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In Silico Analysis and Molecular Docking Studies Of Eumelanin With Estrogen Receptors – A Comparative Study

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Abstract

Eumelanin is a complex biopolymer that is ubiquitous in nature. Eumelanins are reported to exhibit extensive biochemical and functional properties. These properties attribute to the composition and structural organization of the molecules. Eumelanins are composed of Dihydroxy indole (DHI) and Dihydroxy indole 3 carboxylic acid (DHICA) units and their quinone forms. In spite of immense studies the structure of eumelanin is not completely elucidated. Molecular simulation studies had predicted oligomeric/tetrameric structure of eumelanin. Cephalopod ink eumelanins are used in treating several female reproductive disorders. Since estrogen receptors play a crucial role in female reproductive disorders, the present study aims at understanding the binding affinity of the predicted forms of eumelanin with estrogen receptors by docking and simulation analysis. It was observed that eumelanin has better binding affinity than other estrogen receptor ligands and the closed tetrameric structure of eumelanin showed better binding affinity to estrogen receptor α than β.

Key Words: Eumelanin, Estrogen receptor, IMIM, Molecular docking

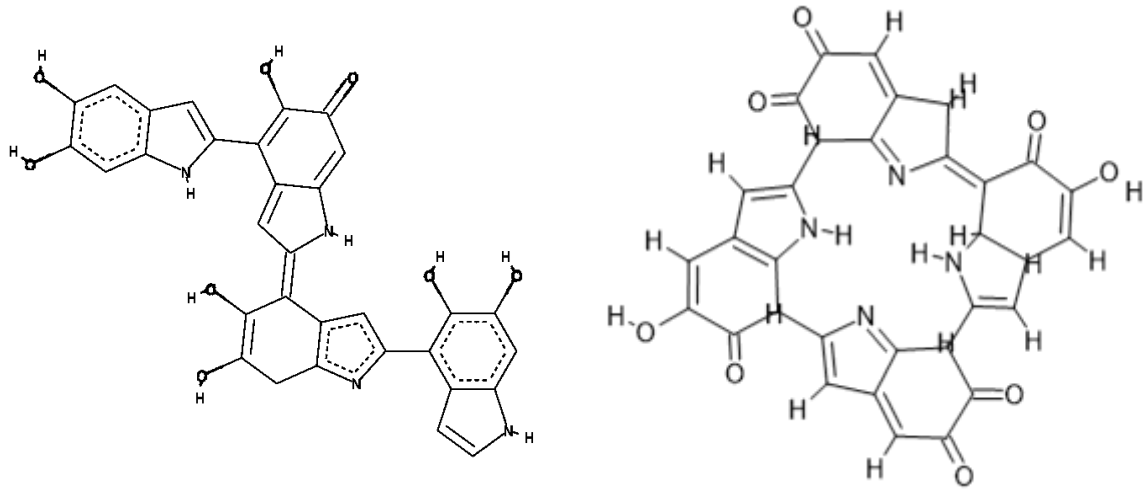
1. Introduction

Melanins are the natural pigments that are widely found in diverse life forms. They are found to have numerous pharmaceutical and industrial applications. Melanins are composed of indoles and phenols that are derived by oxidation of tyrosine [1]. They are classified in to Eumelanin, pheomelanin and allomelanin based on their composition and chemical structure. Eumelanins are present in black brown pigmented components like hair. Pheomelanins are seen as reddish yellow pigmented components in feathers due to the presence of sulphur groups while allomelanin is a heterogenous polymer found in various colored components of plant and fungi [2].

Melanins exhibit various functional roles in different organisms which are unrelated. In humans eumelanin and pheomelanin are predominantly found in varying degrees, they act as photo protectants from harmful UV radiations. Eumelanins that are the prime study of interest plays a crucial role in melanomas apart from being photoprotective in nature [3]. As well eumelanins which were isolated from various sources of bacteria, fungi and marine organisms were reported to exhibit various properties like antioxidant, anti inflammatory, anti carcinogenic, anti bacterial, anti fungal etc [4]. Extracts of eumelanin from marine organisms especially from cephalopod fish Sepia were used in traditional medicine to treat various reproductive disorders [5].

