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Computational Virtual Screening Towards Designing Novel Anticancer Drugs

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1. Introduction

Generally speaking, Docking is most popular and critical issue in this research field, because it contains most important information both Ligands (Drugs) and Receptors (it can be intracellular protein, trans-membrane protein or extracellular protein). However, when the ligand's information is not sufficient, it needs other calculation strategies to design and "modify" the ligands, and theoretically improve the drug effects, and that is called *De Novo* Evolution Drug Design. Current methods for structure-based drug design can be divided roughly into two categories. The first category is about "finding" ligands for a given receptor, which is usually referred as database searching. In this case, a large number of potential ligand molecules are screened to find those fitting the binding pocket of the receptor. This method is usually referred as structure-based drug design. The key advantage of database searching is that it saves synthetic effort to obtain new lead compounds. Another category of structure-based drug design methods is about "building" ligands, which is usually referred as receptor-based drug design. In this case, ligand molecules are built up within the constraints of the binding pocket by assembling small pieces in a stepwise manner. These pieces can be either individual atoms or molecular fragments. The key advantage of such a method is that novel structures, not contained in any database, can be suggested. These techniques are raising much excitement to the drug design community. Above two computational methods, the first is called virtual screening by Docking (the drugs are well prepared and need to be screen out the most suitable candidates), and the other is *De.Novo* Evolution Drug Design (*De Novo* means "creates" or "building" ligands) [1-3]. However, when the targeting protein is unclear, or the factors are complicated, QSAR method is implemented to help user solving these problems. Because QSAR method just needs ligands structures and IC_{50} datasets to unveil an unknown novel drugs. Finally, when both of Ligands and Receptors are unknown, Homology Modeling is the only method for dealing with this problem. By using Homology Modeling, the Receptors 1-D sequences similarities can be used as a tool to reconstruct the 3-D structures.

2. Methods and materials

Docking small molecules (ligands) into larger protein molecules (receptors) is a complex and difficult task. Docking programs include CDOCKER, LibDock, and **LigandFit**. Here, I introduce **LigandFi**t for this research because it bases on an initial shape matched to the binding site and it is easier to observe the interaction of the ligand and the protein.

There are two major parts of the **LigandFit** docking:

1. Specify the region of the receptor to use as the binding site for docking. Site partitioning may be applied to select parts of the binding site during docking.
2. Dock ligands to the specified site. This part consists of the following steps:
   a. Conformational search to generate candidate ligand conformations for docking;
   b. Compare the ligand shape and protein binding site shape by computing their size of possession;
   c. Minimize the rigid body energy of the candidate ligand pose/conformation by using the Dockscore calculation.

In the following steps, we discard the water (because it will be complicated to the calculations) and ligand from the receptor protein, and calculate the score for the ligand docking to protein. Check the interacting force between the receptor protein and drugs (Fig. 1).

