ametoctradin with Cytochrom b; E) Azoxystrobin with Cytochrome b; F) Folpet with Cytochrome b; G) Thiram with Cytochrome b.

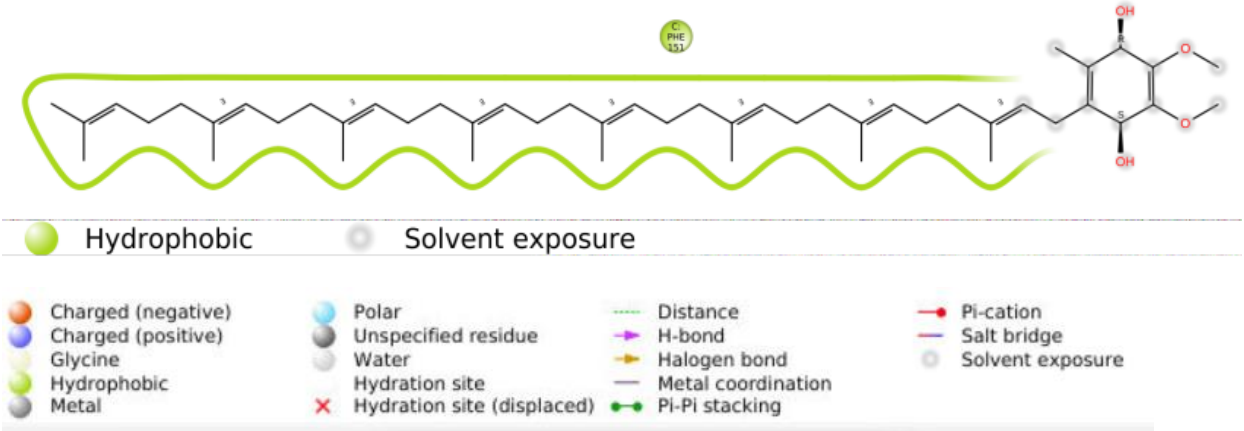
Protein simulation of Cytochrome b for Ubiquinol was equilibrated and Ubiquinol stably bound to the protein. Both protein simulations for Fenamidone and Famoxadone were equilibrated, and these two ligands were in same stable pattern with the protein. Protein simulation for Ametoctradin was equilibrated but Ametoctradin diffused away from the protein after 100 ns. Protein simulations for Azoxystrobin and Folpet were also equilibrated but both ligand RMSD values were significantly larger than the RMSD of protein. Protein simulation for Thiram was equilibrated and Thiram stably bound to the protein. Based on Protein-ligand contact and RMSD value, Fenamidone, Famoxadone and Thiram were suitable treatments for G143A mutated version.

A

Protein-Ligand Contacts

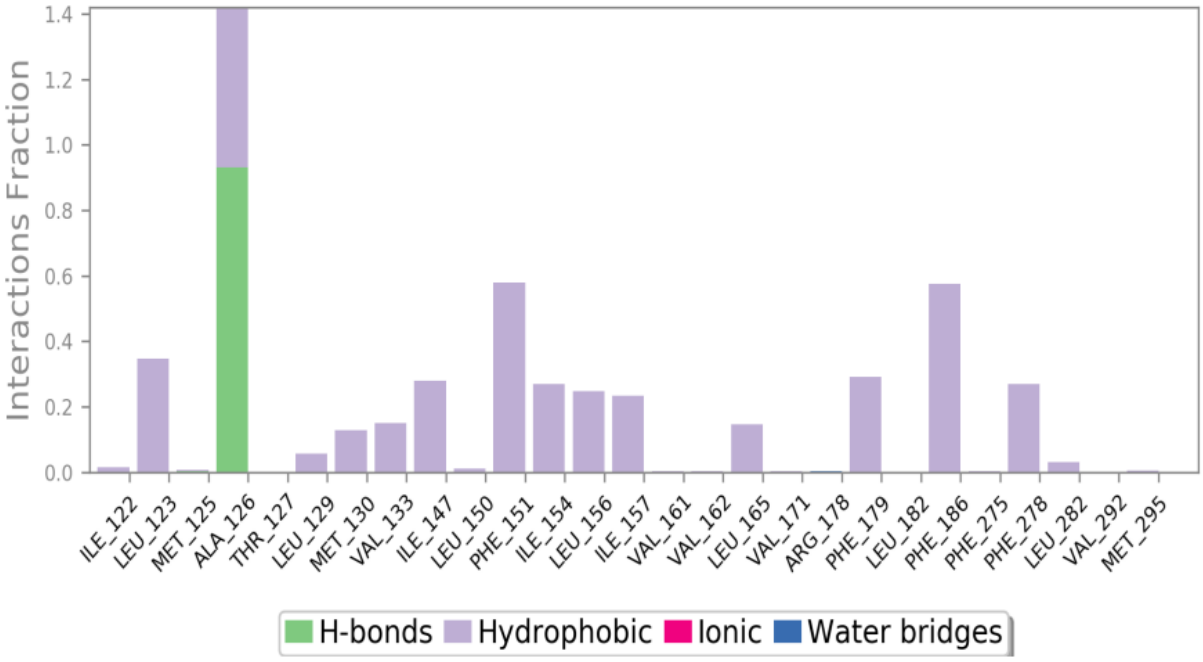


Ligand-Protein Contacts

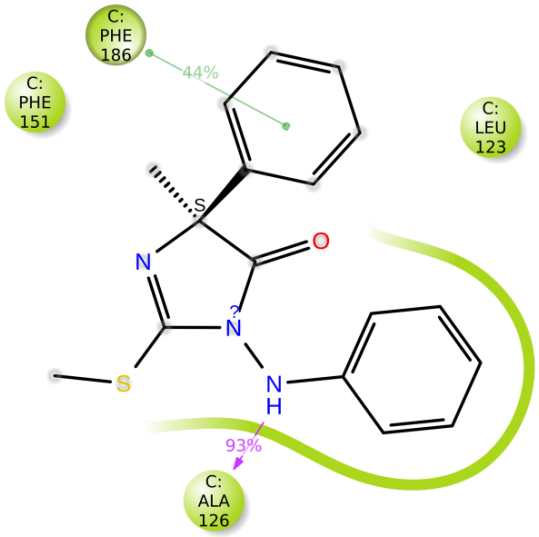


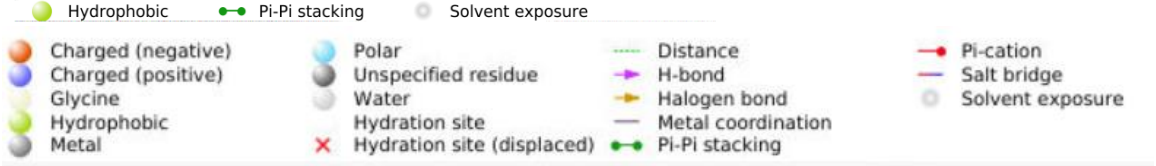
B

Protein-Ligand Contacts



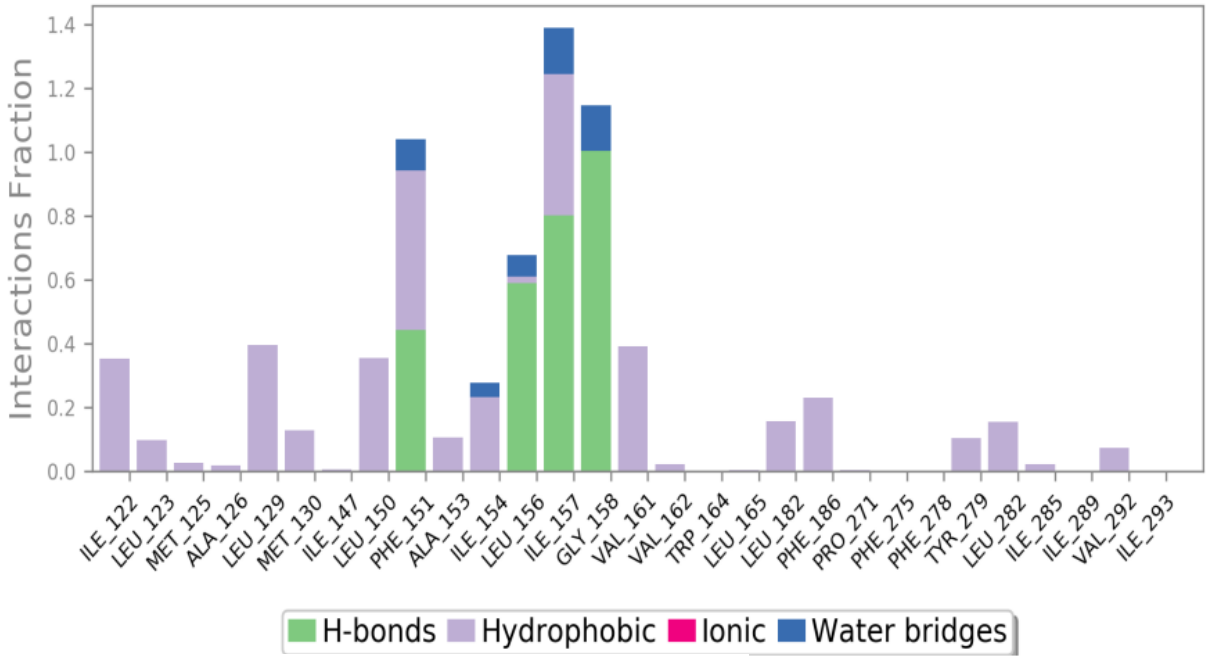
Ligand-Protein Contacts



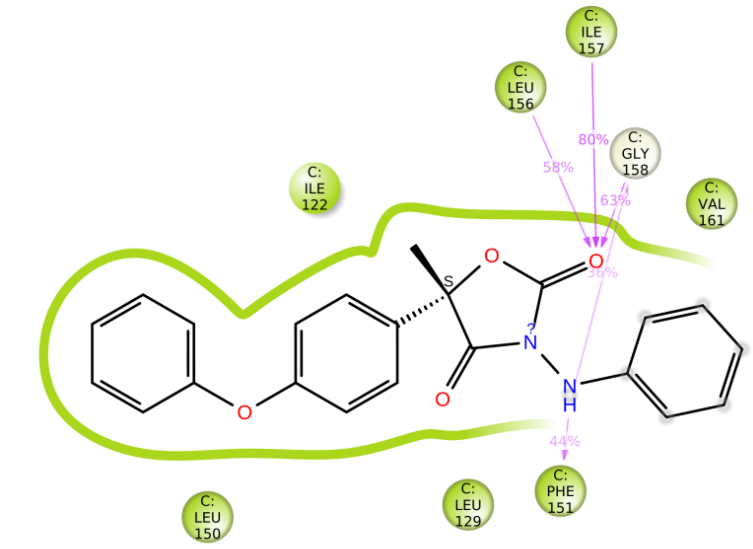


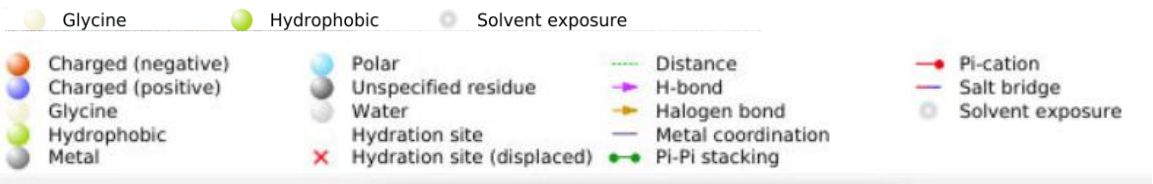
C

Protein-Ligand Contacts



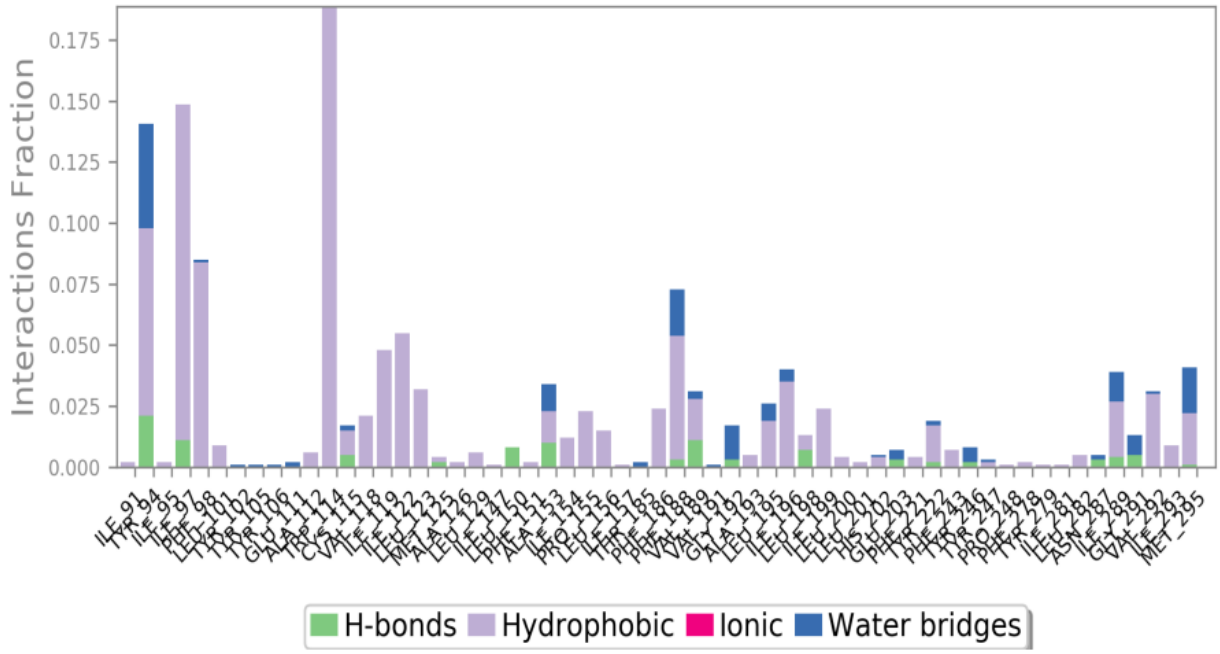
Ligand-Protein Contacts



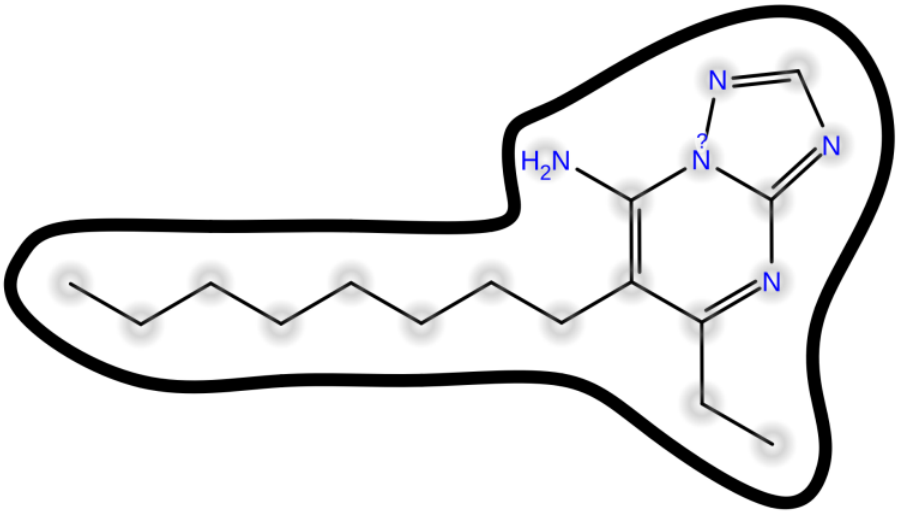


D

Protein-Ligand Contacts

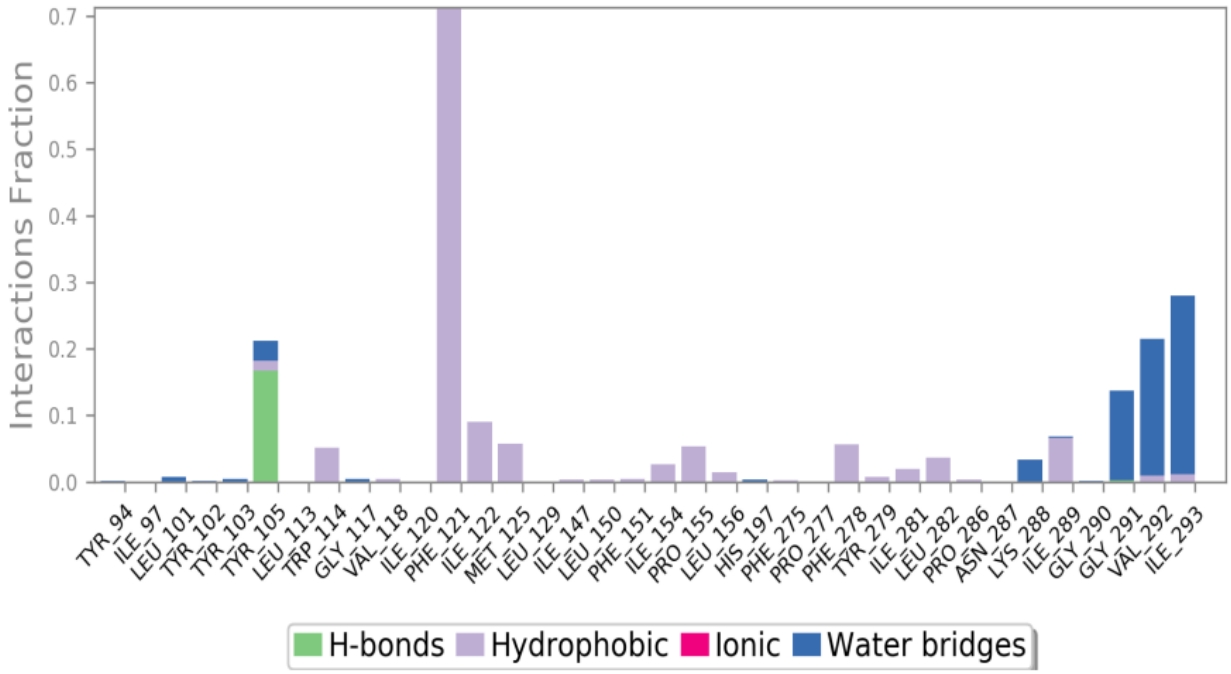


Ligand-Protein Contacts

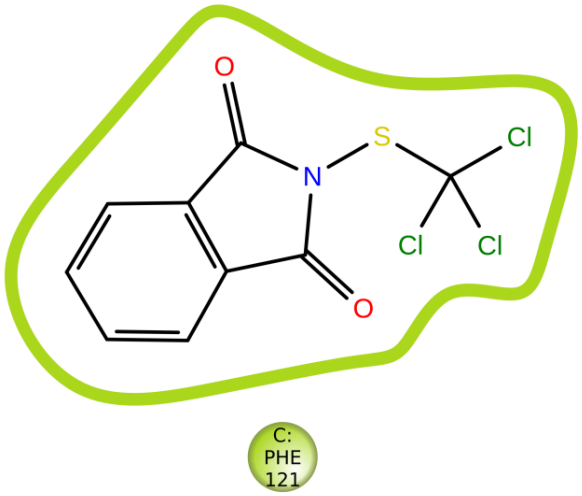


F

Protein-Ligand Contacts



Ligand-Protein Contacts



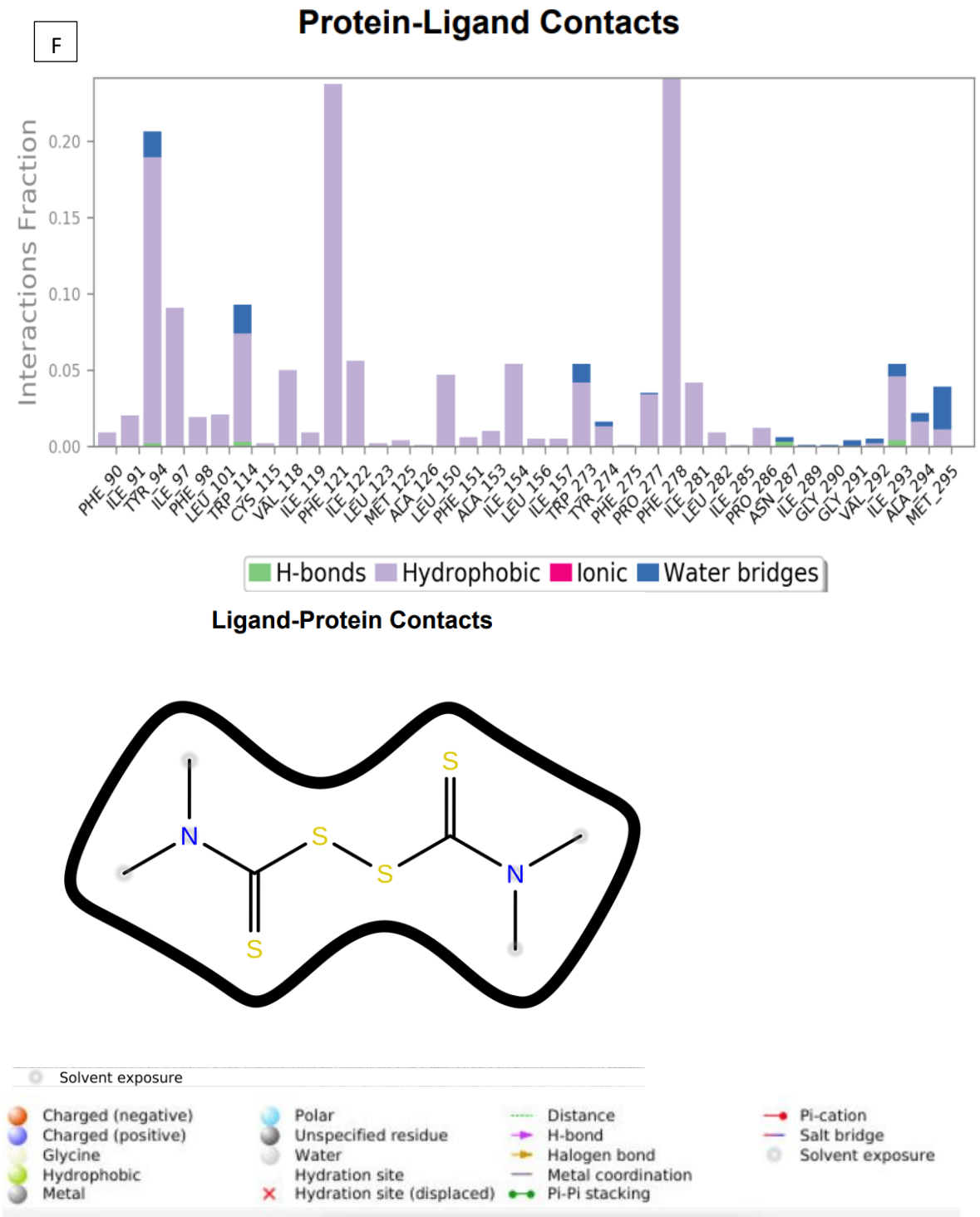
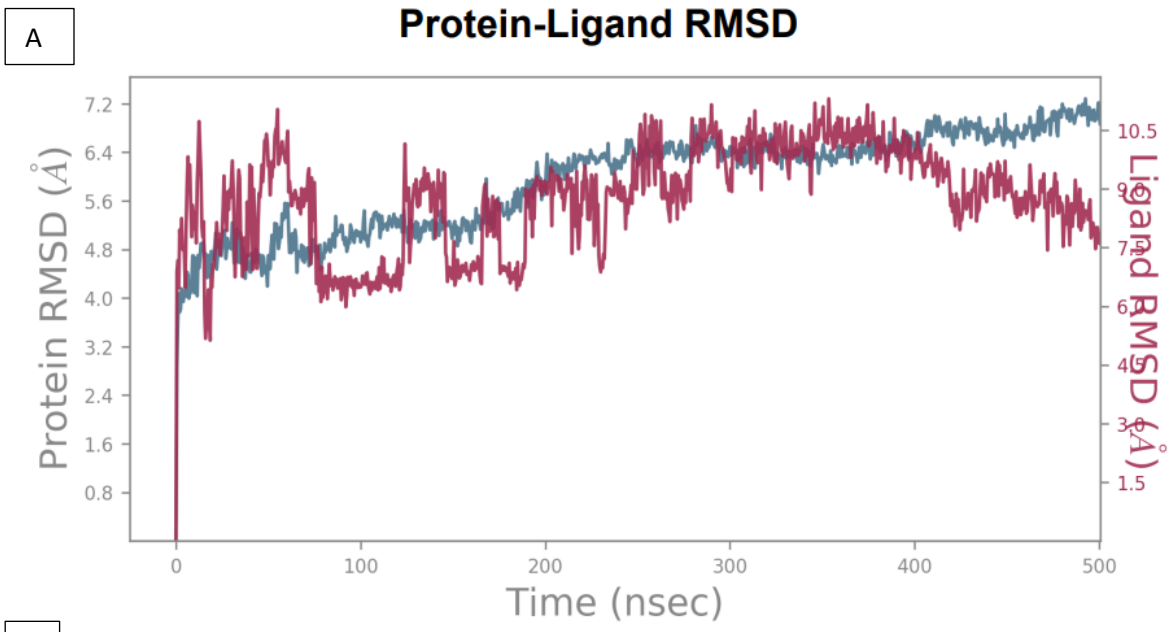


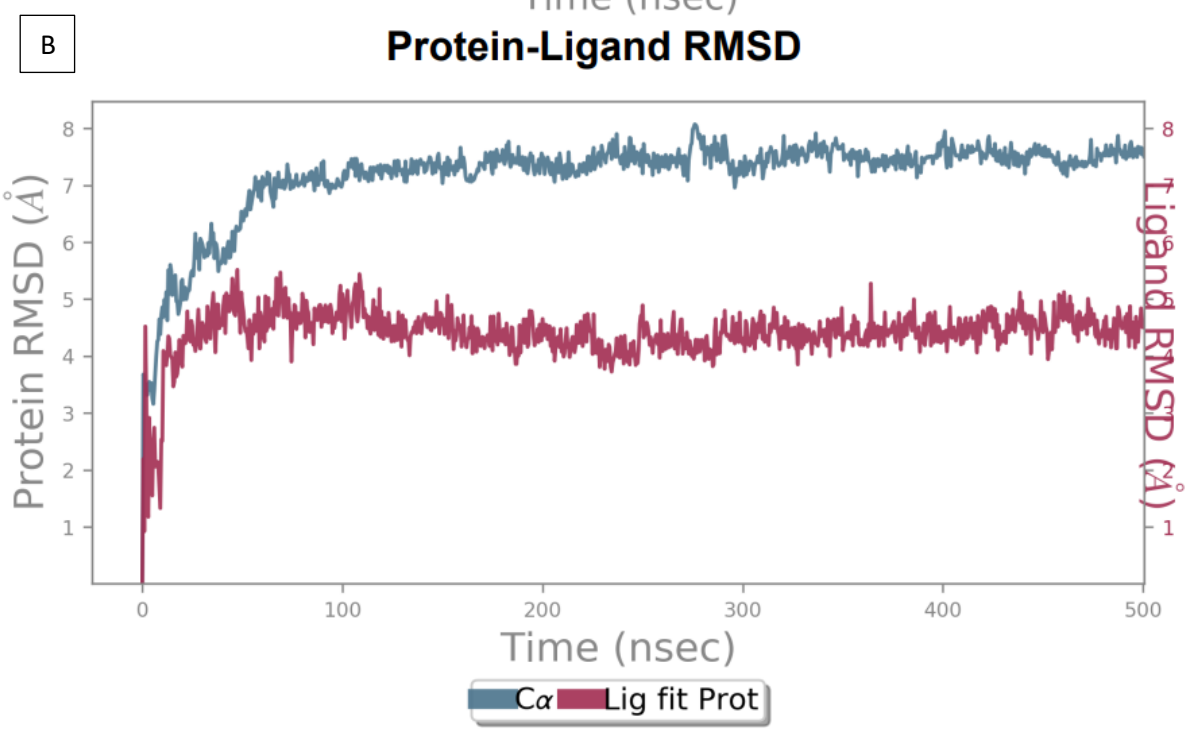
Figure 7. Protein-Ligand Interaction and contact toward active sites G143 and F129L mutated version. A) Ubiquinol with Cytochrome b; B) Fenamidone with Cytochrome b; C) Famoxadone with Cytochrome b; D) Ametoctradin with Cytochrome b; E) Folpet with Cytochrome b; F) Thiram with Cytochrome b. The region started from 79 to 296.