Melanins in their native forms cannot be crystallized and are insoluble in any solvent unless the structure is disrupted [2]. Lack of knowledge on molecular structure of melanins reflects uncertainties in their functional properties. Eumelanin, which is the most abundant type of melanin, is reported to be present in the form of protomolecules containing melanin oligomers. These oligomers constitute 5,6-dihydroxy indole (DHI), 5,6-dihydroxy indole 2 carboxylic acids (DHICA) units and their redox quinone forms [6].Two different qualitative models were proposed for the structure of eumelanin, a cross linked open hetero polymer structure (OPS) and a stacked cyclic tetrameric/ oligomeric structure (CTS) [7] [8].

Sepia ink eumelanin is used in treating several female reproductive disorders where estrogen and its signalling pathways play a crucial role but mechanism of action is not well understood. In the present study we performed: (1) A comparative study of the proposed models of open hetero polymeric structure (OPS) and closed tetrameric structure (CTS) of eumelanin (Figure 1) with estrogen receptors and (2)Molecular docking analysis of eumelanin with estrogen receptors, in comparison with selective estrogen receptor and modulator (SERM), tamoxifen and natural agonist estradiol.(3) Molecular dynamics simulations studies of eumelanin and estrogen receptor alpha to analyze the binding affinity of eumelanin with estrogen receptors.



(a) (b)

Figure 1. Proposed structural models of Eumelanin (a) Open Heteropolymer structure (OPS) of eumelanin (b) Closed tetrameric ring structure (CTS) of eumelanin [7][8]

2. Materials and Methods

All the docking studies were carried using Autodock Vina software 4.2 [9] and the protein PDBs were retrieved from protein data bank.

2.1 Molecular docking study of Eumelanin closed ring (CTS) and open polymer (OPS) structure with estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ)

The CTS structure of eumelanin, which is a tetramer of indolequinone and quinone-methide (IMIM) units was considered based on the theoretical model proposed by Sheng Meng and Efthimios Kaxiras [8]. Docking of CTS - IMIM and OPS of eumelanin were carried out on crystal structure of human estrogen receptor alpha (ERα) ligand binding domain in complex with estradiol, (PDB_ID:1A52) [10] and on crystal structure of estrogen receptor beta(ERβ) ligand binding domain (PDB_ID: 3OLS) [11]. During the docking we used 0.375 Å grid box parameters on human ERα with centre: x =103.86, y = 11.52, z = 97.15 and grid box size: x=74, y=76, z=78, and on human ERβ with centre: x =25.522, y = -34.135, z = -8.139 and grid box size: x=76, y=74, z=84 were used for CTS. The grid box parameters x =105.95, y = 19.83, z = 90.36 and grid box size x = 28, y =28, z =28 on human ERα and grid box parameters x =21.77, y = -22.93, z = -12.63 and grid box size x = 27, y =27, z =27 on ERβ were used for OPS. While docking nine conformations were generated for each ligand by using default genetic algorithm. In this study, input preparation was carried out using MGLtools-1.5.6 [9] and final docking was performed in Autodock4.2 software.

2.2 Molecular docking study of ERα with 4-hydroxyTamoxifen, Estradiol and open structure of Eumelanin.

Proposed open structure of Eumelanin was considered from the review of Noura El-Ahmady El-Naggar and Wesam Eldin I. A. Saber [2]. Docking of Eumelanin was carried out on crystal structure of human ERα ligand binding domain in complex with 4-hydroxytamoxifen, (PDB_ID: 3ERT) [12]. During the docking we have used 1Å grid box parameters with centre: x = 32.957, y = -2.403, z = 23.914 and grid box size: x = 28, y = 28, z = 26. While docking nine conformations were generated for each ligand by using default genetic algorithm. In this study, input preparation was carried out using MGL tools - 1.5.6 [9] and final docking was performed in Autodock Vina.

