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Molecular Docking Studies on TRPV1 (Inhibitors using gold tested on *Rattus* 2nyj)

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Abstract:

The transient receptor potential cation channel, subfamily V member 1 also known as TRPV! Is a protein which in humans is encoded by the TRPV1 gene TRPV 1 receptors are found in the central nervous systems and the peripheral nervous systems and are involved in the transmission and modulation of pain, as well as the integration of diverse painful stimuli. The role of TRPV1 in the regulation of body temperature has emerged in last few years. Current study was to evaluate the ability of Argus Lab 4.0, a relatively new molecular modeling package in which molecular docking is implemented, to reproduce crystallographic binding orientations and to compare its accuracy with that of a well established commercial package, GOLD. The study also aimed to evaluate the effect of the nature of the binding site and ligand properties on docking accuracy. The three dimensional structure of a carefully chosen set of 75 pharmaceutically relevant protein-ligand complexes were used for the comparative study. The study revealed that the commercial package outperforms the freely available docking engine in almost all the parameters tested. However, the study also revealed that although lagging behind in accuracy, results from Argus Lab are biologically meaningful.

Key words: Docking, Ligand, Molecular package, Temperature.

1. Introduction:

The transient receptor potential cation channel, subfamily V member 1 also known as TRPV! Is a protein which in humans is encoded by the TRPV1 gene. TRPV 1 receptors are found in the central nervous systems and the peripheral nervous systems and are involved in the transmission and modulation of pain, as well as the integration of diverse painful stimuli. The role of TRPV1 in the regulation of body temperature has emerged in last few years. Based on number of TRPV 1 selective antagonists casing an increase in body temperature (hyperthermia), it was proposed that TRPV 1 is tonically active in vivo and regulates body temperature. In recent report, it was found that tonically active TRPV1 channels are present in the viscera and keep an ongoing suppressive effect on body temperature. Recently ,it was proposed that predominant function of TRPV1 receptors could potentially be used to treat neuropathic pain associated with multiple sclerosis, chemotherapy, or amputation, as well as pain associated with the inflammatory response of damaged tissue, such as in osteoarthritis.

Molecular docking and virtual screening based on molecular docking have become an integral part of many modern structure – based drug discovery efforts. Hence, it becomes a useful endeavor to evaluate existing docking programs, which can assist in the choice of the most suitable docking algorithm for any particular study. The objective of the current study was to evaluate the ability of Argus Lab 4.0, a relatively new molecular modeling package in which molecular docking is implemented, to reproduce crystallographic binding orientations and to compare its accuracy with that of a well established commercial package, GOLD. The study also aimed to evaluate the effect of the nature of the binding site and

ligand properties on docking accuracy. The three dimensional structure of a carefully chosen set of 75 pharmaceutically relevant protein-ligand complexes were used for the comparative study. The study revealed that the commercial package outperforms the freely available docking engine in almost all the parameters tested. However, the study also revealed that although lagging behind in accuracy, results from Argus Lab are biologically meaningful.

2. Early studies on TRPV1:

Transient Receptor Potential (TRP) cation channels participate in various fundamental processes in cell- and organism-physiology in unicellular eukaryotes, invertebrates and vertebrates. Interestingly, many TRP channels function as detectors of sensory stimuli. The TRPV1 (vanilloid 1) channel serves as an integrator of noxious chemical and physical stimuli known to cause irritation and pain, such as elevated temperatures, acids, and irritant chemical compounds, and its activation has been linked to acute nociceptive pain and neurogenic inflammation. The mechanisms by which the channel detects incoming stimuli, how the sensing domains are coupled to channel gating and how these processes are connected to specific structural regions in the channel are not fully understood, but valuable information is available. Many sites involved in agonist detection have been characterized and gating models that describe many features of the channel's behavior have been put forward. Structural and functional information indicates TRP channels are similar to voltage-activated potassium channels, with a tetrameric organization and six-transmembrane-region subunits, a pore domain with multi-ion binding properties and an intracellular S6 gate that seems to be the point of convergence of the many activation modalities leading to the opening of the ion conduction pathway.