Hydrophobic binding was the main interaction between Cytochrome b and the active site located at PHE121, PHE151 and PHE278. Fenamidone showed very strong hydrogen bonding at ALA126 and strong hydrophobic binding interactions at ALA126, PHE151 and PHE186. Famoxadone showed hydrophobic binding interaction at PHE151 and it also had strong hydrogen bonding at LEU156, ILE157 and GLY158. Ametoctradin had multiply weak hydrophobic contact. Folpet had strong hydrophobic contact at PHE121. Thiram showed hydrophobic contact at TYR94, PHE121 and PHE278.

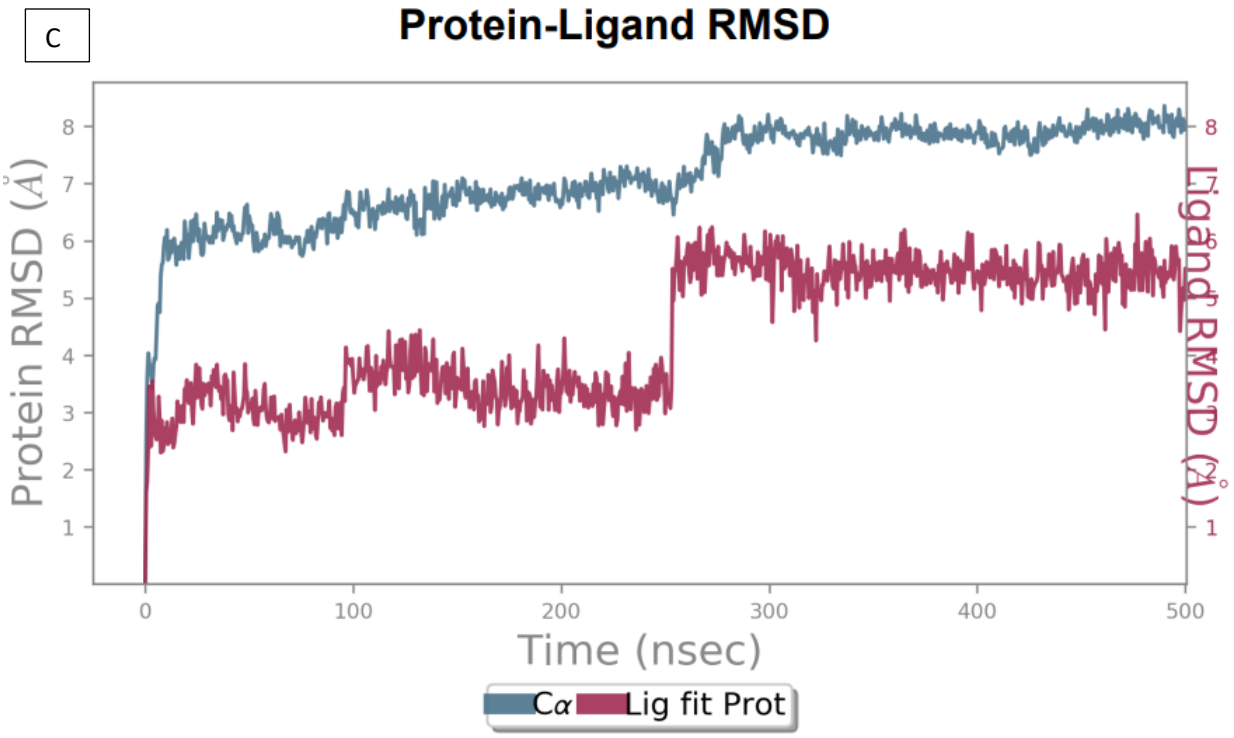
A



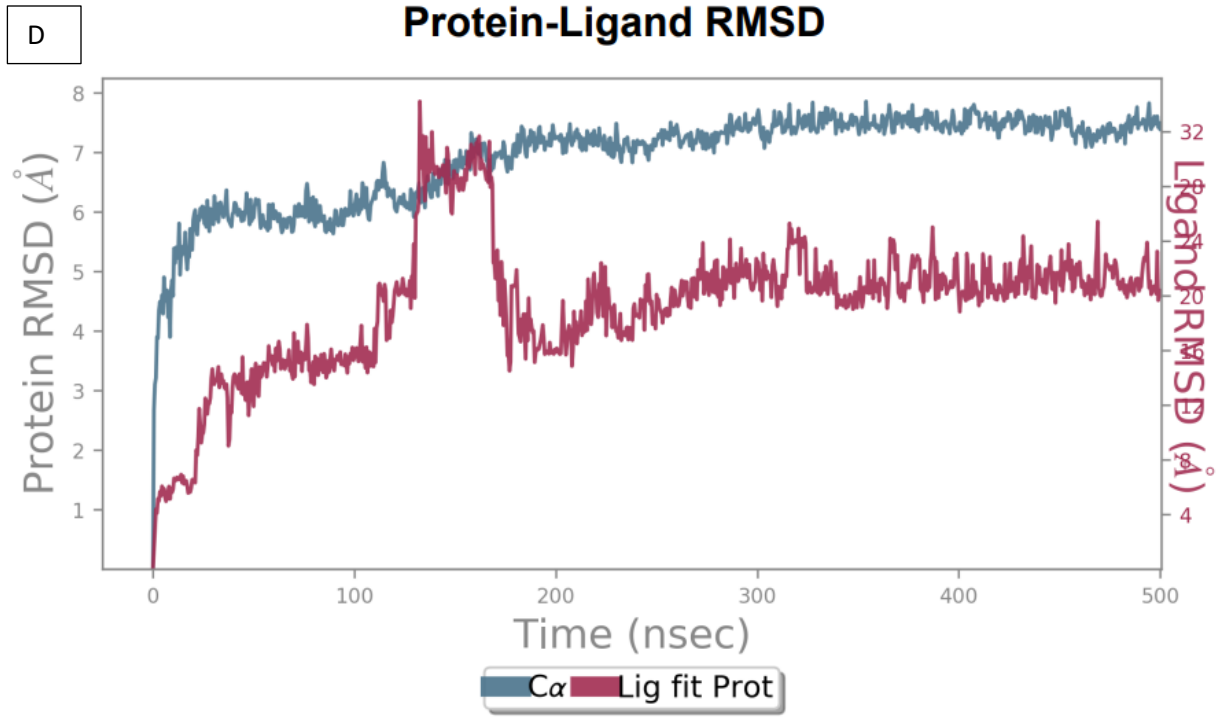
B



C


Protein-Ligand RMSD
Protein RMSD (Å)
Ligand RMSD (Å)
Time (nsec)
Cα
Lig fit Prot

D


Protein-Ligand RMSD
Protein RMSD (Å)
Ligand RMSD (Å)
Time (nsec)
Cα
Lig fit Prot

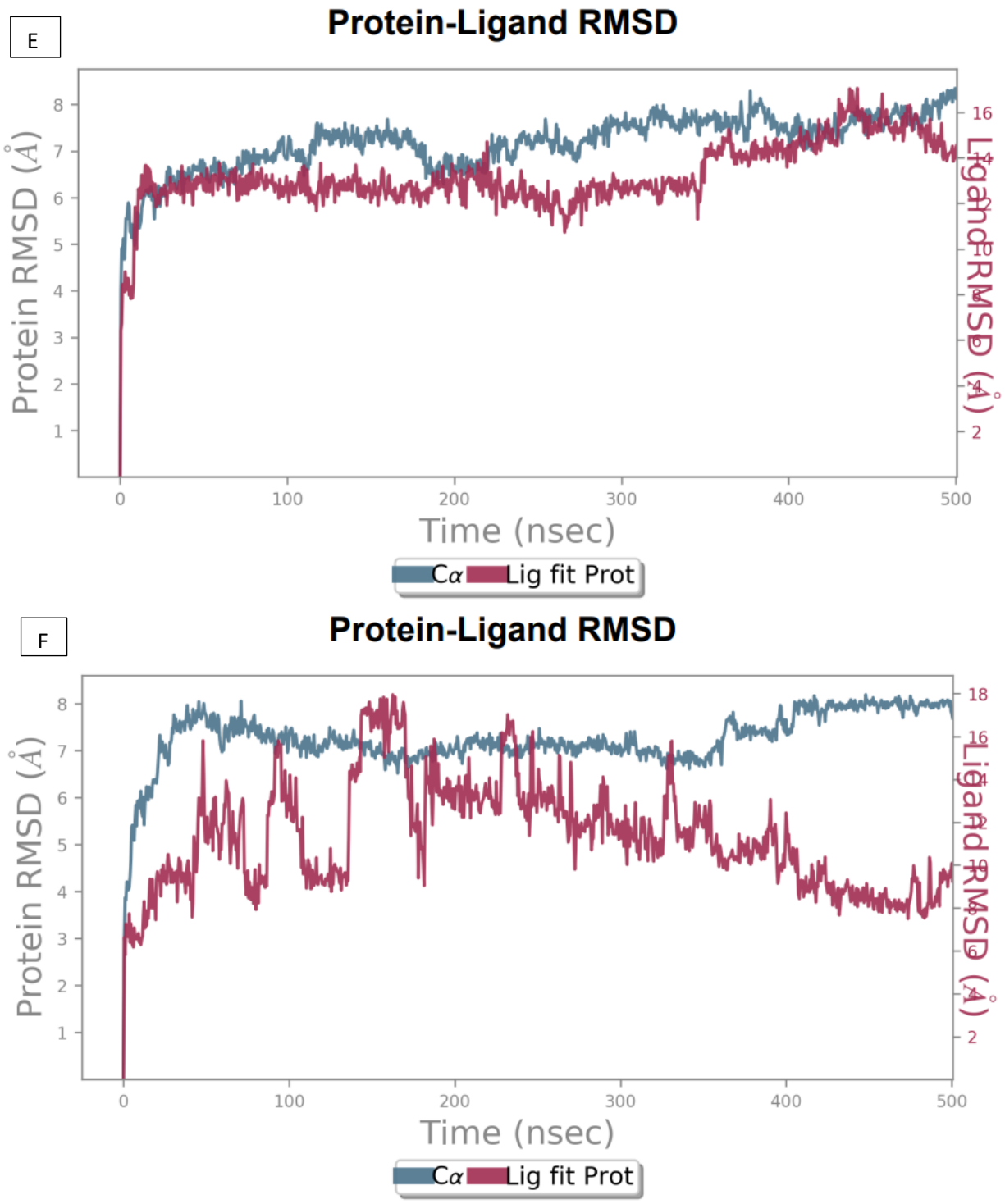
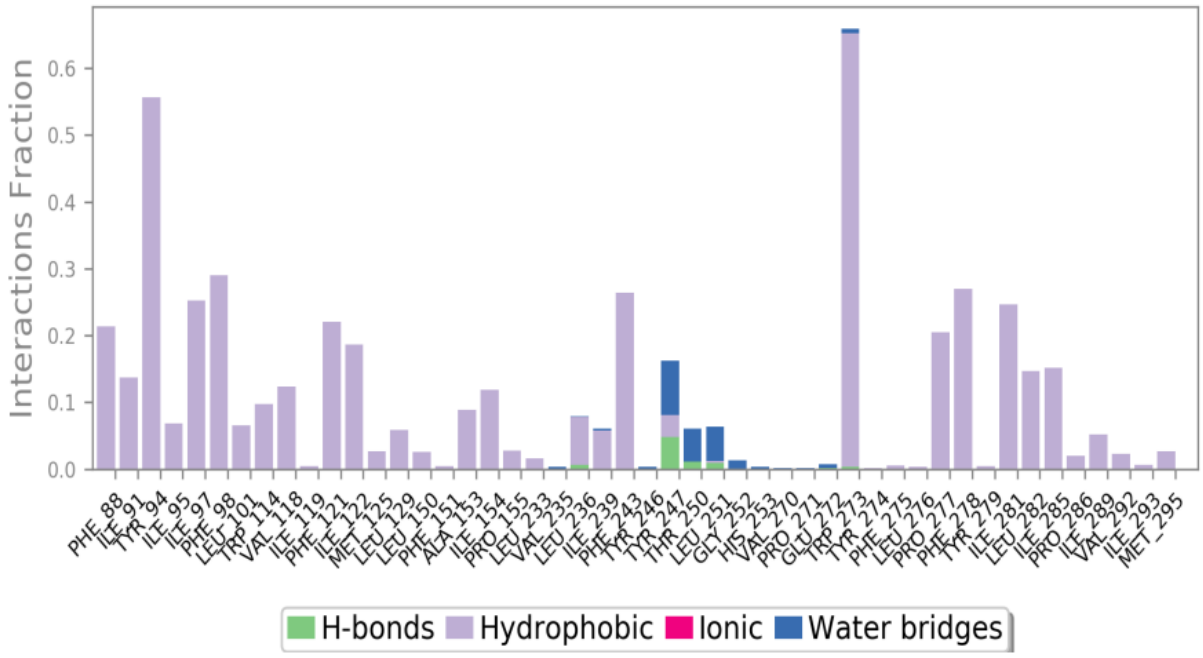


Figure 8. Protein and ligand RMSD (root mean square deviation) for the trajectory of each system toward G143 and F129L mutated version. Protein RMSD is shown in dark blue and Ligand RMSD is shown in red. A) Ubiquinol with Cytochrome b; B) Fenamidone with Cytochrome b; C) Famoxadone with Cytochrome b; D) Ametoctradin with Cytochrom b; E) Folpet with Cytochrome b; F) Thiram with Cytochrome b.

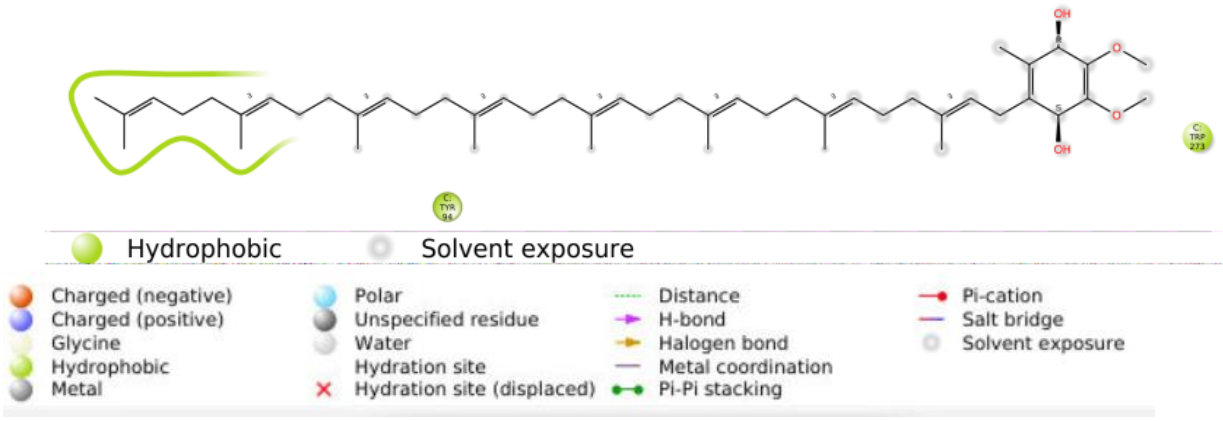
Protein interaction for Ubiquinol was equilibrated and Ubiquinol stably bound to the protein. Protein interactions for Fenamidone and Famoxadone were equilibrated and both ligands stably bound to the protein. Protein interaction for Ametoctradin was equilibrated but RMSD value of Ametoctradin was significantly higher than RMSD of protein and it meant the ligand diffused away from the protein. Protein interaction for Folpet was equilibrated and Thiram stably bound to the protein. Protein interaction for Thiram was equilibrated but Thiram was not stably bound to the protein because there was apparently fluctuation during 500 ns. Based on the protein-ligand contact and protein-ligand RMSD, Fenamidone, Famoxadone and Folpet would be effective treatment toward G143 and F129L mutated version. Ametoctradin and Thiram might be fewer effective options.