2.3 Molecular dynamics simulations studies of eumelanin and ERα

The CTS of eumelanin complexed with ERα was selected to run 100 ns (nanosecond) molecular dynamics simulations using isothermal-isobaric ensembles. All molecular dynamics simulations were performed using Gromacs 2018 on the Linux Ubuntu platform [13, 14] and the protein force fields used were Amber03 [15]. Force fields of inhibitor molecules were generated by the ACPYPE server (https://www.bio2byte.be/acpype/) [16]. A 10 Å cubic box was created around the protein-ligand complex, and the created cubic box was filled with SPC (simple point charge) water model as a solvent. The total charge of the protein inhibitor system was reduced to zero by six Na^+ ions. First, a steepest descent energy minimization was applied and then the position restraint protocol for 100 pico seconds (ps) of equilibrium was performed.

To understand the stability of the complex, we performed molecular dynamics (MD) simulations for a total of 100 ns on the complex using a time step of 0.002 ps. Isothermal and isobaric systems were used to maintain constant pressure and temperature. The constant temperature of 298 K was achieved by using the thermostatic V-rescale method [17] and parrinello-rahman method [18] was used to maintain pressure of 1 bar. During the simulation, hydrogen bonds in the system were constrained using the LINCS algorithm [19]. The output coordinates of the 100 ns simulation of the protein complex were extracted from the MD trajectories and then the root mean square deviation (RMSD) and root mean square fluctuation (RMSF) were calculated from it.

Results and Discussion

Molecular docking studies of CTS and OPS eumelanin with ERα and ERβ

Recent studies on the structure of eumelanin had strongly proposed its existence as an oliogomeric sheets or protomolecules in its quinone forms [20]. Considering the structural ambiguity, binding affinity and docking studies were performed for both the OPS and CTS- (IMIM) of eumelanin.

Human ERα ligand binding domain in complex with Estradiol (PDB 1A52) was selected for molecular docking. We have carried out docking studies of CTS - IMIM into the active site of human estrogen receptor alpha ligand binding site (Figure.2). CTS -IMIM was forming hydrogen bonds with the main chain of Val376, side chains of Lys529 and Tyr526 residues. The other residues are stabilizing the molecule by

hydrophobic interactions and residues are Trp383, Leu354, Met522, Cys381, Ile358, Leu379 and Leu525 residues.

The CTS-IMIM docking into the active site of human ERβ (PDB 3OLS) reveals the affinity of CTS-IMIM with active site residues (Figure.3) CTS-IMIM was forming hydrogen bonds with the side chain of Asn478, Glu474 and a bifurcated hydrogen bonds with Lys480 and Asn496 residues. The other residues are stabilizing the molecule by hydrophobic interactions and residues were Leu477, Leu495, Trp335, Met473, Cys481 and Lys 471 residues.

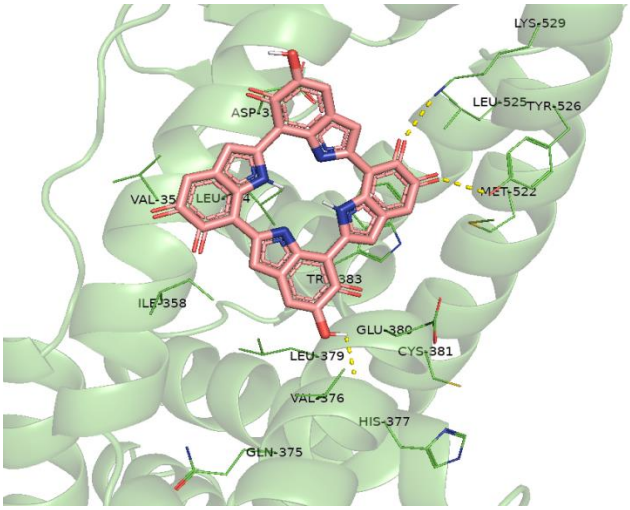


Figure 2: molecular docking of CTS-IMIM molecule in the active site of the ERα (1A52). Protein active site residues shown in lines and the IMIM shown in stick representation. Hydrogen bonds are shown in broken yellow lines.

