CHAPTER 3

Result and Discussion

3.1 Homology modeling

3.1.1 Sequences alignment and homology modeling

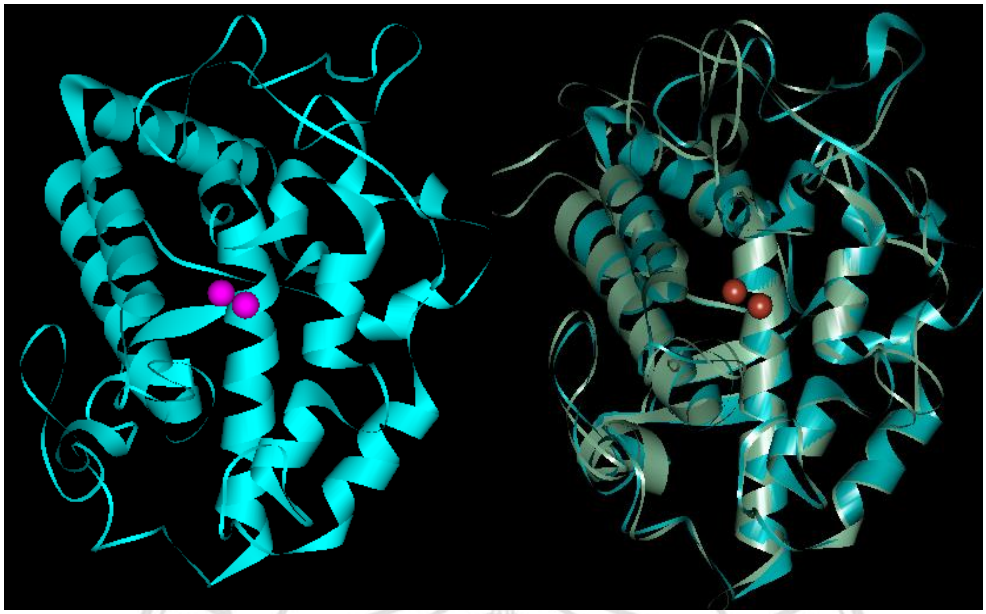
The crystal structure of *B.megaterium* tyrosinase was selected, with the highest resolution of 2.3 Å, by blast searching. The sequence alignment between the query protein sequence (protein id = AAA61242) and template protein sequence (PDB id = 3NQ1) showed highest 33.5% identity and similarity of 50.7%. (**Fig. 3.1**). In homology modeling, the highest sequence similarity to identity percentage is a key factor considered according to the theory; the more sequence the similarity, the higher the accuracy. Otherwise, the resolution is also another factor to indicate the accuracy of the structure, the resolution value should be more than 2.0 Å for a high model prediction or at least 1.0 Å can be accepted in basically. The conserved regions of tyrosinase are six histidine residues in the active site of the target were matched with those of template. Matching residues were highlighted by star symbol.

```
AAA61242        RLLVRRNIFDLSAPEKDKFFAYLTLAKHTISSDYVIPIGTYGQMKNGSTP
3NQ1_A          KYRVRKNVLHLTDTEKRDFVRTVLILKEKGI-------------------

AAA61242        MFNDINIYDLFVWMHYYVSMDALLGGSEIWRDIDFAHEAPAFLPWHRLFL
3NQ1_A          -------YDRYIAWHGAAGKFHTPPG----SDRNAAHMSSAFLPWHREYL
                              *                       *       *

AAA61242        LRWEQEIQKLTGDENFTIPYWDWRDA------EKCDICTDEYMGGQHPTN
3NQ1_A          LRFERDLQSI--NPEVTLPYWEWETDAQMQDPSQSQIWSADFMGGNGNPI

AAA61242        PNLLSPASFFSSWQIVCSRLEEYNSHQSLCNGTPEGPLRRNPGNHDKSRT
3NQ1_A          KDFIVDTGPFAAGRWTTI----------DEQGNPSGGLKRNFGATK--EA

AAA61242        PRLPSSADVEFCLSLTQYESGSMDKAANFSFRNTLEGFASPLTGIADASQ
3NQ1_A          PTLPTRDDVLNALKITQYDTPPWDMTSQNSFRNQLEGFIN---------G

AAA61242        SSMHNALHIYMNGTMSQVQGSANDPIFLLHHAFVDSIFEQWLRRHRPLQE
3NQ1_A          PQLHNRVHRWVGGQMGVVPTAPNDPVFFLHHANVDRIWAVWQIIH-RNQN
                   *   *                       *

AAA61242        VYPEANAPIGHNRESYMVPFIPLYRNGDFFISSKDLGYDYSYLQDSDP
3NQ1_A          YQPMKNGPFGQNFRDPMYPWN--TTPEDV-MNHRKLGYVYDI------
```

Figure 3.1 Sequence alignment between human amino acid sequence (AAA61242) and crystal structure of bacterial tyrosinase (3NQ1) with identity of 33.5% and similarity of 50.7%. Six histidine residues, which are provided by a four helical bundle, coordinate the two copper ions (CuA and CuB) in the active site

3.1.2 The homology modeling construction and evaluation

A superposition of the three dimensional structures of the human homology model and that for *B. megaterium* tyrosinases show template shows a slight deviation from its template of 0.64 Å (**Fig. 3.2**).



(a) **(b)**

Figure 3.2 Superimpose of homology model (green) and its template (blue)

The homology model was validated using PROCHECK and Verify3D. The Ramachandran plot of human tyrosinase (**Fig. 3.3**) found out the most favored regions show as in red, additional allowed, generously allowed, and disallowed regions are shown in yellow, light yellow and white, respectively. In the most favored regions was 81.6% of residues, additional allowed was 12.6% of residues, generously allowed regions was 4.1% of residues and the rest 1.7% are in disallowed regions. The residues in disallowed region are Asp59, Leu74, Trp80, Ser152, and Cys174 which are apart from the active area as shown in **Fig. 3.3 b**. The Verify 3D plot (**Fig. 3.3 c**) shows a compatibility score of the model with its sequence. If more than 70 % of the residues have a score of greater or equal to 0.2, then the protein structure is considered to be of high quality. As shown in **Fig. 3.3 c**, 70 % residues of the generated model have score over 0.2; thus, the quality of the predicted model is suitable for further analysis [1].

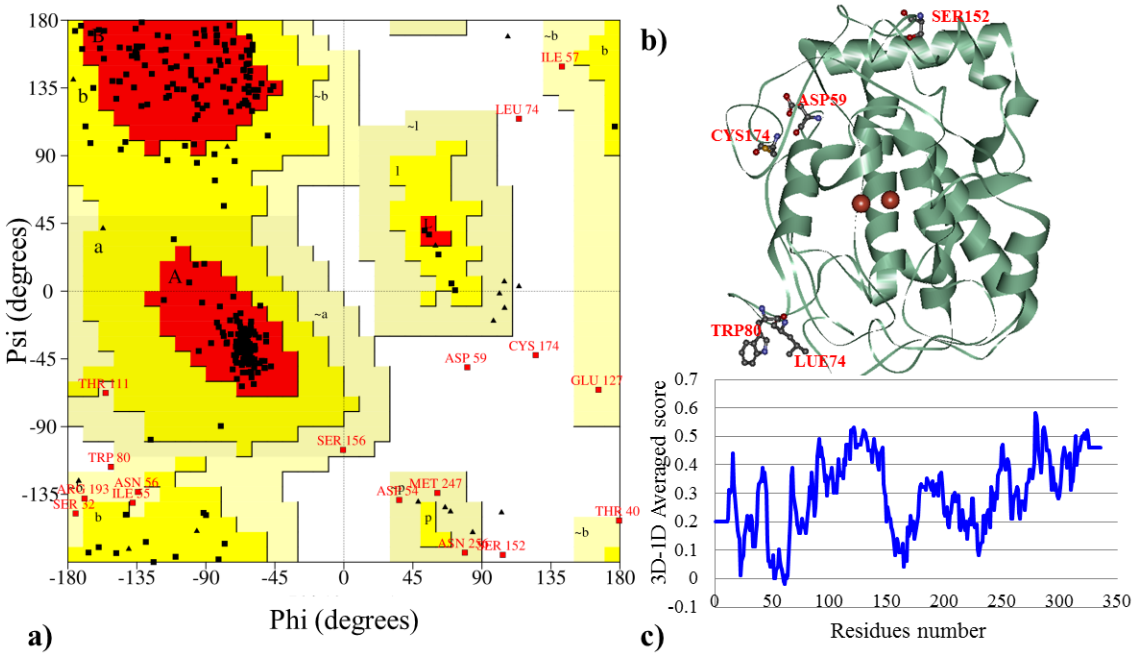


Figure 3.3 Quality of validation of the homology model a) Ramachandran plot of human tyrosinase b) Residue in disallowed region are Asp59, Lue74, Trp80, Ser152, and Cys174 c) Verify 3D plot**.**

3.2 Binding Scaffolds: Docking and MD Simulation

3.2.1 Molecular Docking

From **Fig. 3.4**, the dock energy of mushroom tyrosinase-arbutin complex had the lowest energy at -3.98 kcal/mol and the highest energy at -2.71 kcal/mol. The cluster was classified into 10 clusters and the highest number of docked conformation in a cluster contains 21 conformations and this cluster was chosen in this study. The docking of mushroom tyrosinase-arbutin complex is shown in **Fig. 3.8 a**. The binding structure shown 3 hydrogen bonds with Asn260, Gly281, and Val283, have pi interaction with His263.

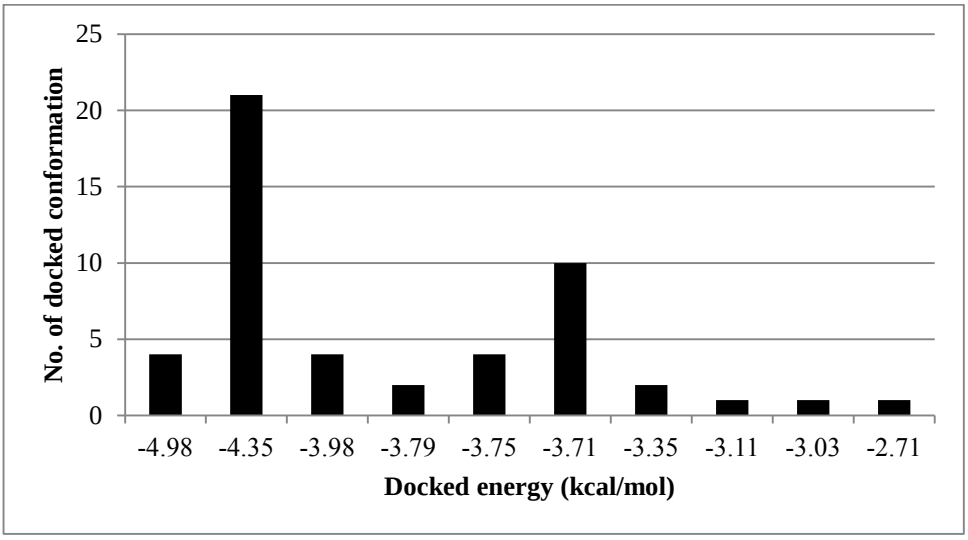


Figure 3.4 The lowest docked energy of each cluster of mushroom tyrosinase-arbutin complex.