A

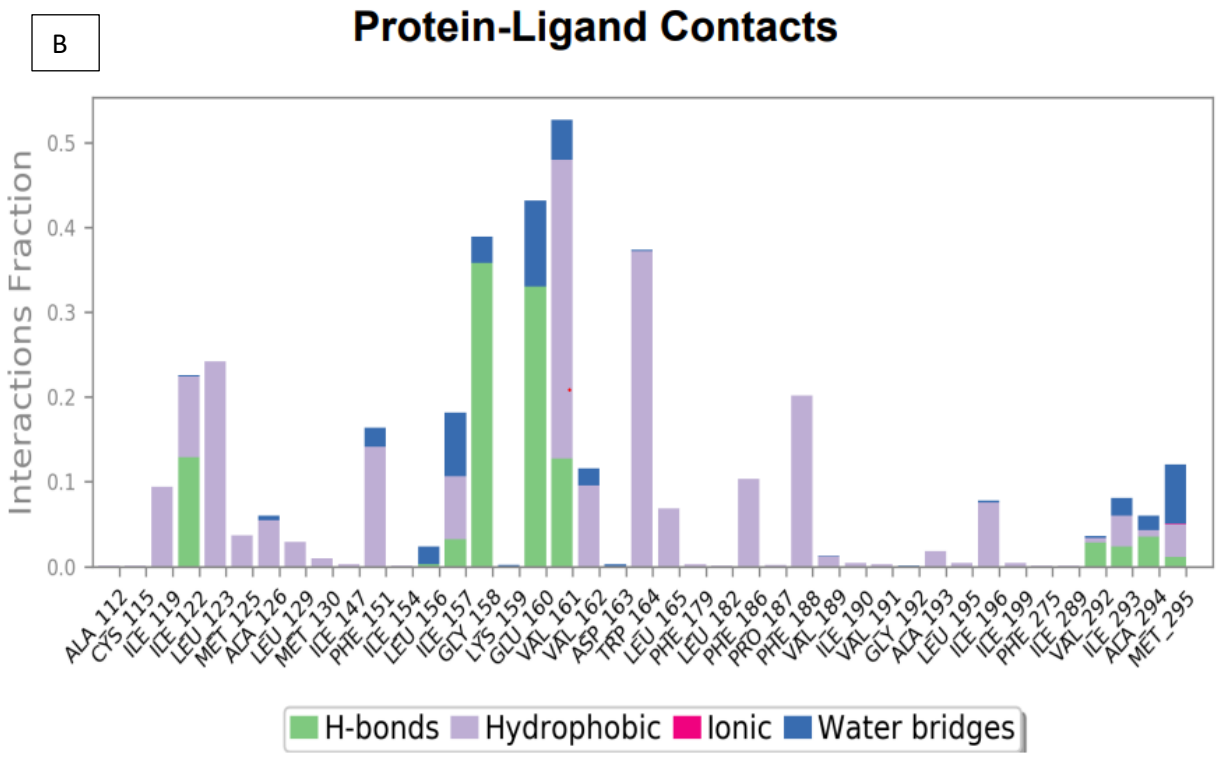
Protein-Ligand Contacts



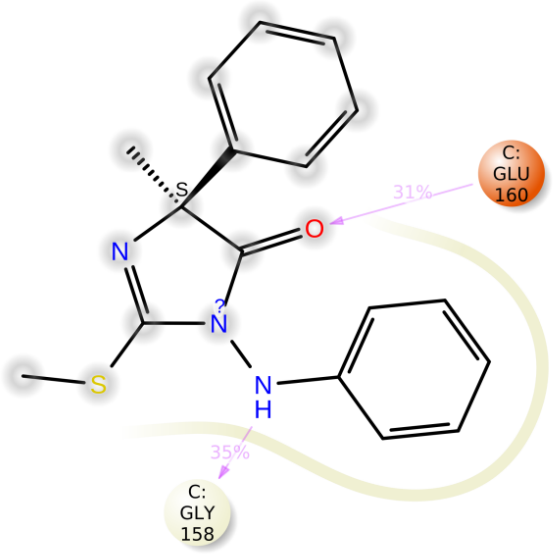
Ligand-Protein Contacts

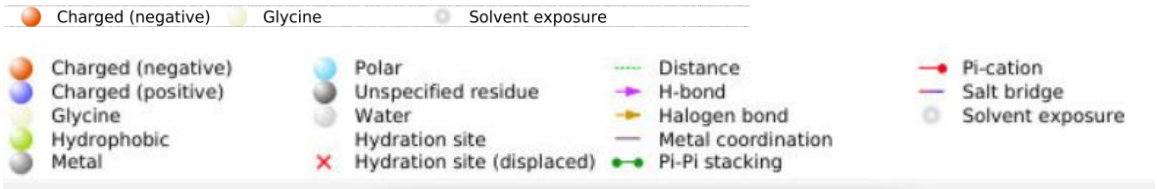


B



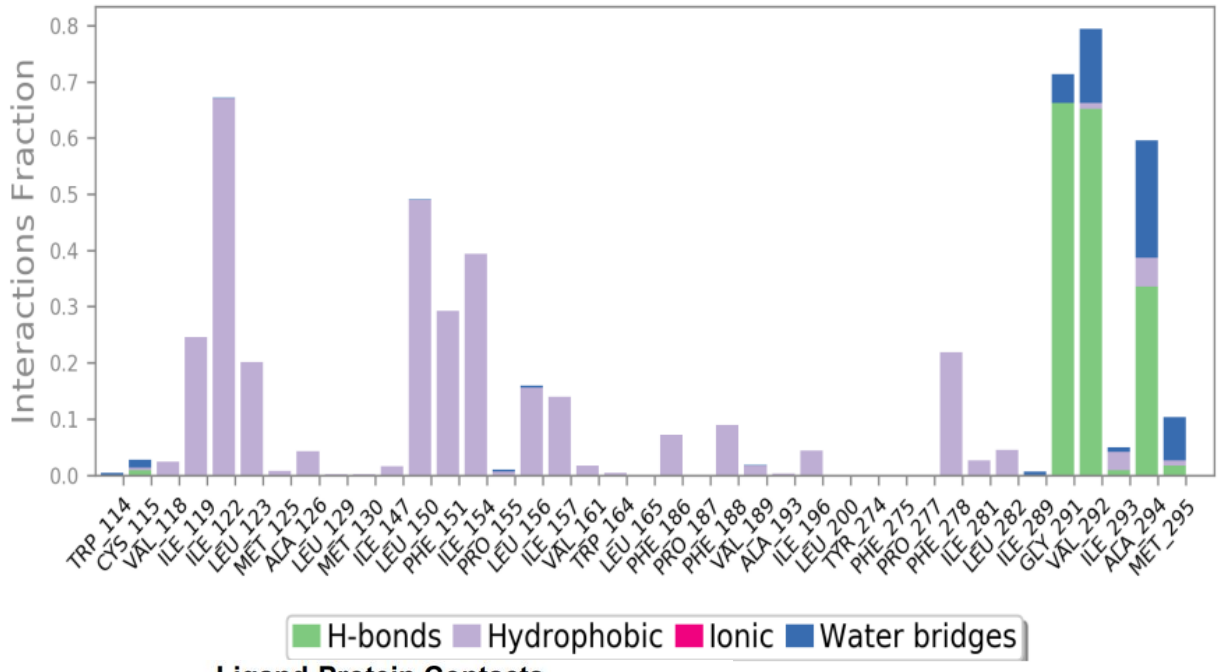
Ligand-Protein Contacts



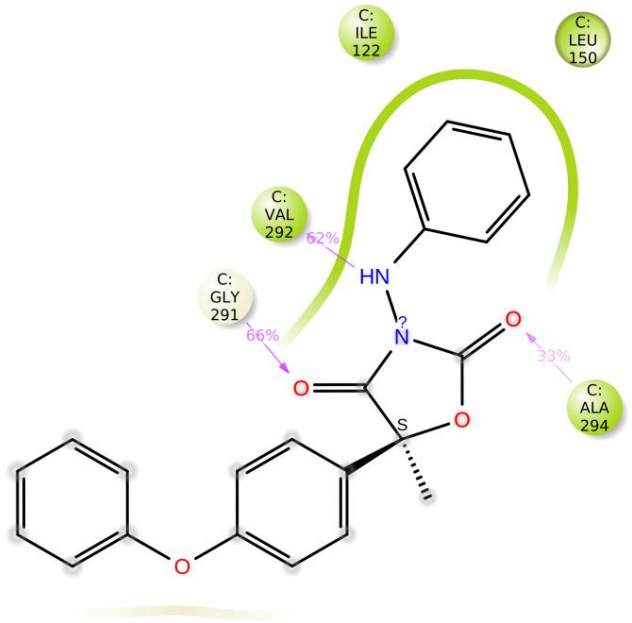


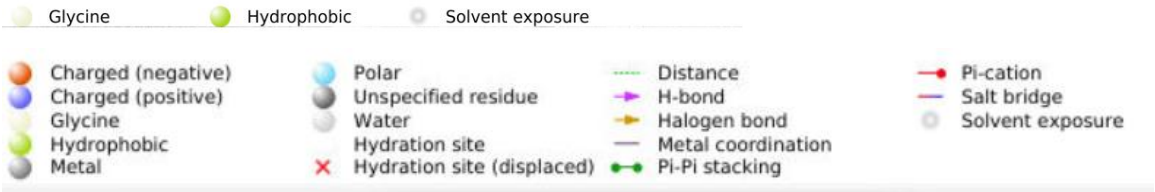
C

Protein-Ligand Contacts



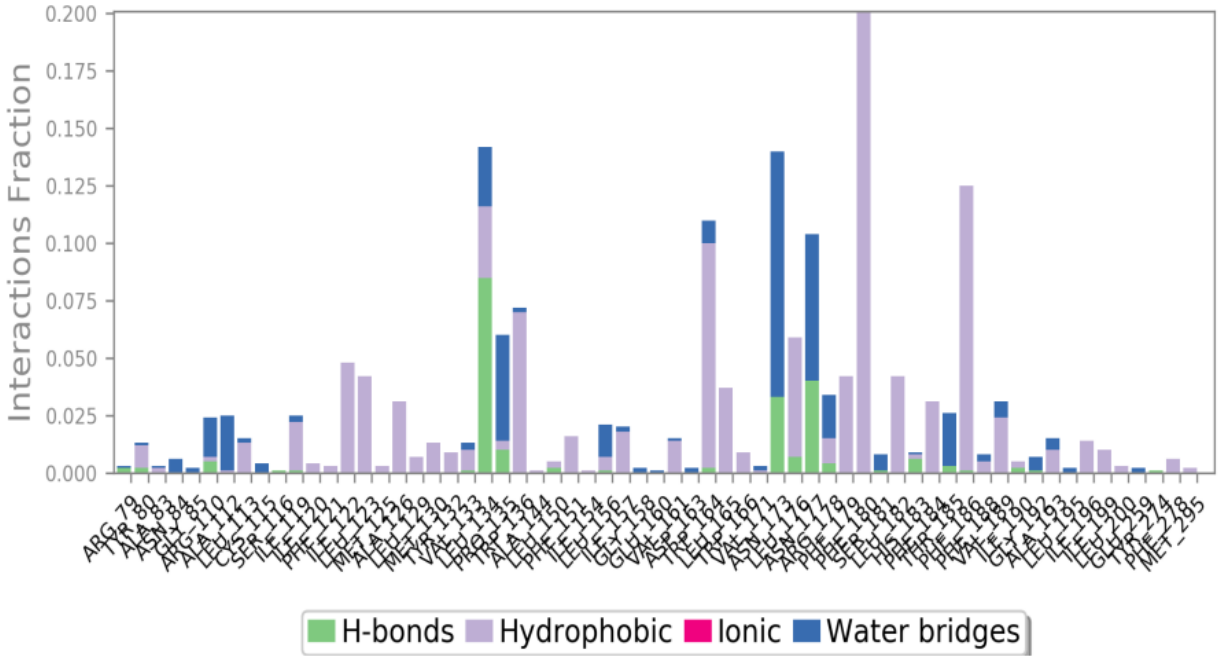
Ligand-Protein Contacts



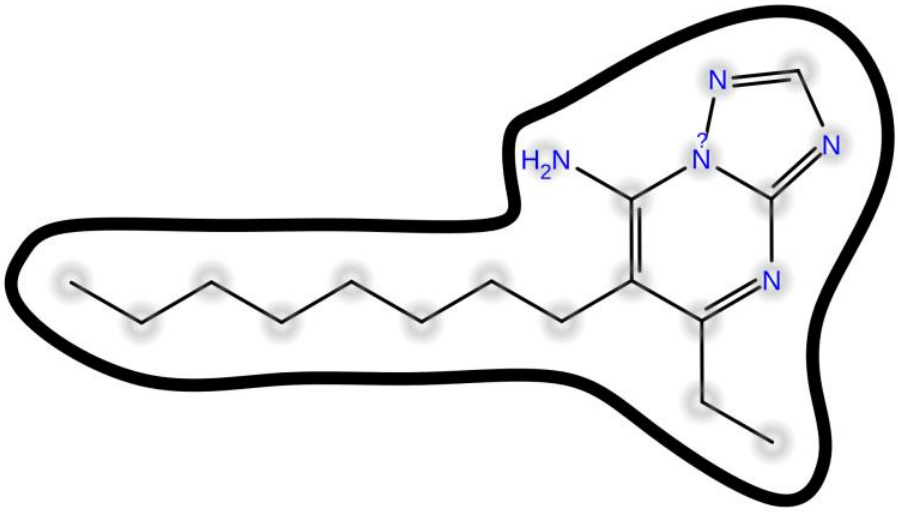


D

Protein-Ligand Contacts



Ligand-Protein Contacts



Solvent exposure

Charged (negative)	Polar	Distance	Pi-cation
Charged (positive)	Unspecified residue	H-bond	Salt bridge
Glycine	Water	Halogen bond	Solvent exposure
Hydrophobic	Hydration site	Metal coordination	
Metal	Hydration site (displaced)	Pi-Pi stacking	

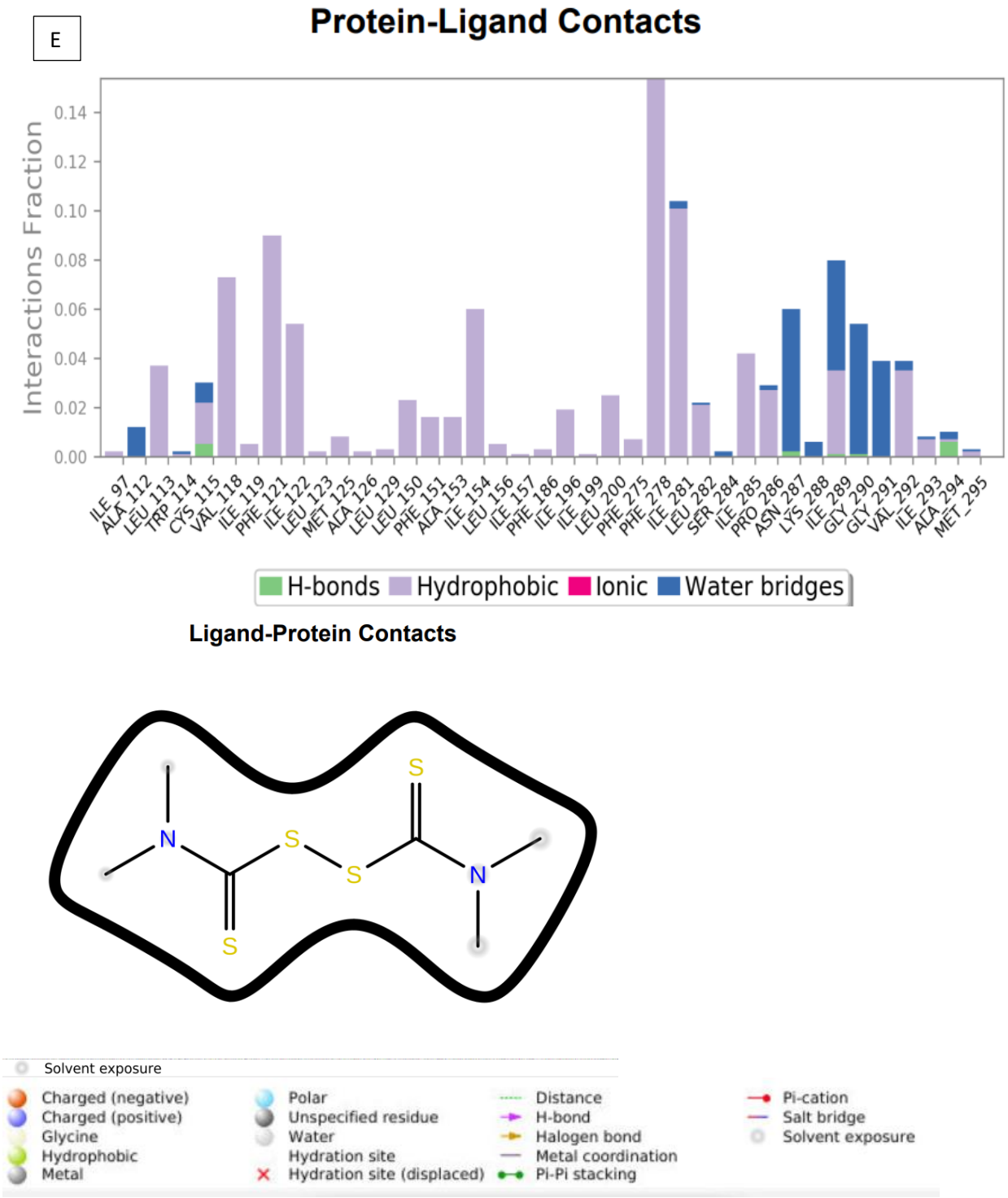
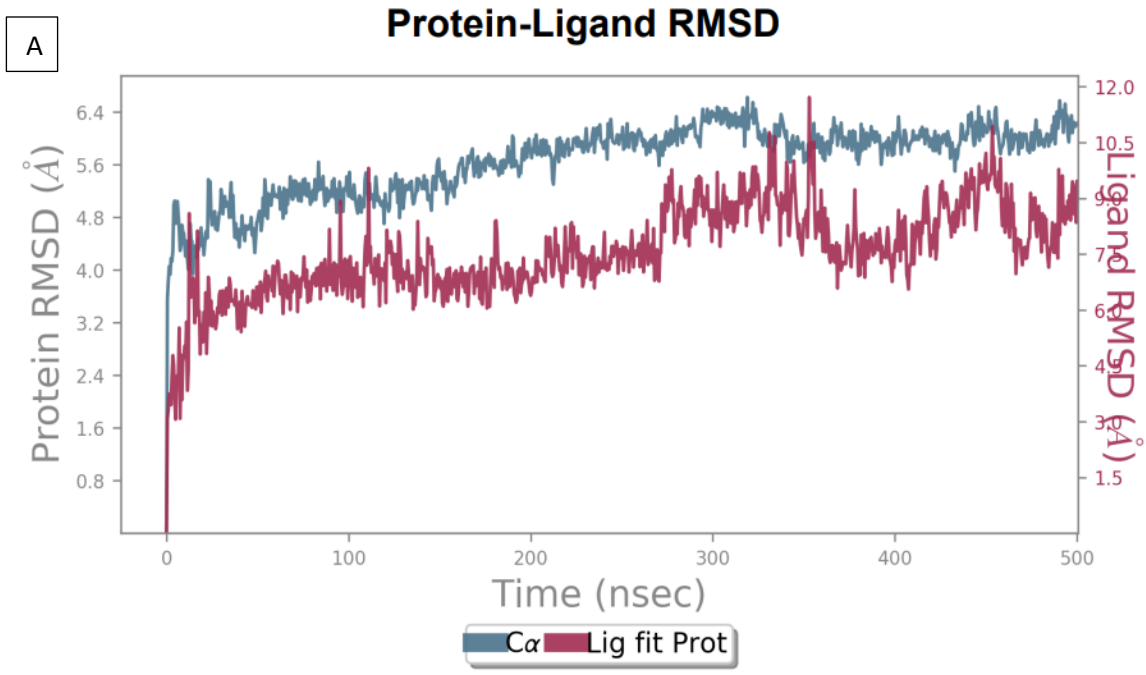


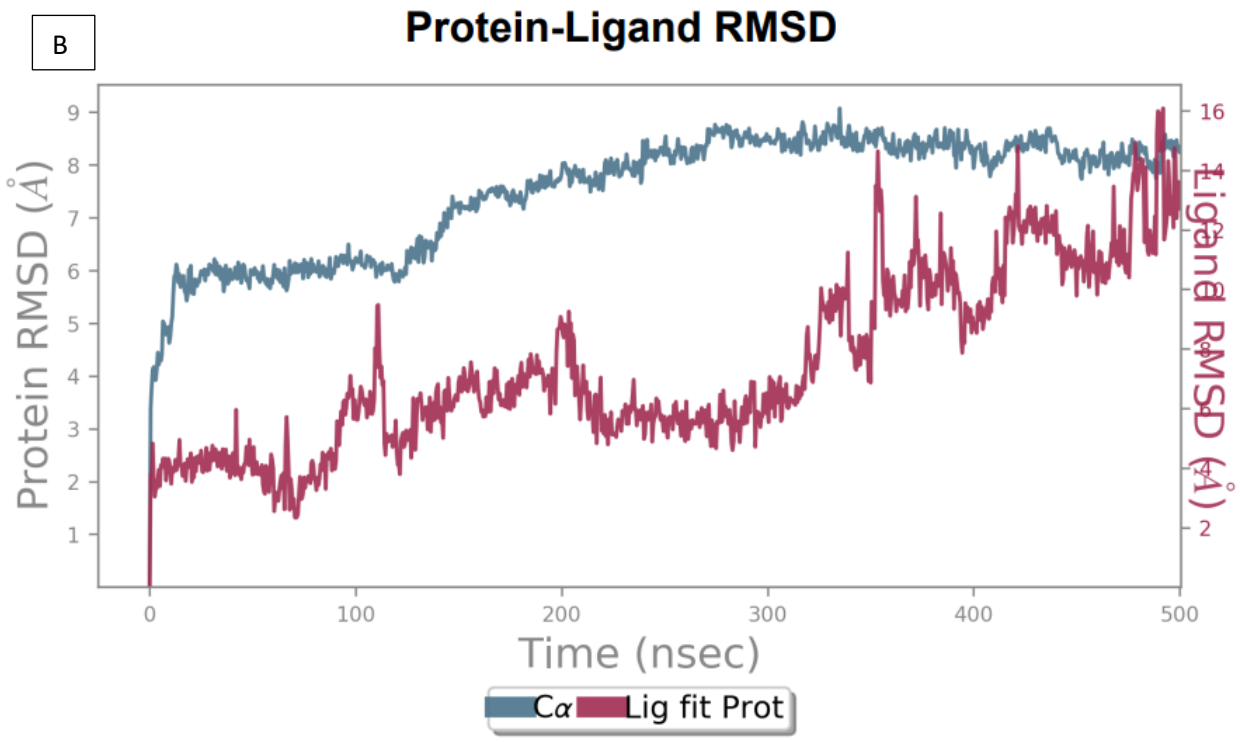
Figure 9. Protein-Ligand Interaction and contact toward active sites G143A and F129L mutated version. A) Ubiquinol with Cytochrome b; B) Fenamidone with Cytochrome b; C) Famoxadone with Cytochrome b; D) Ametoctradin with Cytochrome b; E) Thiram with Cytochrome b. The region started from 79 to 296.

Ubiquinol show strong hydrophobic contact at TYR94 and TRP273. Fenamidone showed hydrogen bonding at GLY158 and GLU160, and it also showed hydrophobic contact at VAL161 and TRP164. Famoxadone had hydrogen bonding at GLY291 and VAL291, and also had hydrophobic contact at ILE122 and LEU150. Ametoctradin had multiply but weak interaction with protein. The strongest interaction for Ametoctradin was at PHE180. Thiram had weaker hydrophobic contact than another three ligands at PHE278.

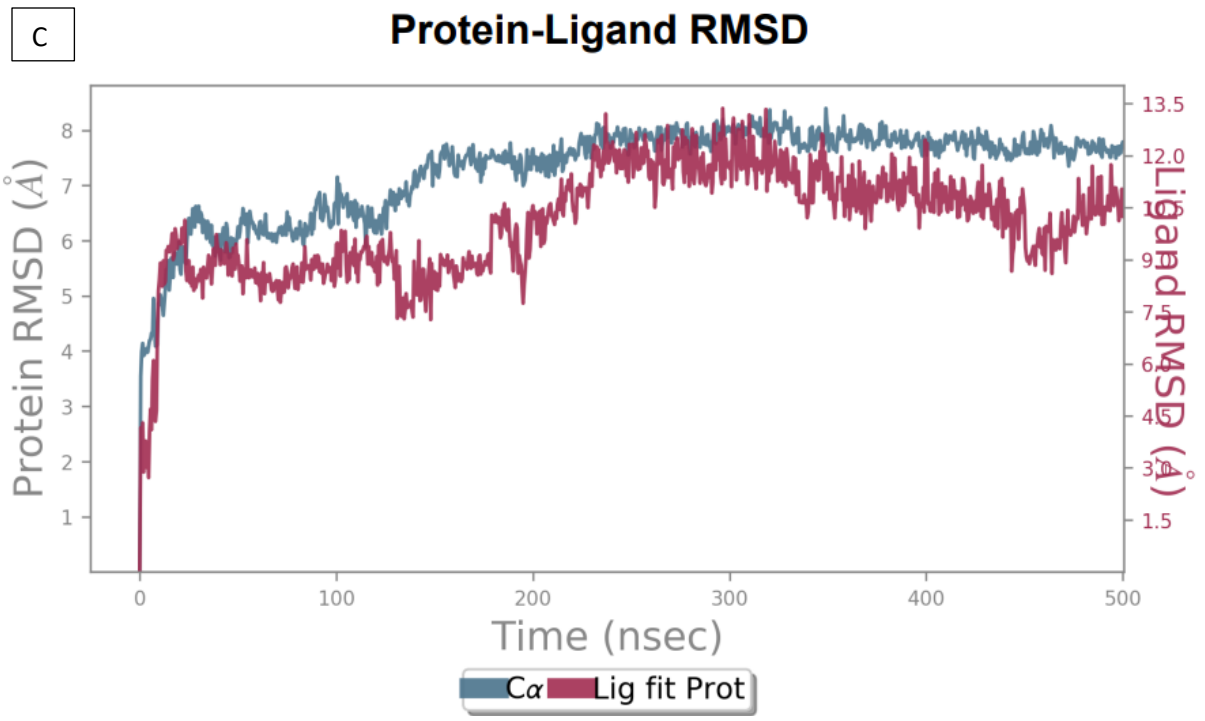
A



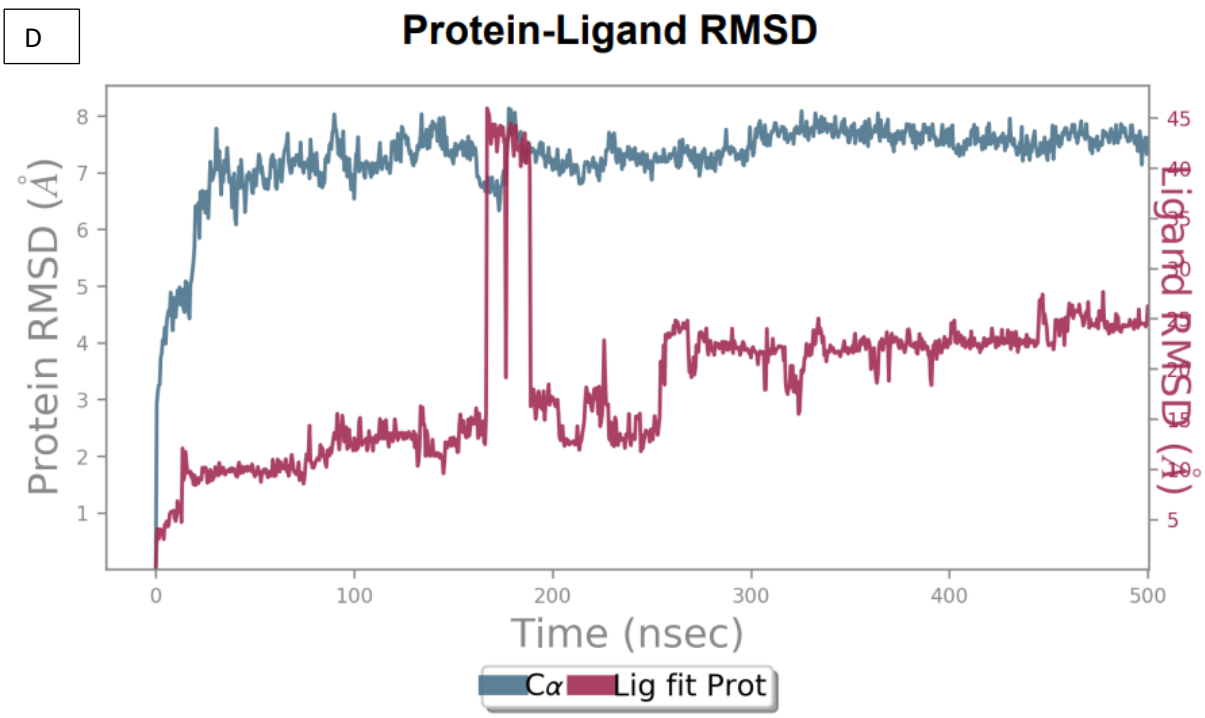
B



C


Protein-Ligand RMSD
Protein RMSD (Å)
Ligand RMSD (Å)
Time (nsec)
Cα
Lig fit Prot

D


Protein-Ligand RMSD
Protein RMSD (Å)
Ligand RMSD (Å)
Time (nsec)
Cα
Lig fit Prot

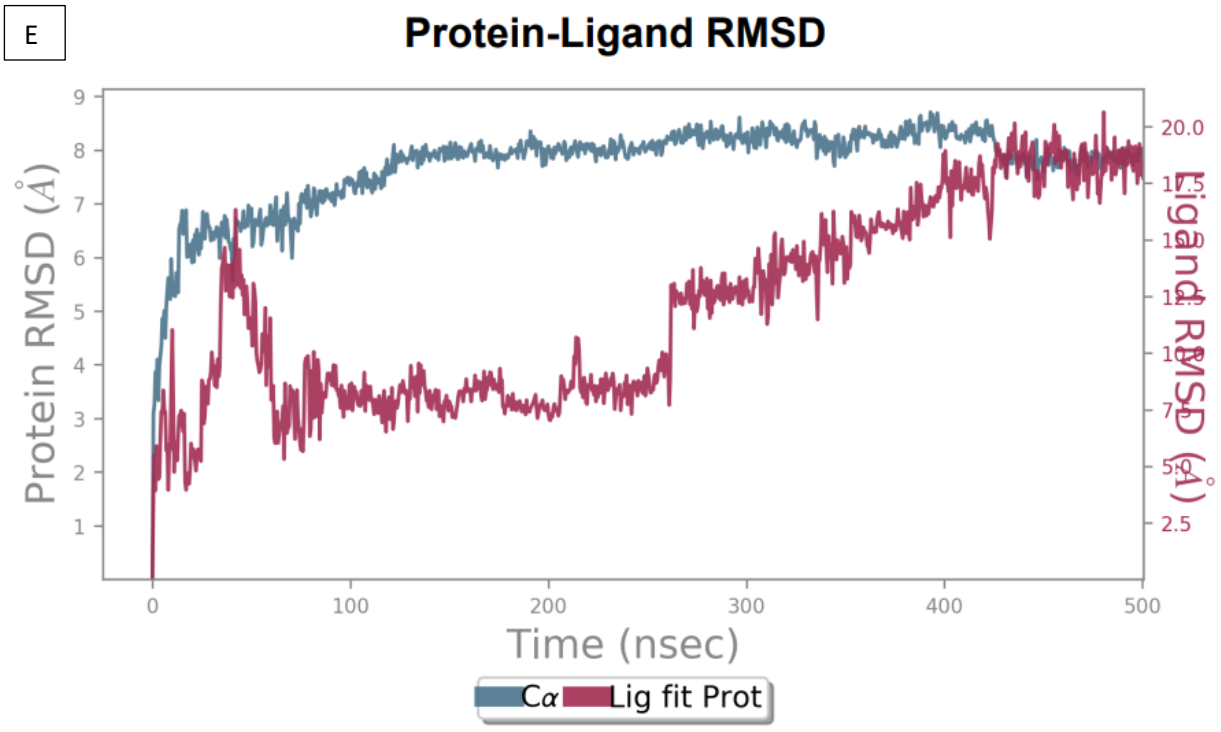
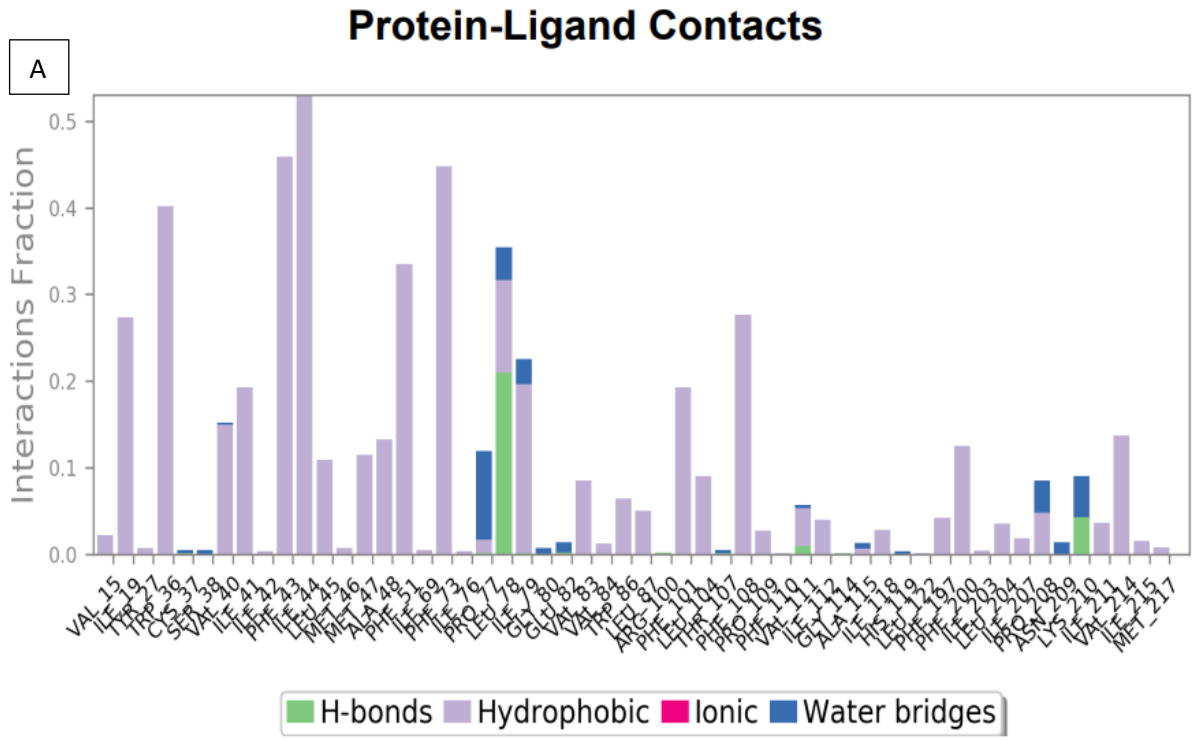


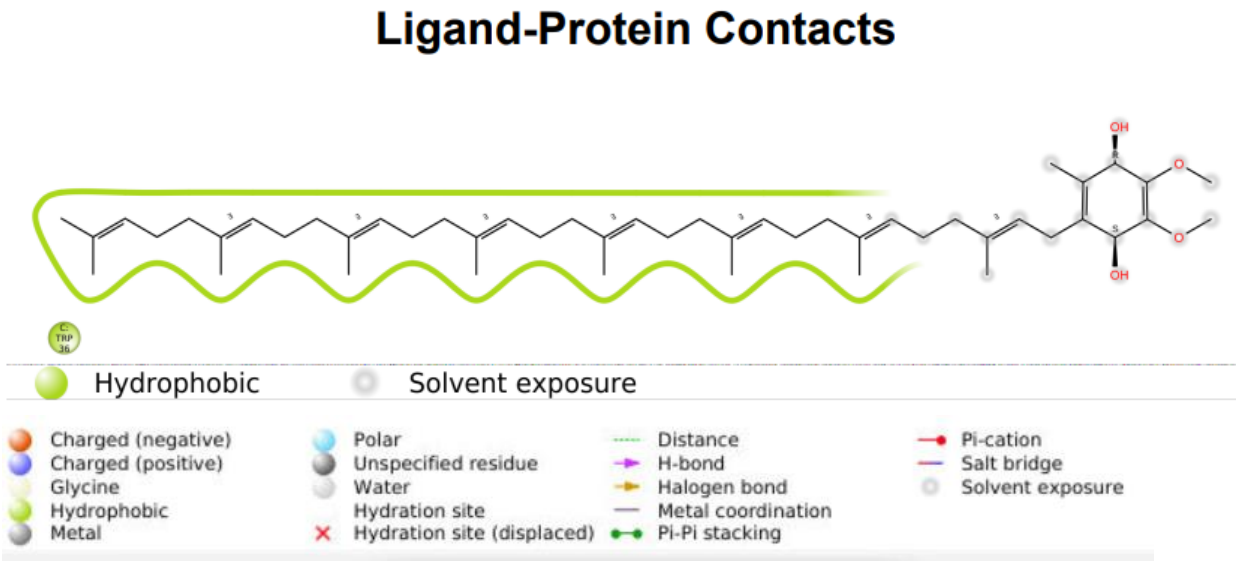
Figure 10. Protein and ligand RMSD (root mean square deviation) for the trajectory of each system toward G143A and F129L mutated version. Protein RMSD is shown in dark blue and Ligand RMSD is shown in red. A) Ubiquinol with Cytochrome b; B) Fenamidone with Cytochrome b; C) Famoxadone with Cytochrome b; D) Ametoctradin with Cytochrome b; E) Thiram with Cytochrome b. The region started from 79 to 296.

