



## LIGAND DOCKING AND BINDING SITE ANALYSIS WITH PYMOL AND AUTODOCK IN ELIMINATION OF LYMPHATIC FILARIASIS

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### ABSTRACT

Docking of small molecule compounds into the binding site of a receptor and estimating the binding affinity of the complex is an important part of the structure-based drug design process. For a thorough understanding of the structural principles that determine the strength of a protein/ligand complex, both, an accurate and fast docking protocol and the ability to visualize binding geometries and interactions are mandatory. Here we present an interface between the popular molecular graphics system PYMOL and the molecular docking suites Autodock and demonstrate how the combination of docking and visualization can aid structure-based drug design efforts, made in the elimination of lymphatic filariasis using mass drug administration.

### INTRODUCTION

**Docking and visualization:** Virtual screening of compound libraries has become a standard technology in modern drug discovery pipelines. Further docking of small molecule compounds into the binding site of a receptor and estimating the binding affinity of the complex is an important part of the structure-based drug design process (Kitchen *et al.*, 2004). Furthermore, the strategy of mass drug administration has moved from a single dose regime of diethylcarbamazine citrate (DEC) to co-administration of albendazole with DEC in areas where onchocerciasis is not co-endemic (Ottesen *et al.*, 1997). Likewise, the replacement of ivermectin by DEC + albendazole combination on visual examination of predicted binding geometries (docking poses) thereby contributes crucially to the further development of a lead compound either or towards enhanced binding affinity towards reduced side effects or towards reduced susceptibility to drug resistance related mutations. Over the last years the PYMOL molecular graphics system of docking (De Lano, 2002) has evolved from being a powerful molecular viewer with exceptional 3D-capabilities into a platform for several programs and applications which make use of PYMOL'S versatile visualization properties (Seeliger and Groot, 2010) in the field of drug design discovery against lymphatic filariasis. Docking of decoquinatone and atovaquone were targeted for plasmodium

liver stages against antimalaria (Filipa *et al.*, 2012). Similarly, co-crystal structures of ligand-bound were targeted for trypsin, thrombin and HIV-1 protease against HIV (Kitchen *et al.*, 2004), syndrome. Further, docking of TNFRSF 10B protein were targeted for head and neck cancers (Tahir *et al.*, 2013). Computational methodologies have become a crucial compound for many drug discovery programmes, from hit identification to lead optimization and beyond (Bajorath, 2002; Walters *et al.*, 1998), approaches such as ligand (Bajorath, 2002) – or structure-based virtual screening (Gohike and Klebe, 2002) techniques that are widely used in many drug discovery efforts. One key methodology such as docking of small molecules of protein binding sites was pioneered during the early 1980s (Kuntz *et al.*, 1982), and remains a highly active area of research (Gohike and Klebe, 2002). When only the structure of a target and its active or binding site is available, high throughput docking is primarily used as a hit-identification tool (Kitchen *et al.*, 2004). Furthermore, docking can also contribute in the analysis of drug metabolism using structures such as cytochrome P<sub>450</sub> isoforms (Venhorst, 2003; Williams *et al.*, 2003). The number of proteins with a known three-dimensional structure is increasing rapidly, and structures produced by structural genomics initiatives are beginning to become publicly available (Berman, 2000; Westbrook *et al.*, 2003). The increase in the number of structural targets is in part due to improvements in techniques for structure determination, such as high-throughput X-ray crystallography (Blundell *et al.*, 2003). With large-scale

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structure determination projects are driven by genomics consortia and many current target have been selected for their therapeutic potential.

PYMOL program provides an easy access to electrostatics calculations and the visualization of potential energy surfaces and charge densities on protein surfaces. CAVER programme (Petrek, 2006; Damborsky *et al.*, 2007) provides calculations for substrate pathways and initial protein structures which are visualized in PYMOL. CASTP (Liang *et al.*, 1998) detects pockets and voids in protein structures to determine and characterize binding sites, and e movie (Hodis *et al.*, 2007) provides a number of functionalities to create animations and movies. In the present study a plugin for PYMOL which allows to carry out molecular docking, virtual screening and binding site analysis with PYMOL are undertaken for the *Wuchereria* protein against DEC+Albendazole. The plugin represents an interface between PYMOL and two popular docking programs, Autodock (Morris, 1998; Huey *et al.*, 2007) and Autodock vina (Trott and Olsen, 2010). These docking programmes are extensively used in a python script collection and autodock tools for the setup of docking runs. Since, visualization is crucial for structure-based drug design; several tools have been developed to add visual support for the autodock suite. The visualize autodock tools offers a complete molecular viewer and a graphical support for all steps required for setup and analysis of docking runs against target protein and its ligand (Seeliger, 2010).