For the docking of mushroom tyrosinase-ascorbic acid complex (**Fig. 3.5** and **Fig. 3.8 b**), the inhibitor forms three hydrogen bonds with Asn81 His85 and Ala323. The dock energy of mushroom tyrosinase-ascorbic acid complex is in range of -4.70 to -3.11 kcal/mol. The cluster was classified into 10 groups and highest number of docked conformations in cluster is 12 conformations and this cluster was chosen.

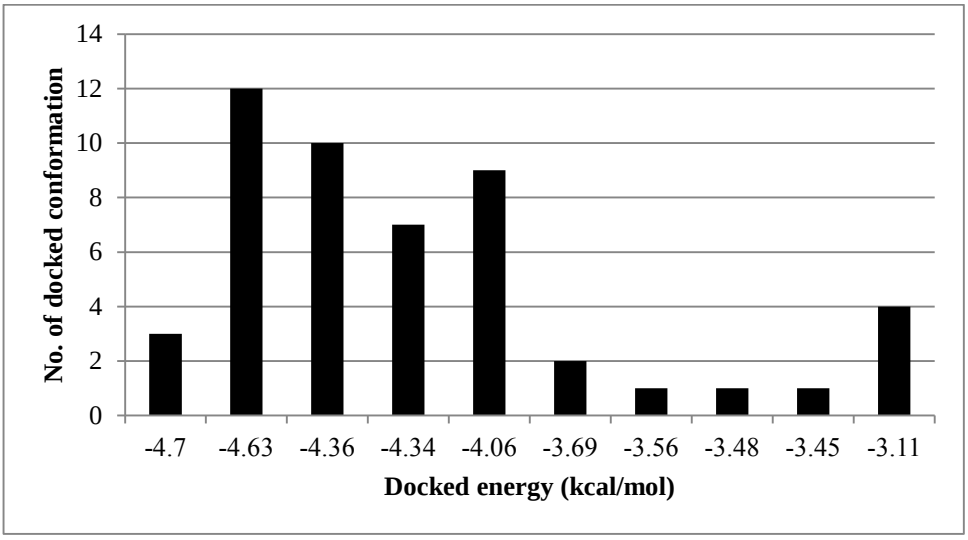


Figure 3.5 The lowest docked energy of each cluster of mushroom tyrosinase-ascorbic acid complex.

The mushroom tyrosinase-kojic acid complex (**Fig. 3.6** and **Fig. 3.8 c**) has pi interaction with His263 and has hydrogen bond with Met280. Results from dock energy of mushroom tyrosinase-kojic acid complex show the lowest energy at -4.62 kcal/mol and the highest energy at -4.05 kcal/mol. The cluster was classified into 6 groups and the chosen one is the highest number of docked conformation cluster with dock energy of -4.45 kcal/mol.

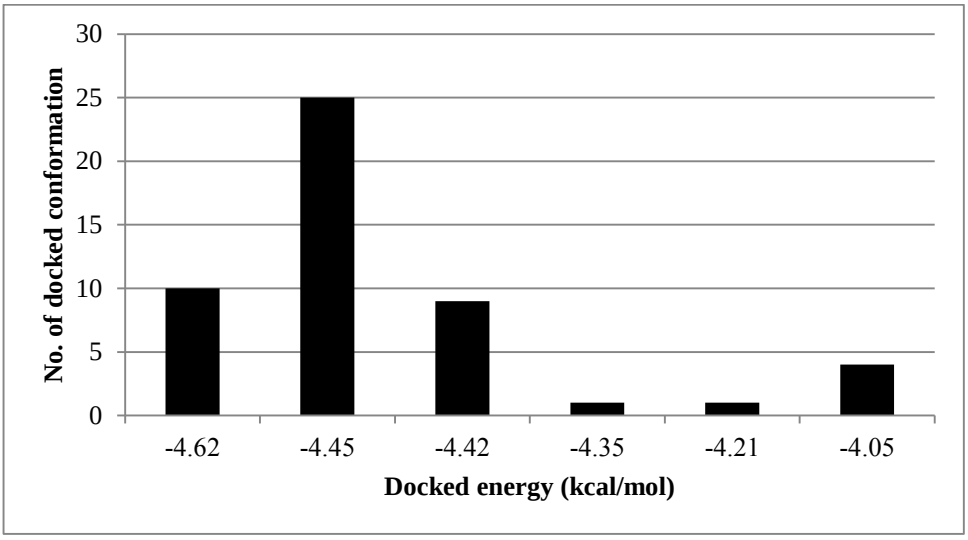


Figure 3.6 The lowest docked energy of each cluster of mushroom tyrosinase-kojic acid complex.

For mushroom tyrosinase-tropolone complex (**Fig. 3.7** and **Fig. 3.8 d**), the pi interaction with His263 remained stable. The result shows that the mushroom tyrosinase-tropolone complex had pi interaction with His263 and had three hydrogen bonds with Asn81, Asn260, and Met280. The dock energy of mushroom tyrosinase-arbutin complex show the lowest energy at -4.86 kcal/mol and the highest energy at -4.17 kcal/mol. The cluster was classified into 3 groups and the highest number of docked conformation in a cluster is 37 conformations and this cluster was chosen.

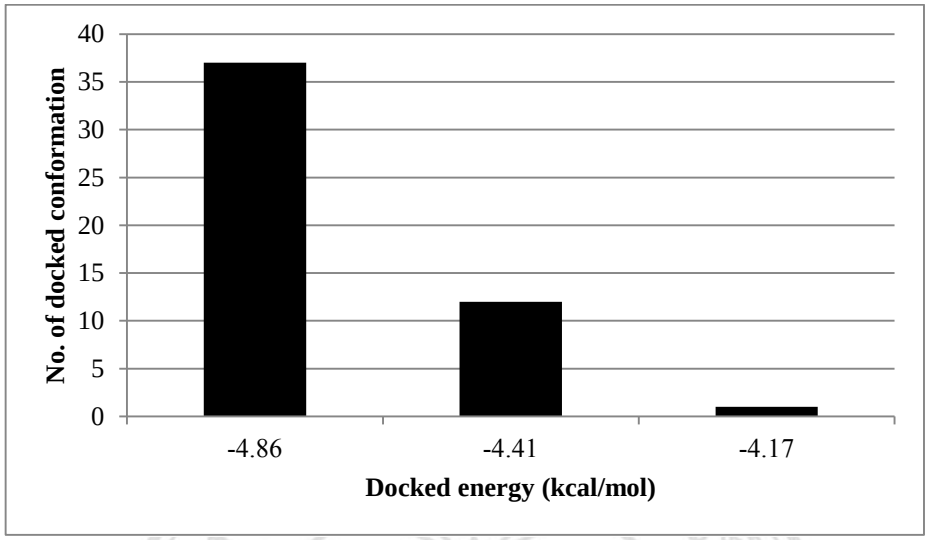


Figure 3.7 The lowest docked energy of each cluster of mushroom tyrosinase-tropolone complex.

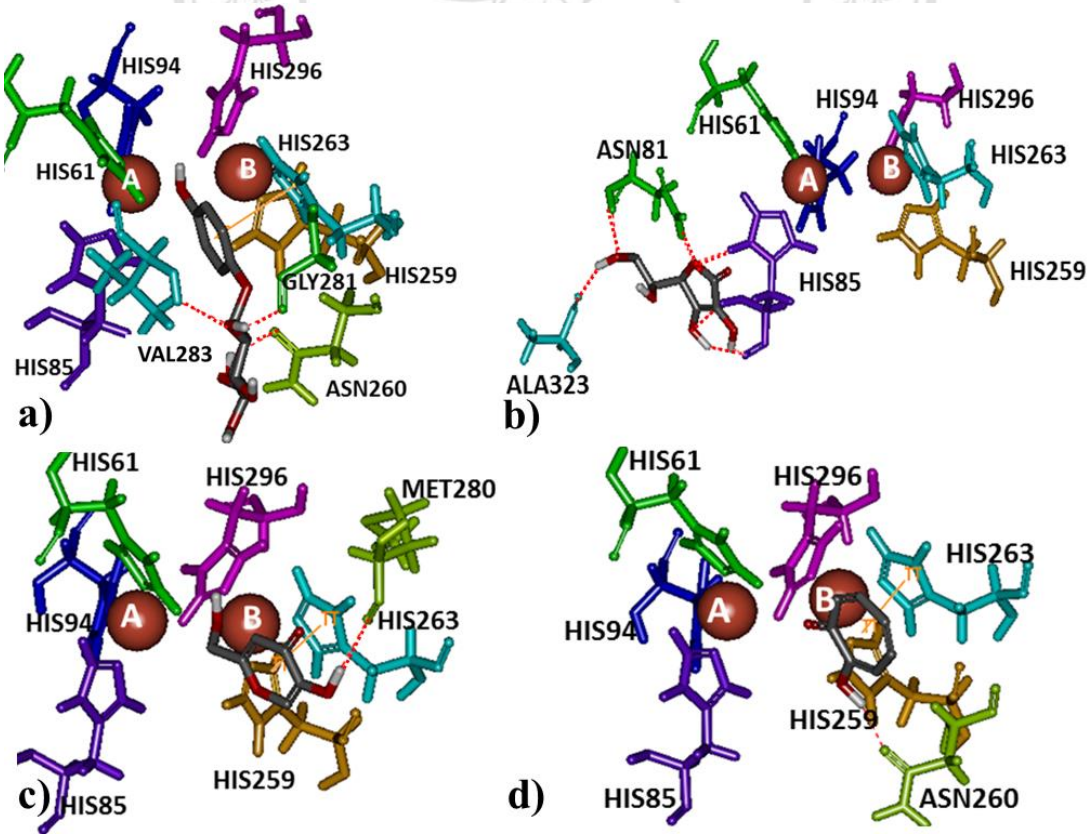


Figure 3.8 Binding structure of mushroom tyrosinase and inhibitors. a) arbutin b) ascorbic acid c) kojic acid d) tropolone

The docking of bacterial tyrosinase-arbutin complex was shown in **Fig. 3.9** and **Fig. 3.12 a**. The binding structure shown the docked structure indicates that arbutin form pi interaction with His208 and had two hydrogen bonds with Asn205 and Val218. The dock energy of bacterial tyrosinase-arbutin complex show the lowest energy at -6.36 kcal/mol and the highest energy at -3.92 kcal/mol. The cluster was classified into 5 groups and the chosen one is the highest number of docked conformation cluster with dock energy of -5.06 kcal/mol.