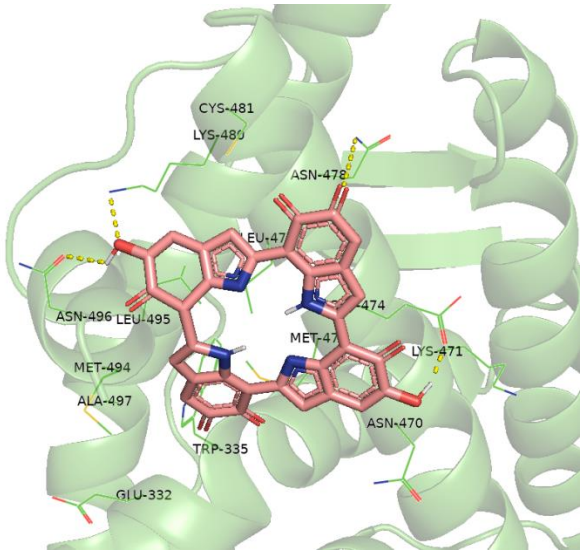


Figure 3: Molecular docking of CTS- IMIM molecule in the active site of the ERβ (3OLS). Protein active site residues shown in lines and the CTS-IMIM shown in stick representation. Hydrogen bonds are shown in broken yellow lines.

Table 1: Binding affinity scores of the CTS eumelanin docked into the active site of ERα and ERβ

Molecule name	Binding affinity (in kcal/mol)
ERα	-6.89
ERβ	-7.27

Docking of open hetero polymer structure of eumelanin with PDBs 1A52 and 3OLS showed a better affinity with ERα but with ER β the binding energy value was very high (Table.2). The binding positions of eumelanin in both the receptors are shown in the figure 4 and 5.

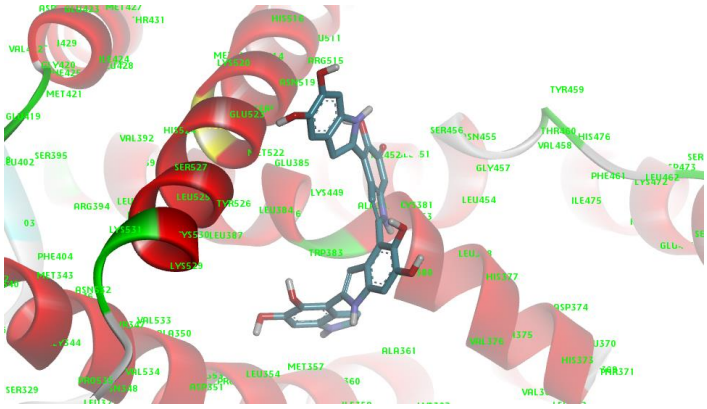


Figure 4: Molecular docking of open heteropolymer molecule in the active site of ERα (1A52)

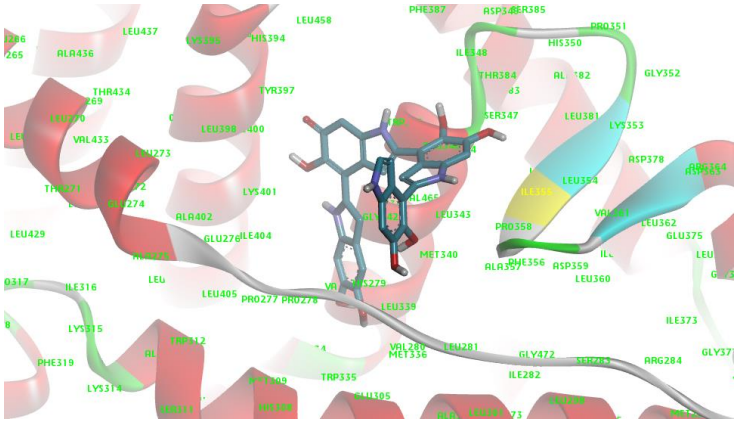


Figure 5: Molecular docking of open heteropolymer molecule in the active site of ERβ (3OLS)

Table.2 Binding affinity scores and amino acid interactions of OPS Eumelanin with ERα and ERβ

S.No	Compound name	Docking score	Amino acid interactions
1	1A52	-7.9	Glu:523, Asn:519, Arg:515, Cys:381, Leu:525, Lys:529, Asp:351-Vanderwaals, Tyr:526, Glu:380-Conventional Hydrogen Bond, Leu:379-Carbon Hydrogen Bond, Met:522-Pi-Sigma, Trp:383-Pi-Pi Stacked, Leu:354-Pi-Alkyl
2	3OLS	0.9	Pro:278, Leu:339, Gly:342, Lys:401, His:350, Val:361, Leu:281-Vanderwaals, Val:280, Glu:305, 276, Tyr:397, His:394-Conventional Hydrogen bond, Pro:358-Pi-Sigma, Arg:279-Unfavorable Bump, Trp:345-Unfavorable Acceptor-Acceptor, Pro:277, Ile:355-Pi-Alkyl