## METHODS

**Binding site definition:** A docking study usually starts with the definition of a binding site that is a restricted region of the protein. The size and location of this binding site is visualized in PYMOL and can be adjusted interactively. Autodock use rectangular boxes for the definition of the binding site. In the plugin, the box center can be defined either by providing explicit co-ordinates or, more user friendly, by defining a PYMOL selection (e.g. a reference ligand). The box center is then calculated from the mean co ordinates of the atoms from the PYMOL selection and the docking box displayed in the PYMOL window. The size and the exact position of the box can also be adjusted to the user's demands. For visualization purposes the plugin furthermore allows to chose between two display options and the color of the box frame (Figure 1a, b).

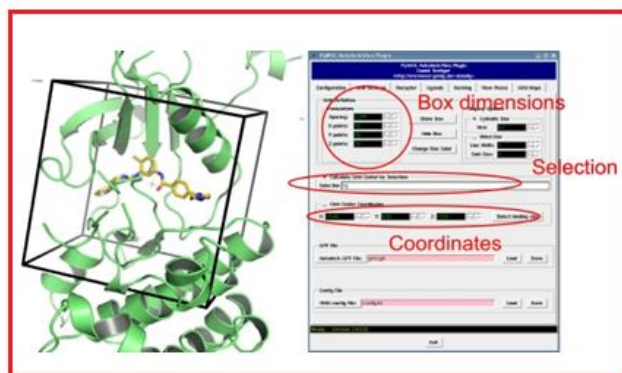
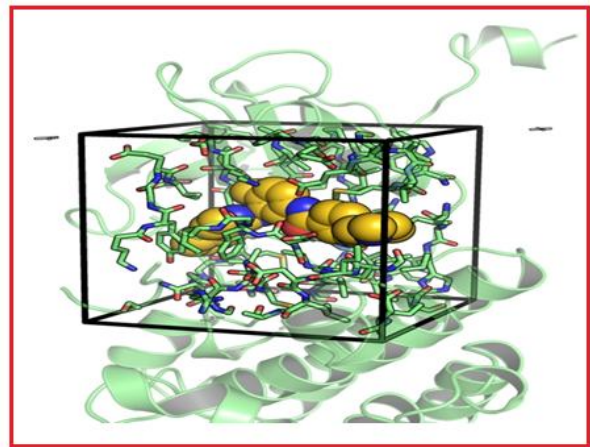


Figure 1. a. Definition of a docking box around a reference ligand. Position, size and visualization properties can be adjusted with the plugin



b. Selection of sidechains within the binding site for the setup of docking runs with flexible sidechains

**Setup and execution of docking runs:** Autodock need receptor and ligand representations in a file format called BTB/POZ domain-containing protein which is a modified protein data bank format containing atomic changes, atom type definitions and for ligands, topological information (rotatable bonds). These file preparations are carried out by the plugin using scripts from the Autodock tools package. Ligands for subsequent docking runs can either be prepared one by one through PYMOL selections or by specifying a directly containing a library of ligands to be docked. The sequence of BTB/POZ domain – containing protein (Swissprot ID: JPEZJ3) was retrieved from swissprot database. The three dimensional structure of BTB/POZ domain-containing protein was modeled using modelle 9V<sub>8</sub> (Figure 2a&b).