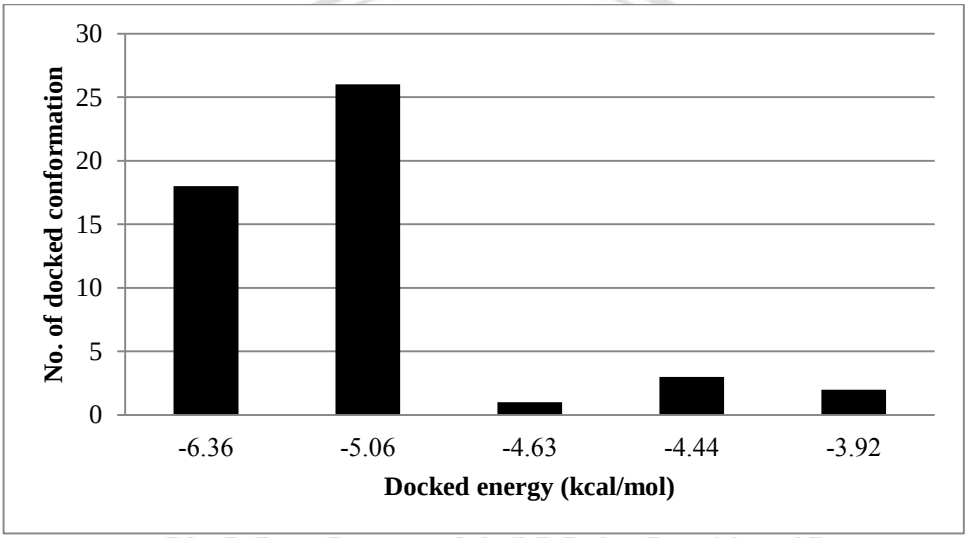


Figure 3.9 The lowest docked energy of each cluster of bacterial tyrosinase-arbutin complex**.**

For the bacterial tyrosinase-ascorbic acid complex (**Fig. 3.10** and **Fig. 3.12 b**), the dock structure forms four hydrogen bonds with His60 and Glu195. The dock energy of bacterial tyrosinase-ascorbic acid complex show the lowest energy at -4.35 kcal/mol and the highest energy at -2.99 kcal/mol. The cluster was classified into 6 groups and the highest number of docked conformations in a cluster is 20 conformations and this cluster was chosen.

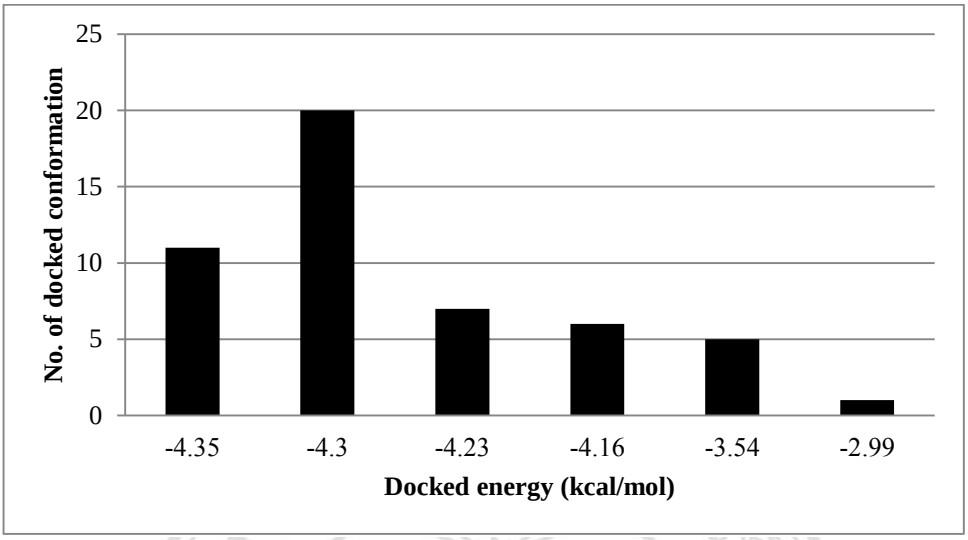


Figure 3.10 The lowest docked energy of each cluster of bacterial tyrosinase-ascorbic acid complex**.**

The bacterial tyrosinase-kojic acid complex (**Fig. 3.11** and **Fig. 3.12 c**) had a pi interaction with His208 and one hydrogen bond with His60. The dock energy of bacterial tyrosinase-kojic acid complex shows the lowest energy at -5.16 kcal/mol and the highest energy at -4.27 kcal/mol. The cluster was classified into 3 groups and the highest number of docked conformations in a cluster is 42 conformations and this cluster was chosen.

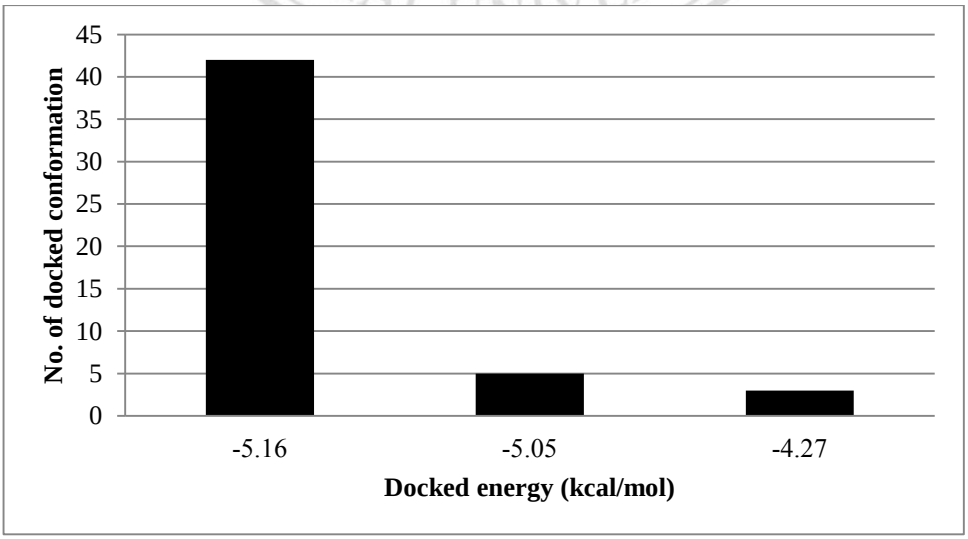


Figure 3.11 The lowest docked energy of each cluster of bacterial tyrosinase-kojic acid complex**.**

For the bacterial tyrosinase-tropolone complex (**Fig. 3.12 d**), the binding structure had pi interaction with His208 and had not hydrogen bond. The dock energy of bacterial tyrosinase-tropolone complex is in range of -4.30 kcal/mol to -5.00 kcal/mol. All of dock structure was located in the one cluster.

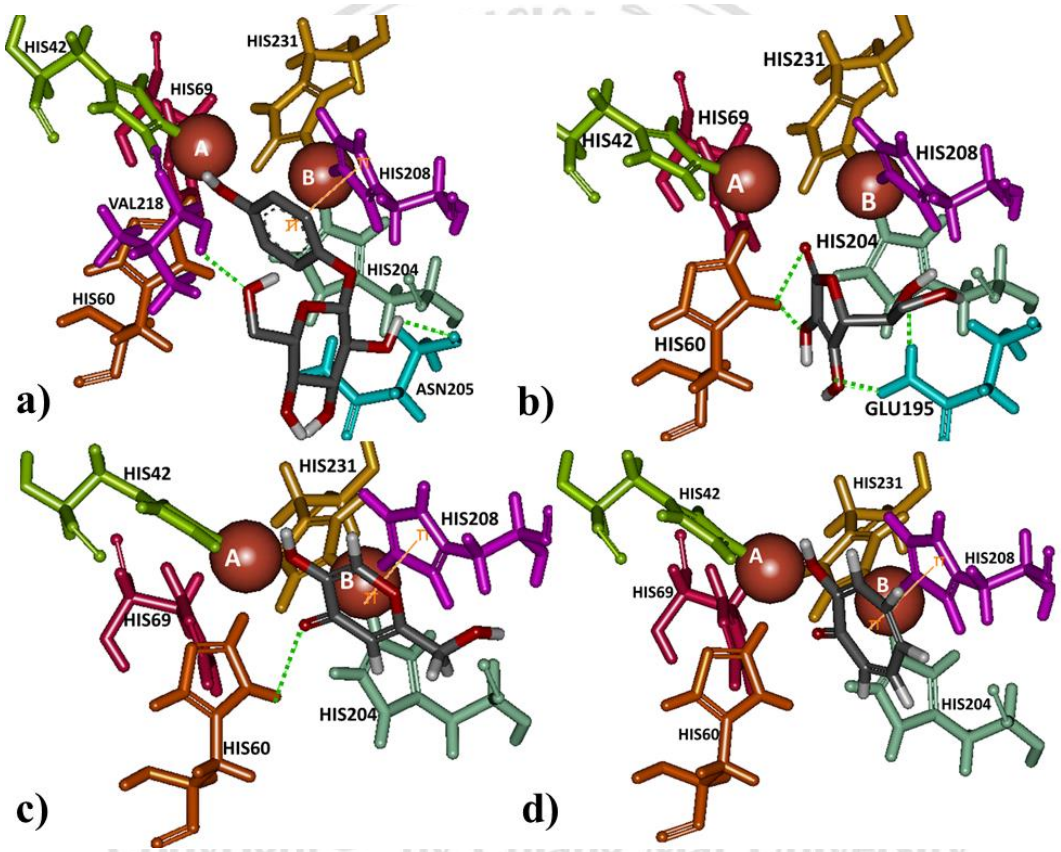


Figure 3.12 Binding structure of bacterial tyrosinase and inhibitors. a) arbutin b) ascorbic acid c) kojic acid d) tropolone

The human tyrosinase-arbutin complex as shown in **Fig. 3.13** and **Fig. 3.16 a**. The docked structure forms 5 hydrogen bonds with Glu88, Ser245, Asn249 and Ser265 and pi interaction with His252. The dock energy of human tyrosinase-arbutin complex show the lowest energy at -4.80 kcal/mol and the highest energy at -3.43 kcal/mol. The

cluster was classified into 6 groups and the chosen one is the highest number of docked conformation cluster with dock energy of -4.80 kcal/mol.