Molecular docking studies of Eumelanin, Tamoxifen and Estradiol with ERα

Human ERα ligand binding domain in complex with 4-hydroxytamoxifen was selected for molecular docking. We have carried out docking studies of eumelanin, tamoxifen and estradiol into the active site of human estrogen receptor alpha ligand binding site. The total active site comprises of Thr347, Met343, Leu346, Ala350, Asp351, Trp383, Leu384, Leu387, Met522, Leu525, Tyr526, Met528, Lys529, Lys531, Asn532, Val533, Val534, Pro535 and Leu536 residues. The Eumelanin molecule is forming hydrogen bond with Thr347 side chain hydroxyl group. The aromatic rings of Eumelanin stabilized by CH-π interactions with Leu525, Lys529 and Ala350 active site residues. The binding affinity of the eumelanin to ERα was observed (-8.9 Kcal/mol) from docking studies (Figure.6).

The reference molecules tamoxifen and estradiol are also docked into the active site to understand and compare the docking pattern and validating the software. Estradiol molecule docked in to the active site of ERα (Figure.7) and the orientation of the molecule compared with known crystal structure with estradiol in estrogen receptor alpha (PDB_ID: 1A52) and the RMSD of the docked molecule was observed to be less than 1 Å compared to crystal molecule. Similarly, the tamoxifen molecule is docked with ERα (Figure 8) and

docking is compared with 4-hydroxytamoxifen in crystal structure (PDB_ID: 3ERT) with less than 1 Å RMSD.

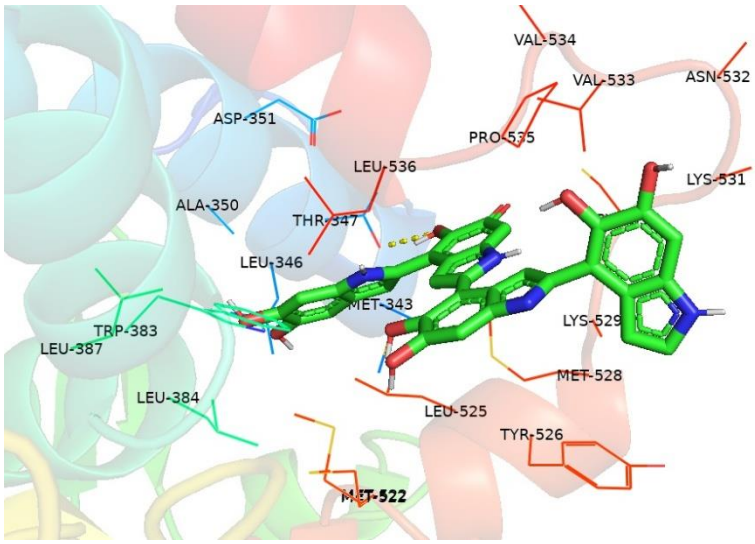


Figure 6: Molecular docking of OPS eumelanin in the active site of the ERα. Protein active site residues shown in lines and the eumelanin are shown in stick representation. Hydrogen bonds are shown in broken yellow lines.

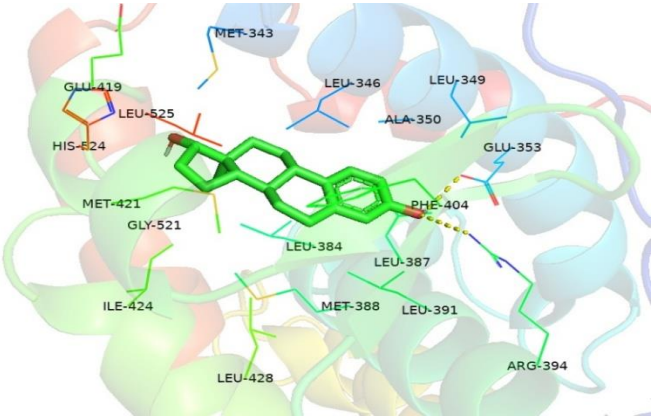


Figure 7: Molecular docking of estradiol molecule in the active site of the ERα . Protein active site residues shown in lines and the estradiol shown in stick representation. Hydrogen bonds are shown in broken yellow lines.