TRPV1 is a channel expressed highly in small sensory neurons. TRPV1 is a ligand-gated, cation channel that is activated by heat, acid and capsaicin, a principal ingredient in hot peppers. Because of its possible role as a polymodal molecular detector, TRPV1 most extensively. In mice lacking TRPV1, thermal hyperalgesia induced by inflammation is reduced, suggesting a role for mediating inflammatory pain. Activity of TRPV1 is modulated by actions of various kinases such as protein kinase A and C. Furthermore, phosphorylation by Ca2+-calmodulin-dependent kinase II is required for its ligand binding. TRPV1 is activated by various endogenous lipids, such as anandamide, N-arachidonoyldopamine, and various metabolic products of lipoxygenases. 12-hydroperoxyeicosatetraenoic acid, an immediate metabolic product of 12-lipoxygenase, activates TRPV1 and shares 3-dimensional structural similarity with capsaicin. Because lipoxygenase products can activate TRPV1 in sensory neurons, upstream signals to lipoxygenase/TRPV1 pathway have been questioned. Indeed, bradykinin, a potent pain-causing substance, is now known to activate TRPV1 *via* lipoxygenase pathway. However, we cannot overlook the sensitizing effect of bradykinin *via* the phospholipase C or protein kinase C pathway. Interestingly, histamine, a pruritogenic substance, also appears to use the lipoxygenase/TRPV1 pathway in order to excite sensory neurons. Because of its role in the mediation of nociception, antagonists of TRPV1 are targeted for development of potential analgesics.

The transient receptor potential vanilloid subtype 1 (TRPV1) is a non-selective cation channel composed of four monomers with six transmembrane helices (TM1–TM6). TRPV1 is found in the central and peripheral nervous system, and it is an important therapeutic target for pain relief. Describe here the construction of a tetrameric homology model of rat TRPV1 (rTRPV1). Experimentally evaluated by mutational analysis the contribution of residues of rTRPV1 contributing to ligand

binding by the prototypical TRPV1 agonists, capsaicin and resiniferatoxin (RTX). performed docking analysis using our homology model. The docking results with capsaicin and RTX showed that our homology model was reliable, affording good agreement with our mutation data. Additionally, the binding mode of a simplified RTX (sRTX) ligand as predicted by the modeling agreed well with those of capsaicin and RTX, accounting for the high binding affinity of the sRTX ligand for TRPV1. Through the homology modeling, docking and mutational studies, we obtained important insights into the ligand-receptor interactions at the molecular level which should prove of value in the design of novel TRPV1 ligands.

Transient receptor potential vanilloid-1 (TRPV1) channels play a role in several inflammatory and nociceptive processes.

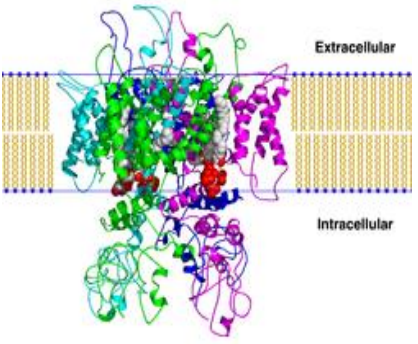
Pain from vertebral or femoral neck fractures is a particularly important problem in clinical orthopaedics. Transient receptor potential vanilloid 1 (TRPV1) is a ligand-gated nonselective cation channel, and there are recent reports on an association between bone pain and TRPV1. However, an increase in TRPV1 activity has not been reported following femoral fracture.

Determining the thermoregulatory phenotype of mice lacking transient receptor potential vanilloid-1 (TRPV1) channels. We used *Trpv1* knockout (KO) mice and their genetically unaltered littermates to study diurnal variations in deep body temperature (*T*b) and thermoeffector activities under basal conditions, as well as thermoregulatory responses to severe heat and cold. Only subtle alterations were found in the basal *T*b of *Trpv1* KO mice or in their *T*b responses to thermal challenges.

Judging the quality of a protein structure based on the distribution of backbone dihedral angles. Inputs to the method are 60 torsion angle distributions extracted from protein structures solved at high resolution; one for each combination of

residue type and tri-state secondary structure. Output for a protein is a Ramachandran Z-score, expressing the quality of the Ramachandran plot relative to current state-of-the-art structures.