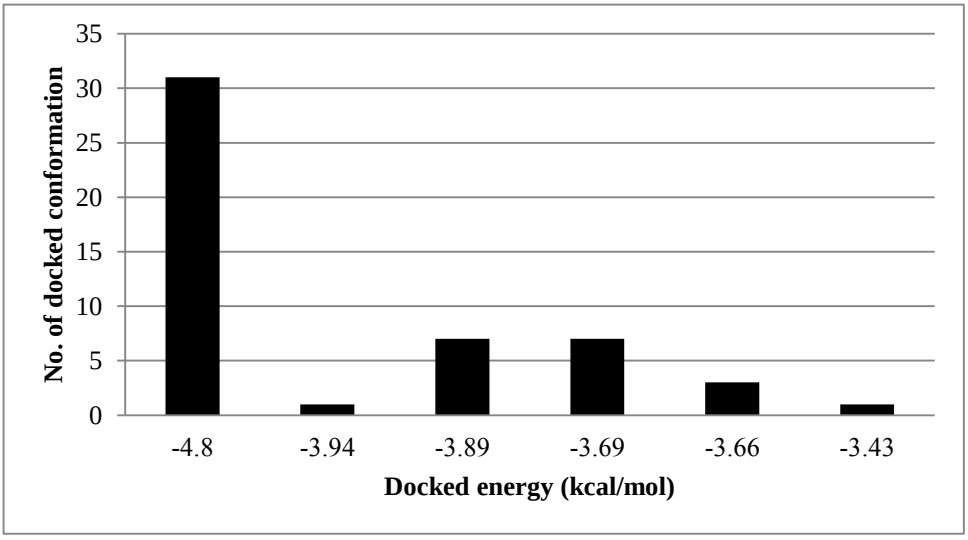


Figure 3.13 The lowest docked energy of each cluster of human tyrosinase-arbutin complex**.**

For the human tyrosinase-ascorbic acid complex (**Fig. 3.14** and **Fig. 3.16 b**), the binding structure forms four hydrogen bond with three amino acid residues (Gln261, Val262 and Ser265). The dock energy of human tyrosinase-ascorbic acid complex shows the lowest energy at -4.94 kcal/mol and the highest energy at -3.20 kcal/mol. The cluster was classified into 6 groups and highest number of docked conformation in a cluster is 30 conformations and this cluster was chosen.

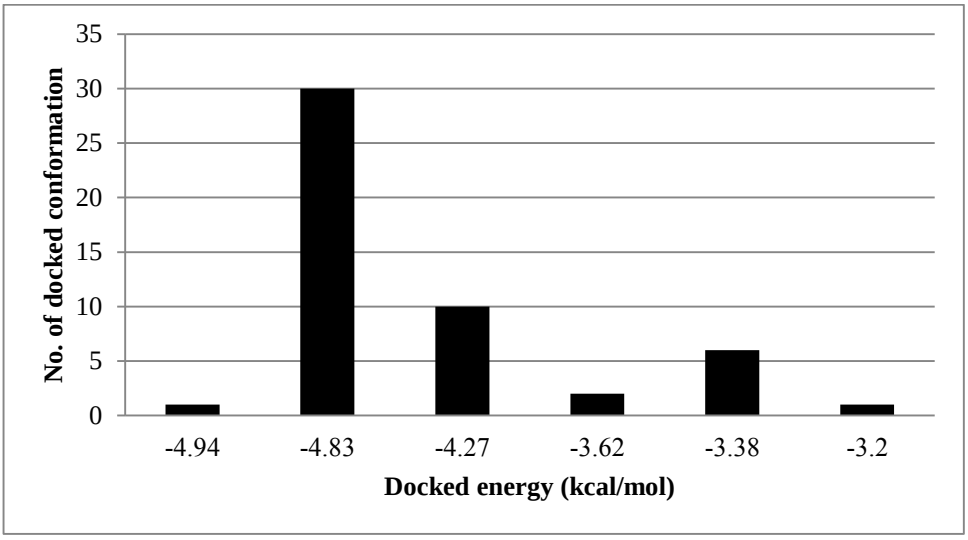


Figure 3.14 The lowest docked energy of each cluster of human tyrosinase-ascobic acid complex**.**

The human tyrosinase-kojic acid complex (**Fig. 3.15** and **Fig. 3.16 c**) had pi interaction with His252 and three hydrogen bonds with Asn249 and Ser265. The dock energy of human tyrosinase-ascorbic acid complex shows the lowest energy at -6.00 kcal/mol and the highest energy at -4.28 kcal/mol. The cluster was classified into 3 groups and the highest number of docked conformation in a cluster is 42 conformations and this cluster was chosen.

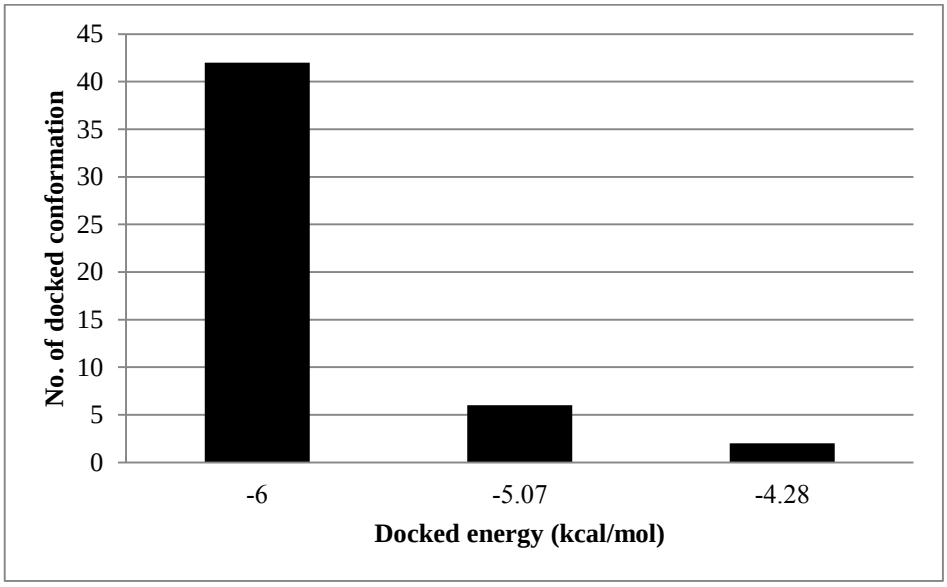


Figure 3.15 The lowest docked energy of each cluster of human tyrosinase-kojic acid complex**.**

For the human tyrosinase-tropolone complex (**Fig. 3.16 d**) had pi interactions with His252 and two hydrogen bonds with Ser265. The dock energy of human tyrosinase-tropolone complex shows the lowest energy at -5.93 kcal/mol and the highest energy at -4.21 kcal/mol. All of dock structure was located in the one cluster.

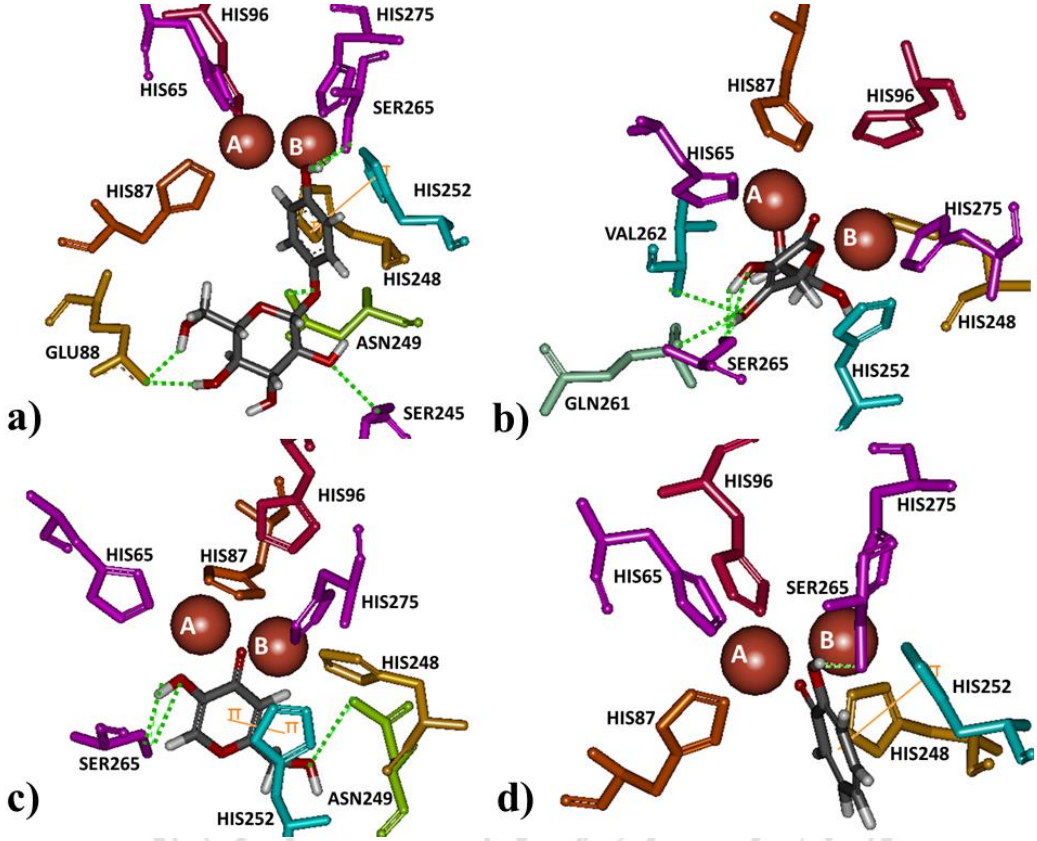


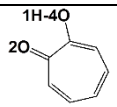
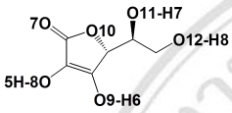
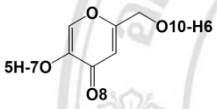
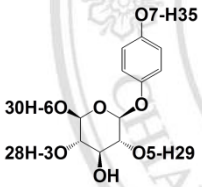
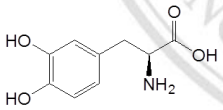
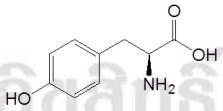
Figure 3.16 Binding structure of human tyrosinase and inhibitors. a) arbutin b) ascorbic acid c) kojic acid d) tropolone