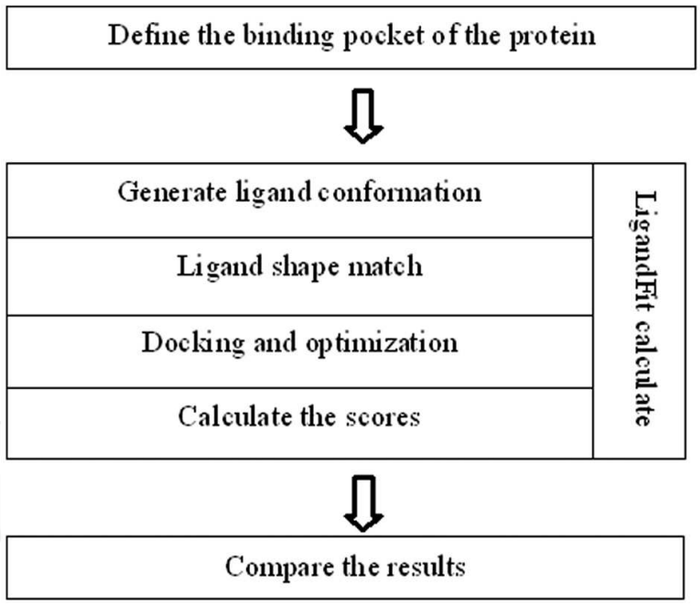


Fig. 1. The procedure of **LigandFit** Docking procedure

LigandFit: Docking and Score using Accelrys Software

The score functions in the Discovery Studio 2.5 which we used were Dock Score, PLP1, PLP2 and PMF. Candidate ligand poses are evaluated and prioritized according to the Dock Score function. There are two types of Dock Score. One is based on a force-field approximation, the other on the Piecewise Linear Potential function (PLP).

$$\text{DockScore (forcefield)} = -(\text{ligand/receptor interaction energy} + \text{ligand internal energy}) \quad (1)$$

$$\text{DockScore(PLP)} = - \text{(PLP potential)} \quad (2)$$

As shown in *Equation1*, there are two energy terms in the force-field version of Dock Score, internal energy of the ligand and the interaction energy of the ligand with the receptor. The interaction energy is taken as the sum of the van der Waals energy and electrostatic energy. The computation of the interaction energy can be quite time consuming. To reduce the time needed for this calculation, a grid-based estimation of the ligand/receptor interaction energy is employed [4].

The van der Waals component of the force-field interaction energy typically exhibits a steep rise at short interatomic distances, which can have undesirable consequences in the context of ligand-receptor docking. In particular, the combination of approximating the receptor structure as rigid and limited sampling of ligand conformational space tends to overly penalize poses with "mild" short contacts between the ligand and receptor, due to the "hard" nature of the van der Waals potential as defined in most standard force-fields.

To overcome this tendency, a softened form of the van der Waals potential is employed with the Dock Score function. This softened potential rises to a large but finite value at zero interatomic separation. To maintain a proper balance between electrostatics and van der Waals, the electrostatic energy is also softened to prevent it from dominating the van der Waals energy at short separations.

The internal energy of the ligand is computed when using the force-field version of Dock Score. The purpose of including the internal energy is to avoid ligand conformations with bad internal non-bond clashes. By default, only the standard (not softened) van der Waals energy is used for the ligand internal energy. Including electrostatic energy as part of the ligand internal energy is optionally available.

The PLP version of Dock Score uses the PLP1 function, because the functional form of PLP1 allows it to be readily represented with a grid-based approach. The PLP2 function has an angular dependence on hydrogen bonding interactions making its representation with a grid considerably more difficult.

In the PLP1 score function, there are four atom types as following: (**1**) hydrogen bond donor only, (**2**) hydrogen bond acceptor only, (**3**) both hydrogen bond donor and acceptor, and (**4**) non-polar. When PLP1 is the docking scores function, the internal energy is calculated for each ligand conformation that the ligand is in the binding site [5].

In the PLP2 score function [6], the atom typing remains the same as in the PLP1 score function. In addition, an atomic radius is assigned to each atom expect for hydrogen [7].

The PMF score function was developed based on statistical analysis of the 3D structures of the protein-ligand complex [8]. They were found to correlate well with protein-ligand binding free energy while being fast and simple to calculate. The scores are calculated by summing pairwise interaction terms over all interatomic pairs of the receptor-ligand complex. The score function of Dock Score is the default function in the Discovery Studio 2.5.

All the simulations were also applied by CHARMM (Chemistry at Harvard Macromolecular Mechanics) Force-field. CHARMM was parameterized by experimental data. It has been used widely for simulations ranging from small molecules to solvated complexes of large

biological macromolecules. CHARMM performs well over a broad range of calculations and simulations, including minima, time-dependent dynamic behavior, and barriers to rotation, vibrational frequencies, and free energy. CHARMM uses a flexible and comprehensive energy function:

$$\mathrm{E}_{(pot)} = E_{bond} + E_{torsion} + \mathrm{E}_{oop} + E_{elect.} + E_{vdW} + E_{constraint} + E_{user}$$

Where, the out-of-plane (OOP) angle is an important torsion. The van der Waals term is derived from rare-gas potentials, and the electrostatic term can be scaled to mimic solvent effects. Hydrogen-bond energy is not included as a separate term as in AMBER. Instead, hydrogen-bond energy is implicit in the combination of van der Waals and electrostatic terms [9].

