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Role of Hydrogen Bonding and Hydrophobic Interactions on the Stabilization of Myoglobin (Globular Protein)-Primaquine-4-Dicyanomethylene-2,6-Dimethyl-4*H*-pyran (DDP) Conformers

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Molecular docking (MD) approach on the binding interaction of two competing ligands with a well-known globular protein, myoglobin (MB) was carried out. Docking studies of 4-dicyanomethylene-2,6-dimethyl-4*H*-pyran (DDP) dye and an anti-malarial drug, primaquine (PRQ) were considered as the guest molecules in the presence of myoglobin as the host molecule. Docking studies of dye-myoglobin conformers reveals that the dye resides predominantly in between the helices C and D, which are stabilized and governed by hydrogen-bonding interactions. Further, the dye is also stabilized through hydrophobic interactions when confined to E and F helices of the protein. However, the drug prefers to reside in all the domains of the polypeptide chain structure of myoglobin resulting in a larger stability when compared to that of dye-protein complex. Interestingly, the displacement of dye from the binding sites of myoglobin was found to be ineffective in the presence of drug. On the contrary, the dye efficiently displaces the drug entirely to a single confined region (between C and D, E and between F and G) of the protein molecule. Docking studies signifies that the hydrogen bonding donor and acceptor sites were involved in the stabilization of complex thereby provides a clear elucidation on the binding nature of two competing guest molecules in the presence of host molecule. Molecular docking technique was used as an efficient and reliable tool to determine the various bimolecular interactions existing between protein-dye and protein- drug complexes.

Keywords: Dye, Myoglobin, Primaquine, Hydrogen bonding, Hydrophobic interactions, Molecular docking.

INTRODUCTION

Molecular docking (MD) is a key tool usually employed in the field of structural molecular biology and computer-assisted drug design. The purpose of ligand-protein docking is to anticipate a ligand's most common and preferred binding sites with a protein of known three-dimensional structure. One of the most basic and important strategies for drug development is molecular docking analysis. It enables in the prediction of molecular interactions that interpret a protein and a ligand linked together with the protein molecules [1-3].

The molecular docking approach can be used to represent the atomic level interaction between a small molecule and a protein, allowing us to characterize small molecule behaviour in target protein binding sites as well as to elucidate key biochemical processes [4]. The relationship between ligand with these probe molecules is usually based on the number of hydrogen bonding donor and acceptor sites available for conventional bonding and also depends upon the hydrophobicity scale. Further, the molecular structure of the binding entities also influences the various possible molecular interactions that contribute and govern the stability.

Binding interaction exist predominantly *via* intermolecular forces such as ionic bonds, hydrogen bonds and van der Waals forces (weaker forces of attraction). The association or docking between host and guest species is actually reversible through dissociation. However, in the presence of a covalent bonding between a ligand and target molecule is observed in biological systems, this type of interaction is of larger significance. Ligand binding to a receptor protein usually results in a variation in the conformation by influencing the three-dimensional shape and orientation. The conformation of a receptor protein composes the functional state. The rate of binding is usually referred as affinity and this measurement signifies a tendency or strength

of the effect of interaction existing between the host and guest molecule. Although, binding affinity is actualized not only governed by host-guest interactions, the role of solvent induced effects presumably play a dominant role that drives the non-covalent binding interactions in solution [4-6]. The solvent generally provides a chemical environment for the ligand and receptor to adapt and thus the mechanism to accept or reject each other as moieties arises. The high-affinity ligand binding results from greater attractive forces between the ligand and its receptor while low-affinity ligand binding involves lesser attractive forces. These attractive forces are important in the concept of drug and fluorophore binding with large proteins and biomolecules. High-affinity binding of the guest molecule usually results in a higher occupancy of the receptor by its ligand than is the case for low-affinity binding; the residence time which is usually refereed to the lifetime of receptor-ligand complex does not correlate in this aspect. High-affinity binding of ligands to receptors is often physiologically important in the concept of site specific and site selective nature such that a quantum of the binding energy (BE) is presumably used to create or produce a conformational change in the receptor thereby resulting in denaturation or renaturation properties in proteins [4-7].

Myoglobin is one of the members of the globular protein superfamily, which also includes haemoglobin. It has resemblance in structurally and functionally to haemoglobin, which four polypeptide chains and four oxygen binding sites. Myoglobin is a single polypeptide chain with one oxygen binding site. Even though they have similarity in the structure and function, but they differ in their binding kinetics. Myoglobin exhibits a higher binding ability for oxygen than haemoglobin and myoglobin can extract oxygen from the blood which plays a crucial role in respiratory systems [8]. Hence, myoglobin is the protein, which can extract oxygen from the blood [9]. The drugs and fluorophores presumably bind and alter the function of the receptor that triggers a physiological response which is called a receptor agonist. In present study, a well-known ICT based fluorophore and an anti-malarial drug primaquine (PRQ) is employed in binding with myoglobin (MB) [9-11].

EXPERIMENTAL

The structures of DDP dye and PRQ structures wer optimized (Fig. 1) using Chemsketch and saved in MDL-mol format and converted to .pdb format using open babel molecular converter program. The SMILES format was generated using Chemsketch and their properties were calculated using Molinspiration tool [12] as provided in Table-1.

Molecular docking studies of myoglobin-DDP dye: The crystal structure of myoglobin was retrieved from protein databank (PDB) (http://www.rcsb.org/pdb, PDB ID: K45R, A Chain) [13]. The water molecules and complexes were removed during the binding interaction studies. Protein preparation [14] was carried out using Autodock software version 4.2. The methodology employed is provided in supporting information which provides the energetics parameter of the confomers generated.

The polar hydrogen and kollman charges were added and saved in .pdbq format. The structure of dye and the drug were uploaded, centre node and torsional bonds were selected and saved in .pdbqt format. In grid preparation, primaquine (PRQ) was saved in .pdbqt format, later grid spacing was set as 0.560 Å with the grid box size of 126 Å × 126 Å × 126 Å, which covers the entire protein and the initial search was carried out. Lamarckian genetic algorithm was applied in docking studies. Ten genetic algorithm runs were performed with the following parameters: population size of 150, maximum number of 2.5 × 10^6 energy evaluations, and maximum number of 27,000 generations and other parameters were default. The region of the most populated of the first ten clusters was selected as the probable binding region, which is universally accepted. The resulting conformations were clustered using a root-mean square deviation (RMSD) of 2.0A and the clusters were ranked in order of increasing binding energy of the lowest binding

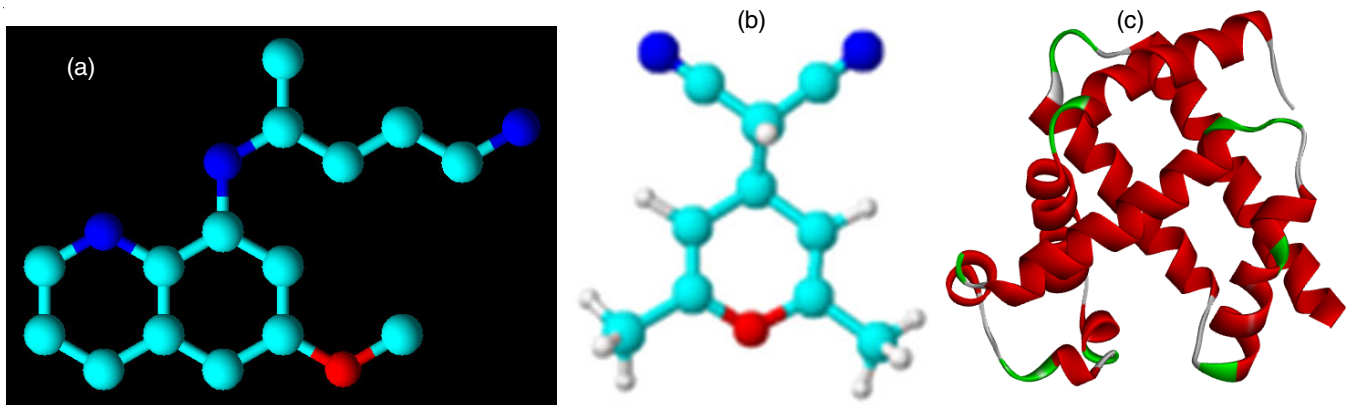


Fig. 1. (a) 3 Dimensional structure of primaquine, (b) Structure of DDP dye, (c) Ribbon structure of myoglobin