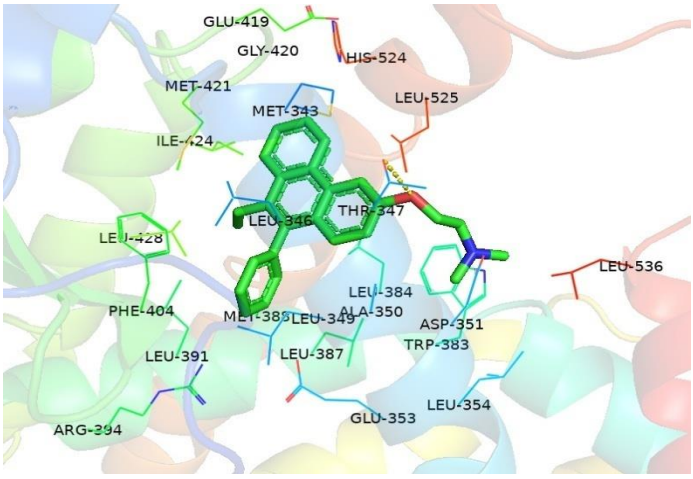


Figure 8: Molecular docking of tamoxifen molecule in the active site of the ERα. Protein active site residues shown in lines and the tamoxifen shown in stick representation. Hydrogen bonds are shown in broken yellow lines.

The expression of the estrogen receptor alpha increases in the reproductive disorders [21]. The selective estrogen receptor modulators (SERMs) like 4-hydroxytamoxifen acts as both agonist and antagonist in uterus, bone marrow and breast cancer respectively [22]. Estradiol is a natural regulator for the expression of estrogen receptor alpha by binding to it [21].

A comparative study of eumelanin, tamoxifen and estradiol with estrogen receptor alpha had shown that the eumelanin has better binding affinity than the tamoxifen and estradiol molecules with the active site residues of Estrogen receptor alpha (Table 3)

Table 3: Binding affinity scores of the molecules docked into the active site of the ERα.

Molecule name	Binding affinity (in kcal/mol)
Eumelanin	-8.9
Tamoxifen	-7.1
Estradiol	-8.8

Molecular dynamics simulation studies of CTS eumelanin and ERα

Molecular dynamics simulations help in understanding the binding affinity and stability of molecular interactions. Since both the proposed structures of eumelanin showed better binding affinity to estrogen receptor alpha compared to beta, we considered CTS - IMIM eumelanin for simulation. Further CTS-IMIM structure is supported by many studies in recent past by simulation and ultra fast vibrational finger printing analysis[8][23][24].

The Eumelanin complexed with ERα was submitted to molecular dynamics simulations for over to 100 nano seconds (ns) of time. The RMSD plot of ERα (Figure 9), eumelanin (Figure 10) and RMSF of ERα (Figure 11) plots that are drawn from simulation trajectories helps us to understand the stability of the protein complexes.

The RMSD plot of the Eumelanin complex protein showed the structural stability at the end of the simulations. The fluctuation range from 0.3 nm to 0.4 nm indicated the limited fluctuation and probable stable structures at end of simulations. The RMSF plot indicate the atomic fluctuation to understand the protein movement and the effect of the residues on the binding molecule.

Few regions of the protein showed high fluctuations than the remaining residues of the protein. The residues Asp332-Ala340 are the connecting loop after a helix structure in protein showing more than 0.3 nm fluctuations to 0.4 nm of maximum. The residues Phe461-Ser468 forming a small connecting loop between two helices region showed fluctuation around 0.31 nm.

These two regions were far from the active site and the C-terminal residues as free tail showing high fluctuations during the MD simulations. The expected uncertainty at C-terminal loop region was observed in the RMSF plot. From the trajectory, the structural changes were observed using graphics visualizer like VMD and Pymol. The Eumelanin molecule was stabilized in the active site and the residues in the active site are stabilizing the Eumelanin.