Protein simulation for Ubiquinol was equilibrated and Ubiquinol bound stably and tightly toward the protein. Protein simulation for Fenamidone was equilibrated and Fenamidone tended to diffuse away from the protein but the difference between RMSD of protein and RMSD of ligand was small. Protein simulation for Famoxadone was equilibrated and Famoxadone bound stably toward the protein. Protein simulation for Ametoctradin was equilibrated but Ametoctradin was apparently diffused away from the protein. Protein simulation for Thiram was equilibrated but Thiram only stably bound to the protein before 270 ns and it started to diffused away from the protein.

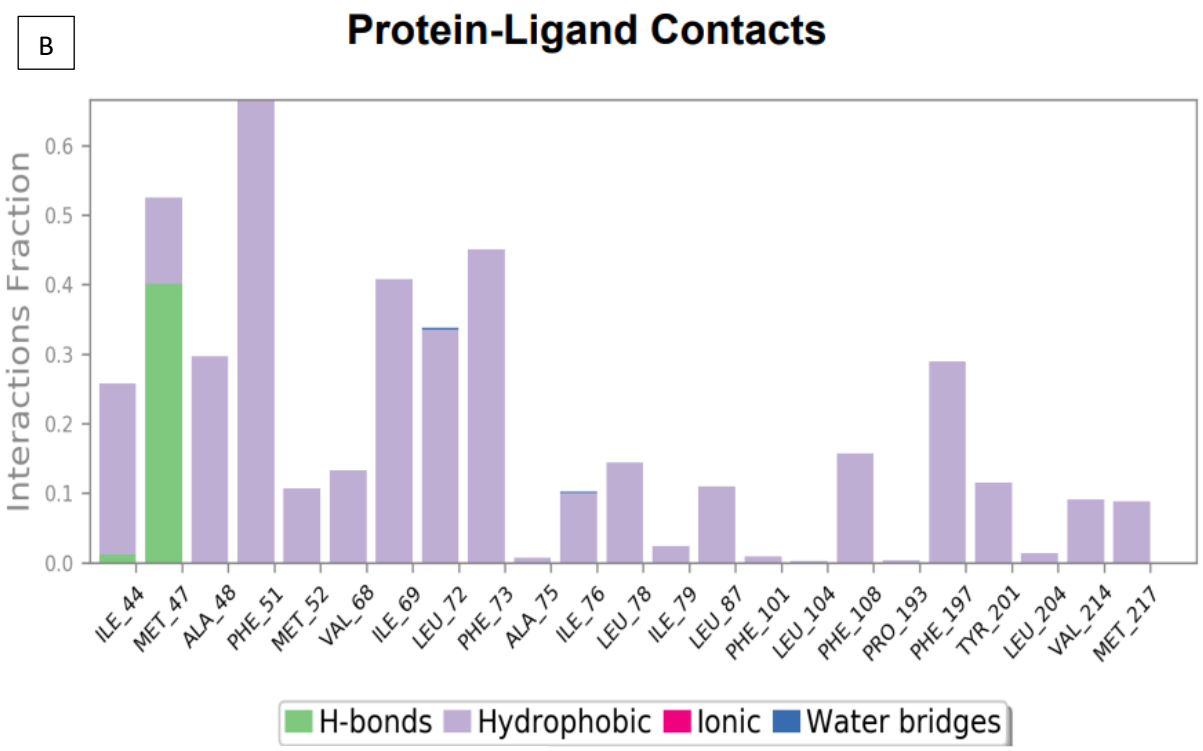
Among four different mutated versions of proteins, hydrophobic contact played a major role in the protein-ligand interaction. There were also few hydrogen bonds occurred in the protein-ligand interaction of Fenamidone and Famoxadone. Ubiquinol as native ligand showed a very strong binding affinity toward Cytochrome b. Two high-risk fungicides selected from the Docking score also verified their strong binding affinity toward the protein. There were stronger hydrogen bonding and hydrophobic contact at specific residues than Ubiquinol. Ametoctradin showed good binding affinity to the wild-type protein but its binding affinity toward G143A, F129L and double mutated version was weak. Azoxystrobin, as a resistant-known fungicide, showed worse protein interaction, proving it was not an effective fungicide against Ubiquinol.

Two low-risk fungicides, Thiram and Folpet, showed weak binding affinity in Ligand-Protein contact, but Thiram had better results than Folpet. Thiram had reasonable hydrogen bonding at ALA153 toward G143A mutated version and it had stable protein interaction during 500 ns. Overall, according to Docking score and MD simulation Fenamidone, Famoxadone and Thiram were suitable fungicides for fungicide combination against *Plasmopara. viticola*. Ametoctradin was not a reasonable choice for fungicide combination.

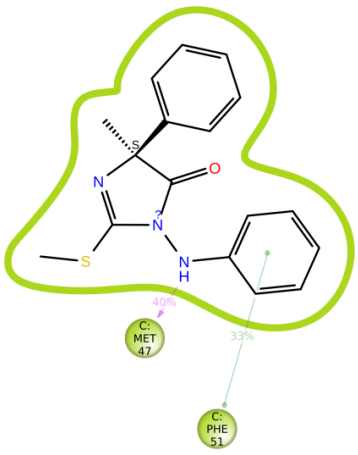




B

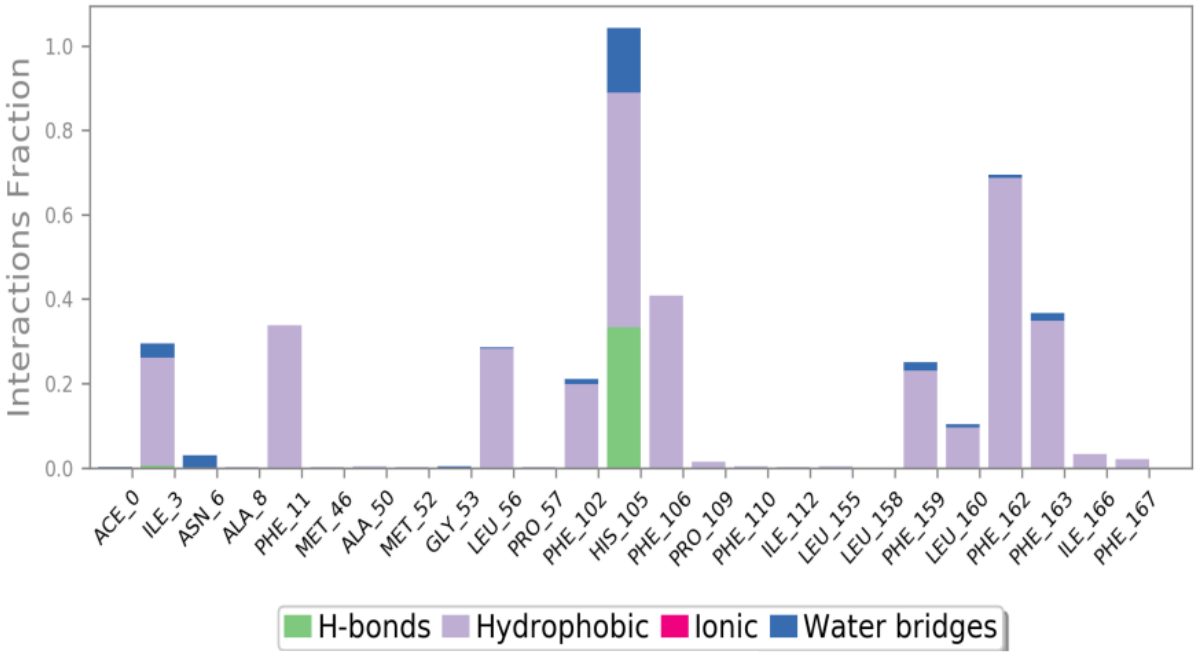


Ligand-Protein Contacts

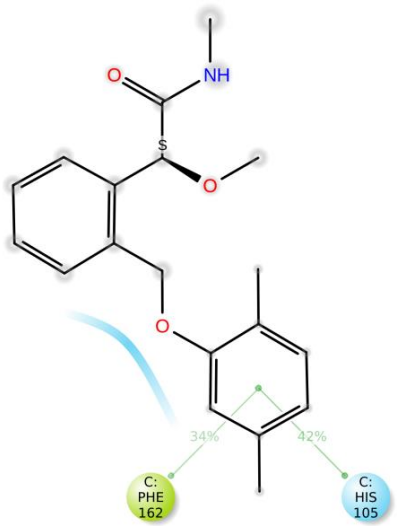


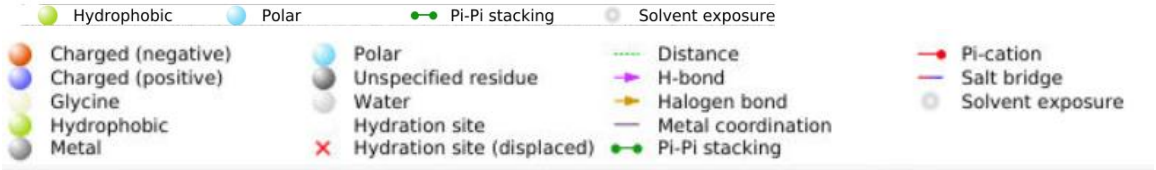
C

Protein-Ligand Contacts



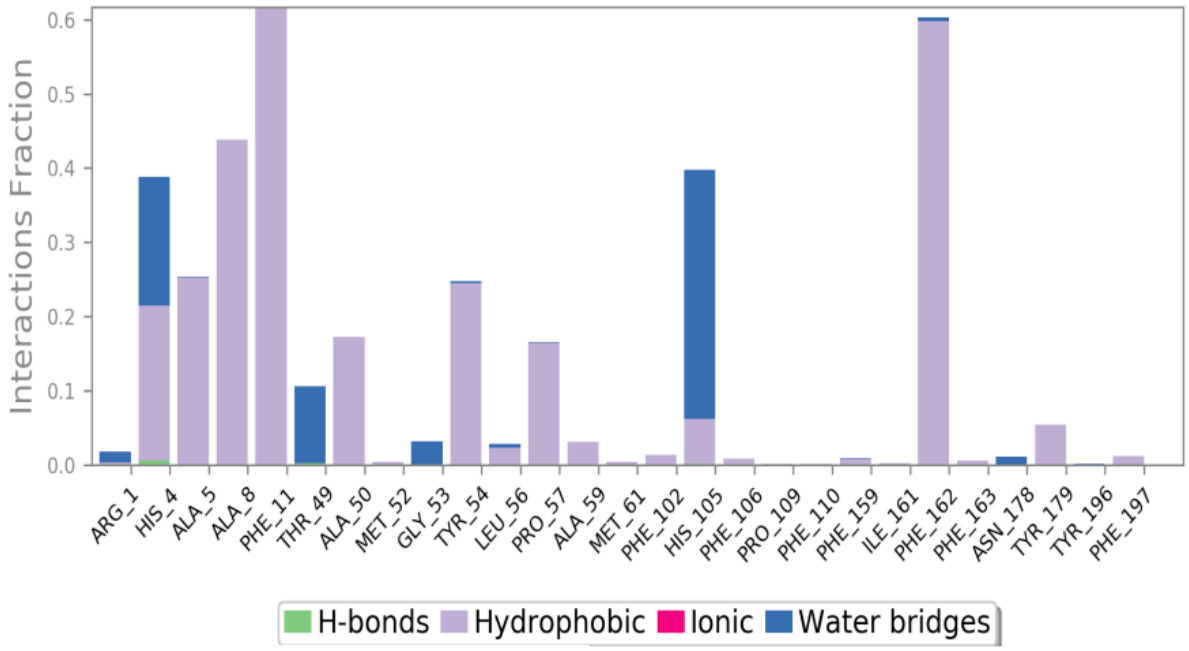
Ligand-Protein Contacts



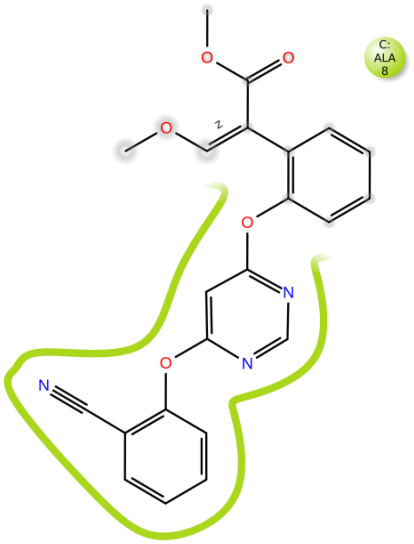


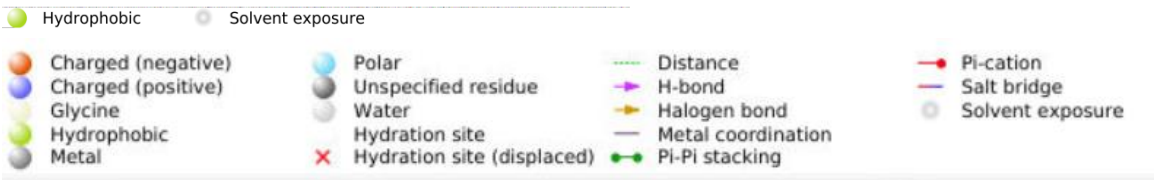
D

Protein-Ligand Contacts



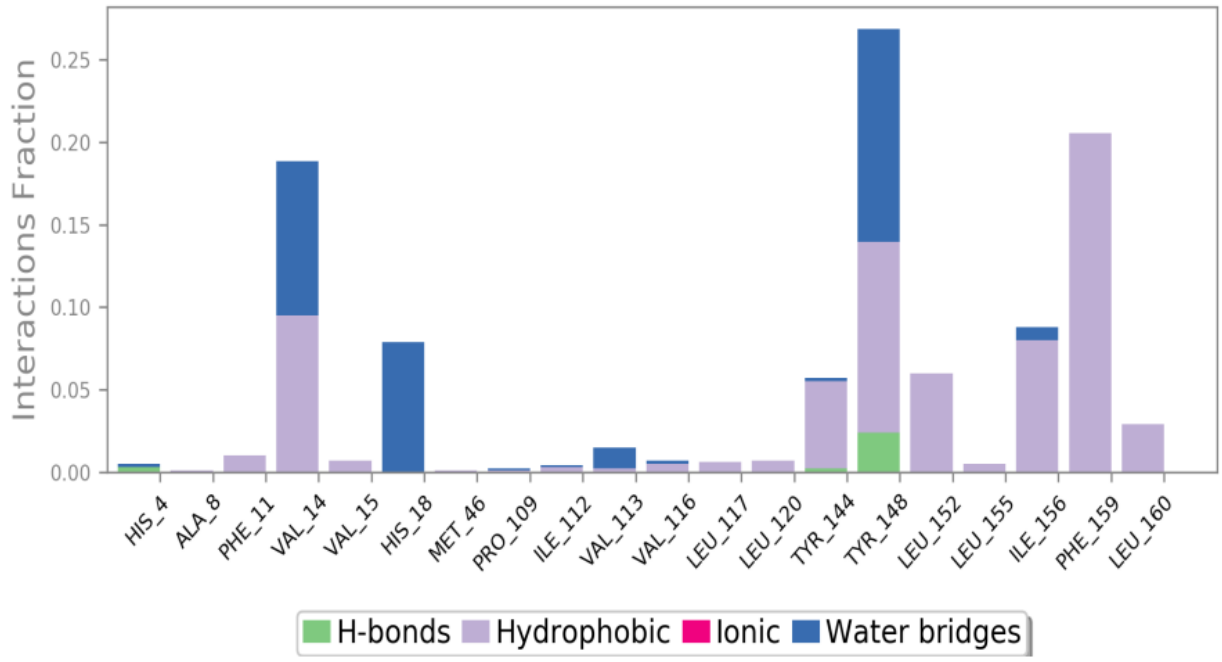
Ligand-Protein Contacts



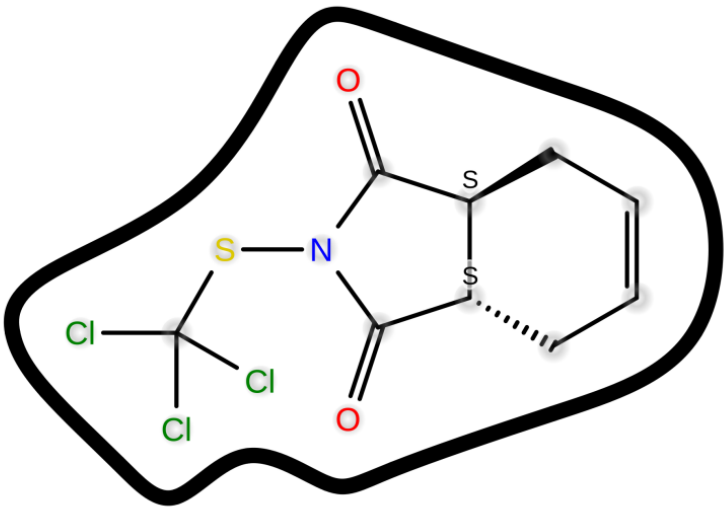


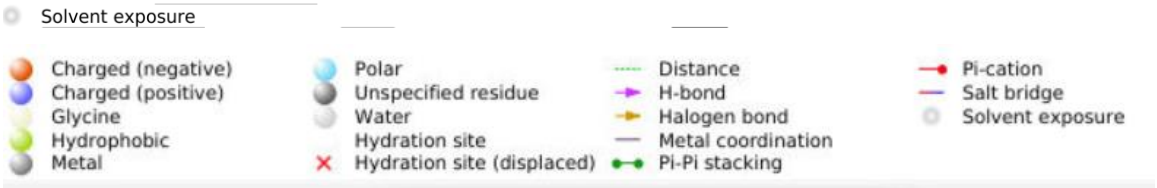
E

Protein-Ligand Contacts



Ligand-Protein Contacts





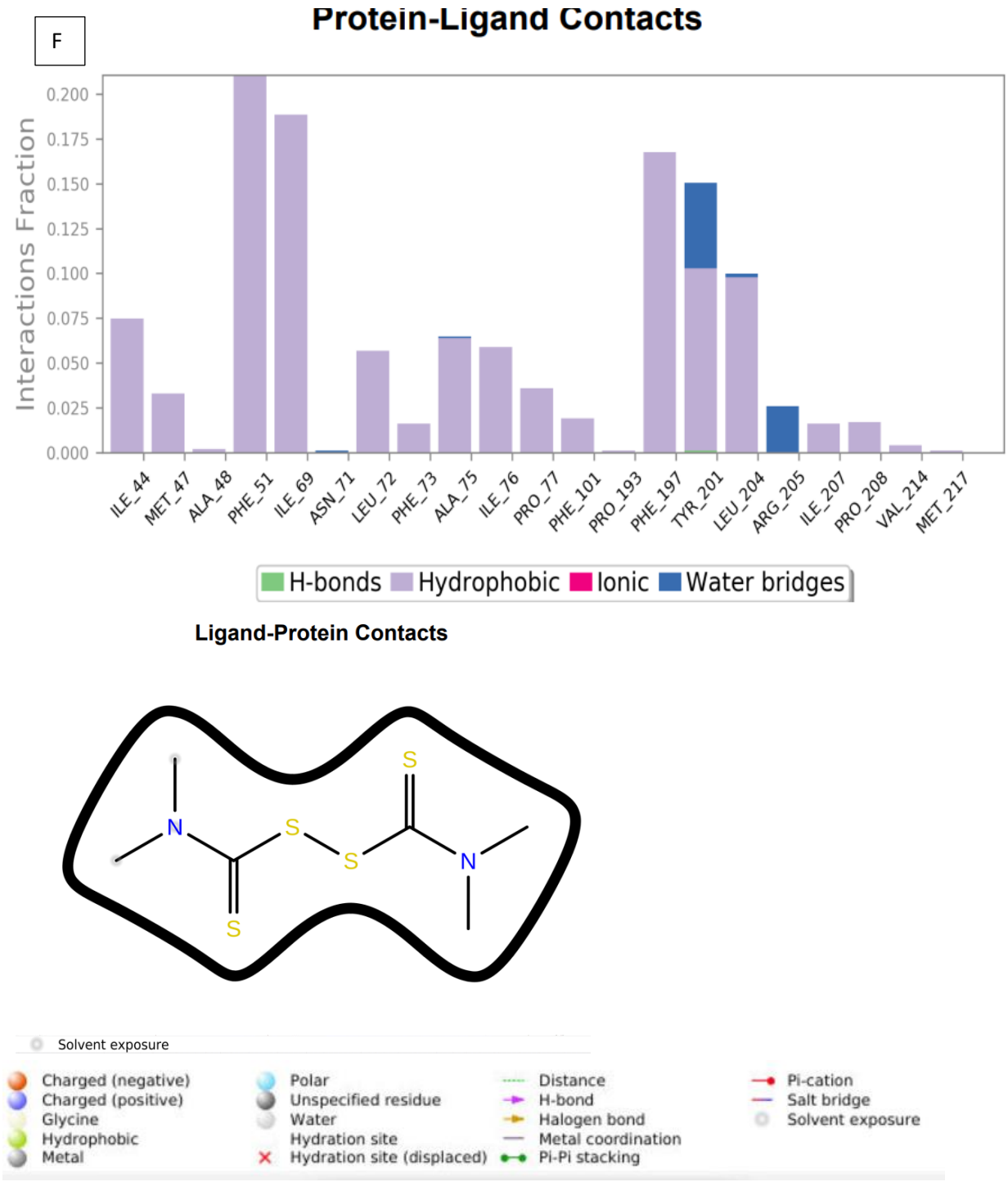
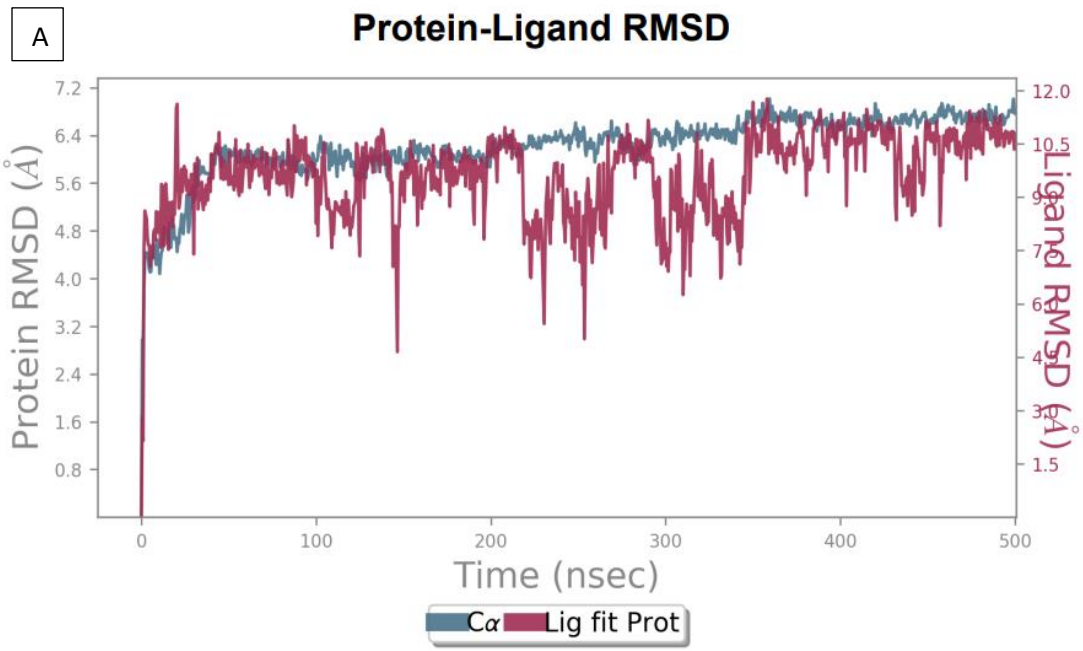


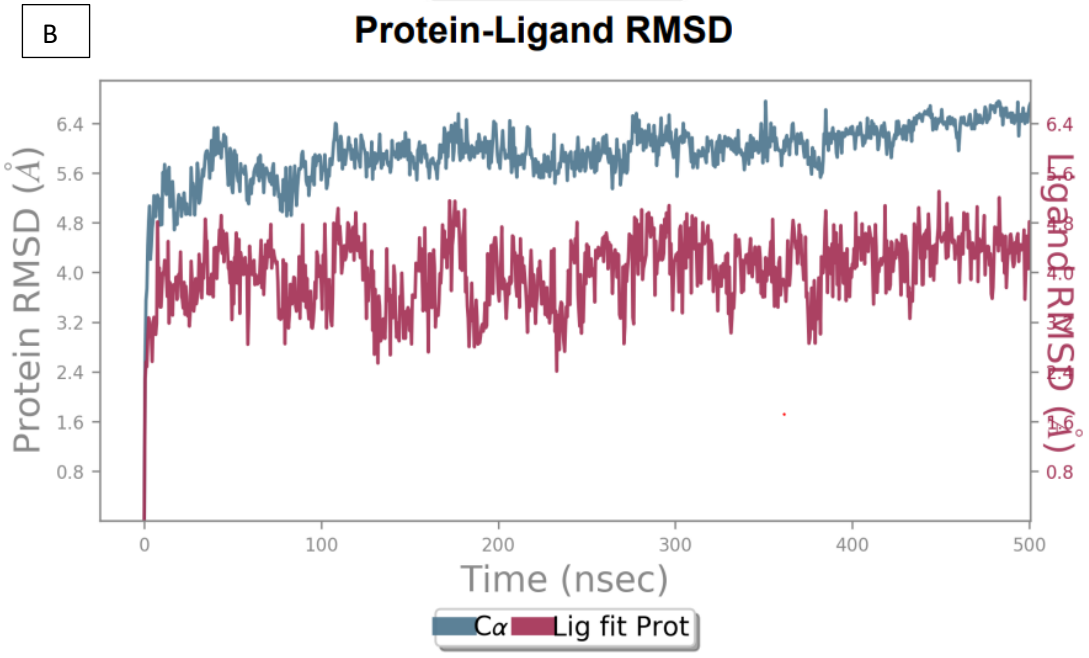
Figure 11. Protein-Ligand Interaction and contact toward active sites L123 and G137A mutated version (L45 and G60A on the figures). A) Ubiquinol with Cytochrome b; B) Fenamidone with Cytochrome b; C) Mandestrobin with Cytochrome b; D) Azoxystrobin with Cytochrome b; E) Captan with Cytochrome b; F) Thiram with Cytochrome b. The region started from 79 to 296 (0 to 217 on the figures).