3. Transient receptor potential cation channel, subfamily V, member 1 (fig 1)



Homology model of the TRPV1 ion channel tetramer (where the monomers are individually colored cyan, green, blue, and magenta respective) imbedded in a cartoon representation of a lipidbilayer. PIP_2 signaling ligands are represented by space-filling models (carbon = white, oxygen = red, phosphorous = orange).
Source image: www.ewikipedea

3.1. Activator of TRPV 1:

2-aminoethoxydiphenyl Bitrate is Common Activator of TRPV1, TRPV2, TRPV3.

The transient receptor potential (TRP) super family contains a large number of proteins encoding cation permeable channels that are further divided into TRPV (canonical), TRPM (melastatin) and TRPV(vanilloid) subfamilies. Among the six TRPV members, TRPV1, TRPV2, TRPV3, and TRPV4 from heat-activated cation channels, which serve diverse functions ranging from nociception to osmolality regulation, in the absence of other stimuli, 2- aminithoxydiphenyl borate (2APB) activates TRPV1, TRPV2 and TRPV3 but not TRPV4, TRPV5 and TRPV6, expressed in HEK293 cells. In contrast, 1APB inhibits the activity of TRPC6 and TRPM8 evoked by 1- oleolyl-2-actyl-sn-Gyycerol and menthol, respectively. In addition low levels of 2APB strongly potentiate the effect of capsaicin,

protons and hear on TRPV1 as well as that of heat on TRPV3 expressed in Xenopus oocytes.

3.2. Inhibitor of TRPV1:

Analgesic compound from sea Anemone Heteractis crispa is the first Polypeptide inhibitor of Vanilloid Receptor 1(TRPV1)

Venomous animals from distinct phyla such as spiders, scorpions, snacks, cone snails, or sea anemones produce small toxic proteins interacting with a variety of cell targets. Their bites often cause pain. One of the ways of pain generation is the cativation of TRPV1 channels. Screening of 30 different venoms from spiders and sea anemones for modulation of TRPV1 activity revealed inhibitors in tropical sea anemone Heteractis crispa venom. Several separation steps resulted in isolation of an inhibiting compound.

This is a56- residue –long polypeptide named APHC1 that has a Bos Taurus Trypsin inhibitor (BPTI)/ Kunitz- type fold, mostly represented by serine protease inhibitors and ion channel blockers. APHC1 acted as a partial antagonist of capsaician- induced currents (32$\pm$ 9% inhibition) with half-maximal effective concentration (EC_{50}) 54$\pm$ 4 nM. In vivo, a 0.1 mg/kg dose of APHC1 significantly prolonged tail-flick latency and reduced capasician- induced acute pain. Therefore, our results can make an help to solve the problem of over activity of this receptor during a number of pathological processes in the organism.

4. Materials and Methods:

A total of 2 entries for TRPV1 were found in RSCB protein data bank. Out of them 1, entries were selected based on:

1. It should contain a ligand
2. Structure should be determined by x- ray diffraction
3. Resolution must be between 2.70-3.2 Angstoms

5. Methodology:

A total of 2 entries for TRPV1 (transient receptor potential vanilloid 1) were selected from RCSB protein data bank, based on the presence of ligand, x ray diffraction and 2.7-3.3 A^0 resolution. Out of the 2 entries, Rattus norvigecus 2NYJ was taken for docking analysis (based on the Ramachandran plot statistics) as it showed 182 residues in allowed regions, 41 in additionally allowed and nine of the residue in other regions. The active residues were found to be TYR199, LYS155, GLN302, ARG211, LEU163, ASN164 and LYS160. A comparative protein- ligand dock analysis was performed using TRPV1 protein 2NYJ, extracted from PDB to evaluate the algorithm and scoring function efficiency using GOLD for docking studies.

PDB ID	EXP METHOD	RESOLUTION(A)	LIGANDS	TITLE
2NYJ	X-RAY DIFFRACTION	3.20A^0	ATP-ADEOSINE 5 PHOSPHATE	CRYSTAL STRUCTURES OF THE ANKYRIN REPEAT DOMAIN OF TRPV1
2PNN		2.70A^0	ATP-ADENOSINE 5 PHOSPHATE	CRYSTAL STRUCTURES OF THE ANKYRIN REPEAT DOMAIN OF TRPV1

From the above table this protein was selected for further analysis 2 NYJ.