TABLE-1
MOLINSPIRATION RESULTS OF DDP AND PRQ

Dye	Hydrogen bond donor	Hydrogen bond acceptor	miLogP	Rotatable bonds	TPSA (Å)	Volume	Number of atoms	Type
DDP	0	3	2.86	1	56.82	166.11	13	ICT
PRQ	2	7	-1.03	6	72.9	256.91	19	Drug

energy conformation in each cluster. Energies were calculated based on autodock scoring function. Finally, all the ten conformation was selected and saved in .pdb format. The myoglobin-DDP dye and myoglobin-PRQ complex formed were visualized using Biovia discovery studio visualizer and analyzed for hydrogen-bonding, hydrophobic, van der Waals interactions as well as for any unfavourable interactions existing during the complex formation [3,15,16].

RESULTS AND DISCUSSION

Myoglobin-DDP dye: Various conformers generated for docking of DDP dye with myoglobin were categorized based on their energetics as well as that of the bimolecular interactions existing between the host and guest molecule. The conformers provide an efficient as well as an informative approach on the factors determining the stability of the dye-protein complex. A quantitative illustration on the bimolecular interactions that governs the stability of the conformers were obtained. Among the conformers of dye with myoglobin, two unique conformers were obtained that significantly differ in nature of the interactions based on the amino acids. Based on binding energy (BE), the variation in the energy of the stable and least stable conformer was found to be 0.26 KJ mol^{-1} only. The difference in the energy reveals that the possibility of existence of the most stable and least stable dye-protein conformers definitely would co-exist such that they differ only through the site and domain of binding (helices and strands). The most stable conformer is MBDDP1. The binding energy of MBDDP1 is –6.17 KJ mol^{-1} (Table-2) and the inhibitory constant value is 30.07. The inhibitory constant value is a crucial factor that determines the binding affinity of host-guest systems. Based on the energetics, the conformers MBDDP1 to MBDDP6 almost possesses a similar energy, whereas the conformers MBDDP7 to MBDDP10 does not vary much on the energetics parameter. Based on the inhibitory constant values, the stability of the conformer has also tabulated in Table-2.

The primary sequence of myoglobin [17-20] reveals that it contains a single long polypeptide chain with several turns and coils. The primary sequence contains 153 amino acids of 8 helices. These helices are coined from helices A-H made up of several polar and non-polar amino acids, which provides various binding pattern with the guest molecule. The helices constituting A, B, C and D contains 60 amino acids and the helices E, F and G also contains 60 amino acids that contribute towards H-bonding and hydrophobic interactions. The sequence of amino acid starts with glycine (GLY1) and ends with glycine (GLY153). All the 20 naturally existing amino acids are present in the peptide chain among, which 76 amino acids are polar amino acids that are hydrophilic in nature. The non-polar amino acids constitute 77 of the peptide structure that are responsible for the hydrophobic interactions.

The molecular interactions of MBDDP conformers are given in Table-2, wherein the dye predominantly binds with the polar amino acids (ASP, ARG, LYS and HIS) compared to the non-polar amino acids. Through docking studies, it is clearly established that MBDDP1-MBDDP6 conformers form conventional H-bonding interaction with ASP44 and ARG45. These amino acids are present in-between the helices C and D. The hydrophobic interactions with the dye molecule are through both polar and non-polar amino acids (LYS42, HIS64, HIS93, ILE99, HIS97, PHE43). Among the amino acids involved in the hydrophobic interactions, LYS and HIS although are hydrophilic in nature, yet they contribute molecular interactions with the protein through both hydrogen-bonding and hydrophobic interactions. The position of the amino acids confined to various helices and the most probable location of the individual conformer are provided in Table-3. Based on the docking of the dye with protein, it was ascertained that the dye is confined to several helices of myoglobin. Interestingly, the dye does not reside in A, B and H, respectively. All the conformers provide the most probable location of the amino acids that are involved in any type of bimolecular interaction. Through docking studies, it is ascertained that the amino acids involved in the interaction with dye are confined to the helices C, D, E, F and G only. Among these conformers, it is authenticated that the hydrogen bonding site is only through the amino acids present in the side chain in between helices C and D. Similar to the docking of dye with protein, a detailed investigation on the most probable location of drug myoglobin was ascertained based on the bimolecular interactions. The unique conformers as established from molecular docking studies are MBDDP1 and MBDDP 7 is provided in Fig. 2 and the DDP dye is represented in line model in pink colour.

Myoglobin-DDP with primaquine: Two unique conformers of myoglobin-DDP were investigated to determine the relative stability when a competing ligand moiety enters the domains

TABLE-2
ENERGETIC VALUES OF MBDDP CONFORMERS

Conformation	Binding energy	Ligand efficiency	Inhibitory constant, K_i (μm)	Intermolecular energy	vdW + H bond + desolv energy	Electrostatic energy	Torsional energy	Total internal unbound energy
MBDDP1	-6.17	-0.47	30.07	-6.17	-6.06	-0.1	0.0	0.0
MBDDP2	-6.15	-0.47	30.93	-6.15	-6.05	-0.1	0.0	0.0
MBDDP3	-6.14	-0.47	31.54	-6.14	-6.04	-0.1	0.0	0.0
MBDDP4	-6.14	-0.47	31.56	-6.14	-6.04	-0.1	0.0	0.0
MBDDP5	-6.14	-0.47	31.54	-6.14	-6.04	-0.1	0.0	0.0
MBDDP6	-6.14	-0.47	31.61	-6.14	-6.04	-0.1	0.0	0.0
MBDDP7	-5.93	-0.46	45.18	-5.93	-5.87	-0.05	0.0	0.0
MBDDP8	-5.91	-0.45	46.69	-5.91	-5.87	-0.04	0.0	0.0
MBDDP9	-5.91	-0.45	46.87	-5.91	-5.85	-0.05	0.0	0.0
MBDDP10	-5.91	0.45	46.31	-5.91	-5.86	-0.06	0.0	0.0