3. Research highlight

In this section, I summarize some of my research works published in Journal of Life Sciences and IEEE *International Conference on Bioinformatics and Bioengineering*, which includes anti-lung cancer and anti-oral cancer research, as illustrated as follows:

3.1 Anti-lung cancer research

The purpose of this research is to use computer docking and screening for the new type MEK1 inhibitor in lung cancer cells through the initiation of receptor tyrosine kinase and Mitogen-activated protein kinase pathway. The influence of lung cancer cell propagation suppressing by the combination of MEK protein and ATP was also discussed. A more active and potential drug molecule which can effectively lower the cost of developing lung cancer drugs can be proposed through the use of bioinformatics software and a series of data comparison, screening, and statistical analysis[10, 11].

In this paper, "Computational Screening of Novel Mitogen-activated Protein Kinase Kinase-1 (MEK1) Inhibitors by Docking and Scoring" [11], we discuss the MEK1 inhibitors by using **LigandFit** method to evaluate the affinity of the drug candidates (**LIGANDS**) towards the target **Protein** (Receptors, MEK1). The best docking poses analysis can be illustrated by Fig. 2 [11]. In this figure, we can reveal which interaction forces are the critical roles in **LIGANDS** and **Protein.**

There are several factors affecting the practical activity, such as the amino acids: LYS97, ASP190 in MEK protein, which are located at the entrance of the **PROTEINs**. When the **LIGAND** enter into the **PROTEIN**, some atoms of the **LIGAND** will bond to the entrance amino acids, moreover PHE209 which is at the terminal of the cavity will produce π-stacking force or H-BOND bonding, and the **LIGAND**s will not leave the **PROTEIN**s. The **LIGAND**s of the high activity group almost have these characters, which will effectively inhibit the MEK PROTEIN. When talking about the **LIGAND**s of the low activity group, they enter a small way into the **PROTEIN**, and can easily leave the **PROTEIN**. PHE209 at the terminal of the cavity of the **PROTEIN** will affect the activity. The authors found the **LIGAND** of the high activity group had the aromatic group at the end of the molecule. So they would arise from the π-stacking force. The medium activity **LIGAND**s formed a small-stacking force. For the low activity **LIGAND**s, they would not form stacking force because they have no aromatic groups [11].

Fig. 2.A PD184352 interacts with the PROTEIN and produces non-competitively with MgATP. It migrates into the PROTEIN and produces HBOND with MgATP and arouses a π stacking force with PHE209. PD184352 holds tightly with the PROTEIN and the MEK1 PROTEIN will lose its function. [11]