The RMSD of the Eumelanin molecule observed between 0.05-0.1 nm and is stabilized with the hydroxy indole group flip movement in the active site. The structural deviation and the hydrogen bond formations were observed from the MD trajectory analysis.

The structural alignment of the random structure sample collection at 25 ns, 50 ns and 100 ns with initial docked conformation are shown in figure 12. During the simulations the hydroxy indole exposed to solvent region of the complex formed hydrogen bonds with main chain CO oxygen of the Cys-530 and main chain carbonyl oxygen of Val-533 residues.

The other hydrogen bond between side chains Thr347 with one of hydroxyindole ring now shifted to main chain carbonyl oxygen of Thr347 and eumelanin. The hydrogen bond count was also observed from the 100 ns trajectory analysis (Figure 13). The molecule is stabilized with other hydrophobic interactions at protein active site.

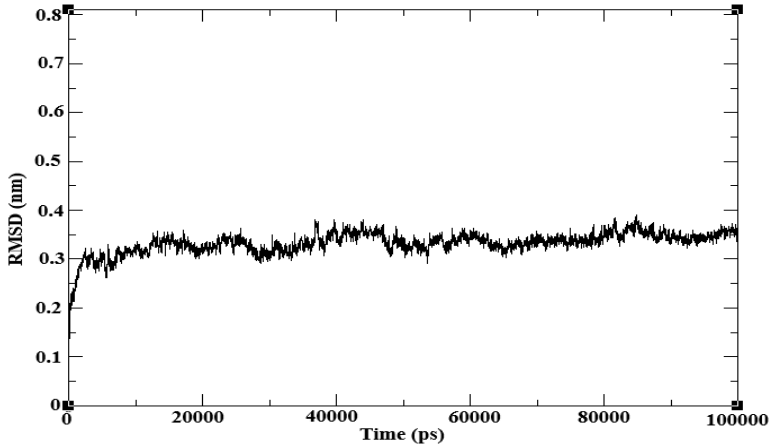


Figure 9. RMSD plot of the ERα in complex with Eumelanin during 100ns of MD simulations

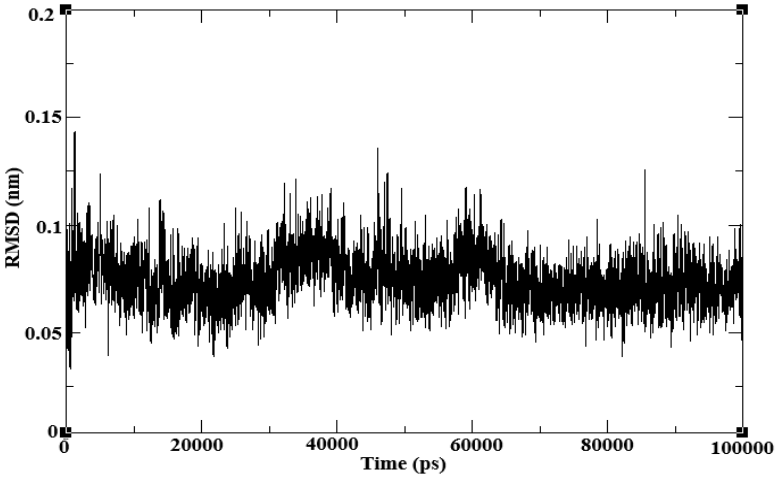


Figure 10: RMSD plot of Eumelanin in complex with ERα during 100 ns of MD simulations