TABLE-3
MOLECULAR INTERACTIONS OF MBDDP CONFORMERS

Conformation	Location of dye (helix)	Hydrogen-bonding interaction donor-acceptor	Bond distance	Hydrophobic interactions	Bond distance	Other interactions
MBDDP-1	between **C** and **D**, **E** and between **F** and **G**	ASP44 (N···N)	2.79	Alkyl		–
		ARG45 (N···N)	3.07	LYS42	4.12	
		ARG45 (NH1···NH)	3.09	HIS64	4.79	
		ARG45 (C···N)	3.17	HIS93	4.15	
				ILE99	4.81	
				HIS97	4.12	
				Pi alkyl		
				HIS97	4.67	
				Pi-Pi		
				PHE43	4.05	
				HIS97	3.93	
MBDDP-2	between **C** and **D**, **E** and between **F** and **G**	ASP44 (N···N)	2.79	Alkyl		–
		ARG45 (N···N)	3.09	LYS42	4.15	
		ARG45 (NH1···NH)	3.09	HIS64	4.79	
		ARG45 (C···N)	3.16	HIS93	4.16	
				ILE99	4.81	
				HIS97	4.10	
				Pi alkyl		
				HIS97	4.63	
				Pi-Pi		
				PHE43	4.08	
				HIS97	3.90	
MBDDP-3	between **C** and **D**, **E** and between **F** and **G**	ASP44 (N···N)	2.80	Alkyl		–
		ARG45 (N···N)	3.09	LYS42	4.06	
		ARG45 (NH1···NH)	3.17	HIS64	4.87	
		ARG45 (C···N)	3.30	HIS93	4.08	
				ILE99	4.68	
				HIS97	4.09	
				Pi alkyl		
				HIS97	4.64	
				Pi-Pi		
				PHE43	4.05	
				HIS97	3.89	
MBDDP-4	between **C** and **D**, **E** and between **F** and **G**	ASP44 (N···N)	2.80	Alkyl		–
		ARG45 (N···N)	3.09	LYS42	4.07	
		ARG45 (NH1···NH)	3.16	HIS64	4.86	
		ARG45 (C···N)	3.29	HIS93	4.09	
				ILE99	4.68	
				HIS97	4.10	
				Pi alkyl		
				HIS97	4.63	
				Pi-Pi		
				PHE43	4.05	
				HIS97	3.90	
MBDDP-5	between **C** and **D**, **E** and between **F** and **G**	ASP44 (N···N)	2.81	Alkyl		–
		ARG45 (N···N)	3.08	LYS42	4.07	
		ARG45 (NH1···NH)	3.15	HIS64	4.81	
		ARG45 (C···N)	3.28	HIS93	4.13	
				ILE99	4.70	
				HIS97	4.14	
				Pi alkyl		
				HIS97	4.66	
				Pi-Pi		
				PHE43	4.02	
				HIS97	3.93	
MBDDP-6	between **C** and **D**, **E** and between **F** and **G**	ASP44 (N···N)	2.79	Alkyl		–
		ARG45 (N···N)	3.08	LYS42	4.07	
		ARG45 (NH1···NH)	3.16	HIS64	4.88	
		ARG45 (C···N)	3.30	HIS93	4.08	
				ILE99	4.68	
				HIS97	4.07	
				Pi alkyl		
				HIS97	4.64	
				Pi-Pi		
				PHE43	4.05	
				HIS97	4.89	

MBDDP-7	**G**	HIS119 (ND1···N)	2.77	Pi-Lonepair		–
				PRO120	2.64	
				Pi-Alkyl		
				PRO120	4.51	
				Alkyl		
				PRO120	4.63	
MBDDP-8	**G**	HIS119 (ND1···N)	2.79	Pi-Lonepair		–
				PRO120	2.64	
				Pi-Alkyl		
				PRO120	4.63	
				Alkyl		
				PRO120	4.61	
MBDDP-9	**G**	HIS119 (ND1···N)	2.81	Pi-Lonepair		–
				PRO120	2.64	
				Pi-Alkyl		
				PRO120	4.54	
				Alkyl		
				PRO120	4.62	
MBDDP-10	**G**	HIS119 (ND1···N)	2.79	Pi-Lonepair		–
				PRO120	2.67	
				Pi-Alkyl		
				PRO120	4.52	
				Alkyl		
				PRO120	4.65	

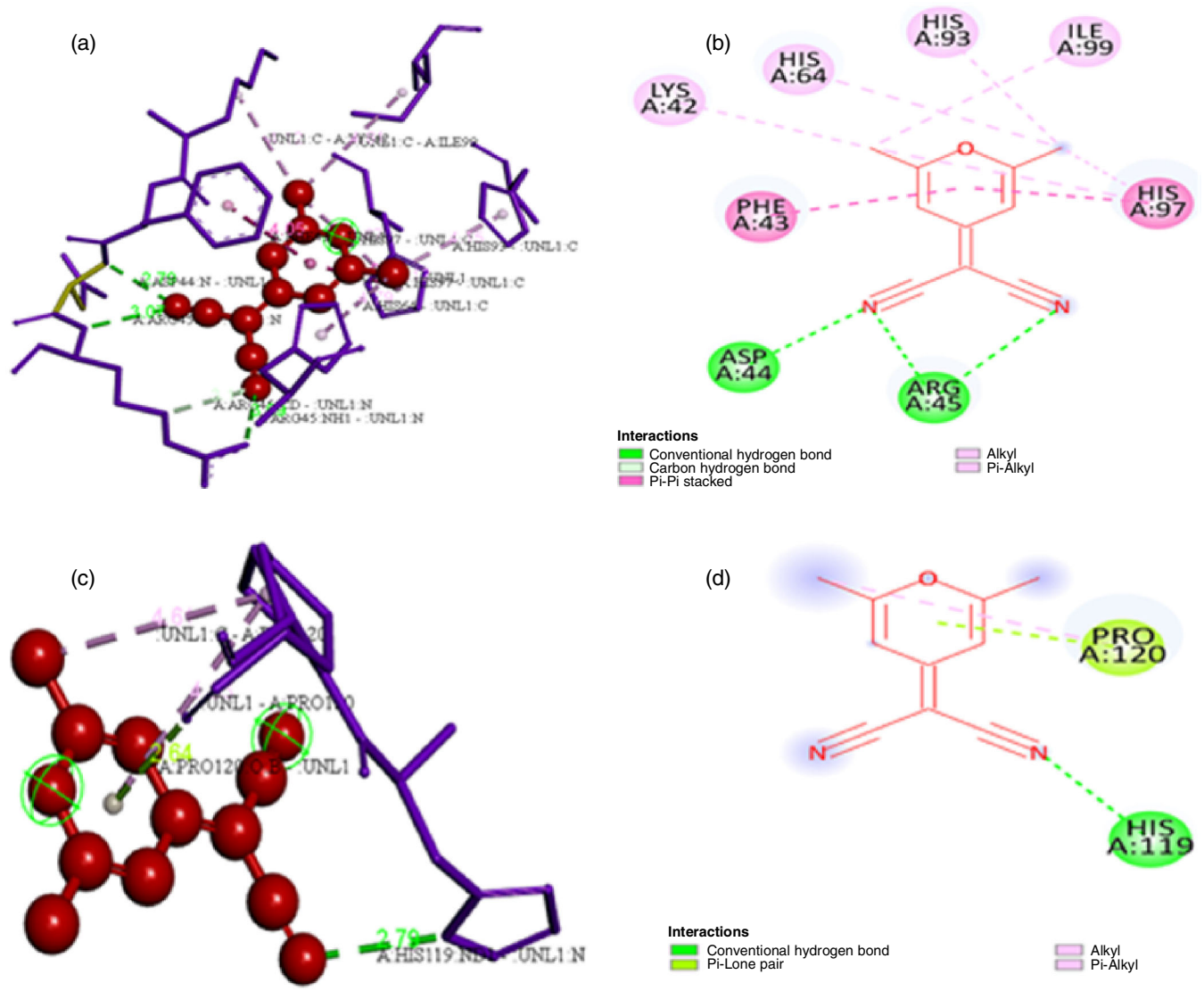


Fig. 2. (a) 3D conformer of MBDDP-1 conformer, (b) 2D representation of MBDDP-1 conformer, (c) 3D conformer of MBDDP-7 conformer, (d) 2D representation of MBDDP-7 conformer