Modeller:

Template ID: 3HQI and A Chain

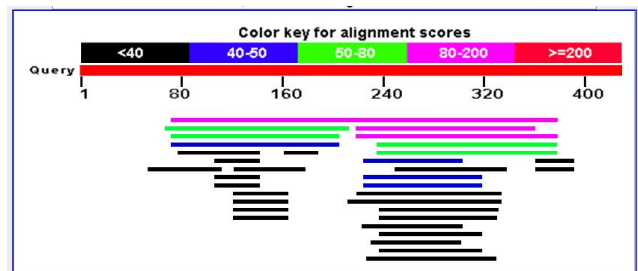


Figure 2. (a) A Pictorial representation of the BLAST Hits

The 3D structure of BTB/POZ domain-containing protein was modeled by modeller version 9v8 with 2608.41016 molpdf

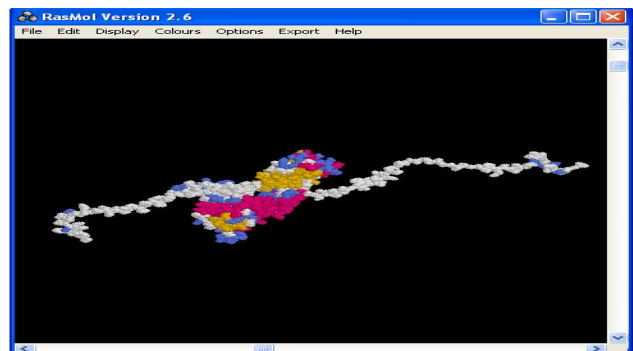


Figure 2. (b) Visualization of modeled structure using Rasmol Tool

The active site of BTB/POZ domain-containing protein was identified using Q-site finder. The drug compound structure was drawn using ACD chemsketch and converted in to PDB format using open Babel. The 3D structure of BTB/POZ domain-containing protein was docked with inhibitors like Albendazole and Diethylcarbamazine using autodock software. The docking results were analyzed using PYMOL visualization tool.

**Binding site analysis with interaction maps:** Autodock uses interaction maps for docking prior to the actual docking run these maps are calculated by the program autogrid. For each ligand atom type, the interaction energy between the ligand atom and receptor is calculated for the entire binding site which is discredited through a grid. This has the advantage that interaction energies do not have to be calculated at each step of the docking process but only in the respective grid map (Figure 3a & b).

