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Molecular Modeling Approach for Identification of Interaction between Doxorubicin and Affibody A Receptor Binding Protein

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Abstract: ***Study of a comprehensive evaluation of interaction mechanism of the doxorubicinwith affibody to prevent the cytotoxicity of the drug when such complex molecule are used as an specific targeted drug delivery, by an in silico approach such as interaction mode,binding constant and binding site.The analysis of DOX binding site to affibody suggested that the types of interactions that contribute in this binding are hydrogen bonding and vanderwall interactions.Our observation is that back bone oxygen atom of Ile31is involved in hydrogen bond interaction with OH atom of the Doxorubicin. The bond distance between donor hydrogen atom and acceptor oxygen was1.994Å.The binding free energy and docked energy of the complexwere-5.72and-13.6kcal/mol.***

Keywords: Affibody, doxorubicin, molecular modelling, Flexible Docking.

1.Introduction

Study of protein interactions plays a key role for investigation of protein complexes and for understanding the various biological processes. A conventional approach for the researchers to studya protein interactions is to perform binding tests in the laboratory. However, thisprocess isa tedious and time-consuming process. Computational methods are now steadilybeing used to predict possible protein interactions.protein-protein interactions differ from protein-ligand interactions dueto the small size of ligand. Because of protein large size, they are usually treated as rigid bodies. However, conformational changes in the protein and the ligand are generally necessary for a successful docking process.

Molecular docking is the methodology which is used to predict the structure of the intermolecular complexformed between two or more molecules. The most interesting study is the protein-ligandinteraction, because of its applications in medicine. Ligand is a smallmolecule, which interacts with proteins at its binding sites. Binding sites are areas of the protein known to be active in forming of compounds. These are commonly calledas binding modes.Usually the smaller molecule involved in the docking is called a ligand and the other is called a receptor. There are two categories of protein docking algorithms rigid- docking and flexible docking. Rigid dockingmeans both ligand and receptor as rigid bodies. The goal of this type of algorithms is to find the relative positions and orientations of the ligand for some possible binding configurations with respect to the receptor.Flexible docking means at least one of the molecules, usually the smaller ligand, as a flexible object that may change shapes during docking. Flexible docking is more meaningful than rigid docking since shape changes occur in protein interactions. Docking simulations and virtual screening are being routinely used in drug design, enabling rapid identification of hits and lead compounds. [1–3]

It is well established that free molecules of drug can act at thetarget site. Protein-drug interactions play an important role in avariety of biological processes and clinically. Thestudies on this generally may provide information of the structuralfeatures of the protein that may determine the therapeuticeffectiveness of drugs, and become an important research inbiomedicine. A high binding affinity for protein has beenobserved for drugs possessing acidic or electronegativefunctional groups [4], which can bind to more than one bindingsite with different specificity. Binding of some ligands to protein induce alterations inthe structure and function of protein. However, selective bindingvarious from protein to protein. Whereby, binding of ligand X at certain site causes aconformational change in the protein so that binding of ligand Yat a different site is altered [5].As an anticancerchemotherapeutic agent utilized therapeutically, DOX maycause strong side effects and has a deleterious influence onmetabolic activity [6].Anthracyclines are widely used in cancer chemotherapy, but the clinicaluses are limited by two major problems: multidrug resistance (MDR) [7, 8] and cardiotoxicity.

Because of its nonspecificinteraction with normal tissues, DOX leads tosignificant normal tissue toxicity and limits dosages of itfar below the tumour sites required to destroy most malignantlesions.[9]The formation of drug–protein adducts can cause cellular and tissuetoxicity which may be either intrinsic or idiosyncratic in nature[10, 11].To overcome the problem, drug targeting and combinedtreatments are tried to investigate through computational tools. Many methods for molecular docking and virtual screening have been developed to date, including AutoDock,[12,13] DOCK,[14 – 16] Flex,[17] Glide,[18] GOLD,[19]RosettaDock,[20] SLIDE[21,22] and Surflex.[23]However, little work is focused on investigating

the interaction mechanismbetween the drug-HER 2 receptor targeting proteincomplex at the molecular level, such as interaction mode, bindingconstant, and binding site number using AutoDock 4.0 software.[12-13]