Comparison of docking result with experimental data is shown in **Table 3.1** The both of two substrates (L-tyrosine and L-DOPA) can bind with mushroom tyrosinase with essentially the same K_m value. The binding of substrate to bacterial and human tyrosinase found that L-tyrosine binds to both human and bacterial tyrosinases with higher affinity than dose L-DOPA. From molecular docking, binding energy analyzed was validated as shown in **Table 3.1**. The binding of L-tyrosine with mushroom and bacterial tyrosinase were compared. The both of simulated binding structure and experimental results were validated at the same condition (temperature). At 25 ºC [13, 15], The K_m value and binding energy of L-tyrosine in complex with mushroom and bacterial tyrosinase are 0.2 mM and -10.00 kcal/mol, and 0.075 mM and -11.09 kcal/mol, respectively. Mushroom tyrosinase has K_m value and binding energy of 0.17 mM and -10.20 kcal/mol, and of 0.35 mM and -10.05 kcal/mol for bacterial tyrosinase

in binding with L-DOPA. At 37 ºC [12], the K_m value and binding energy of L-tyrosine and L-DOPA with mushroom tyrosinase are 0.347 mM and -10.00 kcal/mol and 1.44 mM and -10.20 kcal/mol. For human tyrosinase, K_m value [14] and binding energy of L-tyrosine and L-DOPA are 0.17 mM and -11.66 kcal/mol, and 0.36 mM and -11.15 kcal/mol, respectively.

Among these inhibitors, tropolone is the best inhibitor for inhibit mushroom tyrosinase with the range of the lowest IC_{50} values of 0.0004-0.0017 mM and binding energy of -4.86 kcal/mol. IC_{50} value for ascorbic acid had an greater or equal to 0.02 mM, for kojic acid was 0.0074-0.68 mM and 0.04-7.3 mM for arbutin. The binding energy is at -4.63, -4.45, -4.35 kcal/mol for ascorbic acid, kojic acid and arbutin, respectively. In human tyrosinase, ascobic acid is the best inhibitor with the lowest IC_{50} value and binding energy of $\geq$ 0.1 mM and -4.83 kcal/mol. For kojic acid, IC_{50} value and binding energy were 0.50-2.73 mM and -6.00 kcal/mol, and for arbutin were 1.43-6.50 mM and -4.80 kcal/mol. Tropolone had a binding energy of -5.93 kcal/mol. These results correlate with the higher *in vitro* inhibitory activity on human tyrosinase of kojic acid than that of arbutin reported by Kolbe *et al.* [5].

Table 3.1 Docking score and experimental data in term of binding structure/activity of tyrosinase from mushroom, bacterial, and human.

Inhibitors /Substrate	Chemical structures	Mushroom tyrosinase		Bacterial tyrosinase		Human tyrosinase	
		Binding energy (kcal/mol)	IC_{50} /K_m (mM)	Binding energy (kcal/mol)	IC_{50} /K_m (mM)	Binding energy (kcal/mol)	IC_{50} /K_m (mM)
Tropolone		-4.86 [+ + + +]	0.0004 - 0.0017[7-8]	-4.30 [+]	-	-5.93 [+ + +]	-
Ascorbic acid		-4.63 [+ + +]	≥0.02[4]	-5.16 [+ + +]	-	-4.83 [+ +]	≥0.1[4]
Kojic acid		-4.45 [+ +]	0.0074 - 0.68[2-3]	-5.06 [+ +]	-	-6.00 [+ + + +]	0.50 - 2.73[4-5]
Arbutin		-4.35 [+]	0.04 - 7.3 [9-10]	-6.09 [+ + + +]	-	-4.8 [+ +]	1.43 - 6.50[4-11]
L-DOPA		-10.20 [+ + + + +]	*0.17[15] *1.44[12]	-10.05 [+ + + +]	*0.35[13]	-11.15 [+ + + +]	*0.36[14]
L-tyrosine		-10.00 [+ + + +]	*0.2[5] *0.347[12]	-11.09 [+ + + + +]	*0.075[13]	-11.66 [+ + + + +]	*0.17[14]

Note : 1. Star symbol (*) represents K_m values

2. Cross symbol (+) shows inhibitors rank, the lowest binding energy is [+ + + + +] and the highest binding energy is [+]

3.2.2 Molecular Dynamic Simulation

The change of binding site was determined by demonstrate attribution of thermal motions, The MD simulations were performed, binding configuration was rearranged during simulation to observe conformation changes in each time step in comparison with the initial structure. The root mean square deviation of backbone carbon values of the complexes comparing with the initial structures are shown in **Fig. 3.17**.

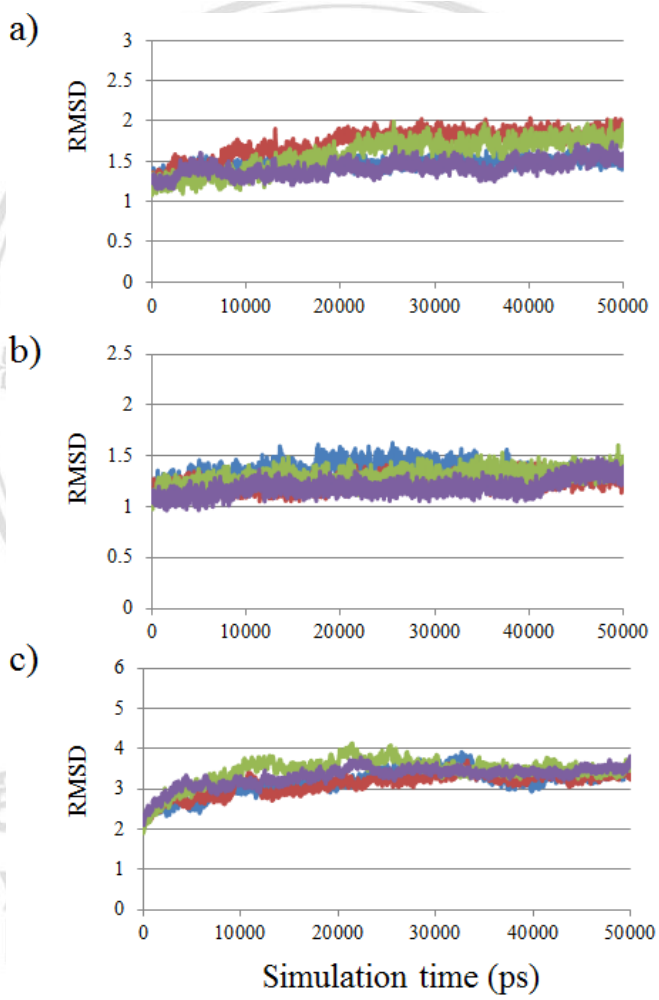


Figure 3.17 RMSD of carbon backbone in complexes a) mushroom tyrosinase-inhibitor comlexes b) bacterial tyrosinase-inhibitor comlexes c) human tyrosinase-inhibitor comlexes; arbutin (blue), ascorbic acid (red), kojic acid (green) and tropolone (purple)

3.2.2 Molecular dynamic simulation

The mushroom tyrosinase-arbutin complex is shown in **Fig. 3.22 a**. The comparison between a docked and MD structure (**Table 3.2**) indicates that, number of hydrogen bonds was decrease from 3 bonds with Asn260, Gly281, and Val283 to only one bond with Asn260, while pi interaction with His263 is still remind. Analysis of hydrogen bonding and *that* of pi distance are shown in **Fig. 3.18**, **Fig.3.22 e** and **Fig. 3.22 f.** The results show that, the mushroom tyrosinase-arbutin complex had hydrogen bond with distance distributing around 2.5 Å between Asn260 residue and O6:hydroxyl group in part of sugar on arbutin and had pi interaction distance distributing between His263 and arbutin with around 5.5 Å and had interaction energy of -1.00 kcal/mol.

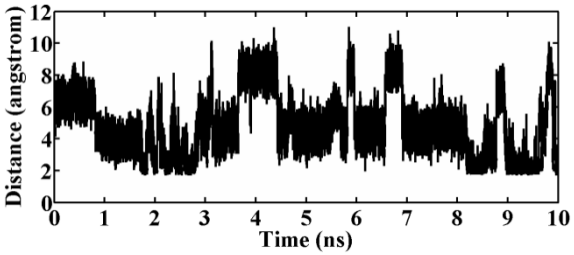
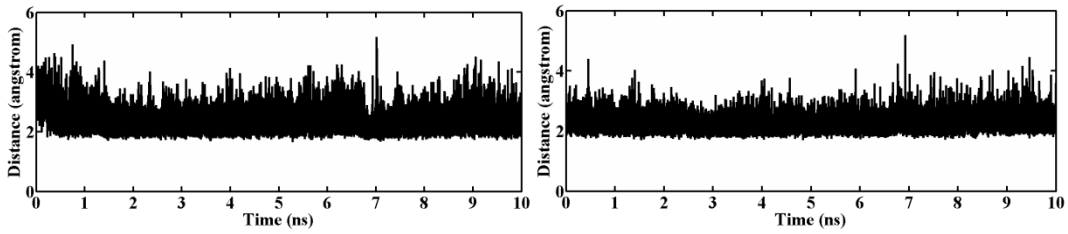
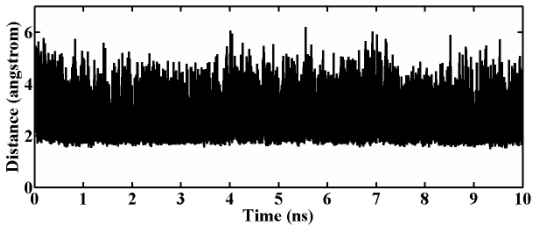