of the protein molecule. The study was carried out in order to determine the binding stability of dye-protein complex in the presence of drug. MBDDP-1 and MBDDP-7 are the two unique conformers as based on the nature of the amino acids involved in interaction with the dye molecule. On the introduction of drug with most stable and least stable conformers of MBDDP, the resultant energetics and subsequent variation in the molecular interactions were investigated. Interestingly, when the drug is docked with the energetically most stable conformer, MBDDP-1, the conformer is found to be destabilized in terms of energy, whereas the stability of other conformer, MBDDP-7 is substantially stabilized. This clearly illustrates that the introduction of a competing ligand disrupts the binding of dye with the protein molecule. The drug promotes the most favourable site of dye in between helices C and D of myoglobin. Table-4 provides the energetics of MBDDP-1PRQ and MBDDP-7PRQ, whereas Table-5 illustrates the variation in their molecular interactions. Docking studies authenticates that the addition of a competing ligand (PRQ) influences the conformer stability such that the forces of contact between dye and protein are disrupted to certain extent in the case of both conformers. In the presence of drug, conformer MBDDP-7PRQ is found to be more stable, as evidenced by the fact that their binding energy and energy parameters related to desolvation and hydrogen bond were found to be stabilized. This is attributed to a significant decrease in the inhibitory constant value, which is of importance in the context of complex stability. Even though, there is no direct dye-drug interaction, it is evident that the binding energy of MBDDP-7 is more stable in the presence of drug rather in the absence of drug. The docking of dye-protein conformers in the presence of drug and the molecular interaction of MBDDP with primaquine is provided in Fig. 3.

Myoglobin-primaquine: The docking of myoglobin with drug results in 9 different unique conformers, which was not observed in the case of dye-protein binding. Based on the binding energy parameters, the conformer MBPRQ-1 is found to possess the maximum stability among all other conformers generated. The conformer MBPRQ-1 has the largest stability on the basis of its binding energy and inhibitory value in comparison with all other conformers which is of significance in the context of stability of the host-guest complex. This conformer is stabilized by hydrogen bonding interactions through GLN128, SER117, PHE123, SER117 amino acid residues. These amino acids are predominantly hydrophilic in nature and also contribute towards conventional hydrogen bonding interactions. Further, these amino acids are confined only to G and H helices of the protein molecule. Apart from these interacting amino acids, the docking studies also exhibits hydrophobic interactions with other residues

TABLE-4
ENERGETICS OF MBDDP WITH PRIMAQUINE

Conformation	Binding energy	Ligand efficiency	Inhibitory constant, K_i (μm)	Intermolecular energy	vdW + H bond + desolv energy	Electrostatic energy	Torsional energy	Total internal unbound energy
MBDDP-1PRQ	-6.12	-0.32	32.51	-8.21	-6.24	-1.97	2.09	-1.03
MBDDP-7PRQ	-6.31	-0.33	23.56	-8.4	-8.22	-0.19	2.09	-0.56

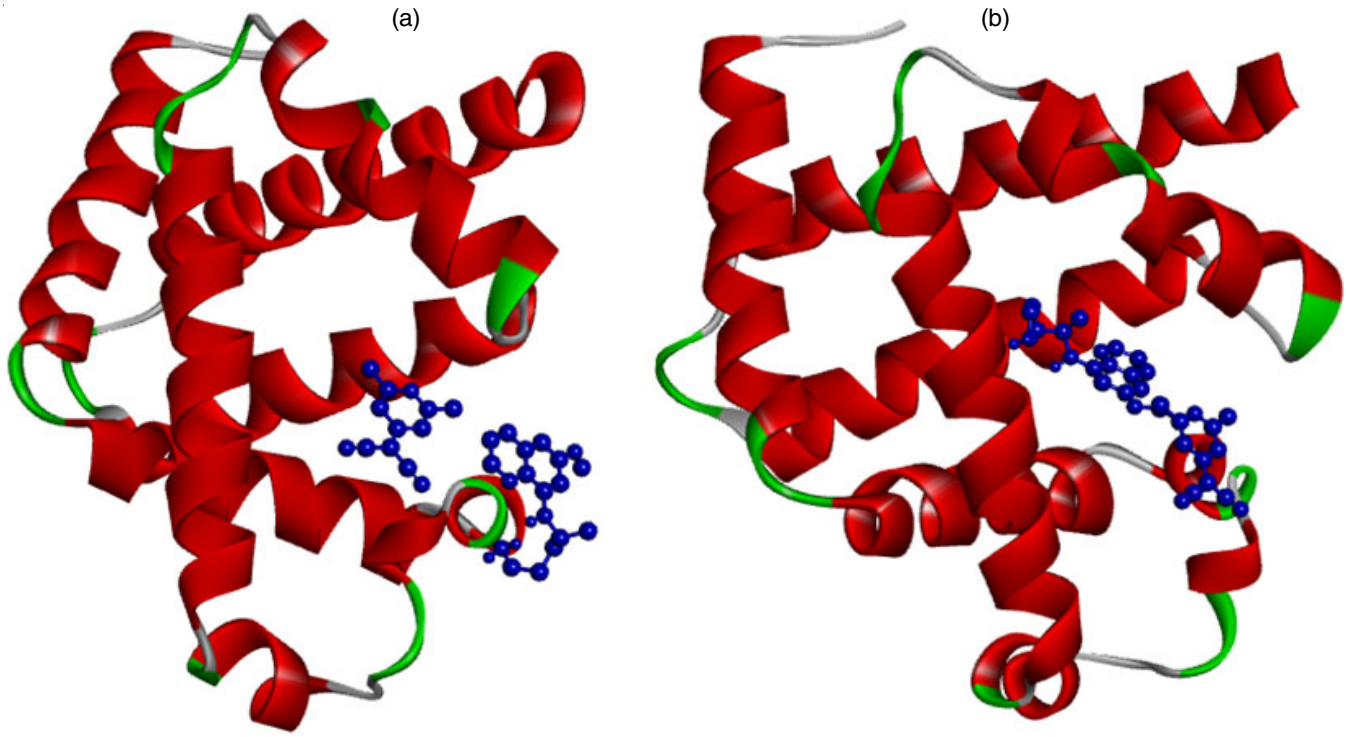
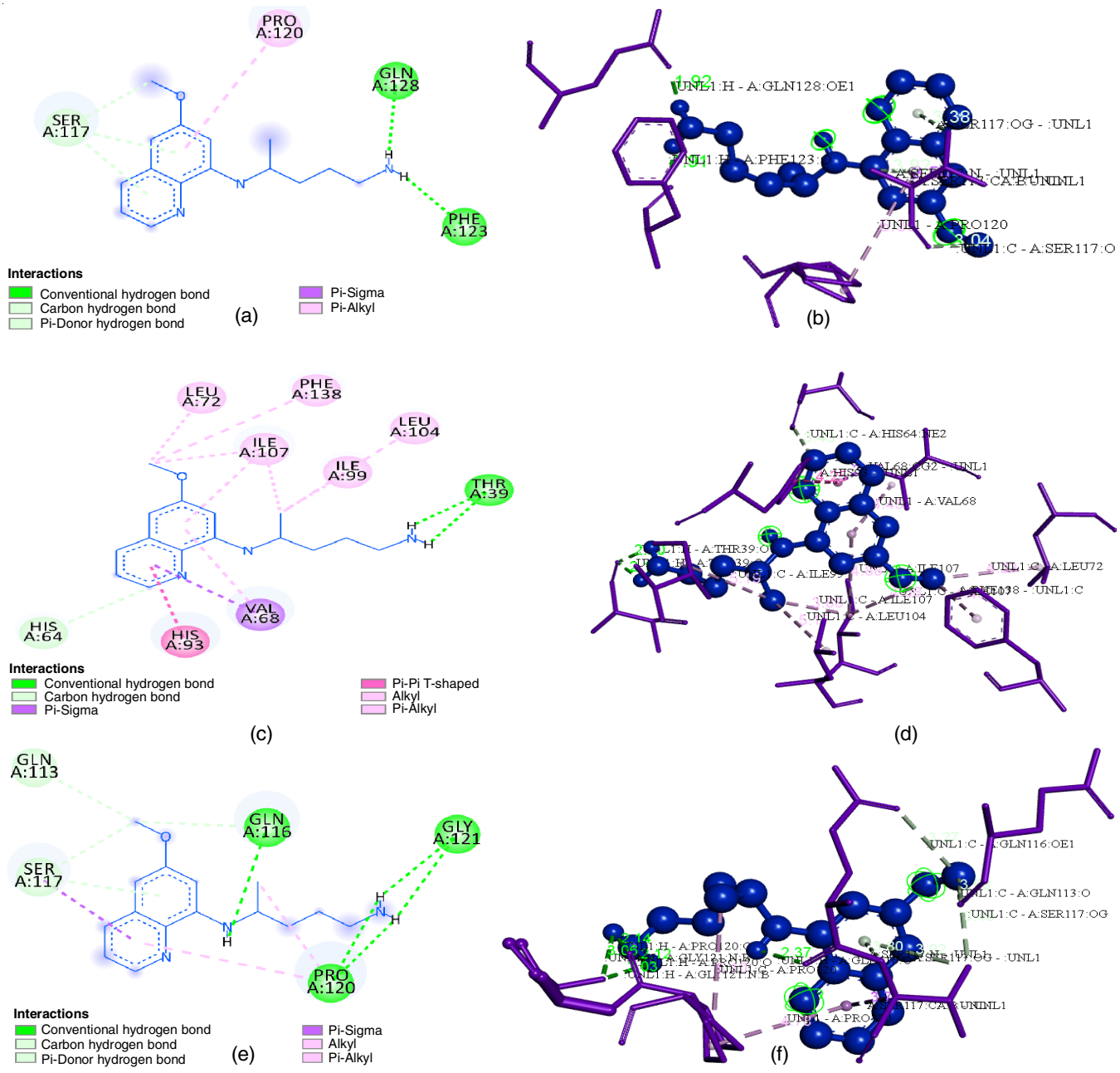
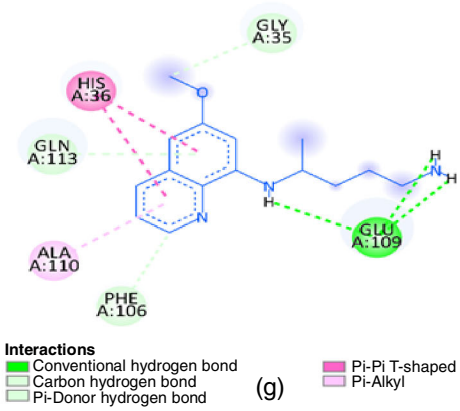