Ubiquinol had strong hydrophobic contact with the protein at TRP114, PHE120, ILE121 and PHE151 (TRP36, PHE43, ILE44 and PHE73 on the figures). Fenamidone showed strong hydrophobic contact at PHE129 and PHE151 (PHE51 and PHE73 on the figures), and it also had strong hydrogen bonding at MET125 (MET47 on the figures). Mandestrobin showed hydrophobic contact at HIS183 and PHE240 (HIS105 and PHE162 on the figures). Azoxystrobin had strong hydrophobic contact at PHE89 and PHE240 (PHE11 and PHE162 on the figures). Captan had weak hydrophobic contact at PHE236 (PHE158 on the figure) and Thiram had weak hydrophobic contact at PHE129 and ILE147 (PHE51 and ILE69 on the figure).

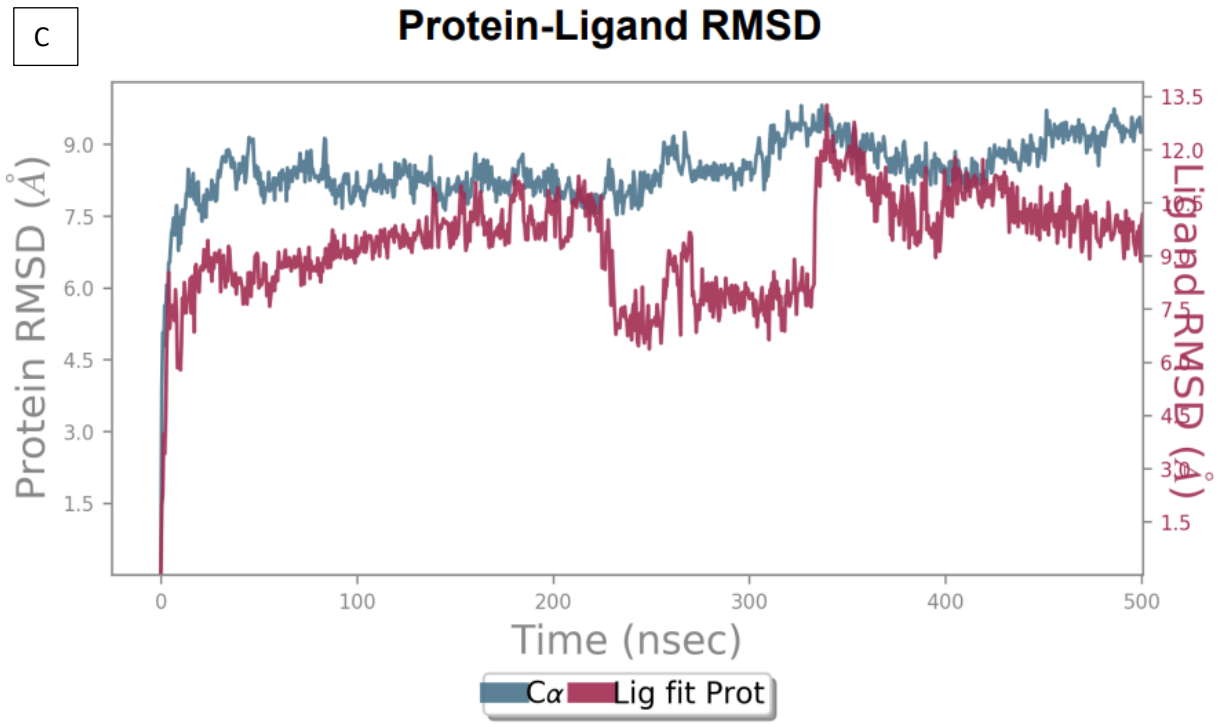
A



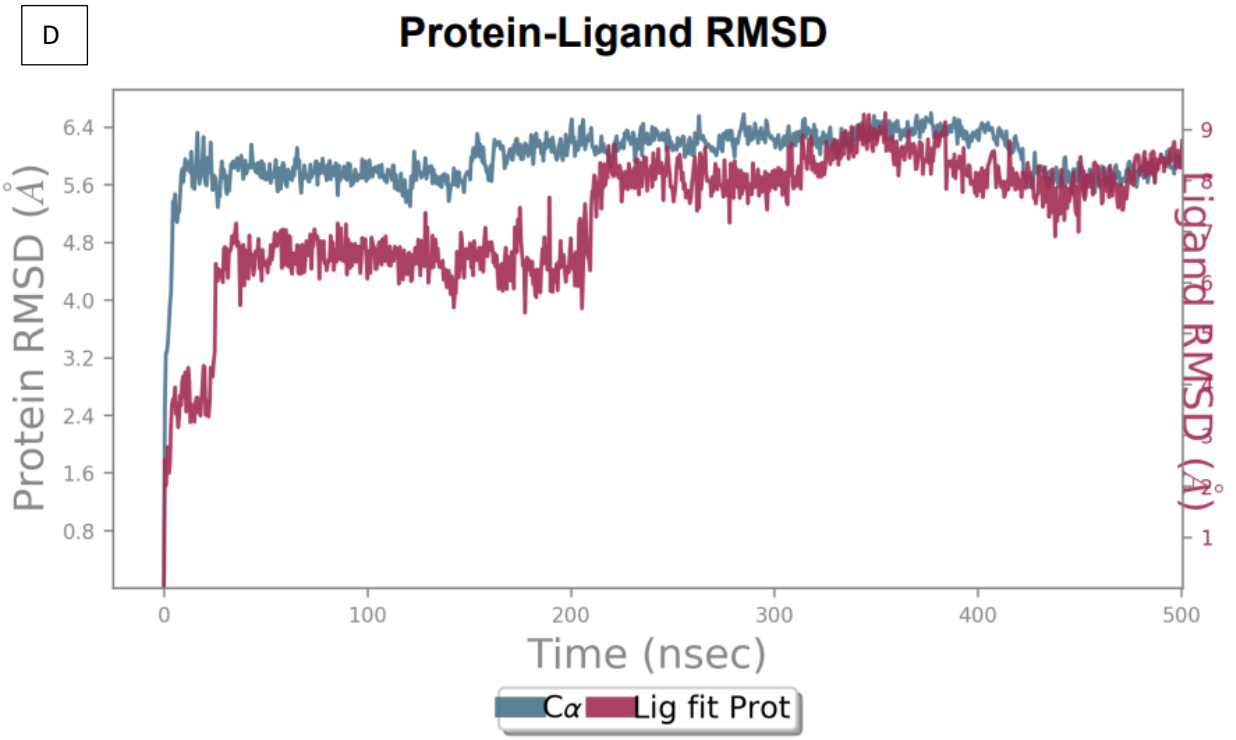
B



C


Protein-Ligand RMSD
Protein RMSD (Å)
Ligand RMSD (Å)
Time (nsec)
Cα
Lig fit Prot

D


Protein-Ligand RMSD
Protein RMSD (Å)
Ligand RMSD (Å)
Time (nsec)
Cα
Lig fit Prot

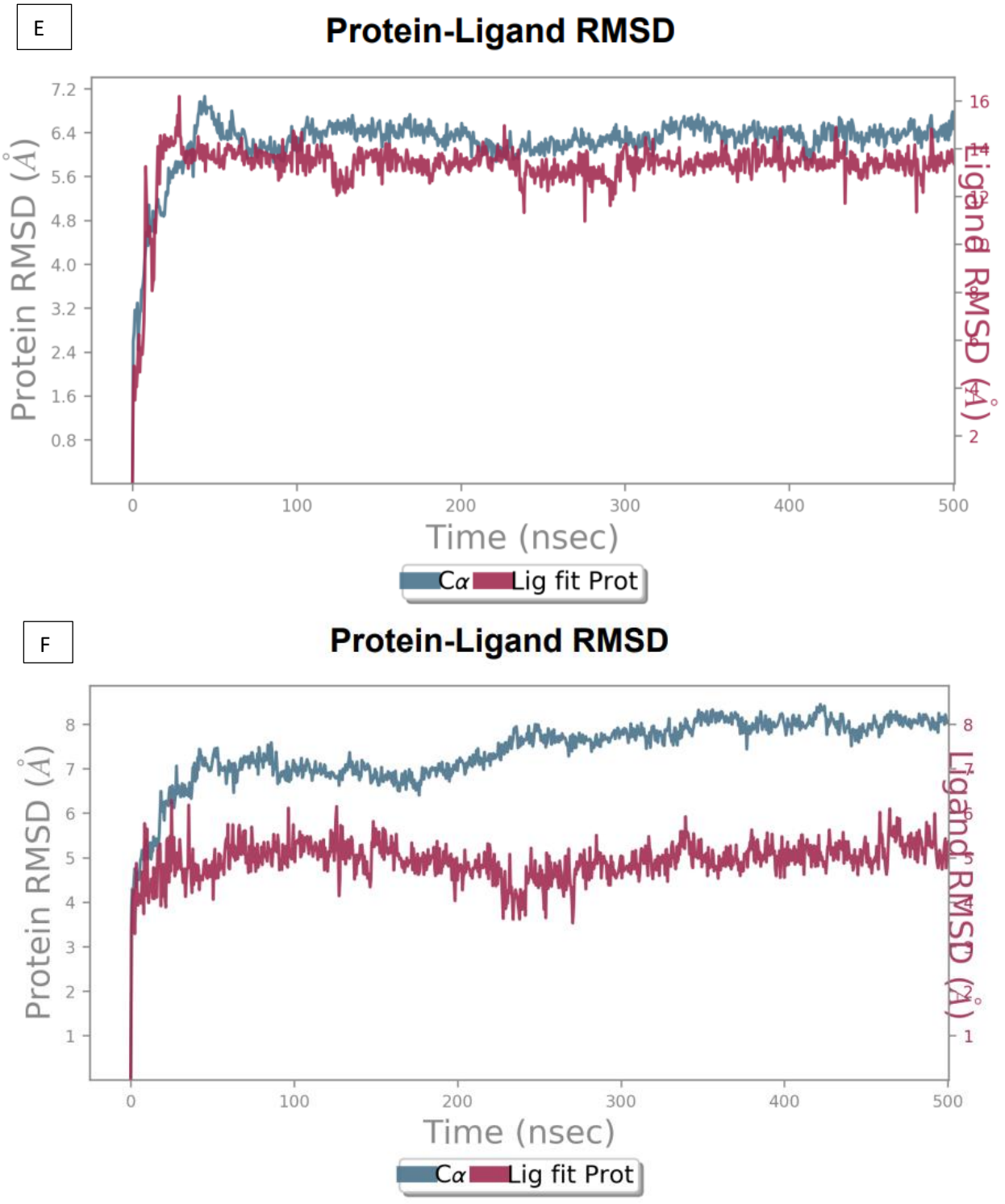
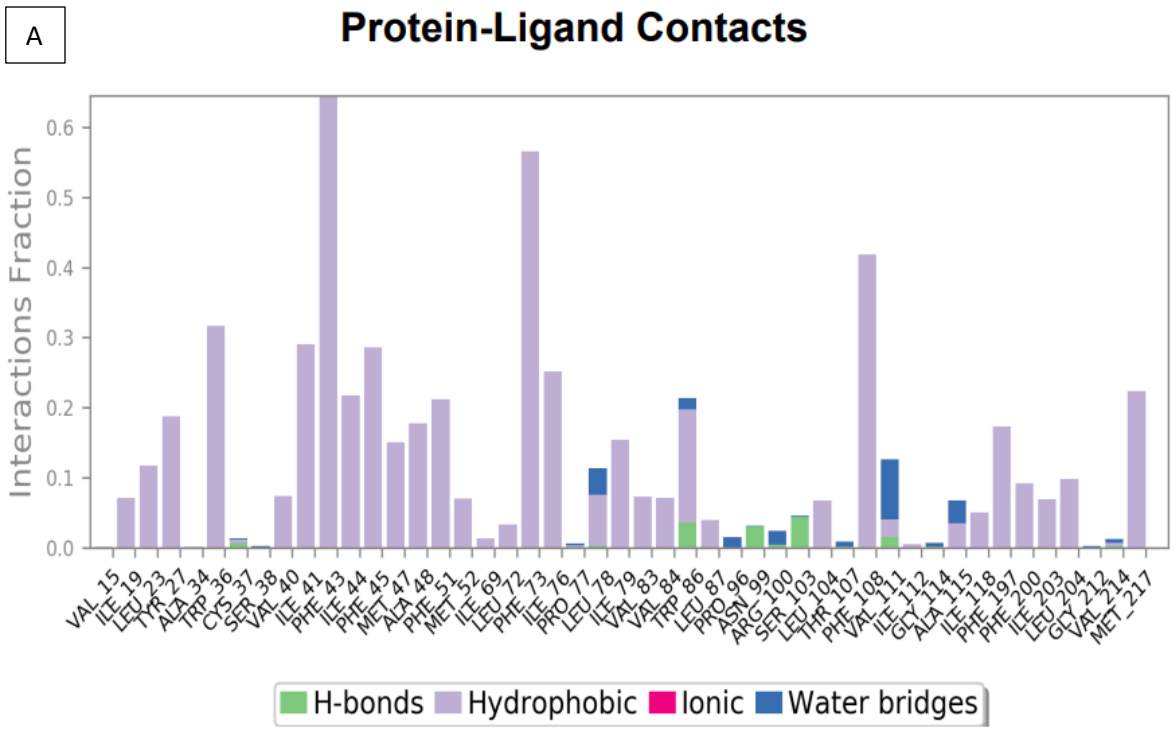


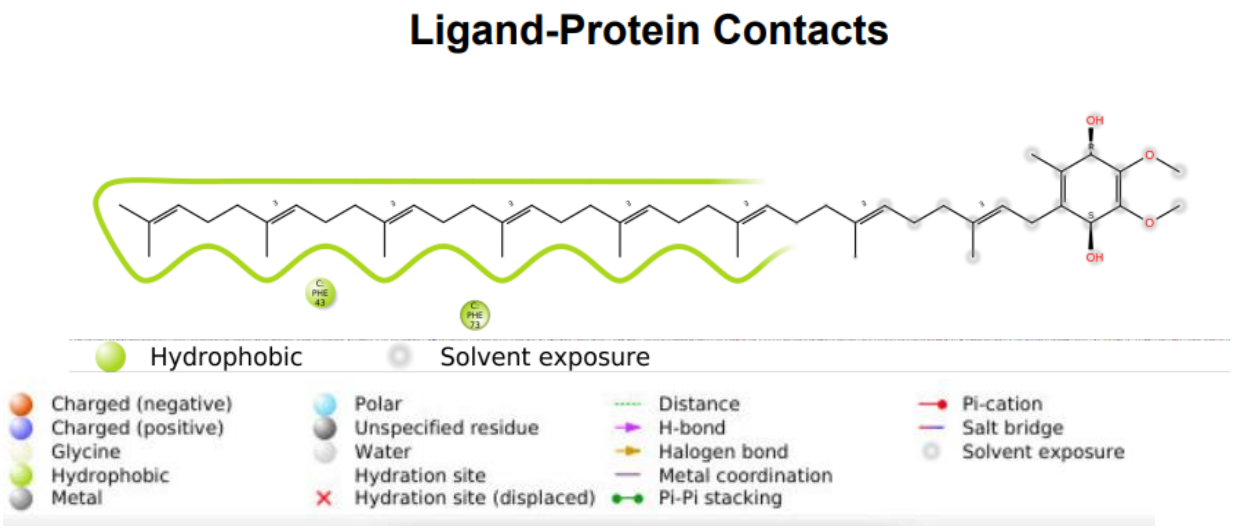
Figure 12. Protein and ligand RMSD (root mean square deviation) for the trajectory of each system toward active sites L124 and G138A mutated type (L45 and G60A on the figures). Protein RMSD is shown in dark blue and Ligand RMSD is shown in red. A) Ubiquinol with Cytochrome b; B) Fenamidone with Cytochrome b; C) Mandestrobin with Cytochrome b; D)

Azoxystrobin with Cytochrome b; E) Captan with Cytochrome b; F) Thiram with Cytochrome b.

All protein interaction of Cytochrome b with six different ligands showed an equilibrate interaction and these six ligands maintained a stable bonding with Cytochrome b. Based on protein-ligand contact and RMSD, Fenamidone, Mandestrobin, Azoxystrobin were effective fungicides against L124 and G138A mutated version. Captan and Thriam showed weak interaction toward the protein but they were still reasonable option for fungicide combination against L124 and G138A mutated version.

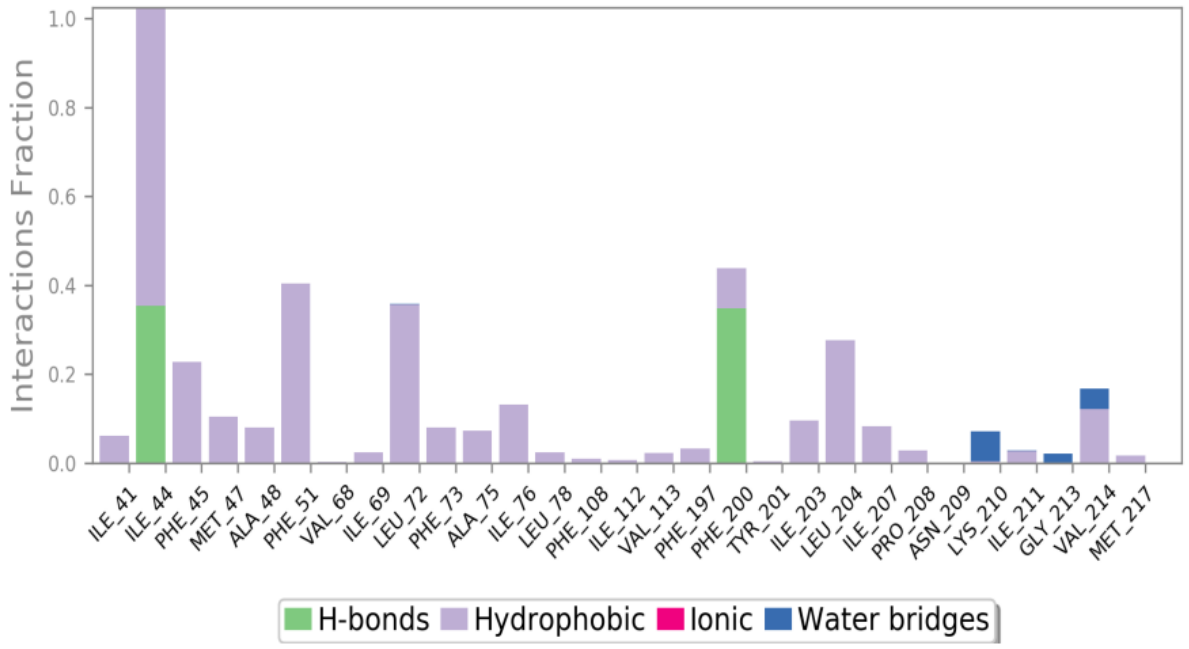
A


Protein-Ligand Contacts
Interactions Fraction
0.0
0.1
0.2
0.3
0.4
0.5
0.6
VAL_15 ILE_19 LEU_23 TYR_27 ALA_34 TRP_36 CYS_37 SER_38 VAL_40 ILE_41 PHE_43 ILE_44 PHE_45 MET_47 ALA_48 PHE_51 MET_52 ILE_69 LEU_72 PHE_73 ILE_76 PRO_77 LEU_78 ILE_79 VAL_83 VAL_84 TRP_86 LEU_87 PRO_96 ASN_99 ARG_100 SER_103 LEU_104 THR_107 PHE_108 VAL_111 ILE_112 GLY_114 ALA_115 ILE_118 PHE_197 PHE_200 ILE_203 LEU_204 GLY_212 VAL_214 MET_217
H-bonds
Hydrophobic
Ionic
Water bridges

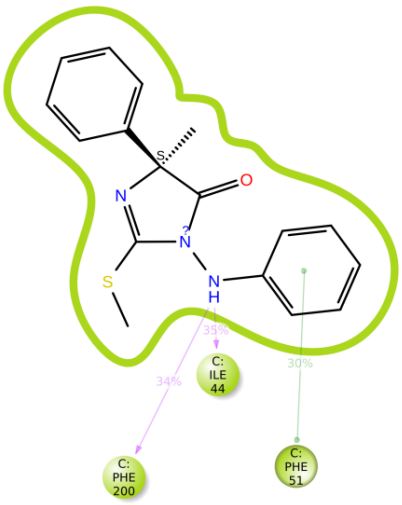

Ligand-Protein Contacts
OH
O
O
OH
C PHE 43
C PHE 73
Hydrophobic
Solvent exposure
Charged (negative)
Charged (positive)
Glycine
Hydrophobic
Metal
Polar
Unspecified residue
Water
Hydration site
Hydration site (displaced)
Distance
H-bond
Halogen bond
Metal coordination
Pi-Pi stacking
Pi-cation
Salt bridge
Solvent exposure