Fig. 3.18 Distance between mushroom tyrosinase and arbutin (Asn260@HD22-ARB@O6) ;

The mushroom tyrosinase-ascorbic acid complex (**Fig. 3.22 b**), these structure had three hydrogen bonds with Asn81 His85 and Ala323 is unchanged but the number of hydrogen bond was decreased from 6 to 3. For mushroom tyrosinase-ascorbic acid complex, the conformation of Asn81 was altered following the position of arbutin to retain hydrogen bonding. **Fig. 3.19 a-c** and **Fig. 3.22 e** found that, the complex had 3 hydrogen bonds including amine groups on Asn81, His85 and carbonyl group on Ala323 with O12, O9, H7:hydroxyl group on ascorbic acid, respectively. All of these hydrogen bonds have distance distribute about 2.5 Å. This result supports the previous study indicated that ascorbic acid reduces *o*-dopaquinone back to L-DOPA, not necessarily at the active site, decreasing melanin formation [16-17].



a) Asn81@H - ASC@O12 **b)** His85@H - ASC@O9



c) Ala323@O - ASC@H7

Fig. 3.19 Distance between mushroom tyrosinase and ascorbic acid

Mushroom tyrosinase-kojic acid complex (**Fig. 3.22 c**) is stabilized through forming of pi interaction and hydrogen bonding with His263 and Met280, respectively. **Fig. 20, Fig. 3.22 e** and **Fig. 3.22 f** shows that the mushroom tyrosinase-kojic acid complex has a hydrogen bond between carbonyl group on Met280 and H5:hydroxyl group on the ring of kojic acid with an distance distribute around 3 Å and has pi interactions distance distribute between His263 and arbutin at 4 Å and had an interaction energy of -0.83 kcal/mol.

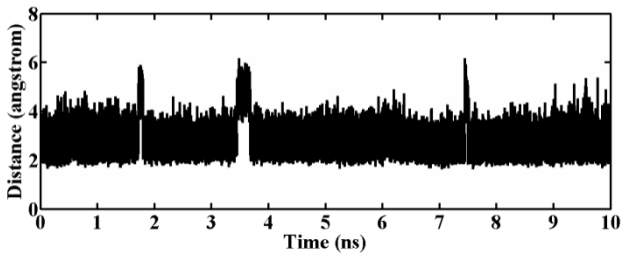
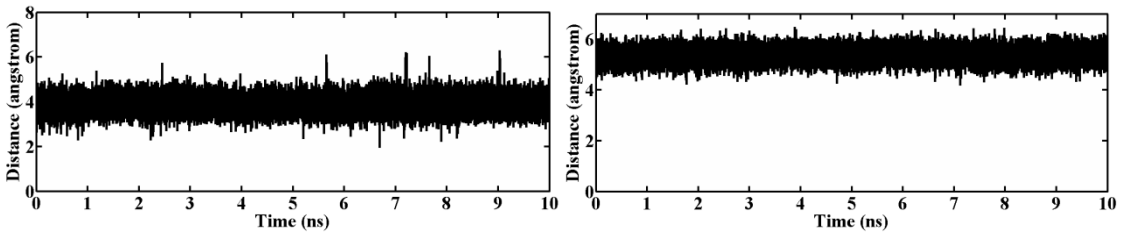
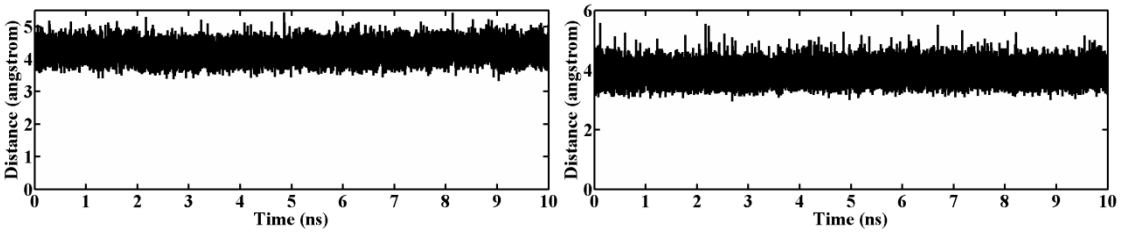


Fig. 3.20 Distance between mushroom tyrosinase and kojic acid (KOJ@H5 - Met280@O)

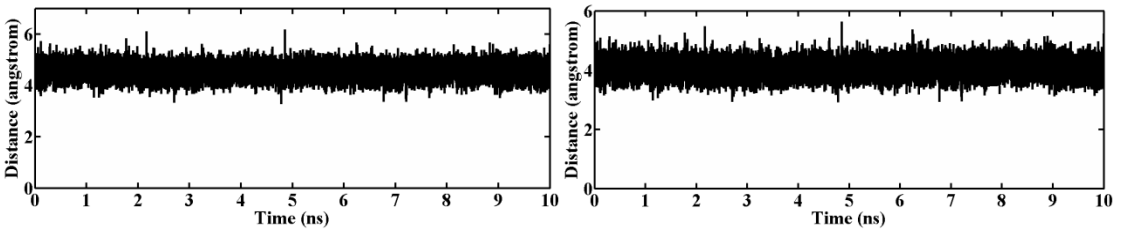
For mushroom tyrosinase-tropolone complex (**Fig. 3.22 d**), the pi interaction with His263 remained stable. The result shows that the mushroom tyrosinase-tropolone complex had pi interaction distance (**Fig. 3.22 f**) between His263 and tropolne at 4.5 Å and had interaction energy of -2.68 kcal/mol. Otherwise, neighbor residues as show in **Fig. 3.21 a-k** found that His61, Asn260, His263, and Met280 had nearly distance with around 4.5 Å. According to this result, Asn81, Asn260, and Met280 are likely to be key amino acids involved in the binding to substrate and His263 forms pi interaction in mushroom tyrosinase. Studies in which Asn260 and Met280 were proposed to play roles in binding substrate [18] and His263 was observed to form pi interaction in mushroom tyrosinase [19].



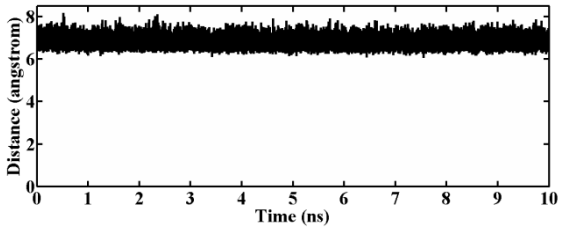
a) TRO@O2 - His259@CE1 **b)** TRO@O2 - His259@NE2



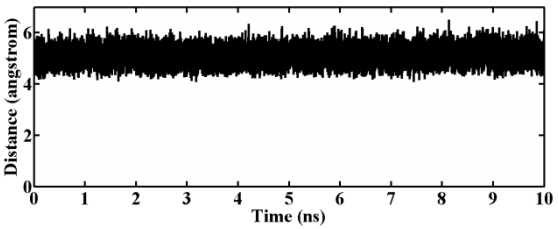
c) TRO@C3 - His263@CE1 **d)** TRO@O4 - His61@CE1



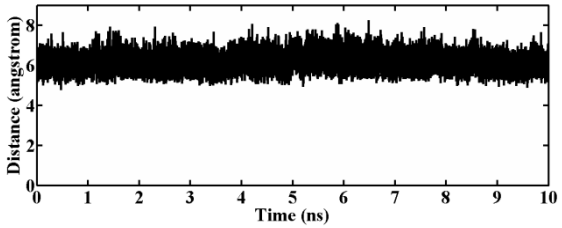
e) TRO@O4 - His263@CE1 **f)** TRO@O4 - His263@NE2



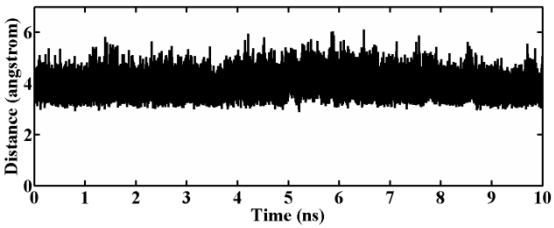
g) TRO@C5 - Ala286@CB



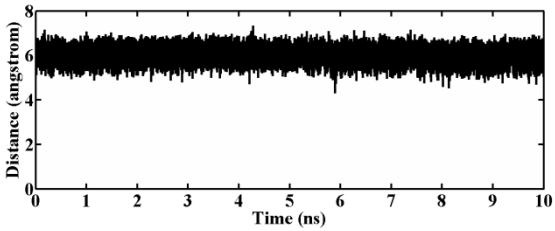
h) TRO@C5 - His263@ND1



i) TRO@C6 - Met280@O



j) TRO@C7- Met280@O



k) TRO@C8 - Asn260@OD1

Fig. 3.21 Distance between mushroom tyrosinase and tropolone

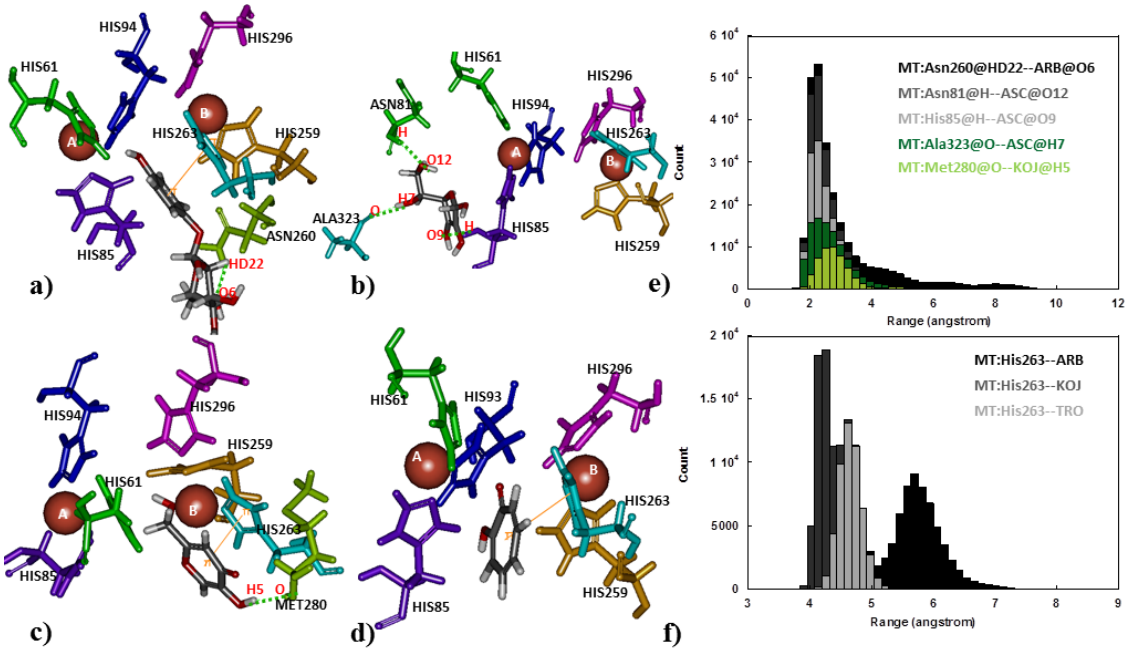


Figure 3.22 Binding structure of mushroom tyrosinase and inhibitors. a) arbutin b) ascorbic acid c) kojic acid d) tropolone e) *distance measurement* of hydrogen bond f) *distance measurement* of pi interaction