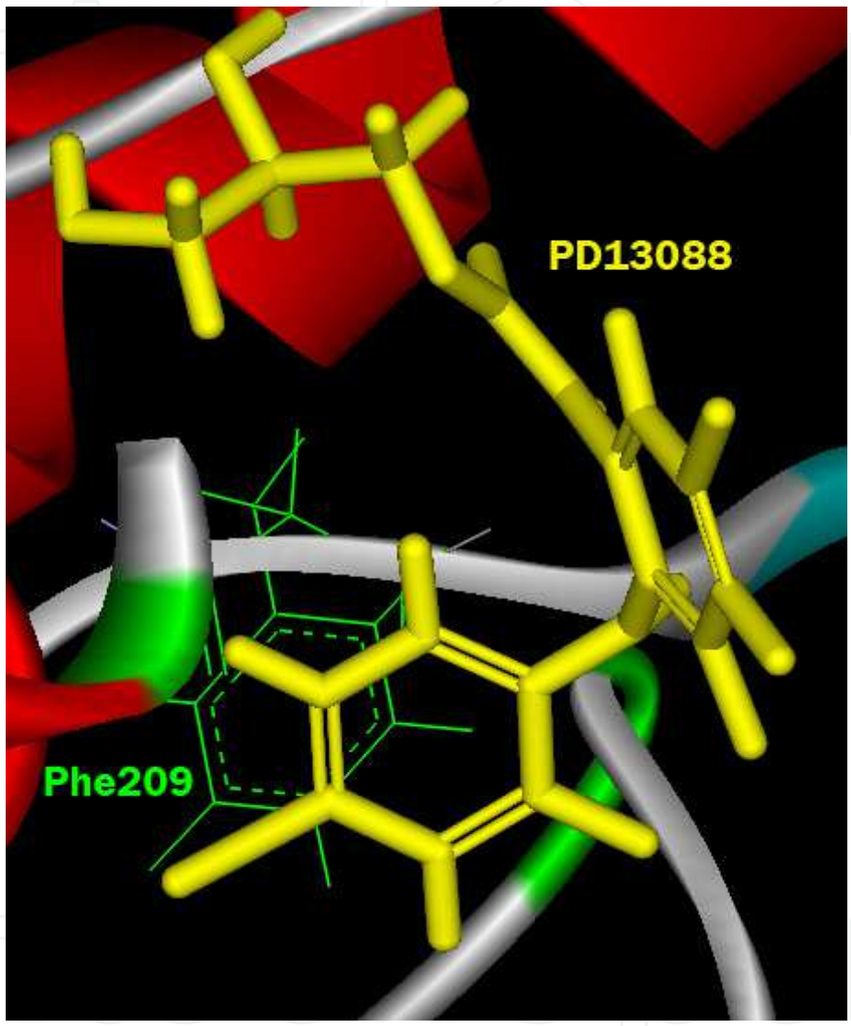


Fig. 2.B This figure shows the location of the aromatic group of PHE209 and the aromatic group of PD13088. It reveals they probably have a π stacking force. [11]

3.2 Anti-oral cancer research

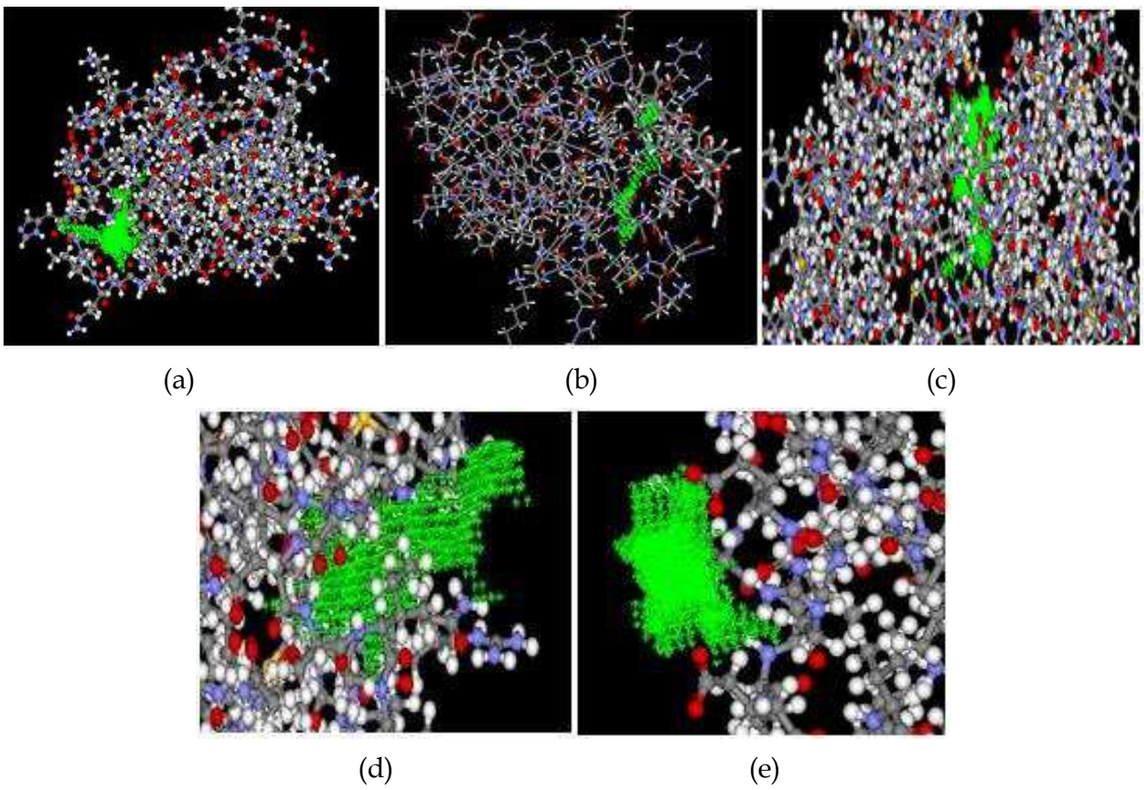
This research paper "Predication the suppressing human oral cancer cell line by curcumin through the research of Fas receptor" [12] which is expected to calculate the activity of *Curcumin* to Fas receptor. Fas receptor is a key receptor which commonly mechanisms caused to oral cancer. The pharmaceutical activity are evaluate by the score from docking procedure perform by simulation program.