B

Protein-Ligand Contacts

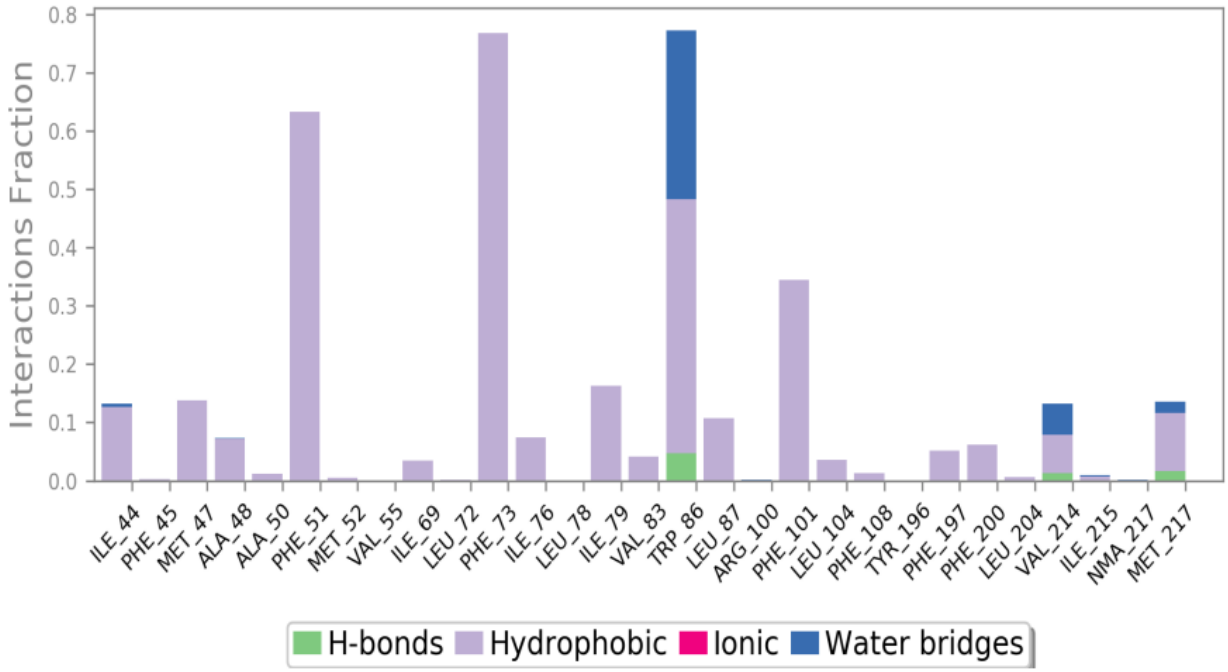


Ligand-Protein Contacts

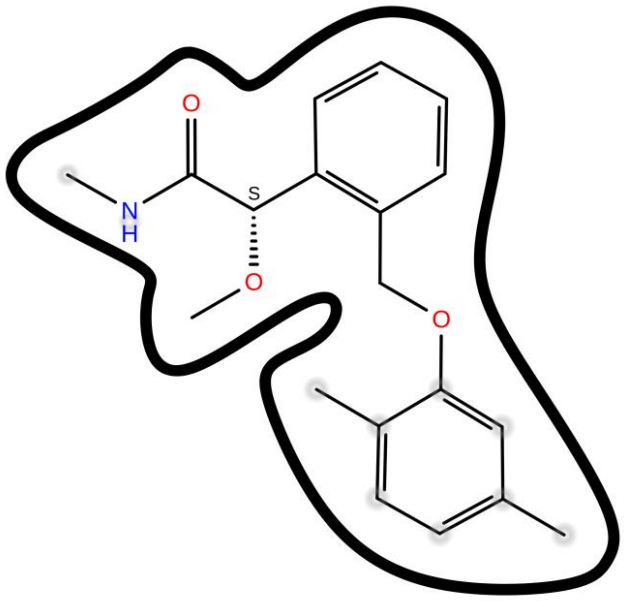


C

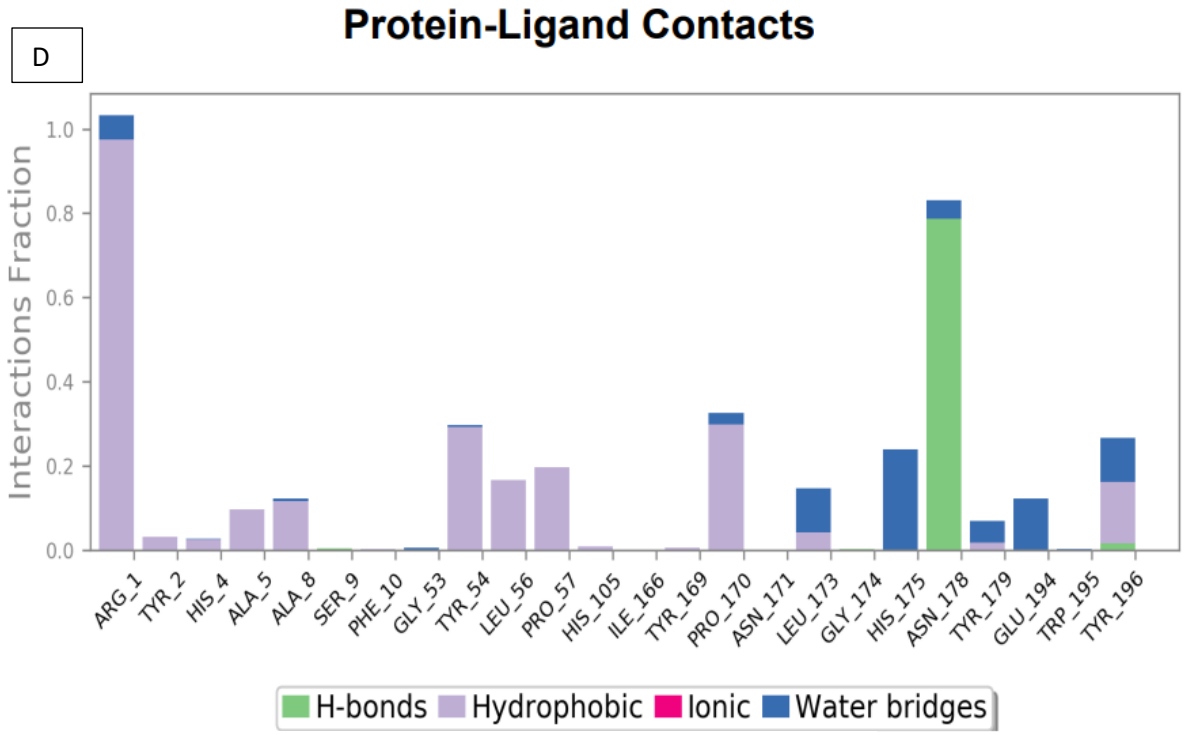
Protein-Ligand Contacts

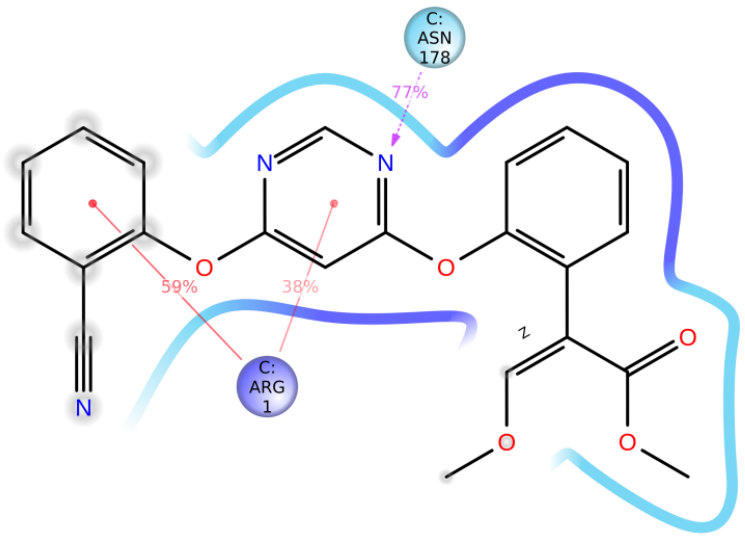


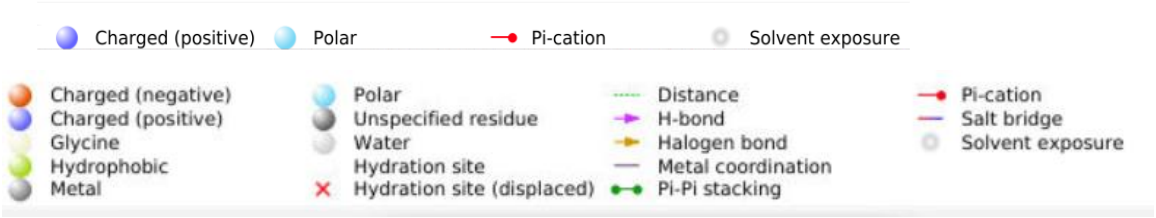
Ligand-Protein Contacts



D

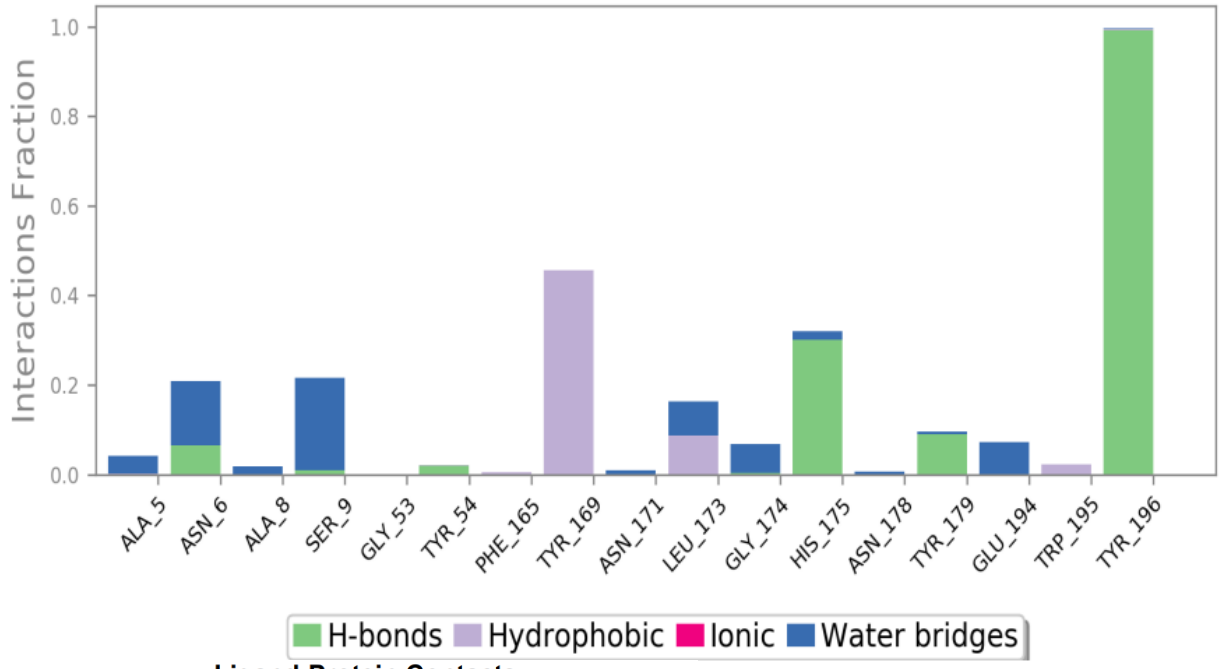

Protein-Ligand Contacts
Interactions Fraction
1.0
0.8
0.6
0.4
0.2
0.0
ARG_1
TYR_2
HIS_4
ALA_5
ALA_8
SER_9
PHE_10
GLY_53
TYR_54
LEU_56
PRO_57
HIS_105
ILE_166
TYR_169
PRO_170
ASN_171
LEU_173
GLY_174
HIS_175
ASN_178
TYR_179
GLU_194
TRP_195
TYR_196
H-bonds
Hydrophobic
Ionic
Water bridges


Ligand-Protein Contacts
C: ASN 178
77%
N
N
O
O
59%
38%
C: ARG 1
N
O
O
O

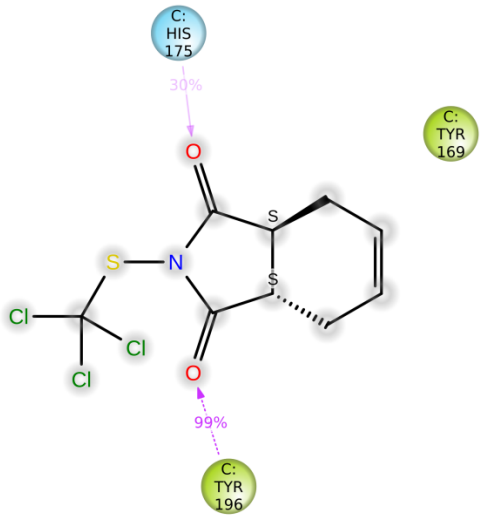

Charged (positive)
Polar
Pi-cation
Solvent exposure
Charged (negative)
Charged (positive)
Glycine
Hydrophobic
Metal
Polar
Unspecified residue
Water
Hydration site
Hydration site (displaced)
Distance
H-bond
Halogen bond
Metal coordination
Pi-Pi stacking
Pi-cation
Salt bridge
Solvent exposure

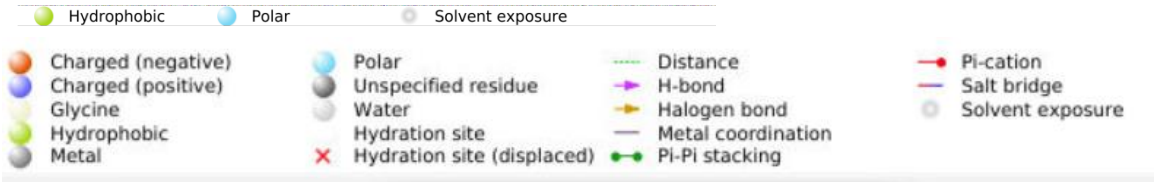
E

Protein-Ligand Contacts



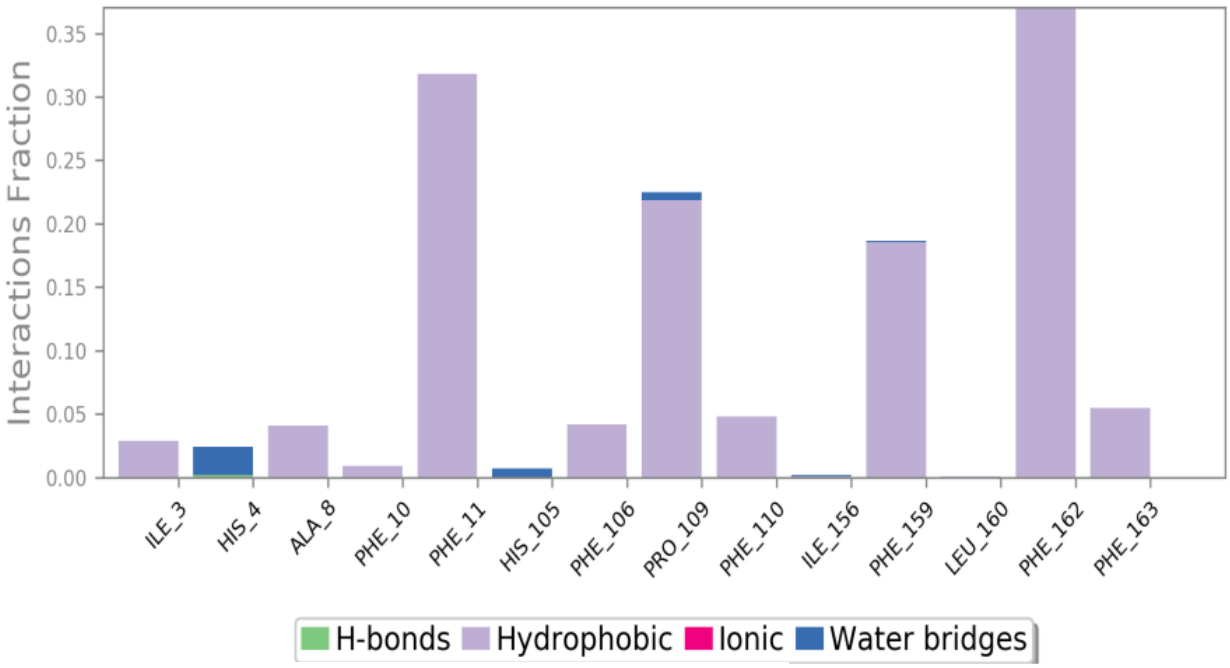
Ligand-Protein Contacts



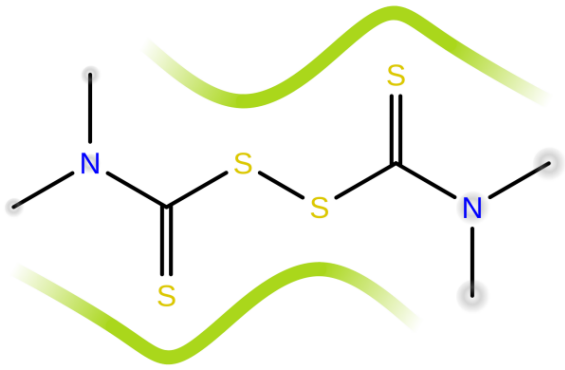


F

Protein-Ligand Contacts



Ligand-Protein Contacts



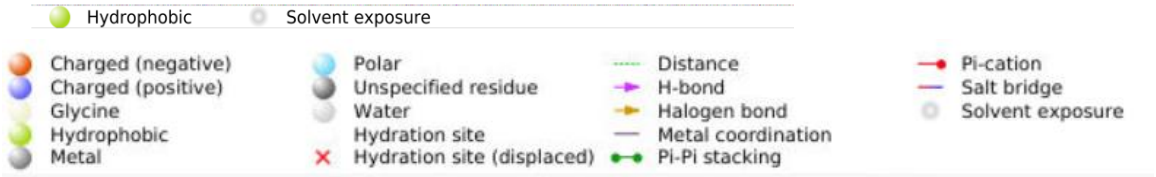
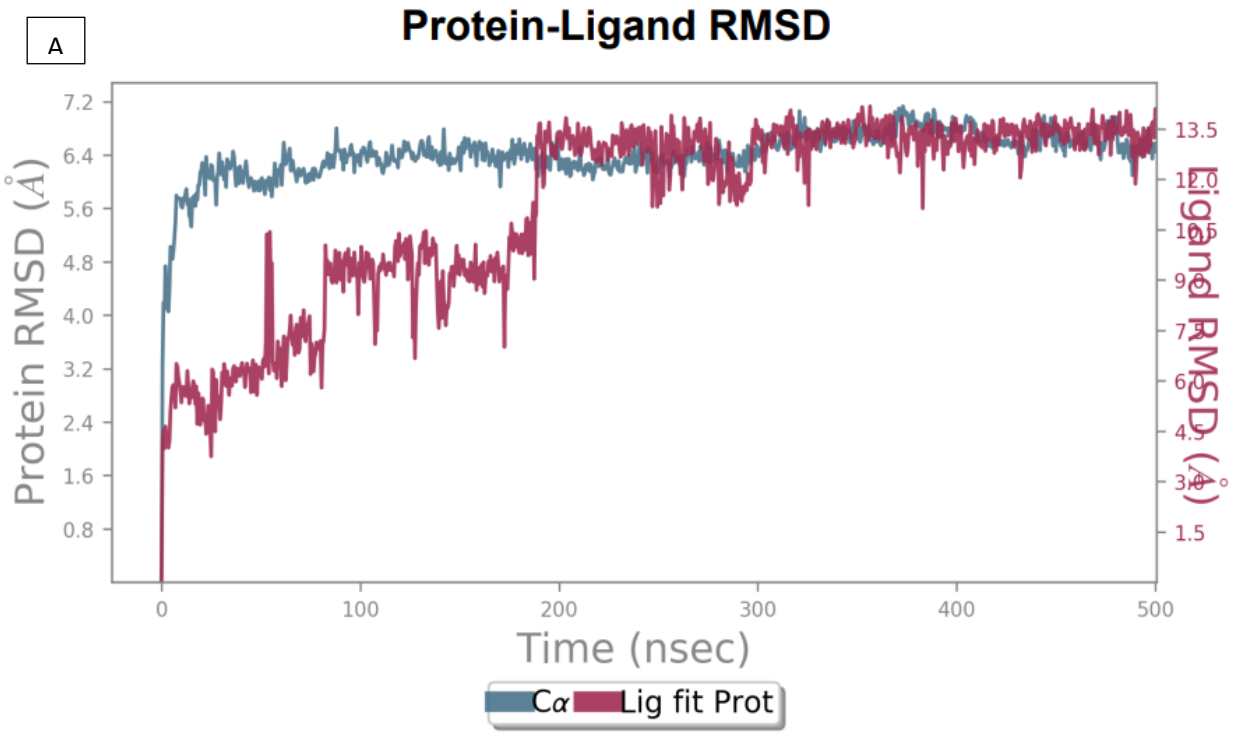
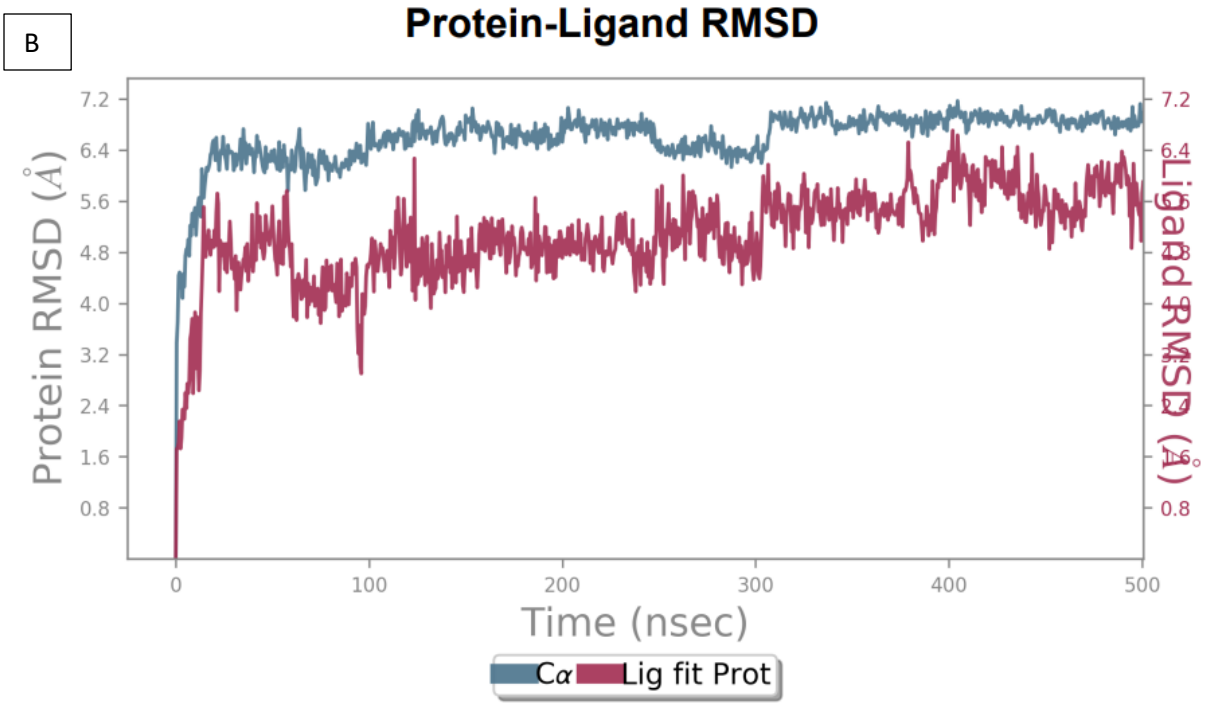


Figure 13. Protein-Ligand Interaction and contact toward active sites G137 and L123F mutated version (L45F and G60 on the figures). A) Ubiquinol with Cytochrome b; B) Fenamidone with Cytochrome b; C) Mandestrobin with Cytochrome b; D) Azoxystrobin with Cytochrome b; E) Captan with Cytochrome b; F) Thiram with Cytochrome b. The region started from 79 to 296 (0 to 217 on the figures).

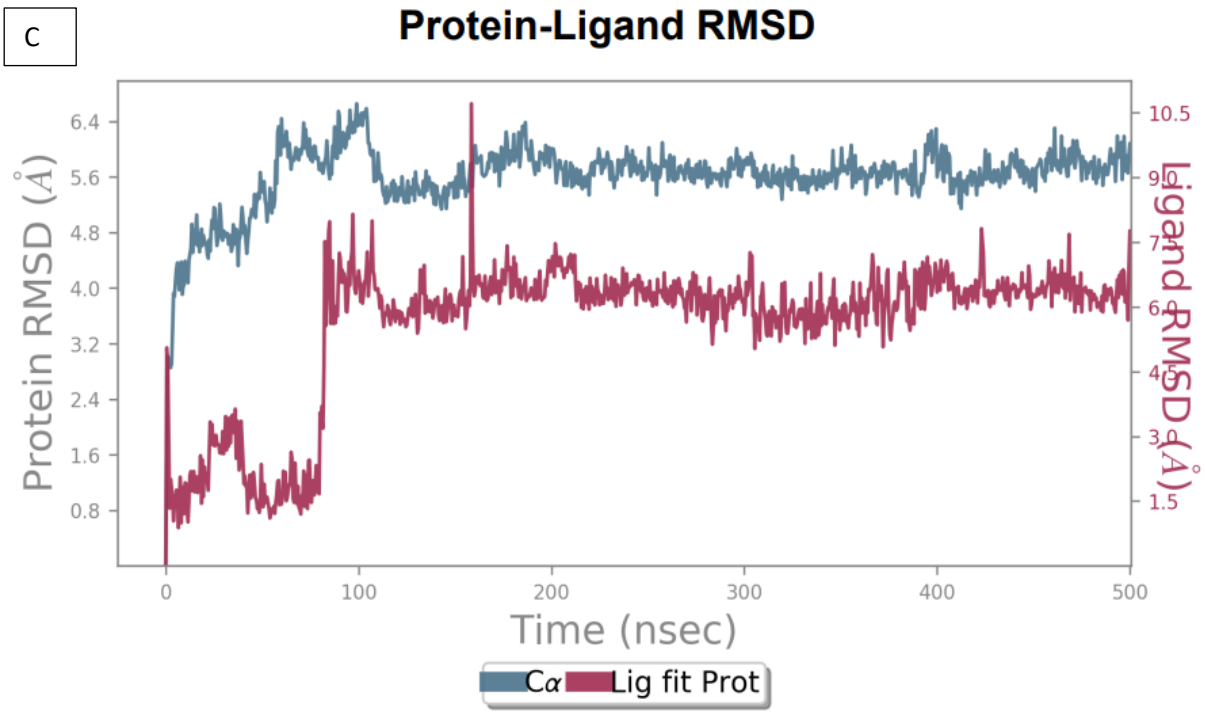
Ubiquinol had strong hydrophobic contact at PHE121, PHE151 and PHE186 (PHE43, PHE73 and PHE108 on the figures). Fenamidone had strong hydrophobic contact at ILE122 (ILE44 on the figure) and hydrogen bonding at ILE122 and PHE278 (ILE44 and PHE200 on the figure). Mandestrobin showed strong hydrophobic contact at PHE129, PHE151 and TRP164 (PHE51, PHE73 and TRP86 on the figure). Azoxystrobin showed very strong hydrophobic contact at ARG79 and ASN256 (ARG1 and strong hydrogen bonding ASN178 on the figure). Captan showed very strong hydrogen bonding at TRP274 (TRP196 on the figure). Thiram had weak hydrophobic contact at PHE89, PRO187 and PHE240(PHE11, PRO109 and PHE162 on the figure).