2.Methods

The structural coordinates of the ZHER2affi body (PDBID: 2KZI) was retrieved from Protein Data Bank (PDB)database. This structure was determined by solution NMR (Eigenbrot et.al., 2010).Similarly, the structure of doxorubicin was retrieved from Drug Bank (Drug Bank ID: DB00997) database. The molecular docking of doxorubicin into ZHER2 affibody was carried out using AutoDock 4.0 software. The protein structure was prepared by adding Kollman charges and polar hydrogen atoms, whereas ligand molecule was prepared by adding Gasteiger charge. The grid generation was carried on center of the macro molecules at 60×62×94Åinthex, y and z axis by which to covers entire fold of the protein. Grid points pacing was kept at0.375Å.The docking was performed using the Lamarckian genetic algorithm with 50 runs. Finally, the docked conformations were clustered using root-mean square deviation of2.0Å with reference to starting geometry of the ligand.

The output from ligand docking programs usually includes a coordinate file (in PDB format or other standard formats) and supplementary data, such as estimated binding affinity, clustering of alternative poses, etc. To view coordinate files, commonly used macromolecular visualisation programs, such as Jmol, [24] Py Mol,[25] Visual Molecular Dynamics (VMD)[26]

3. Results

Preparation of the protein target and ligand

Known the structure of the protein fromPDBID: 2KZI which was obtained from the RCSB Protein Data Bank. ThestructureofdoxorubicinwasretrievedfromDrugBank(Drug BankID:DB00997)database.

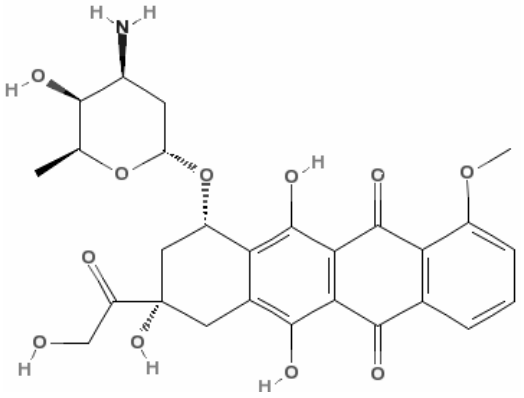

Figure 1: Two dimensional structure of doxorubicin

Water molecules near the surface and ions were removed and hydrogen atoms were added at appropriate geometry. Groups within the protein were ionized as required behaves at physiological P^H.The structure of protein was protonated. [27] The genetic algorithm was implemented in Autodock that was applied to calculate the possible conformations of the drug binding to protein. A maximum of 17 different conformations was considered for the drug during the docking process.table 1 The conformer with the lowest binding free energy was used for further analysis.

Table 1: Clustering histogram of docked complexes of doxorubicin with ZHER2 affibody

Cluster Rank	*Lowest Binding Energy*	*Run*	*Mean Binding Energy*	*Number in Cluster*
1	-5.72	25	-5.39	14
2	-5.01	47	-4.44	15
3	-4.84	20	-4.66	2
4	-4.74	8	-4.47	3
5	-4.40	38	-4.40	1
6	-4.05	41	-3.98	3
7	-4.04	17	-4.04	1
8	-3.75	28	-3.75	1
9	-3.70	27	-3.70	1
10	-3.66	42	-3.66	1
11	-3.65	1	-3.65	1
12	-3.65	2	-3.65	1
13	-3.54	39	-3.54	1
14	-3.30	7	-3.14	2
15	-3.19	35	-3.19	1
16	-3.08	37	-3.08	1
17	-2.38	21	-2.38	1

Molecular docking simulations

All the conformations were evaluated by X-Score. RMSD values which concert as a best scored conformations of this protein-ligand complexes were calculated. The binding energy of docked complexes was calculated using X-Score [28]. The scoringfunctions have all the necessary elements that correspond to the non-covalent interactions in a conventional force field, such asthe van der Waals interaction. Scoring functions are calibrated through multivariate regression analysis of protein-ligand complexes and these resultsin the binding free energies of the entire complex.fig 2