Fig. 3. Two unique conformers of MBDDP with PRQ

TABLE-5
MOLECULAR INTERACTION OF MBDDP WITH PRIMAQUINE

Conformation	Location of dye (helix)	Hydrogen-bonding interaction donor-acceptor	Bond distance	Hydrophobic interactions	Bond distance	Other interactions
MBDDP PRQ-1	Between **C** and **D**	ASP44 (OD1···H)	1.87	Pi alkyl		–
		ASP44 (OD1···H)	1.95	LYS42	4.41, 5.25	
		ASP44 (OD1···H)	2.18	Alkyl		
				LYS42	4.38	
				Pi-lonepair		
				LYS42	2.90	
MBDDP PRQ-2	Between **C** and **D**, **E**, between **F** and **G**	LYS42 (O···H)	1.89			–
		GLU41 (O···H)	2.07	Pi alkyl		
		ASP44 (OD1···H)	1.84	HIS64	4.52	
				HIS97	4.92	
				Pi-Pi		
				HIS97	4.15	
				HIS97	4.26	
				PHE43	3.88	
				PHE43	4.39	

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HIS A:36
GLN A:113
ALA A:110
PHE A:106
GLU A:109
Interactions
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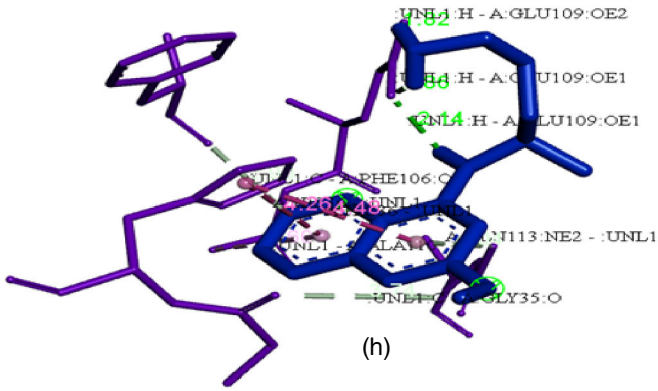
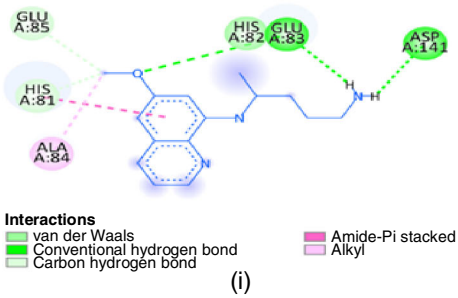
Carbon hydrogen bond
Pi-Donor hydrogen bond
Pi-Pi T-shaped
Pi-Alkyl

(g)



(h)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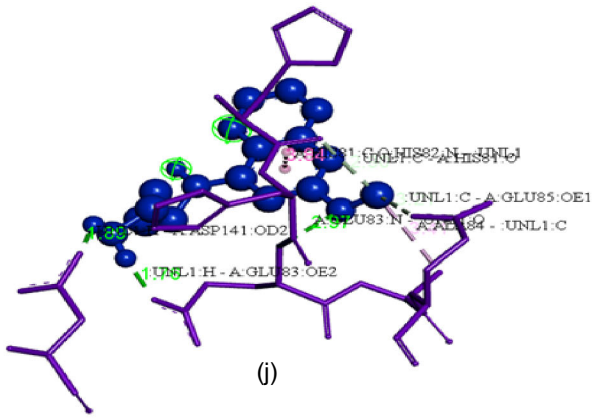
(i)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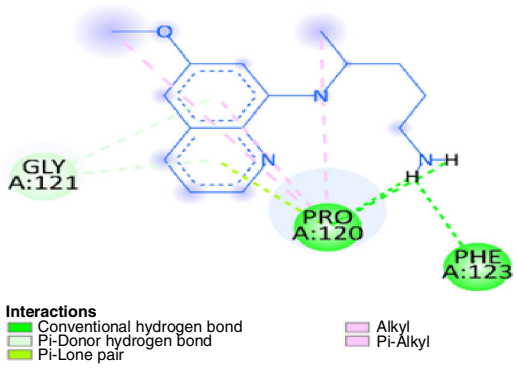
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Alkyl
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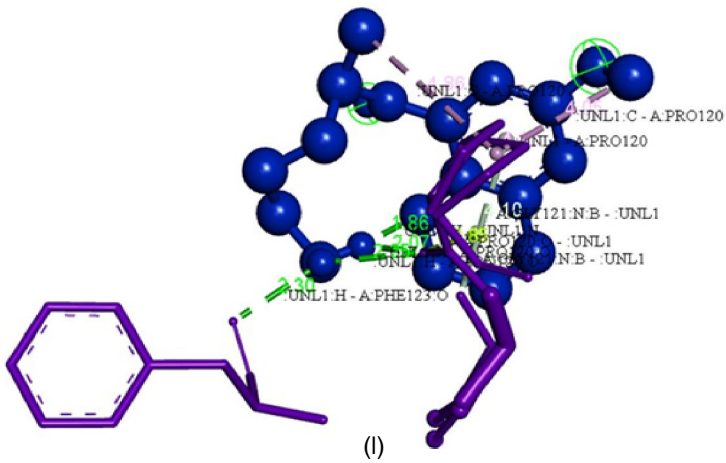
(k)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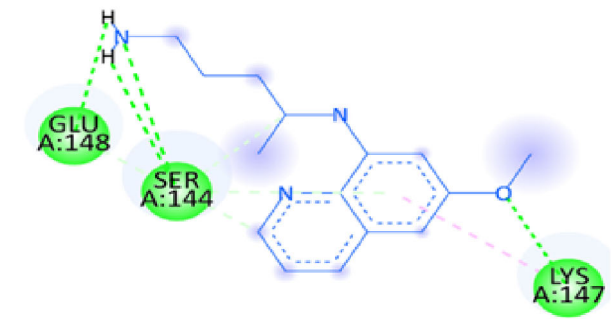
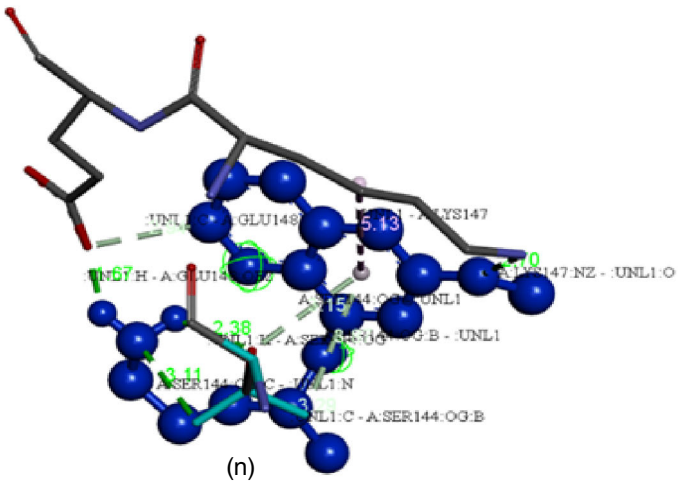
:UNL1:H - A:PHE123:O