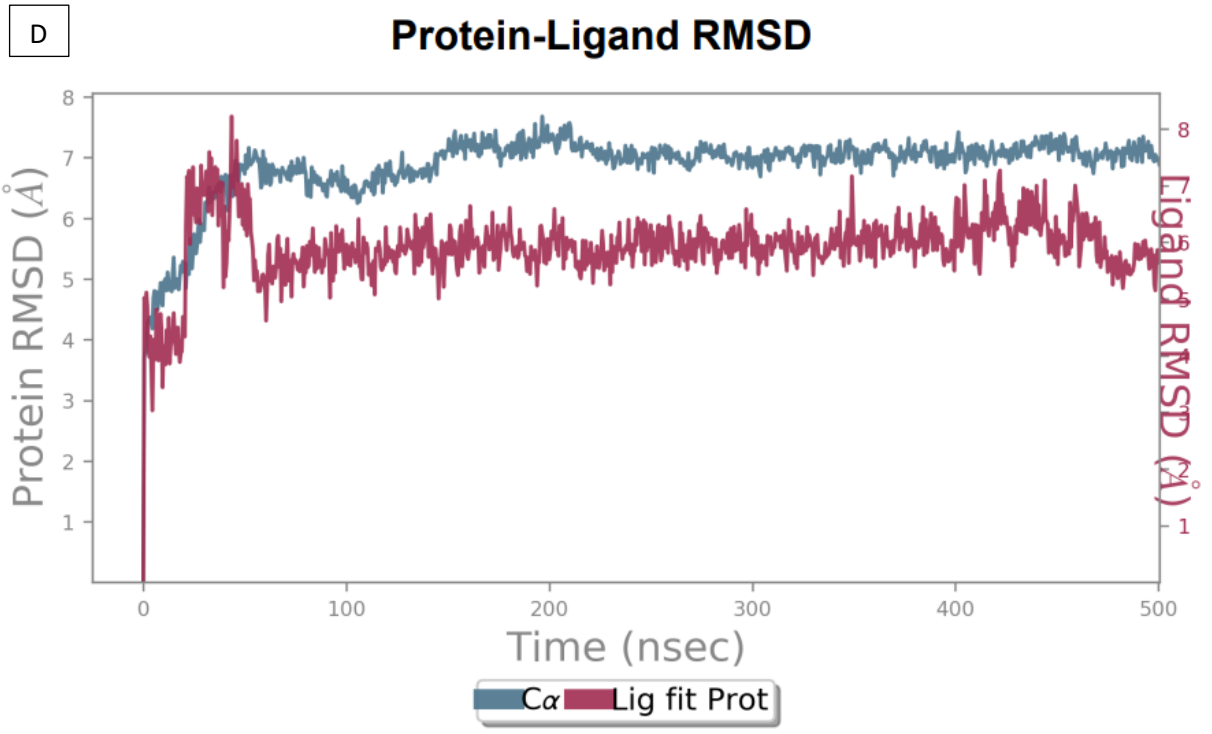
B


Protein-Ligand RMSD
Protein RMSD (Å)
Ligand RMSD (Å)
Time (nsec)
Cα
Lig fit Prot

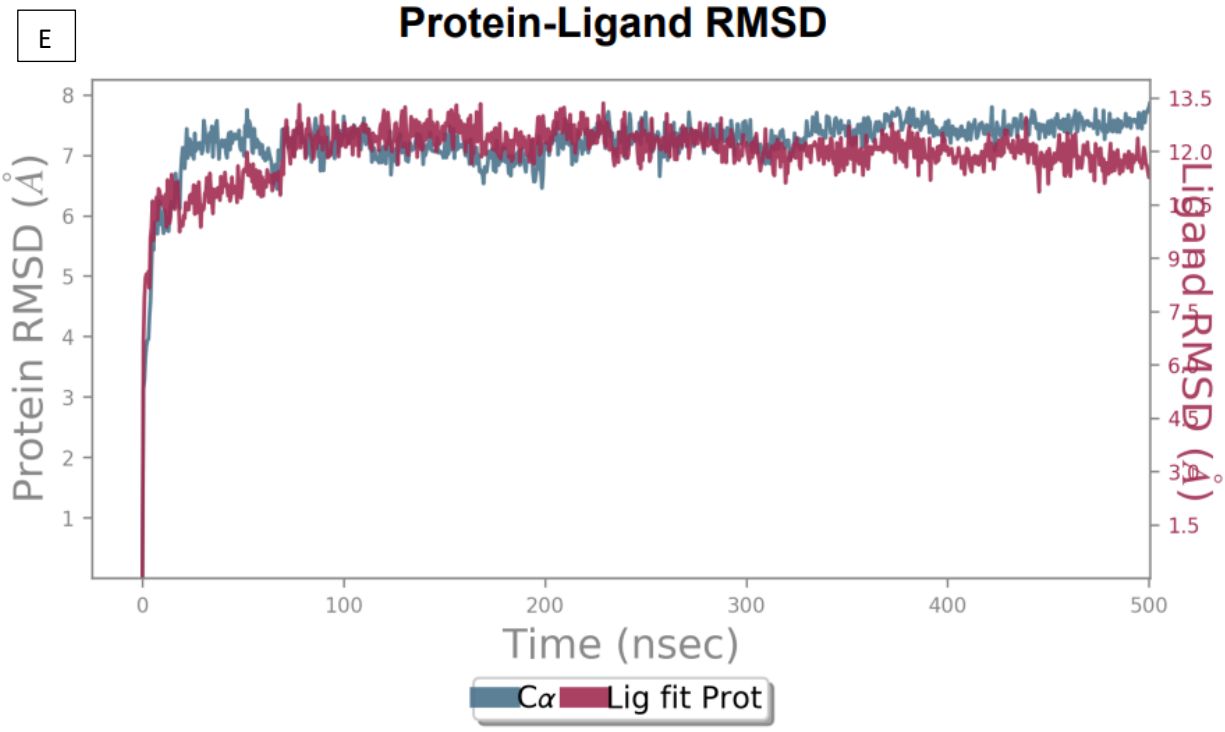
C


Protein-Ligand RMSD
Protein RMSD (Å)
Ligand RMSD (Å)
Time (nsec)
Cα
Lig fit Prot

D


Protein-Ligand RMSD
Protein RMSD (Å)
Ligand RMSD (Å)
Time (nsec)
Cα
Lig fit Prot

E


Protein-Ligand RMSD
Protein RMSD (Å)
Ligand RMSD (Å)
Time (nsec)
Cα
Lig fit Prot

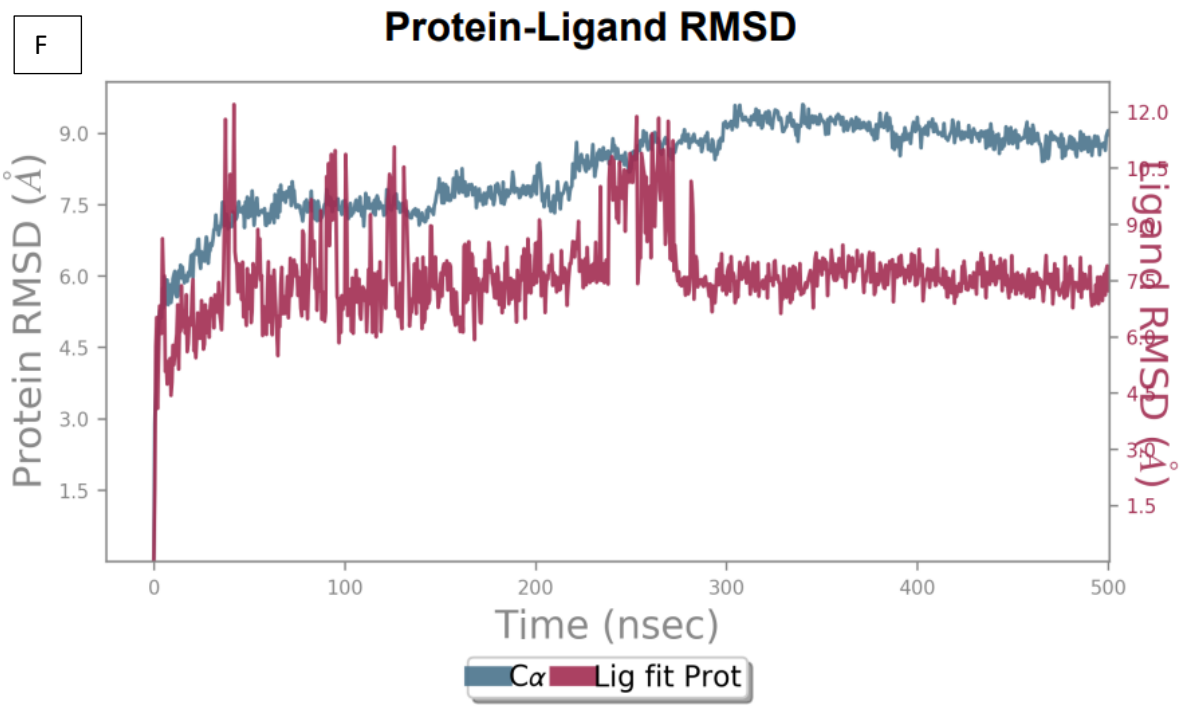
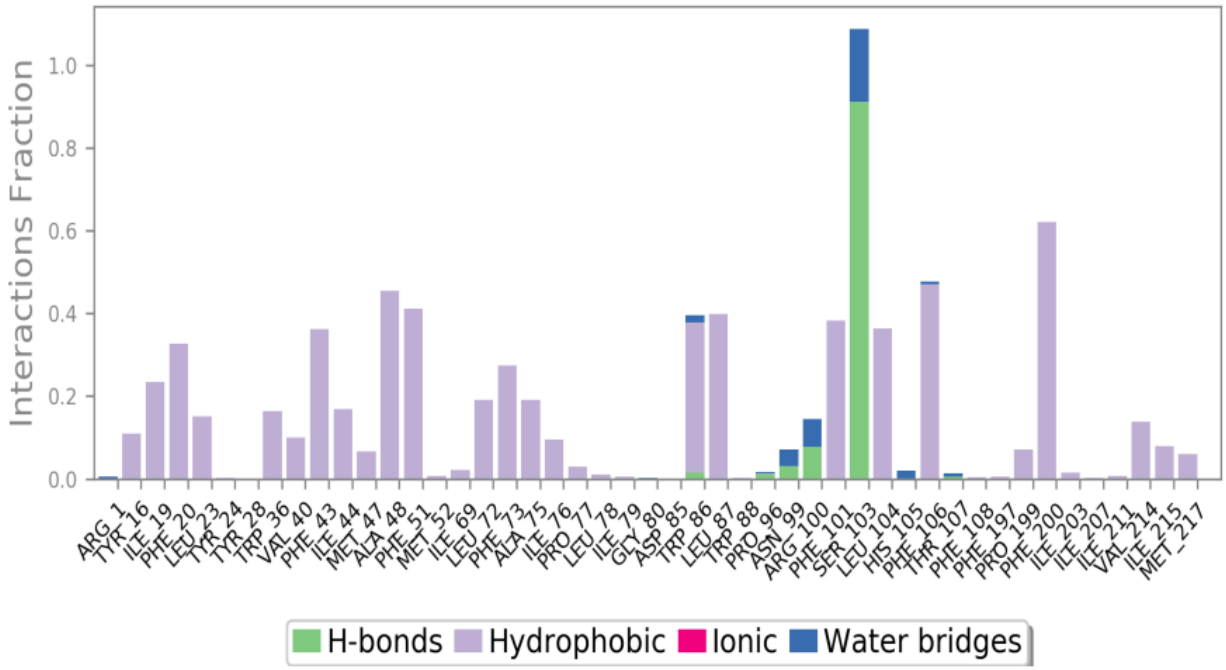


Figure 14. Protein and ligand RMSD (root mean square deviation) for the trajectory of each system toward active sites G138 and L124F mutated type (L45F and G60 on the figures). Protein RMSD is shown in dark blue and Ligand RMSD is shown in red. A) Ubiquinol with Cytochrome b; B) Fenamidone with Cytochrome b; C) Mandestrobin with Cytochrome b; D) Azoxystrobin with Cytochrome b; E) Captan with Cytochrome b; F) Thiram with Cytochrome b.

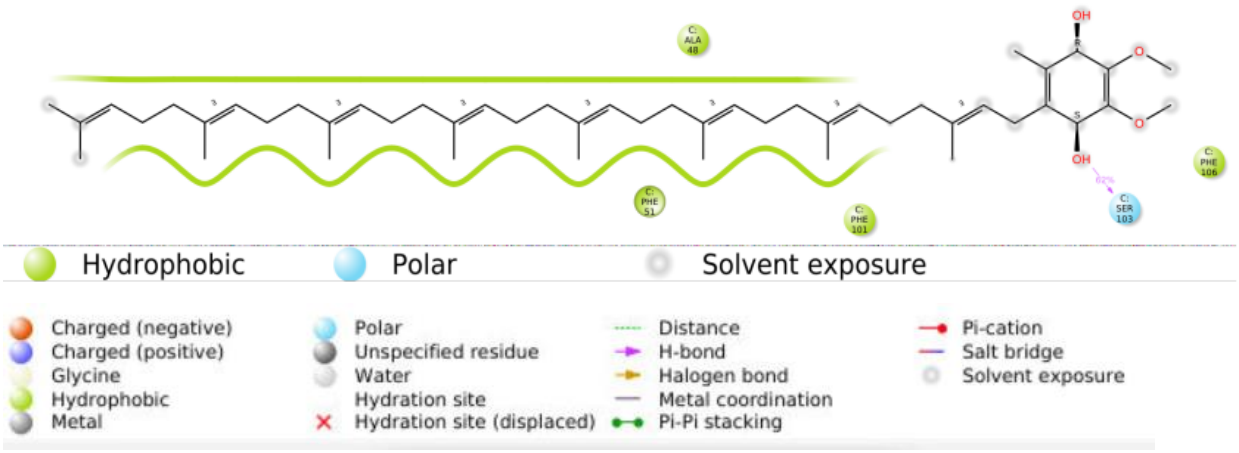
Protein interaction for Ubiquinol was equilibrated and Ubiquinol stably bound to the protein. Protein interactions for Fenamidone, Azoxystrobin and Captan were equilibrated and these three ligands stably bound to the protein. Mandestrobin had equilibrate protein interaction and it stably bound to the protein after 100 ns. Protein interaction for Thiram was equilibrated and Thiram stably bound to the protein after 300 ns. All five fungicides were stably maintained the interaction with Cytochrome b, showing they were effective fungicides against the G138 and L124F mutated version.

A

Protein-Ligand Contacts

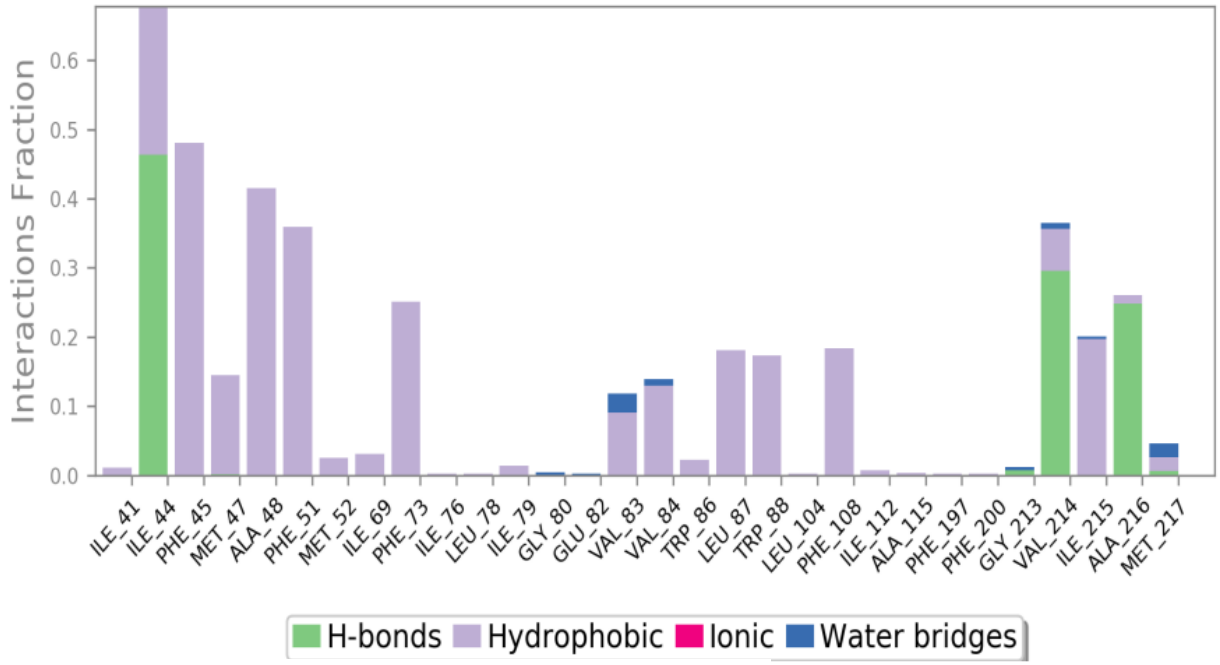


Ligand-Protein Contacts

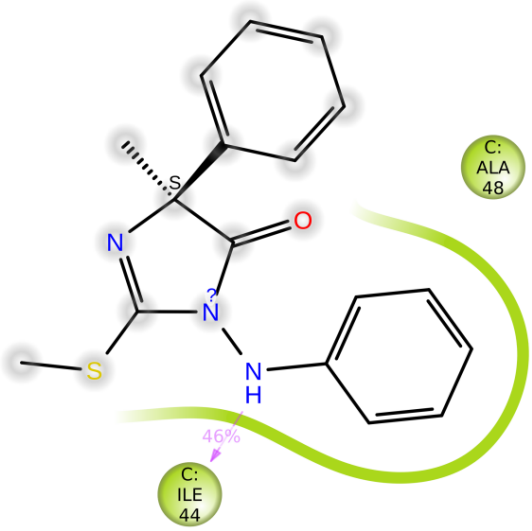


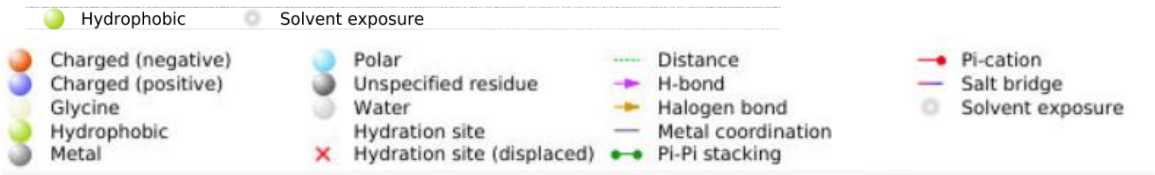
B

Protein-Ligand Contacts



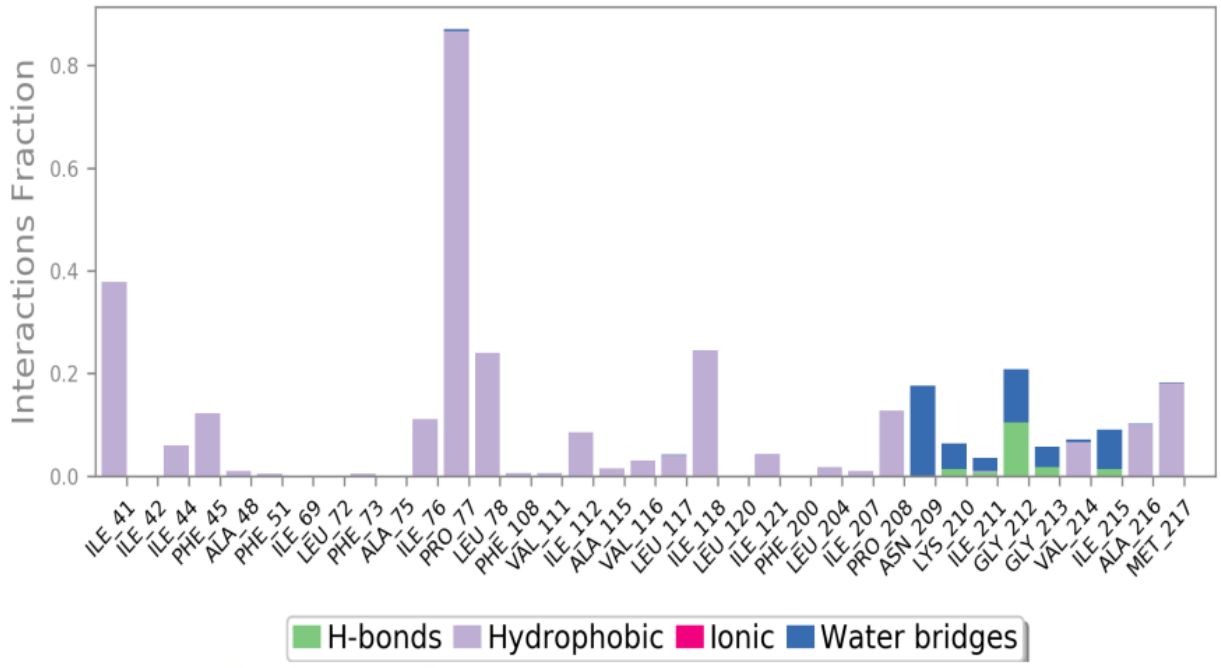
Ligand-Protein Contacts



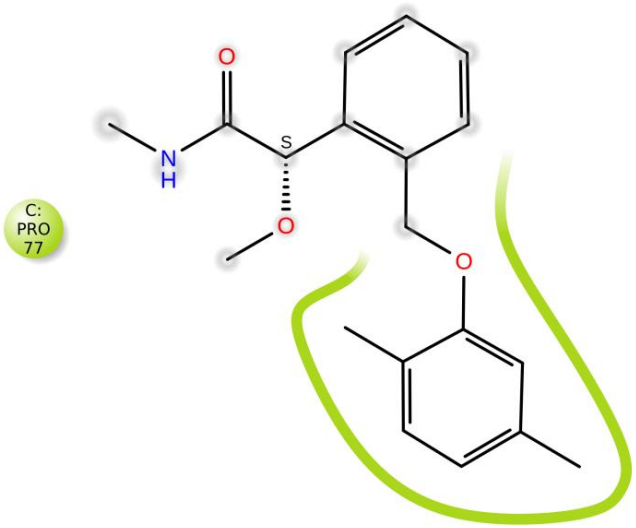


C

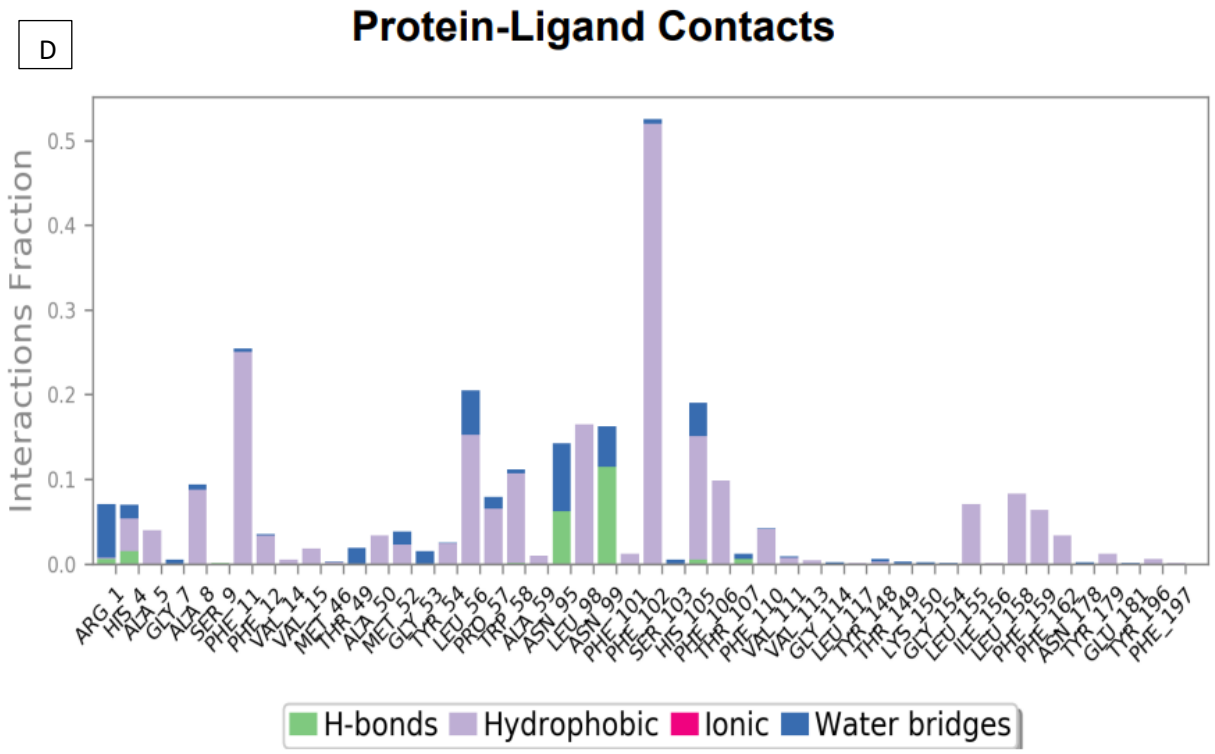
Protein-Ligand Contacts



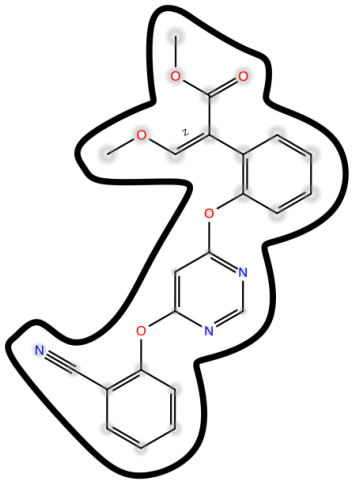
Ligand-Protein Contacts



D



Ligand-Protein Contacts

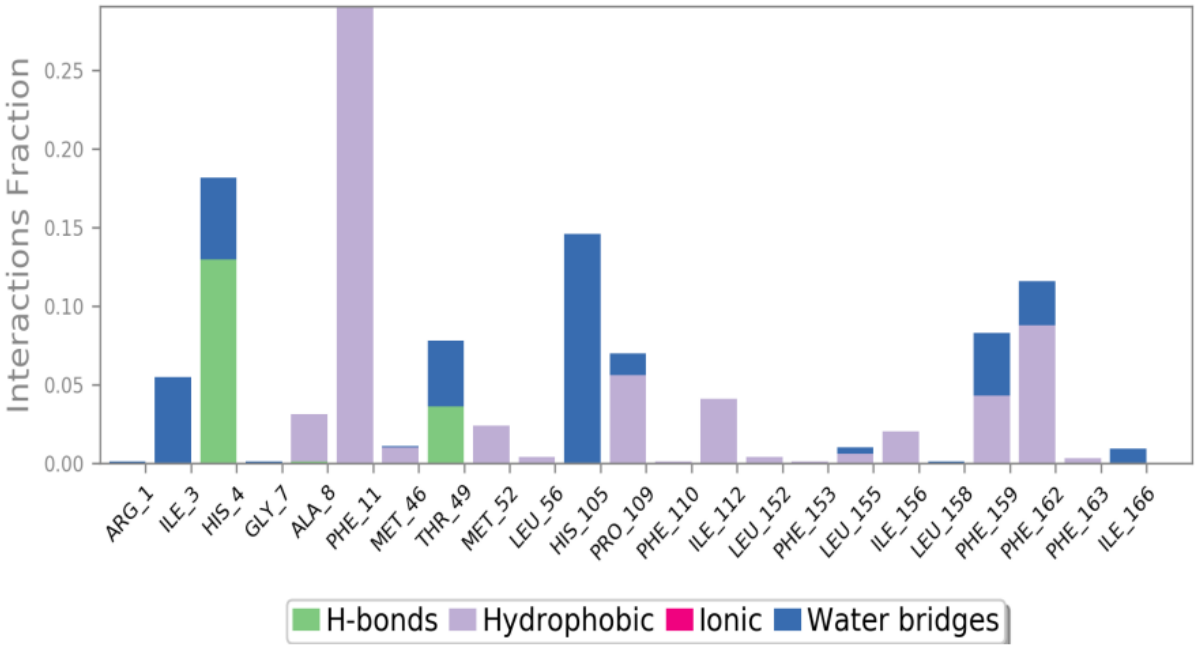


Solvent exposure

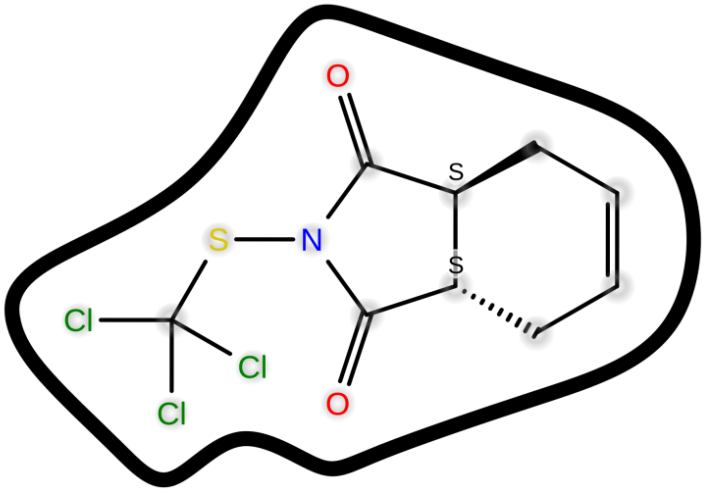
Charged (negative)
Charged (positive)
Glycine
Hydrophobic
Metal
Polar
Unspecified residue
Water
Hydration site
Hydration site (displaced)
Distance
H-bond
Halogen bond
Metal coordination
Pi-Pi stacking
Pi-cation
Salt bridge
Solvent exposure