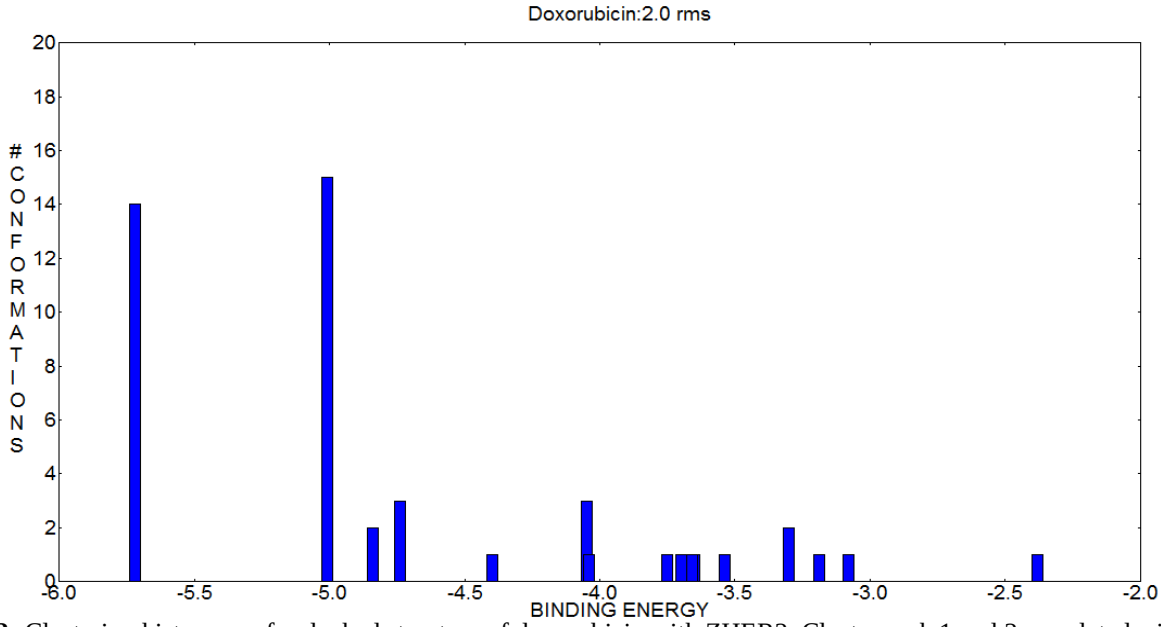

Figure 2: Clustering histograms for docked structure of doxorubicin with ZHER2. Cluster rank 1 and 2 populated with more number of conformations.

HER2-Drug binding

The complementary applications of molecule modeling have been employed by computer methods to improve theunderstanding of the interaction of DOX and protein The best docking result is shown in Figure 2. As predicted from the
docking procedure the DOX binding site is situated on the alpha subunit of protein. The binding free energy (ΔG°) and the binding affinity (Ka) were shown in the table 2

Table 2: Binding energy and RMSD values of docked complexes of doxorubicin with ZHER2 affibody

Rank	*Sub Rank*	*Run*	*Binding Energy*	*Cluster RMSD*	*Reference RMSD*
1	1	25	-5.72	0.00	11.26
1	2	12	-5.69	0.80	11.37
1	3	29	-5.68	0.31	11.39
1	4	4	-5.68	0.90	11.31
1	5	5	-5.63	0.69	11.42
1	6	48	-5.63	0.51	11.50
1	7	31	-5.58	0.37	11.38
1	8	13	-5.57	0.53	11.41
1	9	24	-5.34	0.43	11.34
1	10	26	-5.33	0.51	11.41
1	11	44	-5.32	0.73	11.37
1	12	15	-5.16	0.54	11.38
1	13	23	-4.81	0.97	11.51
1	14	14	-4.28	1.06	11.20
2	1	47	-5.01	0.00	10.42
2	2	33	-4.94	0.92	10.38
2	3	36	-4.63	0.66	10.54
2	4	40	-4.59	0.84	10.12
2	5	10	-4.59	1.22	10.23
2	6	46	-4.54	1.02	10.56
2	7	6	-4.46	0.98	10.56
2	8	9	-4.43	0.72	10.39
2	9	34	-4.43	1.01	10.17
2	10	16	-4.40	0.91	10.51
2	11	18	-4.33	0.81	10.21
2	12	19	-4.19	0.49	10.65
2	13	45	-4.06	0.67	10.79
2	14	50	-4.04	0.60	10.41
2	15	22	-3.91	0.88	10.25
3	1	20	-4.84	0.00	10.45
3	2	32	-4.48	1.99	11.01
4	1	8	-4.74	0.00	11.47
4	2	49	-4.37	0.32	11.40
4	3	3	-4.30	1.88	11.49
5	1	38	-4.40	0.00	11.33
6	1	41	-4.05	0. 00	8.80
6	2	11	-4.01	1.72	9.27
6	3	43	-3.87	1.67	9.23
7	1	17	-4.04	0.00	11.05
8	1	28	-3.75	0.00	11.55
9	1	27	-3.70	0.00	9.89
10	1	42	-3.66	0.00	9.71
11	1	1	-3.65	0.00	10.63
12	1	2	-3.65	0.00	12.50
13	1	39	-3.54	0.00	9.64
14	1	7	-3.30	0.00	12.67
14	2	30	-2.99	1.76	12.49
15	1	35	-3.19	0.00	9.30
16	1	37	-3.08	0.00	10.75
17	1	21	-2.38	0.00	11.02