(l)


GLU A:148
SER A:144
LYS A:147
Interactions
Conventional hydrogen bond
Carbon hydrogen bond
Pi-Donor hydrogen bond
Pi-Alkyl

(m)


:UNL1:H - A:GLU148:OE1
:UNL1:C - A:SER144:OG:B

(n)

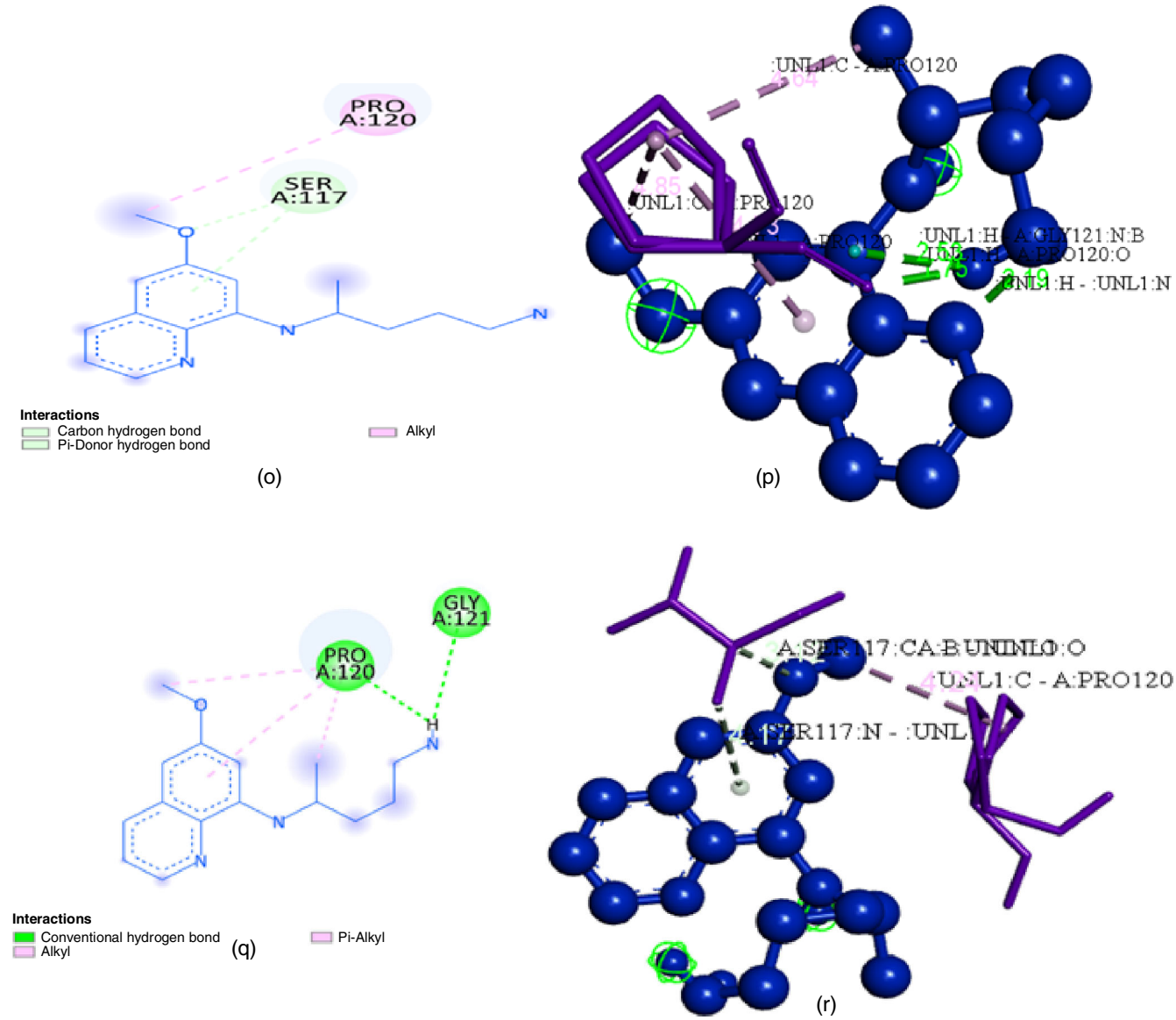


Fig. 4. (a-r) 2D and 3D representation of all the nine conformers of MBPRQ1 to MBPRQ10

PRO120 and SER117 that are confined to G helix of the protein sequence. The least stable conformer among the ten conformers generated for protein-drug is MBPRQ-10, which has a very high unfavourable BE compared to other MBPRQ conformers. The conformers MBPRQ-3, MBPRQ-4 and MBPRQ-5 possess almost similar stability in terms of BE and inhibitory constant values. MBPRQ-9 and MBPRQ-10 conformers formation is attributed to very high inhibitory constant value than all other conformers are responsible for the unfavourable interaction arising in this conformer. Based on the binding energy (BE), inhibitory constant and intermolecular energy, the stability of the conformers is of the order MBPRQ-1 > MBPRQ-2 > MBPRQ-3 > MBPRQ-4 > MBPRQ-5 > MBPRQ-6 > MBPRQ-7 > MBPRQ-8 > MBPRQ-9 > MBPRQ-10.

The energetics and molecular interactions of MBPRQ conformers is shown in Tables 6 and 7, respectively. Compared to the dye-protein interaction (Table-8), wherein the dye is predominantly residing in between the helices C and D, the drug enjoys a larger stability and it is confined to G strand only. Docking studies clearly visualises that the most probable location of guest molecules when docked with protein is entirely different even though both dye and drug pose several HB donor and acceptor moieties. It is further carried out the role of binding stability of drug-protein complex in the presence of dye. The nine unique conformers of MBPRQ are provided in Fig. 4, where the blue colour line model represents the primaquine drug.