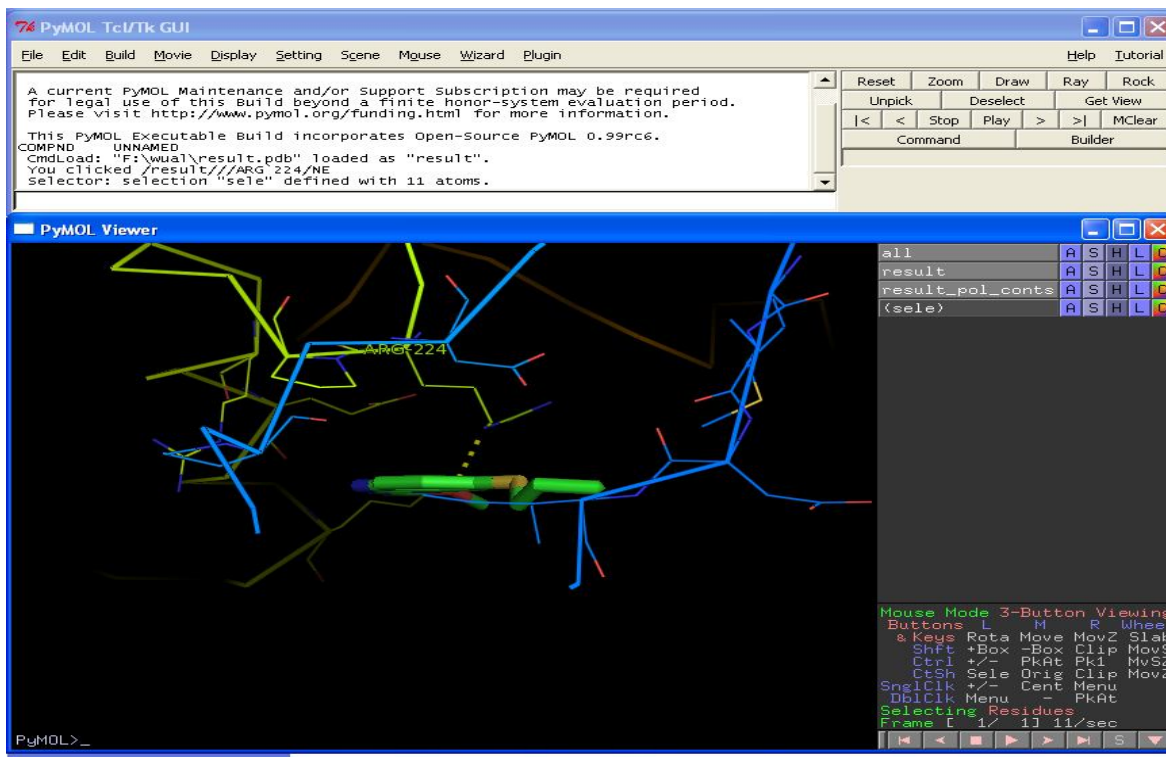
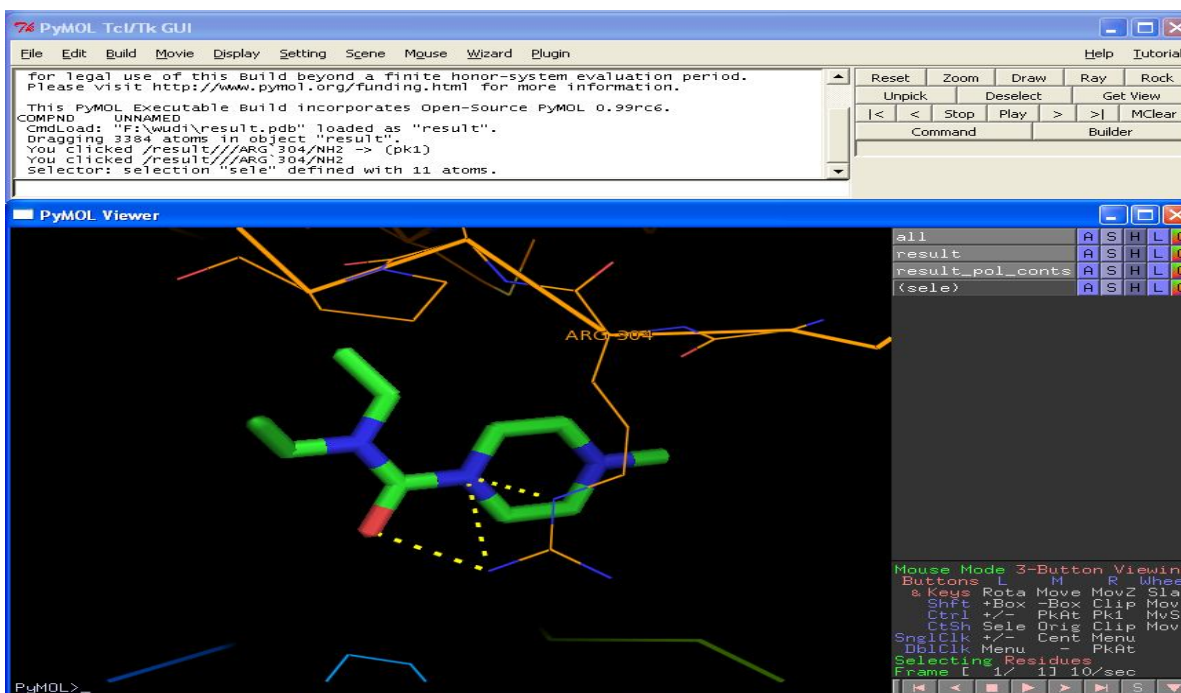


Figure 3. a. Visualization of docked complex of Albendazole in Pymol tool



b. Visualization of docked complex of diethylcarbamazine in Pymol tool

In addition to speeding up a docking runs the grid maps on their own can also provide value hints for ligand optimization. Since a grid map represents the interaction energy as a function of the co ordinates their visual inspection may reveal potential unsaturated hydrogen acceptors or donors or unfavourable overlaps between the ligand and the receptor. The plugin therefore provides the functionality to visualize these grid maps in PYMOL. The maps generated by autogrid are converted to a file format readable by PYMOL (PDB Format). The maps generated by autogrid are converted to a file format readable by PYMOL which allows drawing iso surfaces and iso meshes analogues to electron density maps. Since several maps can be loaded and controlled simultaneously, a rapid inspection of several interaction types is made very easily.