The occurrence rate and death rate of oral cancer increased year by year according to the statistic value from Department of Health. The statistical report published by DOH in 2009 indicates that the death rate of oral cancer rank number 6 in Taiwan among all 10 kinds of cancer and the occurrence rate rank number 4 in male among 10 kinds of cancer. These values persist very high in recent years. Tongue cancer and buccal cancer are the cancer with highest frequency in oral cancer. These two cancers take almost 90% of oral cancer and tongue cancer take 60% among all which is the highest. The effect of treating tongue cancer is far less than buccal cancer which makes the five year survival rate of oral cancer decrease gradually. Curcumin is able to activate the apoptosis pathway to make cancer cell apoptosis and achieve the anti-cancer effect. The purpose of this research is to simulate the pharmaceutical activity and evaluate the degree of activity to determine: (1) whether curcumin is able to activate cell apoptosis to oral cancer cells? (2) Is the pharmaceutical activity of curcumin to oral cancer cells higher than those medicines on market?

Fas receptor (FasR) is a kind of death receptor on cell surface which can initiate the programmed cell death (cell apoptosis) through extrinsic pathway. Another one is the intrinsic pathway (mtirochondira). These are two types of cell apoptosis. FasR can also be called as CD95 which is the member of superfamilty of Apo-1 and TNF receptor [13, 14]. FasR is located on the 10^{th} chromosome in human and 19^{th} chromosome in rate. There are similar sequences in most mammal chromosomes [15]. The death-inducing signaling complex (DISC) formed by Fas can combine with receptor (FasL). FasL trimer makes the adjacent FasR to shape into trimer on the membrane and active the DISC below to attract and combine with Fas associsted death dimain(FADD). FADD will further attarct pro-caspase 8 for combination and cut the pro-caspase 8 into caspase 8. The caspase 8 will further cut and activate pro-caspase, therefore the caspase cascase magnifying effect is aroused to reinforce the activation of caspase 3. The caspase 3 will destruct the structural proteins such as cytokeletal protein and finish the apoptosis (reference: Eksp Klin Farmakol. 2010 Dec;73(12):44-9).

Curcumin, which is a kind of yellowish pigment extract from roots of turmeric. 70% of Curcumin is composed of curcuminoid which takes 3%~6% of turmeric (http://curcumin-turmeric.net/). The application of curcumin is far from now which curcumin were used as nature pigment in food industry. Beside, curcumin is stable to reductant and with good coloring ability but sensitive to light, heat and iron ions. The major application the coloring for canned food, sausages, and stewed soy sauce produce. Curcumin is also applied as acid-base indicator [pH 7.8 (yellow)- 9.2 (reddish brown)] (http://www.lookchem.cn/4150/productproperty.html). Curcumin have critical value and pharmaceutical action such as decreasing the blood fat, anti-oxidation, anti-inflammatory, and anti-atherosclerosis. Research in 2004 even found that curcumin can be use to suppress the activity of HIV-1 integrase and applied in AIDS clinical trial [16]. Beside the above functions, curcumin is also proved to have the pharmaceutical activity to anticancer and the effect of suppressing carcinoma have been verified repeatedly during many animal experiments [17, 18].