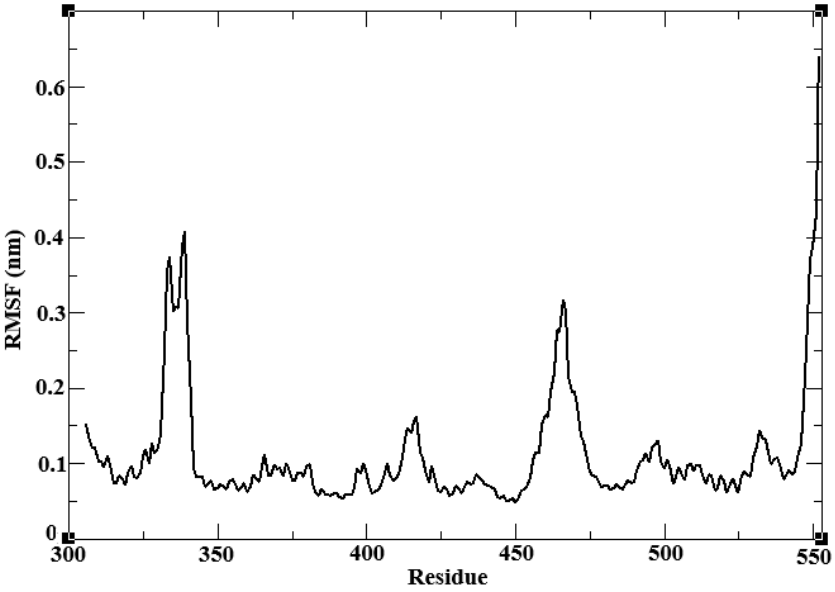


Figure 11. RMSF plot of the ERα in complex with Eumelanin during 100 ns of MD simulations

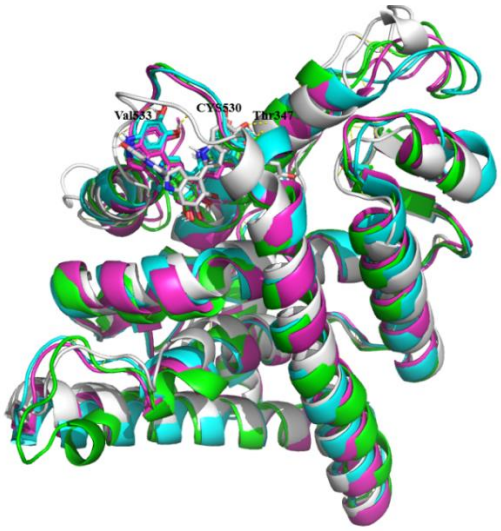


Figure12. The structural changes during the MD simulations docked conformation at 0 ns (white), 25 ns (cyan blue), 50ns (hot pink), 100 ns (green). The Eumelanin molecule conformational changes were observed in stick format. The ERα structures are in cartoon representation.

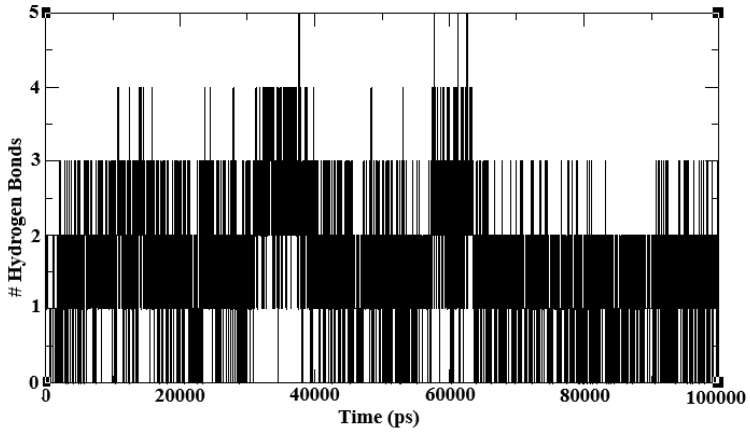


Figure 13. The number of hydrogen bonds between ERα and eumelanin molecule through 100 ns of MD simulations.

Conclusion

Eumelanins are versatile molecules in terms of their existence and chemical structure. Lack of knowledge on the structure reflects uncertainties in their functional properties. Its extensive properties and usage in traditional medicine for treating many female reproductive disorders is an interesting area of research. Estrogen signalling plays a pivotal role in the pathophysiology of several gynecological diseases. The present study aims to understand if this biomolecule has any binding affinity to estrogen receptor alpha and beta. Our results indicates a positive output in both the docking and simulation studies. Docking analysis with SERMs and estradiol showed that eumelanin has better binding ability compared to tamoxifen and estradiol. This study indicates that eumelanin might play a key role in altering the function of estrogen receptor alpha. Further studies on other steroid receptors and in vivo analysis might provide a better understanding about the dynamic properties of this molecule to be considered as a potential drug target against reproductive disorders.

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