## RESULTS

**Docking and Visualization:** In Fig. 4a & b an iso surface at a contour level of 1 Kcal/mol for the interaction of the protein with aliphatic carbon atoms is shown, such a setting may be used to get a visual impression of the overall shape of the binding site. Ligand modifications which cause a penetration of such a wall will most likely not enhance the affinity. In Fig. 2a the same map is visualized at a contour level of 2.09 Kcal/mol (Table 1). As can be seen, the shape of the surface, here shown as isomech, roughly describes an envelope of the ligand and reveals putative spots of attractive interactions that may guide further ligand optimization. Likewise, hydrogen bond donor or acceptor interaction maps can guide ligand optimization since they might reveal unsaturated acceptor or donor positions (Fig. 2b).

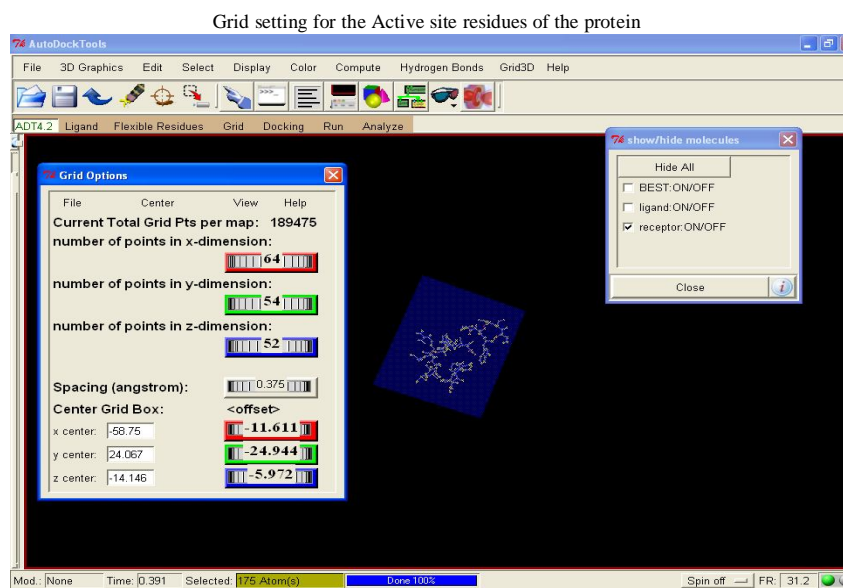
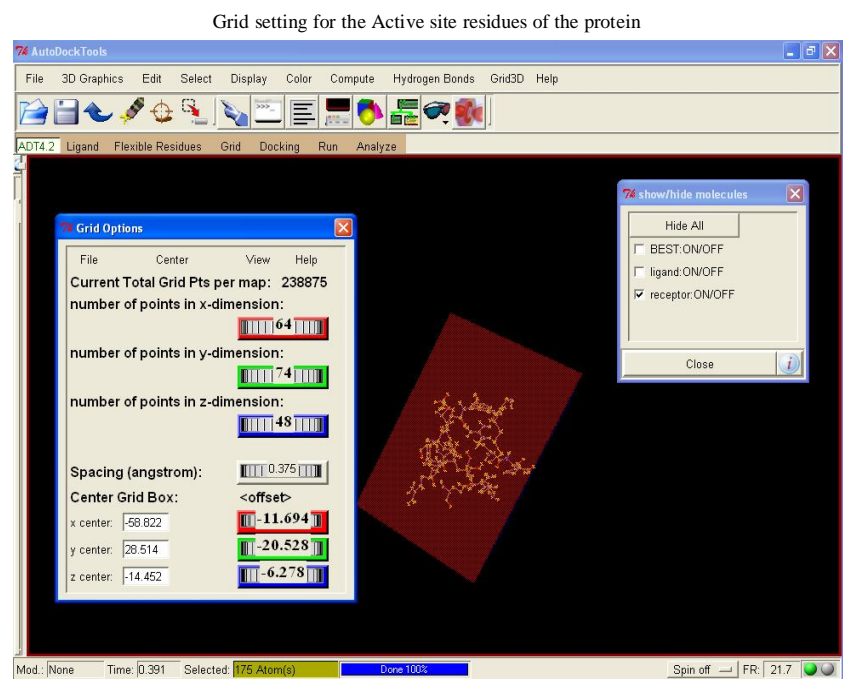