This research is to calculate and simulate the pharmaceutical activity of anticancer effects of *Curcumin* to oral cancer and get quality evaluation results. The preliminary results are listed as figures and tables below. The receptor applied is curcumin-derivatives: bisdemethoxy curcumin; target protein: FAS/FADD death domain assembly (*Protein PDB ID*: 3OQ9) to perform Docking and Scoring:



(a) (b) (c)

(d) (e)

Fig. 3. (a) Bind Site-A & Ligand poses (I) (b) Bind Site-A & Ligand poses (III) (c) Bind Site-B & Ligand poses (I) (d) Bind Site-B & Ligand poses (II) (e) Bind Site-B & Ligand poses (III)

Bind Site-A & Ligand poses (I)			Bind Site-A & Ligand poses (III)			Bind Site-B & Ligand poses (I)		
Name	Index	DOCK_SCORE	Name	Index	DOCK_SCORE	Name	Index	DOCK_SCORE
Molecule-1	1	36.04	Molecule-1	1	24.895	Molecule-1	1	18.071
Molecule-1	2	32.879	Molecule-1	2	17.943	Molecule-1	2	17.132
Bind Site-B & Ligand poses (II)			Bind Site-B & Ligand poses (III)					
Name	Index	DOCK_SCORE	Name	Index	DOCK_SCORE			
Molecule-1	1	24.895	Molecule-1	1	39.984			
Molecule-1	2	17.943	Molecule-1	2	39.777			

Table 1. Docking Score data

4. Conclusions

In this chapter, I introduce the simple docking method: **LigandFit.** Without consider the water-containing environment (water free), and flexible situations (**PROTEINs** vibration), the estimations will be worthy of discussion. However, because of its fast screening and

effective for most Receptor cases, it can be applied to many anti-cancer drugs candidate virtual screening at many situations.

5. Acknowledgement

This work was supported by a grant from China Medical University (CMU95-173, CMU96-107). The corresponding authors would like to thank their parents, all colleagues and friends who contributed to this study. We thank the editor and series editor for constructive criticisms and comments.

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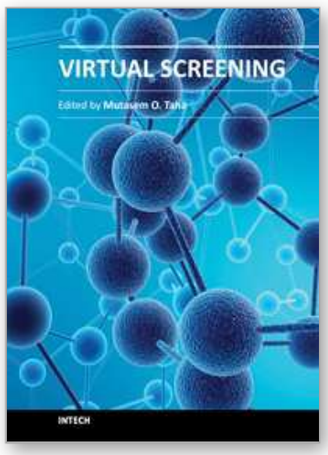

Virtual Screening

Edited by Prof. Mutasem Taha

ISBN 978-953-51-0308-0

Hard cover, 100 pages

Publisher InTech

Published online 14, March, 2012

Published in print edition March, 2012

Pharmacophore modeling, QSAR analysis, CoMFA, CoMSIA, docking and molecular dynamics simulations, are currently implemented to varying degrees in virtual screening towards discovery of new bioactive hits. Implementation of such techniques requires multidisciplinary knowledge and experience. This volume discusses established methodologies as well as new trends in virtual screening with aim of facilitating their use in drug discovery.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Po-Yuan Chen (2012). Computational Virtual Screening Towards Designing Novel Anticancer Drugs, Virtual Screening, Prof. Mutasem Taha (Ed.), ISBN: 978-953-51-0308-0, InTech, Available from: http://www.intechopen.com/books/virtual-screening/computational-virtual-screening-of-antivirus-and-anticancer-drugs-design

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