Myoglobin-PRQ with DDP dye: Dye was incorporated with all the unique conformers of drug-protein complex as carried out for drug docked with dye-protein complex. The energetics and molecular interactions of drug-protein complex in the presence of dye is provided in Tables 8 and 9, respectively. Interestingly, the presence of dye resulted in contradicting nature of the location of drug with protein. The identities of all the unique conformers of drug-protein were completely lost such that the dye governs the binding domains efficiently.

TABLE-6
ENERGETICS OF MBPRQ CONFORMERS

Conformation	Binding energy	Ligand efficiency	Inhibitory constant, K_i (μm)	Intermolecular energy	vdW + H bond + desolv energy	Electrostatic energy	Torsional energy	Total internal unbound energy
MBPRQ1	-6.18	-0.33	29.42	-8.27	-7.75	-0.52	2.09	-0.83
MBPRQ2	-6.10	-0.32	33.52	-8.19	-8.14	-0.06	2.09	-0.64
MBPRQ3	-5.83	-0.31	52.98	-7.92	-7.82	-0.10	2.09	-0.92
MBPRQ4	-5.58	-0.29	81.38	-7.67	-5.44	-2.23	2.09	-0.94
MBPRQ5	-5.58	-0.29	81.28	-7.67	-5.01	-2.26	2.09	-0.97
MBPRQ6	-5.19	-0.27	155.83	-7.28	-6.96	-0.33	2.09	-1.74
MBPRQ7	-5.17	-0.27	163.17	-7.25	-6.53	-0.72	2.09	-0.70
MBPRQ8	-5.11	-0.27	181.12	-7.19	-5.16	-2.03	2.09	-1.54
MBPRQ9	-4.77	-0.25	320.68	-6.85	-6.72	-0.13	2.09	-2.10
MBPRQ10	-4.32	0.23	678.48	-6.41	-6.24	-0.17	2.09	2.11

TABLE-7
MOLECULAR INTERACTION OF MBPRQ CONFORMERS

Conformation	Location of dye (helix)	Hydrogen-bonding interaction donor-acceptor	Bond distance	Hydrophobic interactions	Bond distance	Other interactions
MBPRQ-1	**G** and **H**	GLN128 (OE1···H)	1.92	Pi alkyl		–
		SER117 (O···C)	3.04	PRO120	5.35	
		PHE123 (O···H)	1.91	Pi-Sigma		
		SER117	3.38, 3.92	SER117	3.50	
MBPRQ-2	**C**, **E**, **H**, **G**, **F** and between **F** and **G**	THR39 (O···H)	2.20, 2.10	Alkyl		–
		HIS64 (NE2···C)	3.43	LEU72	4.23	
				PHE138	4.45	
				ILE107	3.88, 4.58	
				ILE99	5.19	
				LEU104	5.17	
				Pi alkyl		
				ILE107	4.88	
				VAL68	4.45	
				Pi-Pi		
				VAL 68	3.55	
				Pi-Pi		
				HIS93	3.43	
MBPRQ-3	**G**	PRO120 (O···H)	2.14	Alkyl		–
		PRO120 (O···H)	2.12	PRO120	5.10	
		GLY121 (N···H)	3.03	Pi alkyl		
		GLY121 (N···H)	3.06	PRO120	4.78	
		GLN116 (O···H)	2.37	Pi-Sigma		
		SER117 (O···C)	3.48	SER117	3.71	
		GLN113 (O···C)	3.58			
		GLN116 (OE1···C)	3.27			
		SER117	3.92, 3.80			
MBPRQ-4	**G** and between **B** and **C**.	GLU109 (OE1···H)	1.86	Pi alkyl		–
		GLU109 (OE1···H)	2.14	ALA110	4.60	
		GLU109 (OE2···H)	1.82	Pi-Pi		
		GLY35 (O···C)	3.74	HIS36	4.48	
		PHE106 (O···C)	2.90	HIS36	4.26	
		GLN113	4.04			
MBPRQ-5	**F** and between **E** and **F**	GLU83 (OE2···H)	1.76	Alkyl		Vander waals
		ASP141 (OD2···H)	1.89	ALA84	3.41	HIS82
		GLU85 (N···O)	2.97	Pi amide		
		GLU85 (OE1···C)	3.21	HIS81	3.84	
		HIS81 (O···C)	2.90			
MBDDP-6	**G**	PRO120 (O···H)	2.07	Alkyl		–
		PRO120 (O···H)	2, 25	PRO120	4.05	
		PHE123 (O···H)	2.30	PRO120	4.86	
		GLY121	4.10	Pi alkyl		
		GLY121	3.41	PRO120	4.63	
				Pi-Lonepair		
				PRO120	2.85	
MBDDP-7	**G**	PRO120 (O···H)	2.88	Pi-Alkyl		Donor-donor
		PRO120 (O···C)	2.89	PRO120	3.59, 4.10	ASP122
		ASP122 (OD2···H)	2.14	Alkyl		
		GLY121	4.18	PRO120	5.27	

MBDDP-8	**H**	GLU148 (OE2···H)	1.67	Pi-Alkyl		–
		LYS147 (NZ···O)	2.70	LYS147	3.13	
		SER144 (OG···H)	2.38			
		SER144 (OG···N)	3.11			
		SER144 (OG···C)	3.29			
		SER144	3.87			
		SER144	3.15			
MBDDP-9	**G**	PRO120 (O···H)	1.75	Pi-Alkyl		–
		GLY121 (N···H)	2.53	PRO120	4.85	
				Alkyl		
				PR0120	4.95	
				PRO120	4.64	
MBDDP-10	**G**	SER117 (O···C)	3.12	Alkyl		–
		SER117	4.17	PRO120	4.24	

TABLE-8
ENERGETICS OF MBPRQ WITH DDP DYE

Conformation	Binding energy	Ligand efficiency	Inhibitory constant, K_i (μm)	Intermolecular energy	vdW + H bond + desolv energy	Electrostatic energy	Torsional energy	Total internal unbound energy
MBPRQ-1DDP	-6.17	-0.47	29.97	-6.17	-6.05	-0.12	0.0	0.0
MBPRQ-2DDP	-6.05	-0.47	36.97	-6.05	-5.95	-0.10	0.0	0.0
MBPRQ-3DDP	-6.16	-0.47	37.6	-6.16	-6.03	-0.13	0.0	0.0
MBPRQ-4DDP	-6.18	-0.48	39.6	-6.18	-6.07	-0.11	0.0	0.0
MBPRQ-5DDP	-6.14	-0.47	39.41	-6.14	-6.02	-0.13	0.0	0.0
MBPRQ-6DDP	-6.14	-0.47	39.57	-6.14	-6.02	-0.12	0.0	0.0
MBPRQ-8DDP	-6.14	-0.47	38.39	-6.14	-6.01	-0.13	0.0	0.0
MBPRQ-9DDP	-6.17	-0.47	39.93	-6.17	-6.05	-0.12	0.0	0.0
MBPRQ-10DDP	-5.91	0.45	36.31	-5.91	-5.86	-0.06	0.0	0.0

The dye displaces the drug entirely from all other helices completely to one particular helix of the protein molecule. Further, the dye displaces the drug from all the helices of protein located between the helices C and D, E and in between F and G, respectively, even though there exist no direct dye-drug interaction. Docking studies portray that the dye definitely influences the bimolecular interaction existing between drug and protein in such a way that the protein doesn't favour all the binding domains exclusively either for drug or with the dye. Even though both dye-protein and drug-protein possess similar binding energy the influence of dye on drug-protein interaction is visualized clearly through docking studies. A