The binding site of the bacterial tyrosinase-arbutin complex is shown in **Fig. 3.26 a**. The comparison between docked and MD structure (**Table 3.2**) show that arbutin form pi interaction with His208 and lack hydrogen bonding. The MD simulation results indicated the distance between carbonyl group on Asn205 and the hydroxyl group in the sugar part of arbutin was far to form hydrogen bond (**Fig. 3.23**) and show the pi interaction distance between His208 and arbutin at 5.5 Å with an interaction energy of -1.84 kcal/mol (**Fig. 3.26 f**).

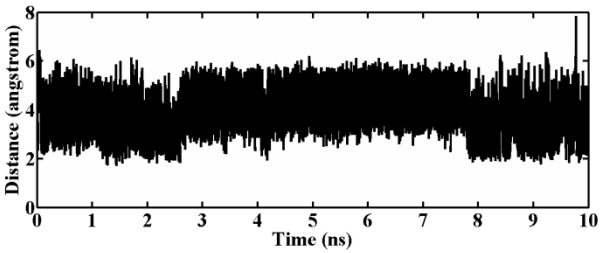
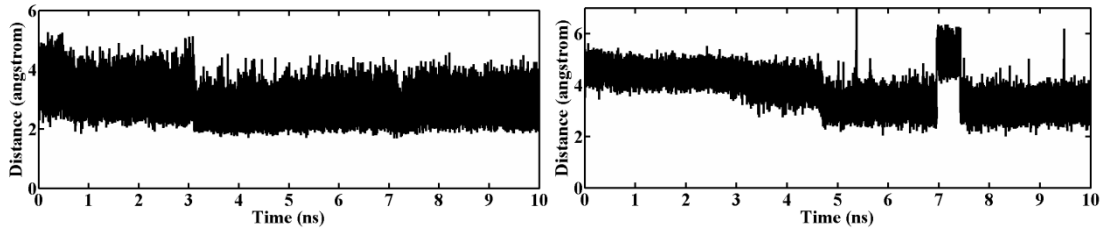
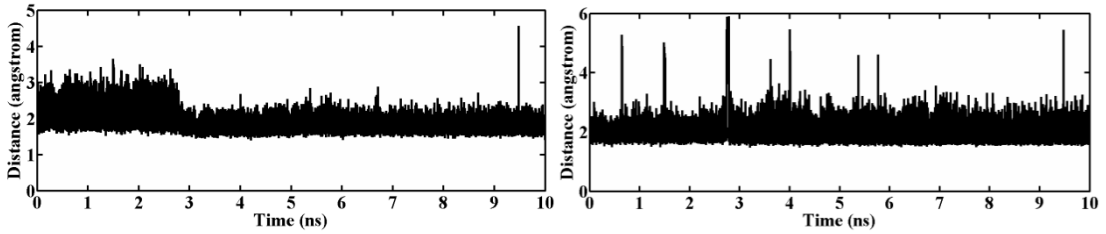


Fig. 3.23 Distance between bacterial tyrosinase and arbutin (ARB@H29 - Asn205@O)

For bacterial tyrosinase-ascorbic acid complex (**Fig. 3.26 b**), the MD structure was decreased number of hydrogen bonds from 4 hydrogen bonds with His60 and Glu195 to only 2 hydrogen bonds with Glu195. From the hydrogen bond distance between Glu195 and H5, H6:hydroxyl group on the ring of ascorbic acid are around 2 Å (**Fig. 3.26 e**) that indicating a strong interaction.



a) Asn205@HD21 - ASC@O9 **b)** Asn205@HD21 - ASC@O11



c) ASC@H5 - Glu195@OE1 **d)** ASC@H6 - Glu195@OE1

Fig. 3.24 Distance between bacterial tyrosinase and ascorbic acid

The bacterial tyrosinase-kojic acid complex (**Fig. 3.26 c**) had a pi interaction with His208 that is quite stable with distance around 4 Å and had an interaction energy of -4.17 kcal/mol (**Fig. 3.26 f**). These complex structures was absent hydrogen bonding in MD simulation, but observe one hydrogen bond in docked structure with His60 (**Fig. 3.25**).

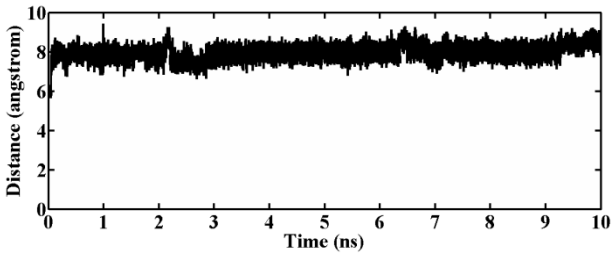


Fig. 3.25 Distance between bacterial tyrosinase and kojic acid (His60@HD1 - KOJ@O8)

The bacterial tyrosinase-tropolone complex (**Fig. 3.26 d**), pi interaction form between His208 in dock to His60 in MD simulation and had pi interaction distances between His208 and His60 and tropolone were at 7 and 5.5 Å, respectively as shown in **Fig. 3.26 f**. His60 had an interaction energy of -0.52 kcal/mol. From this result, we suggest that Glu195 is important in binding to the substrate, while His208 plays a role in forming pi interactions in bacterial tyrosinase as these interactions still stable during simulation. Above mentioned results correlated well with molecular docking results suggested by Kang *et al.* [20]. In their work, arbutin can interact with Asn205 and His208 using hydrogen bonding and pi interaction, respectively.

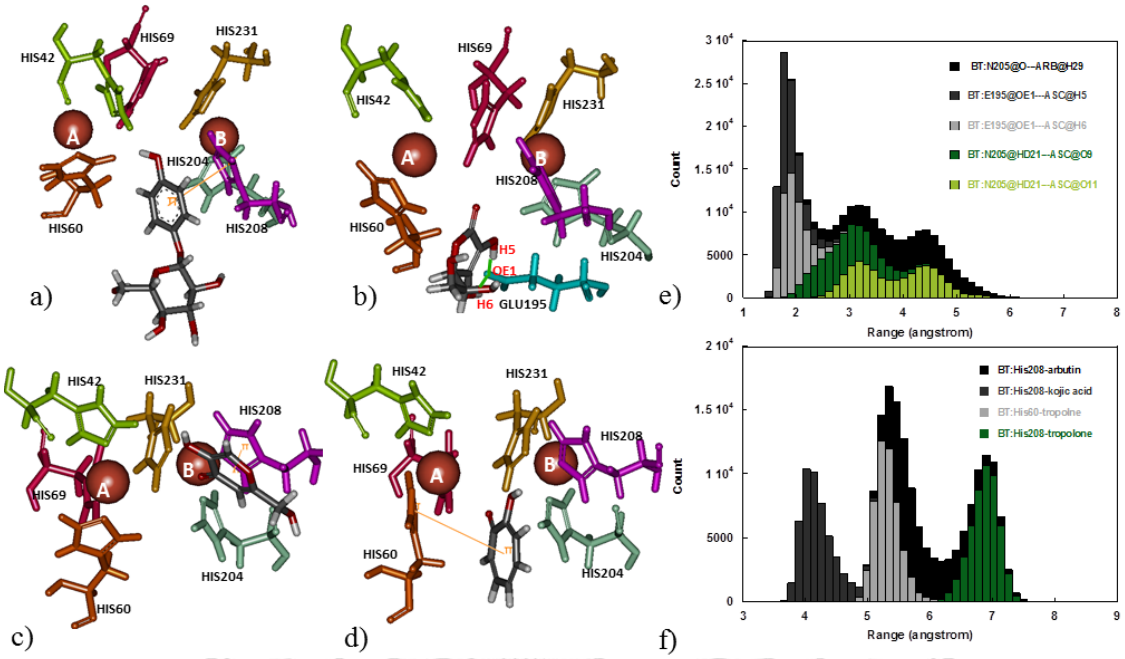
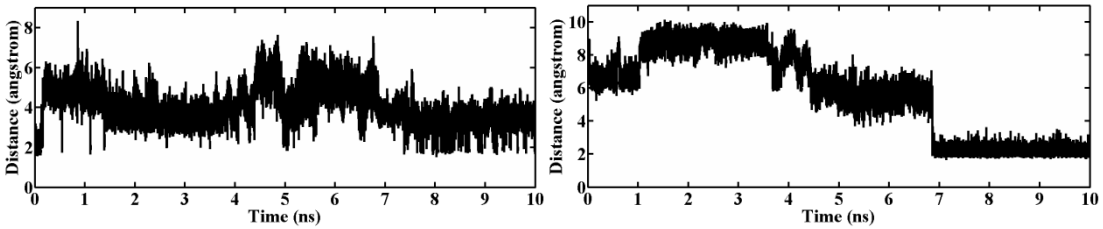
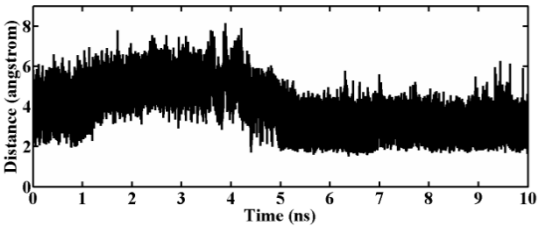


Figure 3.26 Binding structure of bacterial tyrosinase and inhibitors. a) arbutin b) ascorbic acid c) kojic acid d) tropolone e) *distance measurement* of hydrogen bond f) *distance measurement* of pi interaction

The human tyrosinase-arbutin complex is shown in **Fig. 3.31 a**. The comparison between the docked and MD structures (**Table 3.2**) show that the number of hydrogen bonds was decrease from 5 with Glu88, Ser245, Asn249 and Ser265 to 3 with Ser245, Asn249 and Val262 while pi interaction with His252 is still remained. The hydrogen bonds between Ser245, Val262, Asn249 and O5, O6, H29:hydroxyl group in part of the sugar on arbutin were all at around at 2 Å (**Fig. 3.27 a-c, 3.31 e**). The pi interaction was stable formed in the docked structure was analyzed in the MD trajectory in **Fig. 3.31 f**. The distance between His252 and arbutin was 6.5 Å and had an interaction energy of -0.21 kcal/mol.