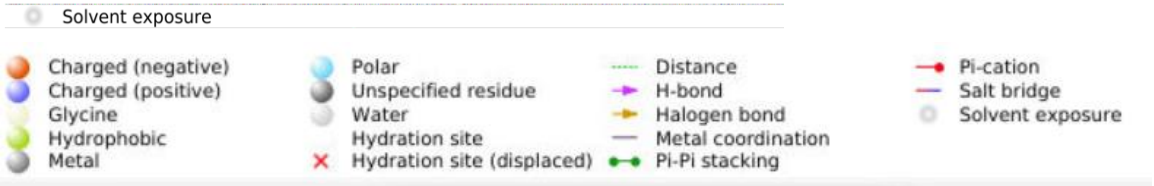
E

Protein-Ligand Contacts



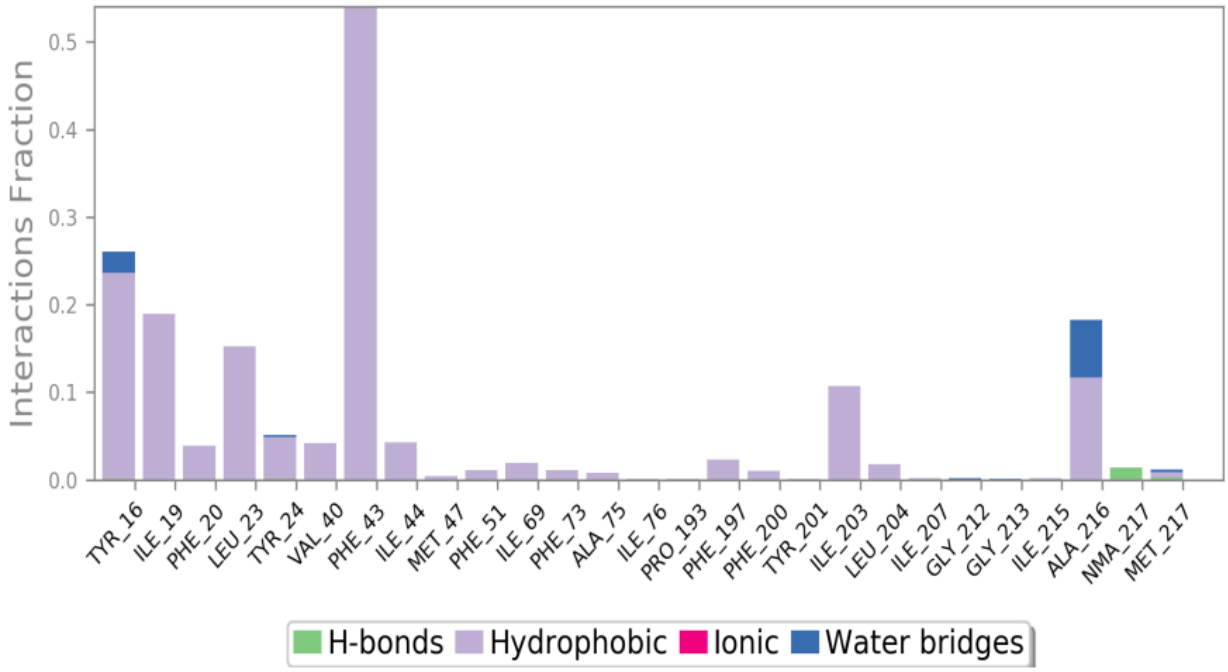
Ligand-Protein Contacts



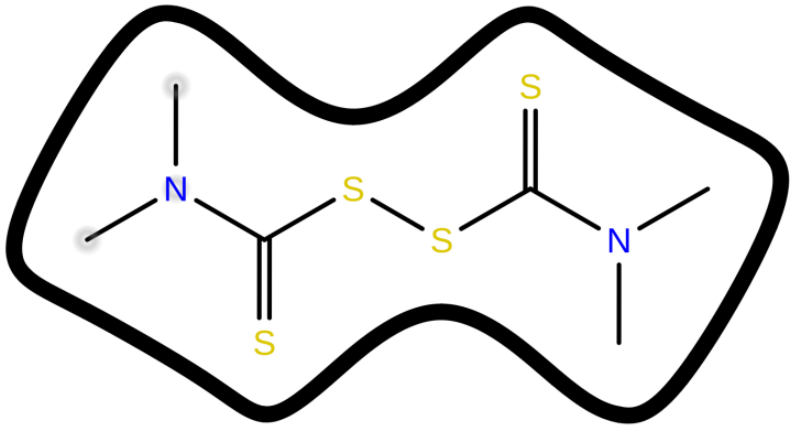


F

Protein-Ligand Contacts



Ligand-Protein Contacts



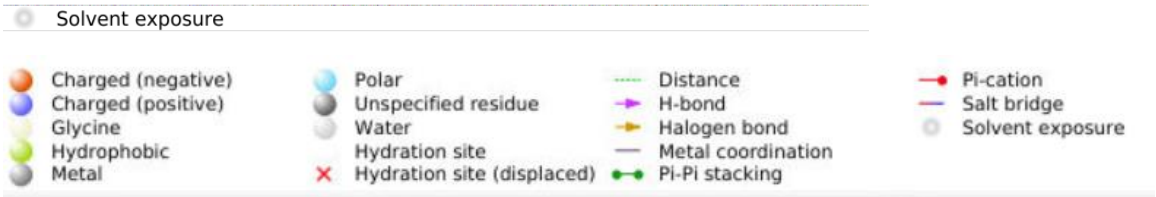
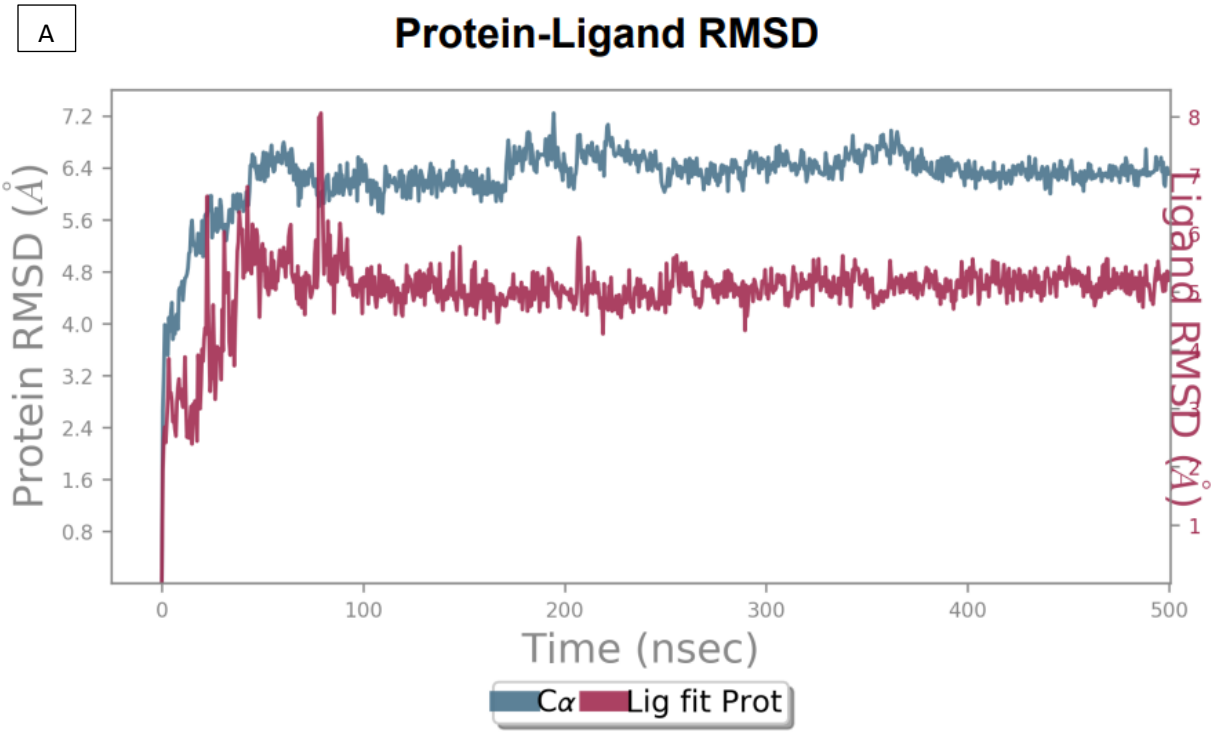
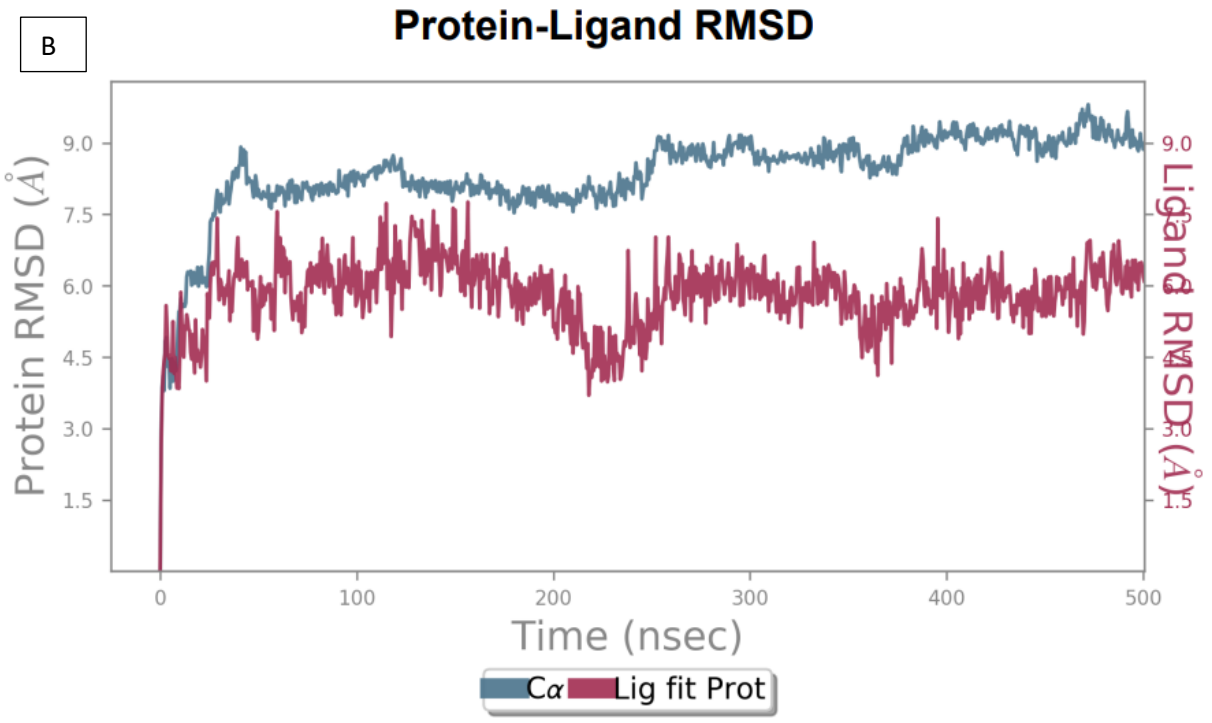


Figure 15. Protein-Ligand Interaction and contact toward active sites G137A and L123F mutated version (L45F and G60A on the figures). A) Ubiquinol with Cytochrome b; B) Fenamidone with Cytochrome b; C) Mandestrobin with Cytochrome b; D) Azoxystrobin with Cytochrome b; E) Captan with Cytochrome b; F) Thiram with Cytochrome b. The region started from 79 to 296 (0 to 217 on the figures).

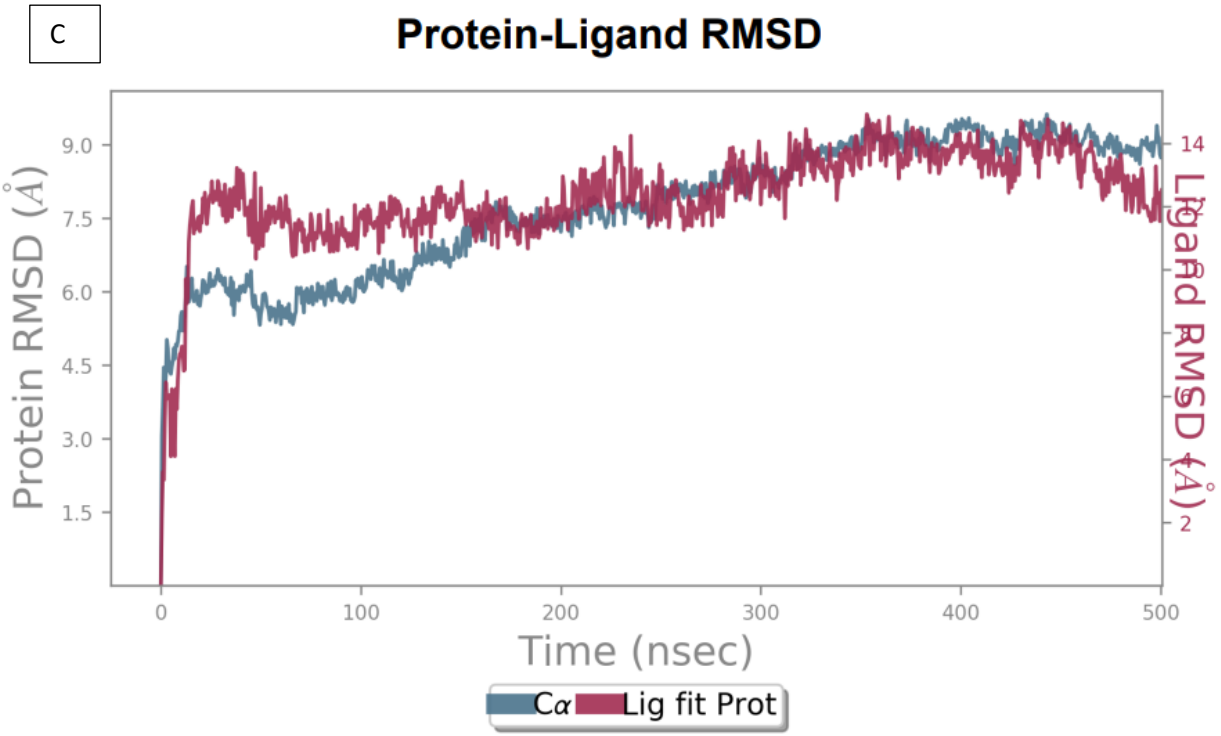
Ubiquinol had very strong hydrogen bonding at SER181 (SER103 on the figure) and strong hydrophobic contact at PHE278 (PHE200 on the figure). Fenamidone had strong hydrogen bonding at ILE122 (ILE44 on the figure) and hydrophobic contact at PHE123 (PHE45 on the figure). Mandestrobin only showed a very strong hydrophobic contact at PRO155 (PRO77 on the figure). Azoxystrobin only had strong hydrophobic contact at PHE179 (PHE102 on the figure). Captan showed weak hydrophobic at PHE89 (PHE11 on the figure) and weak hydrogen bonding at HIS82 (HIS4 on the figure). Thiram had weak hydrophobic contact at PHE120 (PHE43 on the figure).



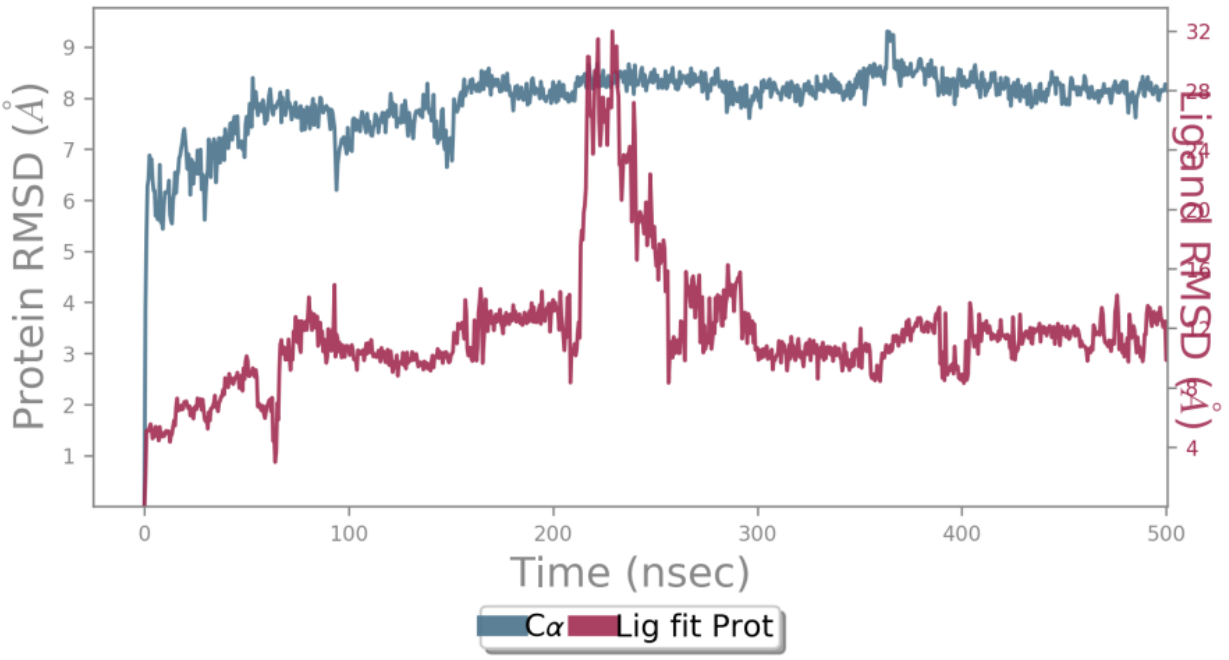
B


Protein-Ligand RMSD
Protein RMSD (Å)
Ligand RMSD (Å)
Time (nsec)
Cα
Lig fit Prot

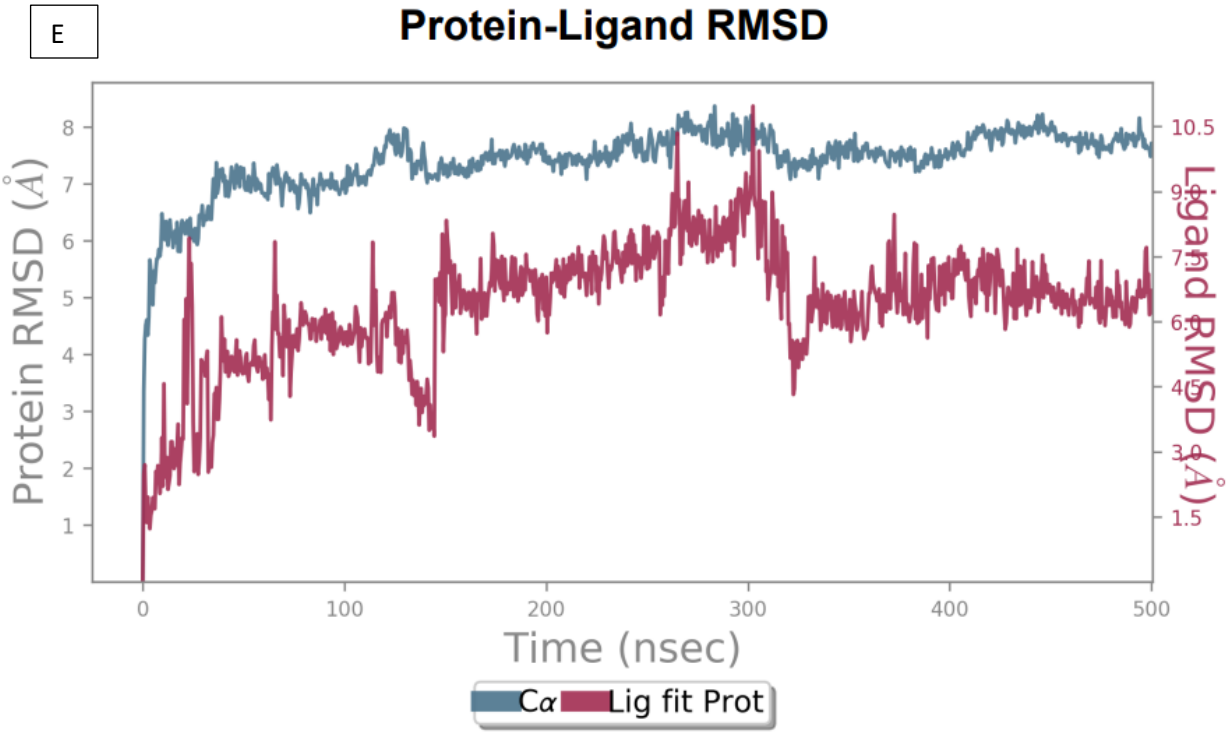
C


Protein-Ligand RMSD
Protein RMSD (Å)
Ligand RMSD (Å)
Time (nsec)
Cα
Lig fit Prot

D


Protein-Ligand RMSD
Protein RMSD (Å)
Ligand RMSD (Å)
Time (nsec)
Cα
Lig fit Prot

E


Protein-Ligand RMSD
Protein RMSD (Å)
Ligand RMSD (Å)
Time (nsec)
Cα
Lig fit Prot

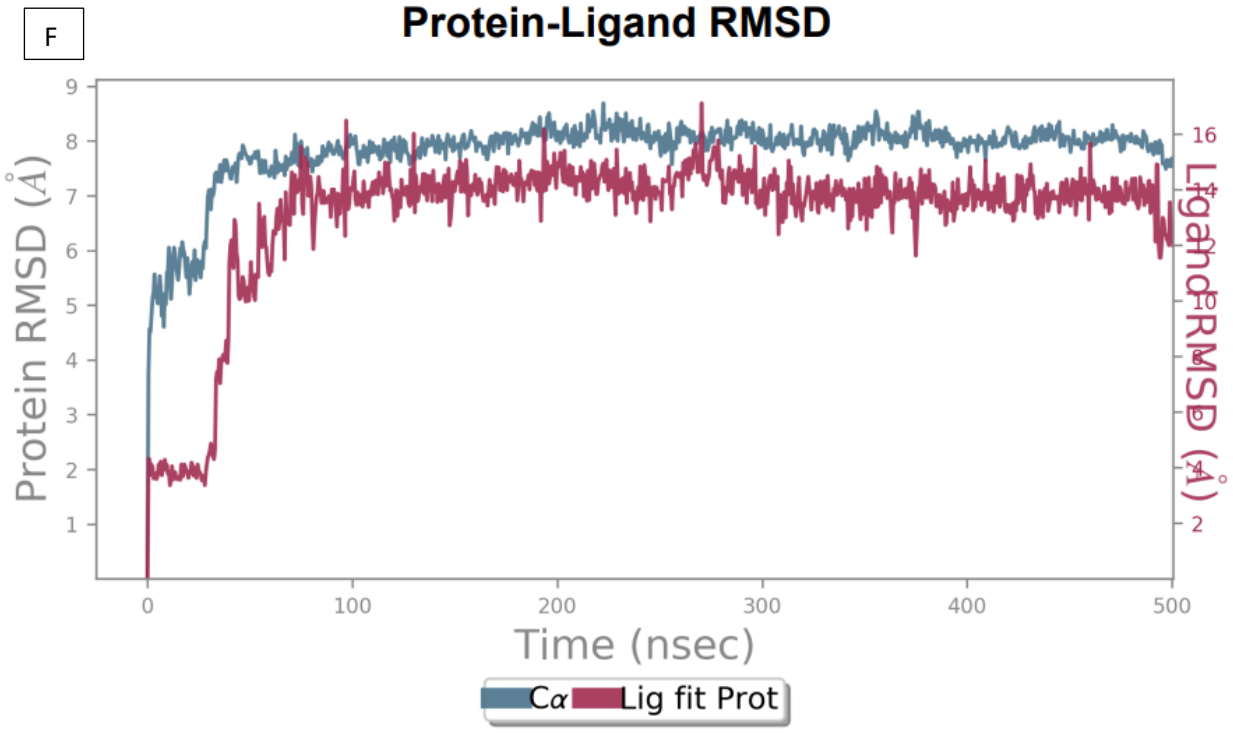


Figure 16. Protein and ligand RMSD (root mean square deviation) for the trajectory of each system toward active sites G138A and L124F mutated type (L45F and G60A on the figures). Protein RMSD is shown in dark blue and Ligand RMSD is shown in red. A) Ubiquinol with Cytochrome b; B) Fenamidone with Cytochrome b; C) Mandestrobin with Cytochrome b; D) Azoxystrobin with Cytochrome b; E) Captan with Cytochrome b; F) Thiram with Cytochrome b.

Protein interaction for Ubiquinol was equilibrated and Ubiquinol stably bound to the protein. Protein simulation for Fenamidone and Mandestrobin were equilibrated and these two ligands stably bound to the protein. Azoxystrobin had equilibrate protein interaction but Azoxystrobin was not stable in the range from 200 ns to 260 ns. Captan and Thiram had equilibrate protein interaction and both ligands stably bound to the protein.

Among all the protein-ligand contact, hydrophobic contact still was the major interaction and the second interaction was hydrogen bonding. The protein-ligand RMSD toward different mutated version at active sites L124 and G138 was more stable than RMSD of protein-ligand interaction on F129 and G143, which meant active sites L124 and G138 had less influence on the protein than F129 and G143.