Figure 4. a. Docking of BTB/POZ domain-containing protein responsible for Lymphatic Filariasis with the ligand Albendazole



b. Docking of BTB/POZ domain-containing protein responsible for Lymphatic Filariasis with the ligand Diethylcarbamazine

The plugin provides functionally to handle different interaction maps and representations at different contour levels at the same time and hence, offers the possibility to visualize different binding site properties which may provide valuable insights for structure – based drug design.

**Analysis of docking results (Fig. 5):** Docking poses generated by the docking programs can be directly loads into PYMOL through the plugin. Poses for multiple ligands may be handled simultaneously using an intuitive notebook layout. For each docking pose, meta information containing the docking score is displayed in a small text viewer, allowing direct analysis of configuration/ score relationships. Moreover, results from multiple docking runs are summarized in a Table 1. The interactions of BTB/POZ domain-containing protein with albendazole form one hydrogen bond with energy 2.09 Kcal/mol; with diethylcarbamazine forms 3 hydrogen bonds with energy 1.94 Kcal/mol. The key interacting sites of BTB/POZ domain-containing protein with both ligands are APG 224 and ARG 304 respectively. The docking poses are ranked according to their docking scores and both the ranked list of docked ligands and their corresponding binding poses may be exported. For instance the ranked list of docking results can be exported in a PDB file format which can be directly imported into programs like excel.