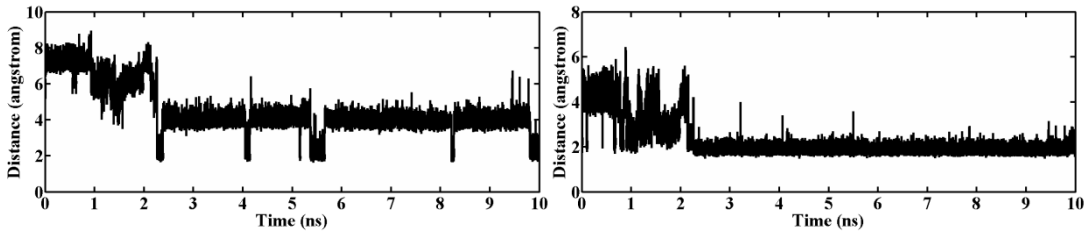
a) Ser245@HG - ARB@O5 **b)** Val262@H - ARB@O6



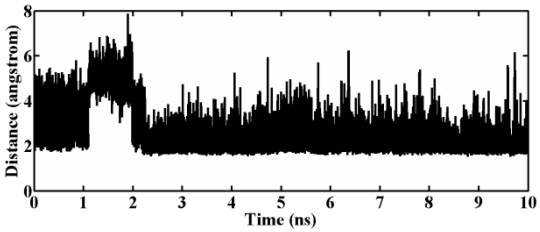
c) ARB@H29 - Asn249@O

Fig. 3.27 Distance between human tyrosinase and arbutin

For the human tyrosinase-ascorbic acid complex (**Fig. 28** and **Fig. 3.31 b**), the number of hydrogen bonds is decreased from 4 to 3 and a hydrogen bond with Ser265 is unchanged. **Fig. 3.31 e** found that the hydrogen bond distances between Ser265 and H18, H20:hydroxyl group on the ring of ascorbic acid, and Glu230 and H19:hydroxyl group in ring of ascorbic acid, with distances distributed around 2 Å. Binding in the active site of tyrosinase with ascorbic acid was shown the molecular docking in this study corresponding to previous tyrosinase inhibitory effect proposed by Senol *et al.* [21].



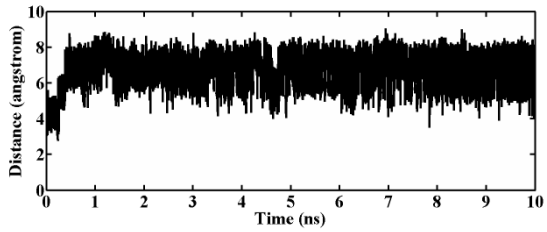
a) ASC@H6 - Ser265@OG **b)** ASC@H5 - Ser265@OG



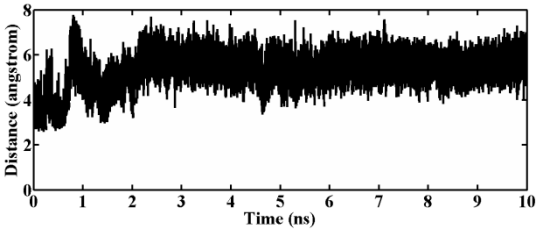
c) ASC@H8 - Glu230@OE2

Fig. 3.28 Distance between human tyrosinase and ascorbic acid

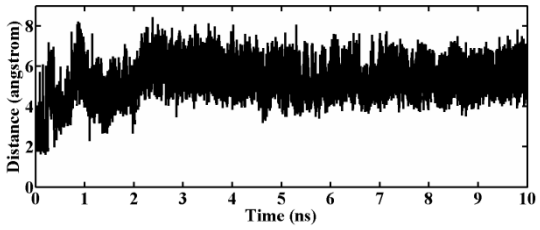
For the human tyrosinase-kojic acid complex (**Fig. 3.31 c**) structure had stable pi interaction with His252 at a distance of 6.5 Å and had an interaction energy of -0.25 kcal/mol (**Fig. 3.31 f**) and hydrogen bond distance was far for bonding as show in **Fig. 1.29 a-c**.



a) Asn249@ND2 - KOJ@O10



b) Ser265@OG - KOJ@O7

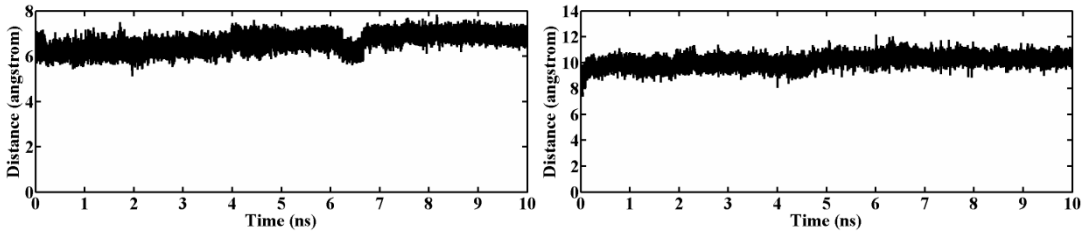


c) KOJ@H5 - Ser265@OG

Fig. 3.29 Distance between human tyrosinase and kojic acid

The human tyrosinase-tropolone complex (**Fig. 3.31 d**) both of pi interaction hydrogen bonding was not observed. **Fig. 3.30 a-b** shows hydrogen bonds distance between His265 and tropolone was far to form bonding. Therefore some residues that are far apart are able to form pi interaction. From this result, we suggest that Glu230,

Ser245, Asn249, Val262, and Ser265 are key amino acids essential in binding to substrate and His252 is involved in forming pi interactions in human tyrosinase**.**



a) Ser265@OG - TRO@O4 **b)** TRO@H1 - Ser265@OG

Fig. 3.30 Distance between human tyrosinase and tropolone

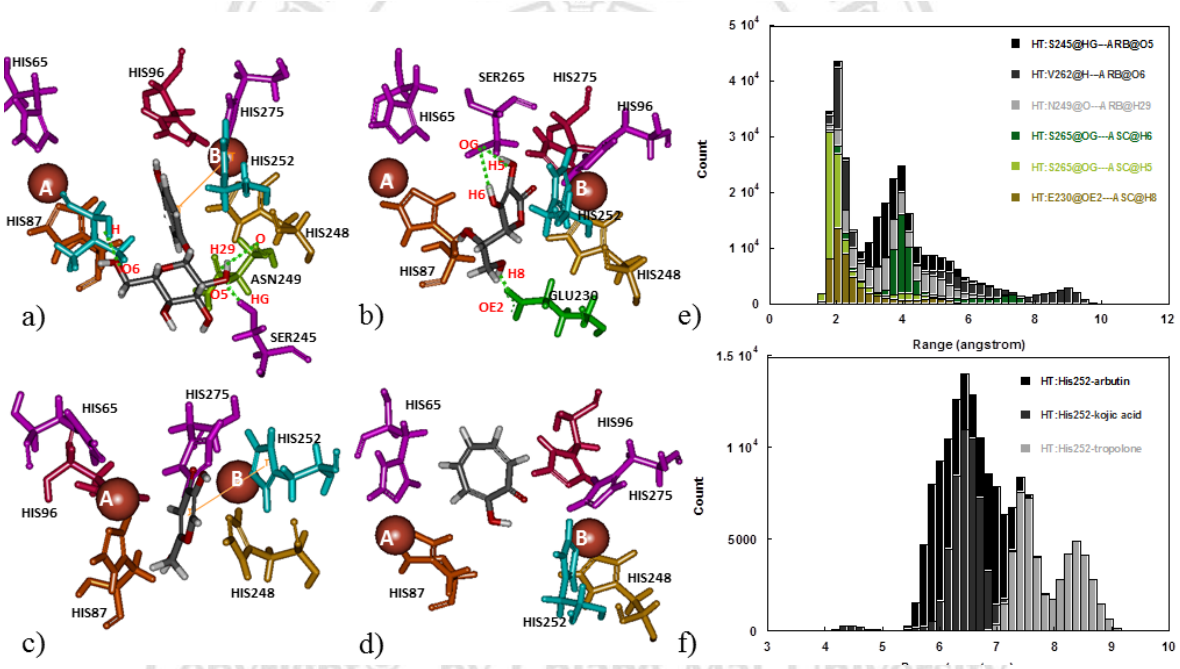


Figure 3.31 Binding structure of human tyrosinase and inhibitors. a) arbutin b) ascorbic acid c) kojic acid d) tropolone e) *distance measurement* of hydrogen bond f) *distance measurement* of pi interaction

Table 3.2 The comparison of interaction site found in docked (in parenthesis) and MD structures

Inhibitors	Mushroom tyrosinase		Bacterial tyrosinase		Human tyrosinase	
	H bonding	Pi interaction	H bonding	Pi interaction	H bonding	Pi interaction
Kojic acid	1:M280 (1:M280)	H263 (H263)	- (1:H60)	H208 (H208)	- (1:N249 2:S265)	H252 (H252)
Tropolone	- (3:N260)	H263 (H263)	- (-)	H60 (H208)	- (2:S265)	- (H252)
Ascorbic acid	1:ASN81 1:HIS85 1:A323 (2:ASN81 4:HIS85 1:A323)	- (-)	2:E195 (2:H60 2:N205)	- (-)	1:E230 2:S265 (1:Q261 1:V262 2:S265)	- (-)
Arbutin	1:N260 (1:N260 1:G281 1:V283)	H263 (H263)	- (1:N205 1:V218)	H208 (H208)	1:S245 1:N249 1:V262 (2:E88 1:S245 1:N249 1:S265)	H252 (H252)

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