TABLE-9
MOLECULAR INTERACTIONS OF MBPRQ WITH DDP DYE

Conformation	Location of dye (helix)	Hydrogen-bonding interaction donor-acceptor	Bond distance	Hydrophobic interactions	Bond distance	Other interactions
MBPRQ DDP-1	between **C** and **D**, **E** and between **F** and **G**	ASP44 (N···N)	2.95	Alkyl		–
		ARG45 (N···N)	2.96	LYS42	4.15	
		ARG45 (NH1···N)	2.89	HIS64	4.76	
		ARG45 (CD···N)	3.22	HIS93	4.13	
				ILE99	4.84	
				HIS97	4.24	
				Pi alkyl		
				HIS97	4.64	
				Pi-Pi		
				PHE43	3.95	
				HIS97	4.02	
MBPRQ DDP-2	between **C** and **D**, **E** and between **F** and **G**	ARG45 (N···N)	2.85	Alkyl		–
		ARG45 (NH1···N)	3.05	LYS42	4.64	
		ARG45 (C···N)	3.32	HIS64	4.95	
				HIS93	4.37	
				ILE99	4.82	
				HIS97	4.51	
				PHE43	4.22	
				Pi alkyl		
				HIS97	3.78	
				Pi-Pi		
				PHE43	4.32	
				HIS97	3.84	

MBPRQ DDP-3	between **C** and **D**, **E** and between **F** and **G**	ASP44 (N···N) ARG45 (N···N) ARG45 (NH1···N) ARG45 (CD···N)	2.95 2.95 2.88 3.21	Alkyl LYS42 HIS64 HIS93 ILE99 HIS97 Pi alkyl HIS97 Pi-Pi PHE43 HIS97	 4.15 4.76 4.12 4.84 4.24 4.64 3.95 4.02	–
MBPRQ DDP-4	between **C** and **D**, **E** and between **F** and **G**	ASP44 (N···N) ARG45 (N···N) ARG45 (NH1···N) ARG45 (CD···N)	2.91 3.07 3.01 3.25	Alkyl LYS42 HIS64 HIS93 ILE99 HIS97 Pi alkyl HIS97 PHE43 Pi-Pi PHE43 HIS97	 4.04 4.88 4.04 4.87 4.74 4.16 5.47 3.99 3.96	–
MBPRQ DDP-5	between **C** and **D**, **E** and between **F** and **G**	ASP44 (N···N) ARG45 (N···N) ARG45 (NH1···N) ARG45 (CD···N)	2.84 2.92 2.89 3.07	Alkyl LYS42 HIS64 HIS93 ILE99 HIS97 Pi alkyl HIS97 PHE43 Pi-Pi PHE43 HIS97	 4.19 4.62 4.27 4.83 4.61 4.29 5.46 3.93 4.06	–
MBPRQ DDP-6	between **C** and **D**, **E** and between **F** and **G**	ASP44 (N···N) ARG45 (N···N) ARG45 (NH1···N) ARG45 (CD···N)	2.89 3.06 2.95 3.16	Alkyl LYS42 HIS64 HIS93 ILE99 HIS97 Pi alkyl HIS97 PHE43 Pi-Pi PHE43 HIS97	 4.08 4.94 4.00 4.90 4.17 4.62 5.43 4.01 3.94	–
MBPRQ DDP-7	between **C** and **D**, **E** and between **F** and **G**	ASP44 (N···N) ARG45 (N···N) ARG45 (NH1···N) ARG45 (CD···N)	2.82 3.08 3.02 3.14	Alkyl LYS42 HIS64 HIS93 ILE99 HIS97 Pi alkyl HIS97 Pi-Pi PHE43 HIS97	 4.15 4.76 4.14 4.79 4.19 4.65 4.02 3.95	–
MBPRQ DDP-8	between **C** and **D**, **E** and between **F** and **G**	ASP44 (N···N) ARG45 (N···N) ARG45 (NH1···N) ARG45 (CD···N)	2.93 2.92 2.85 3.15	Alkyl LYS42 HIS64 HIS93 ILE99 HIS97 Pi alkyl HIS97 Pi-Pi PHE43 HIS97	 4.19 4.69 4.18 4.85 4.27 4.62 3.94 4.04	–

MBPRQ DDP-9	between **C** and **D**, **E** and between **F** and **G**	ASP44 (N···N) ARG45 (N···N) ARG45 (NH1···N) ARG45 (CD···N)	2.97 2.96 2.89 3.23	Alkyl LYS42 HIS64 HIS93 ILE99 HIS97 Pi alkyl HIS97 Pi-Pi PHE43 HIS97	 4.15 4.77 4.11 4.84 4.63 4.23 3.96 4.01	–

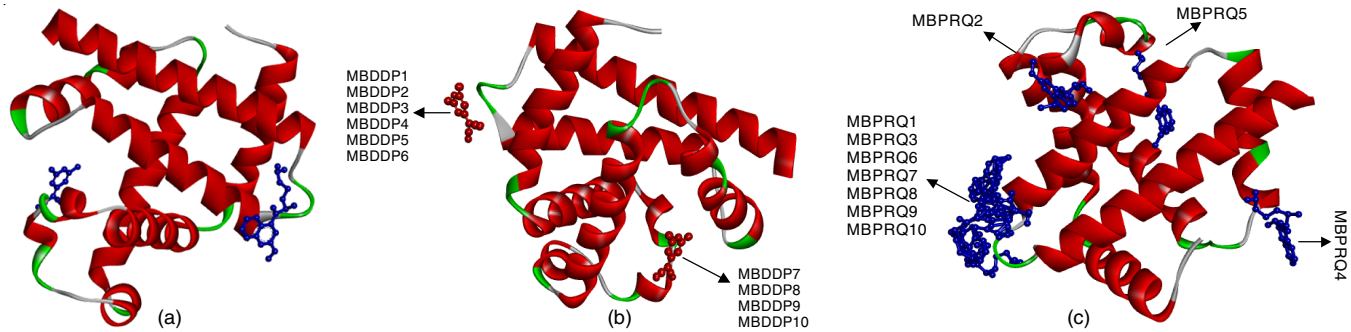


Fig. 5. Unique conformer of MYOPRQ with DDP (a), DDP dye docked with protein myoglobin (b) and PRQ drug docked with protein myoglobin (c)

contrasting and interesting phenomenon was observed after the simultaneous docking of DDP dye to the protein-drug complex, such that only one unique conformer of dye-myoglobin-drug was obtained. The above observation reflects that the dye efficiently displaces the drug from the binding sites in protein.

Earlier studies involving the docking of sulfamethazine and sulfadiazine drugs with myoglobin results in 32 ligand binding sites with protein which reveals that the host molecule provides several binding options for the guest molecules [21]. However, in present study DDP dye effectively displaces the drug from several binding domains towards to a single confined region of the protein is visualized through docking studies.

The complete visualization of protein docked with dye DDP and drug PRQ is provided in Fig. 5. The red colour ribbon structure in the figures represents the protein myoglobin.

Conclusion

Even though two competing ligands were employed in docking with myoglobin, there is no direct dye drug interaction such that each guest molecule prefers to orient away from each other. Although both the dye-protein and drug-protein complex are stabilized in one particular helix when docked individually, but docked in the presence of competing ligands, the dye effectively displaces the drug from all its binding domains than the drug displacement towards the dye. The stability is predominantly attributed to hydrogen bonding interactions wherein along the coexistence of hydrophobic interactions also contribute towards the stability of the conformers and binding domains.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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