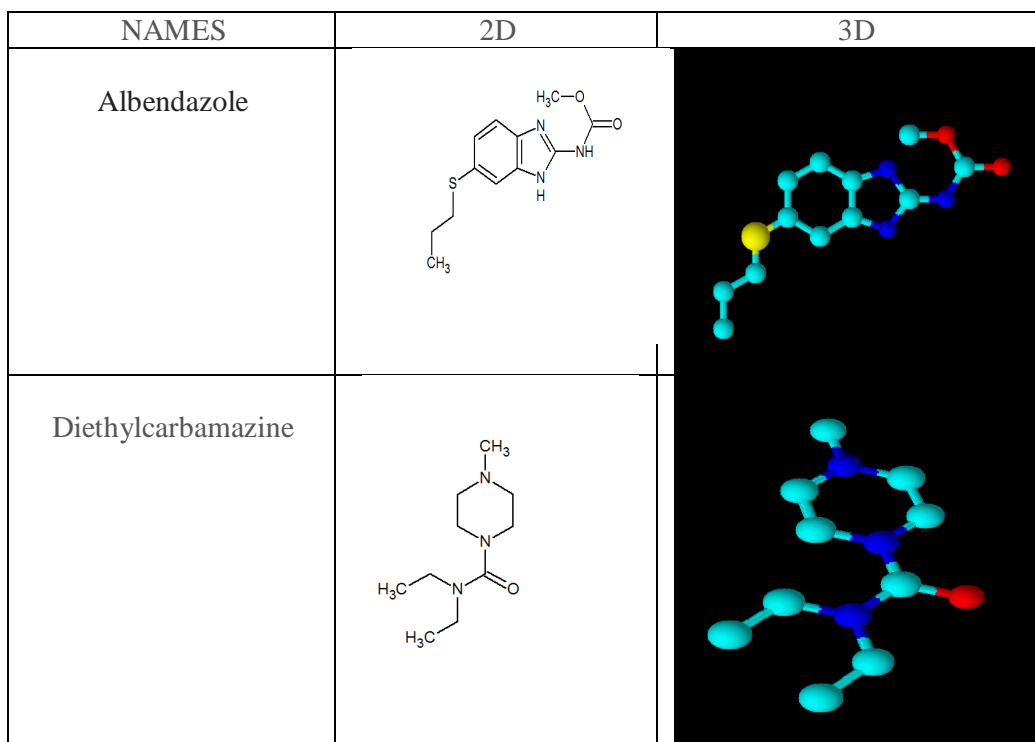
## DISCUSSION

Clinical study on the comparative tolerability and efficacy of either a single dose of albendazole or its co-administration with DEC in *Wuchereria bancrofti* microfilaraemic patients in India are widely analyzed. The study has clearly shown that single dose regimens of either albendazole, diethylcarbamazine (DEC) or albendazole + DEC are well tolerated and efficacious. Although no individual patient in any of the drug groups showed complete clearance of microfilaraemia (Ismail *et al.*, 1998). The faster decline of mean microfilaria density in the DEC and albendazole + DEC groups also correlated with the higher mean score of adverse reaction intensity as compared to albendazole alone. The delayed reduction in mf density due to albendazole alone suggests a different mode of action of this drug, either by damage or sterilization of the adult worm (Pani *et al.*, 1991). The statistical analysis of score distributions resulting from docking of large compound databases into different target sites has enabled scoring ranges to be determined that are most likely to reflect 'nonspecific' binding events (Godden *et al.*, 1999). Similarly, docking of compound collections into arbitrarily selected targets can provide information about background or 'noise' scoring levels, regardless of the scoring functions that are applied. This type of strategy has its roots in

**Table 1. Docking Score and Number of Hydrogen Bonds formed between BTB/POZ domain-containing protein With Albendazole and Diethylcarbamazine**

S.NO	PROTEIN	Albendazole		Diethylcarbamazine	
		H-BOND	ENERGY	H-BOND	ENERGY
1	BTB/POZ domain-containing protein	1	2.09	3	1.94

Drawn using ACD Chemscketch and Converted to 3D using Open Babel



**Figure 5. Ligands Structure**

earlier investigations designed to determine similarity measures for ligands on the basis of docking against panels of at least partly irrelevant receptor sites (Briem and Kuntz, 1996). Compound ranking has also been improved by the classification of databases into groups of similar molecules prior to docking and final selection of only best scoring representative of each group (Su, 2001). Knowledge – based approach is the use of three – dimensional similarity information from co – crystallized ligands as an additional constraint or scoring term (Rognan *et al.*, 1999). Docking of small molecule compounds into the binding site of a receptor and estimating the binding process facilitate in drug discoveries. For a thorough understanding of the structural principles that determine the strength of a protein / ligand complex both, an accurate and fast docking protocol and the ability to visualize binding geometries and interactions are mandatory. An interface between the popular molecular graphics system PYMOL and the molecular docking suites autodock demonstrate the combination of docking and visualization can aid structure-based drug design efforts (Groot, 2010). Docking score and number of hydrogen bonds formed between BTB/POZ domain – containing protein with Albendazole and Diethylcarbamazine were analyzed using PYMOL tool. BTB/POZ domain – containing protein from *Wuchereria bancrofti* is responsible for lymphatic filariasis. From the above docking results, both ligands docks were suited well to the proteins responsible for lymphatic filariasis and is said to be the best compounds. The result of Lipinski's rule suggests that the analyzed compounds act as best therapeutic drug. Docking study and *insilico* toxicity results proves the application of compounds as potential and synthetic therapeutic agents to treat lymphatic filariasis (Helmy *et al.*, 2006).

### Conclusion

PYMOL act as an important bioinformatic tool allowing to perform docking studies using autodock against drug discovery for lymphatic filariasis. The plugin covers all functionalities for the entire workflow of a docking run plus additional functionality to prepare, execute and analyze virtual screening tasks. Since visual support is an important aspect of structure-based drug design, the plugin is expected to enhance these efforts by allowing the combined use of two widely used docking programs and PYMOL. Docking of small molecule compounds of DEC and albendazole into the binding site of the receptor and estimating binding affinity of the complex is an important part of the mass drug administration design process. Docking helps in the production of compound of DEC with albendazole against *Wuchereria bancrofti* by identifying the molecular features and two improve the potency of the drug. PYMOL and molecular docking findings highlight the versatile tool in drug design efforts.

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