Binding site

The amino acid residues involved in the binding sites of HER2 to DOX were predicted and their respective molecular distances from thebound drug have been evaluated. The presented data revealed that Leu 35 is the closest residue to be found in thevicinity (5 Å) of the drug molecule and val 1 was found to be
far away.fig 3

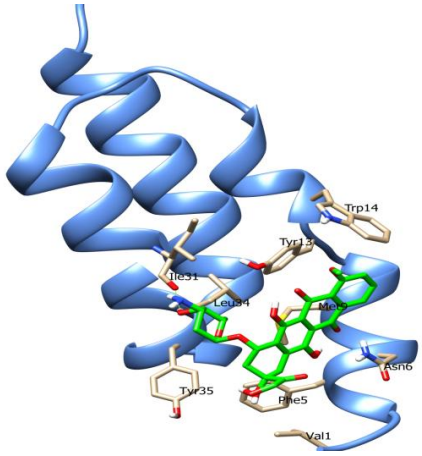

Figure 3: Docked structure of doxorubicin with ZHER2 affibody (Cluster rank 1). Ribbon model of the protein is colored by blue. Amino acid residues which are involved in molecular interactions are represented in stick model and colored their carbon atoms by tan. This structure was prepared using UCSF Chimera software.

Binding mode

The validation of the binding mode as per the amino acidresidue predicted to be part of the binding site is shown Figure4.

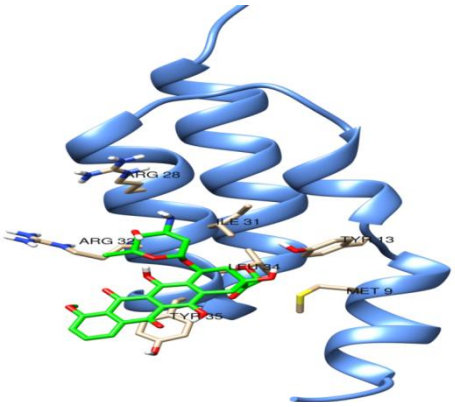

Figure 4: Docked structure of doxorubicin with ZHER2affibody (Cluster rank 2)

The amino acid residues involved in the binding of protein toDOX were predicted are Val1,Phe5,Asn6,Met9,Tyr13,Trp14,Leu34andTyr35.

4. Conclusions

The docked simulation of doxorubic into ZHER2 affibody created seventeen clusters of conformers, in which first ranked cluster was populated with 14 conformations in RMSD tolerance of 2.0 Åout of 50 runs of LGA. The lowest binding free energy complex of cluster was taken to analyze for binding site residues. We have observed that backbone oxygenatomofIle31is involved in hydrogen bond interaction with OH atom of the ligand. The bond distance between donor hydrogen atom and acceptor oxygen was 1.994Å. In addition, the docked complex was more stabilized by vander Waal's interactions by amino acid residues viz., Val1, Phe5, Asn6, Met9, Tyr13, Trp14, Leu34 and Tyr35 in the scaling factor of 1.00Å around the ligand molecule. The binding free energy and docked energy of the complex were-5.72 and-13.6kcal/mol, respectively. This work not only indicates that the mechanism of affibody interact with DOX but also provides an approach for the evaluation of protein conformation. Nevertheless, the degeneration of physiological functions of proteins is a determinant when it comes to analyze the toxic effect of drugs on proteins [29].Finally wants to conclude that instead of free drug which can be tagged to such targeting proteins, and can be used as a drug delivery.This research provides a novel angle for the evaluation of nano phase drug delivery.

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