5.1. GOLD Docking:

Although ZINC database provide ligands in ready-to-dock formats, an energy minimization routine was performed to

generate three dimensional structures of all the molecules using cornia make 3D option, derived charges and the geometries were optimized using cosmic module of Tsar software. Water molecules were discarded from the pdf file, and missing side chains were reconstructed using the option "prepare file for docking programs "available at the WHAT-IF web interface. Hydrogen's were added and then the structure was converted to mol 2 forlat using Mercury (v. 1.4.2 Cambridge crystallographic data centre (CCDC)). Automated docking studies were then performed using the genetic algorithm GOLD (genetic optimization for ligand docking) (v. 3.1; CCDC). The algorithm had been previously validated and successfully tested on a dataset of over 300 complexes extracted from the PDB.

The GOLD program uses a genetic algorithm (GA) and the binding site was defined as a spherical region which encompasses all protein atoms within 5.0 A^0 of each crystallographic ligand atom. Default setting were used for all calculatios. For each of the 10 independent GA runs, a maximum number of 10000 GA operator were performed on a single population of 50 individuals. Operator weights for crossover, mutation, and migration were set to 100, 100 and 0, respectively. To further speed up the calculation, the GA docking was stopped when the top three solutions were within 1.5 A^0 RMSD (root mean square deviation) of each other.

The nine inhibitors (active, moderately active and inactive) were selected from the article Bioorganic and Medicinal chemistry Letters 15 (2007) 6043- 6053 for docking studies. All these molecules as well as the bound ligand of the protein 1NYJ were docked by using the software GOLD and the score values are predicted. GOLD is a program for calculating the GOLD suite, a package of programs for structure visualization and manipulation, for protein –ligand docking (GOLD) and for post- processing and visualization of docking results. The product of collaboration between the University of

Sheffield, GlaxoSmithKline Plc and CCDC, GOLD is very highly regarded within the molecular modeling community for its accuracy and reliability.

6. Results:

Table show the activities, scores, H-bonds and the interacting residues of molecules.

Compounds	Activity(IC_{50}) micro M	GOLD score (kcal/mol)	NO of bonds	Interacting Residues
2NYJ bond LIGAND	-------	53.5	4	LYS160,ASN164,TYR199,GLN202
LIGAND_ ML5	520	27.52	3	LYS160.ASN164,PHE118
LIGAND_ ML6	14.7	27.31	2	PHE118,LYS160
LIGAND_ ML2	9.2	28.72	2	LYS160,ASN164
LIGAND_ ML1	54	28.57	1	PHE118
LIGAND_ ML7	700	23.82	-----	--------
LIGAND_ ML3	860	25.19	3	LYS160,PHE118,ASN164
LIGAND_ ML9	60	30.12	3	ASN164,PHE118,LYS160
LIGAND_ ML4	980	21.46	4	ASN164,ASN119
LIGAND_ ML6	663	25.4	4	PHE118,LYS160,ASN164
LIGAND_ ML7	243	29.74	4	ASN164,LYS160,PHE118

All molecules were drawn using ISIS draw tool and energy minimized using TSAR SOFTWARE. ISIS/ Draw is a chemical drawing program. The draw and beautified structure of the molecule should have minimized energy for docking process because the protein being considered for docking is extracted from PDB, the structure with lowest possible energy. Therefore the molecules should be energy minimized in order to meet the

requirements as the docking should be performed with lowest energy states of protein and ligand. The calculation was performed for the entire search space and the orientation with the lowest possible energy is reported in terms of Kcal/mol. This energy minimized molecule was then saved used for docking step.

7. Conclusion:

- By using GOLD for docking a positive correlation was observed between experimental values and computational dock scores. RMSD value was less than 2 A^0. Dock runs resulted in binding energy scores that ranges from 21.46 to30.12 kcal/mol.
- Among the nine inhibitors (active, moderately active and inactive, from the docking studies, an excellent correlation was observed in all cases, for instance, experimentally reported most active molecule ligand57 (69μM) showed a high dock score (30.12 kcal/mol) and was reported to be active than the remaining inhibitors. Similarly the most inactive molecule in this series with the experimental activity 980 μM was reported to very inactive in the docking studies with the dock score (21.46 kcal/mol) therefore our dock analysis using GOLD software suggests the importance of evaluating the prediction accuracy of scoring functions adopted in docking and represents that reproducibility of experimental values computation.

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