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In-silico molecular docking of RET proto oncogene by optimized inhibitor

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Abstract
Molecular docking screens large databases of small molecules by orienting and scoring them in the binding site of a protein. Top-ranked molecules may be tested for binding affinity *in vitro*, and may become lead compounds, the starting point for drug development and optimization.
Here, I have focused on the RET proto-oncogene. The RET gene provides instructions for producing a protein that is involved in signaling within cells. This protein appears to be essential for the normal development of several kinds of nerve cells. The RET proto-oncogene encodes a receptor tyrosine kinase for members of the glial cell line-derived neurotrophic factor family of extracellular signalling molecules. Inappropriate activation of the RET receptor tyrosine kinase causes development of papillary and medullary thyroid cancer.
I made inhibitor library for finding optimized inhibitor. Initially I focused on the RET and worked on computational docking to fit the model ligand Pyrazolopyrimidine (PP2) to the 2X2M protein. Here computational methodology useful in drug design based on model ligand 4-amino-5-(4-chloro-phenyl)-7-(t-butyl) pyrazolo [3, 4-d] pyrimidine) (PP2). I have collected 40 ligand libraries for finding minimum energy score through virtual screening. Through molecular docking, I blocked the active site of the targeted protein. However the results achieved demonstrate that high power computer clustering software services technology (HPCCSN), networking technology and Linux platform can be useful in applied drug design.
Pyrazolopirimidines containing drugs are used to prevent activation of RET Proto-oncogene. The Pyrazolopirimidines are a class of sedative and anxiolytic drugs related to benzodiazepines. Most of the drugs from this class marketed to date are intended to induce sleep, and are prescribed for people suffering insomnia.

Keywords: RET proto-oncogene, docking, inhibitor

Methodology and Observation

Following methodologies were adapted for various types of exercises done under the current project work:

- NCBI and PDB home page were opened to find the list of organism protein and related sequences.
- The RET proto oncogene (2X2M) was selected for molecular docking.
- On clicking the hyperlink on Accession / gi number, information related to amino acid sequence and 3D structure of the gene can be seen which can be downloaded.
- The downloaded structure of 2X2M protein was visualized in PyMOL visualizing software.

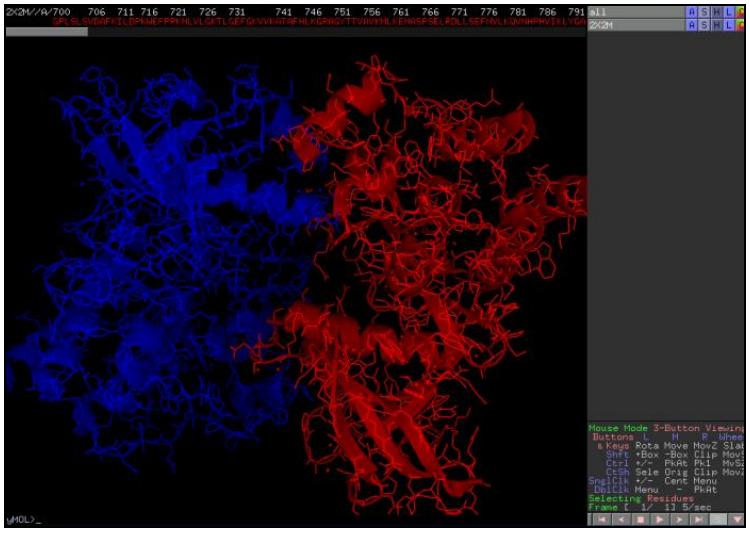

Fig 1: chain A and B of 2X2M protein in red and blue color respectively

- The downloaded structure of 2X2M protein was then opened in FRED receptor software for identifying the active sites present in the protein.

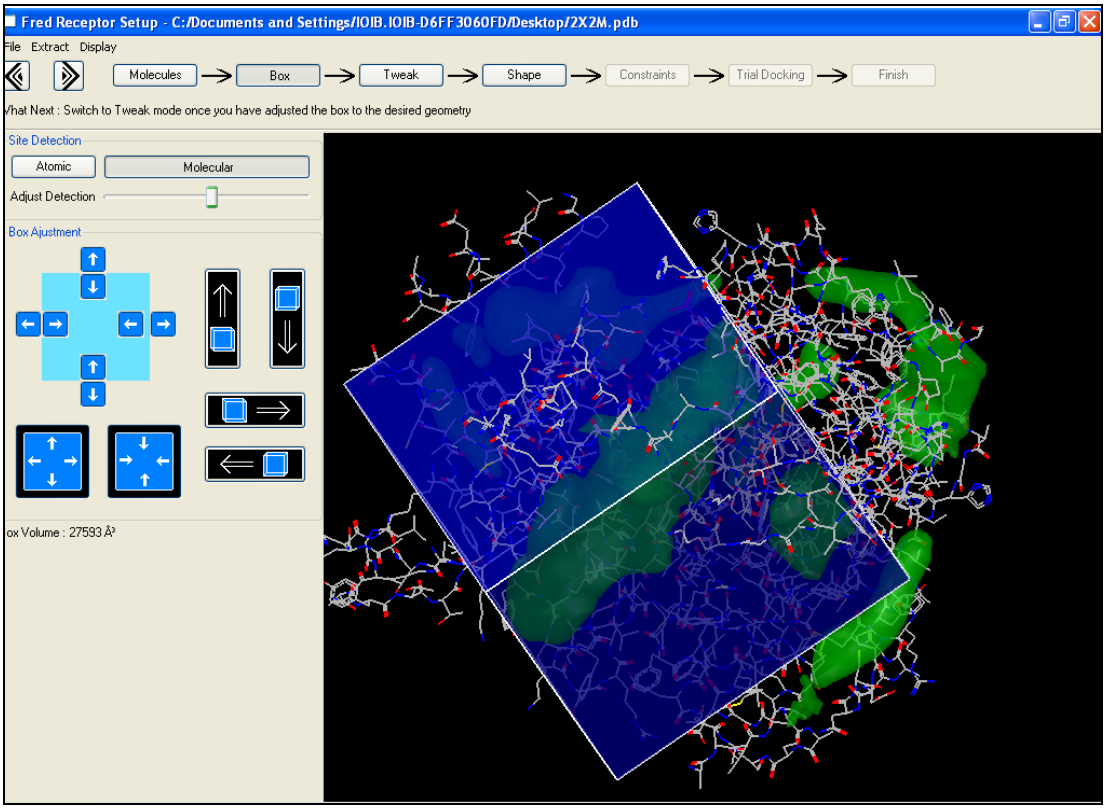

Fig 2: shows all the active sites present in the 2X2M protein

After the collection of sheet, helix, loop, chain A and B, I used the FRED software to identify the active site of 2X2M 3-D structure. I submitted the model to the active site prediction programme "FRED Receptor" to identify the active site present in the target protein structure that can be further utilized by the ligand (inhibitors).

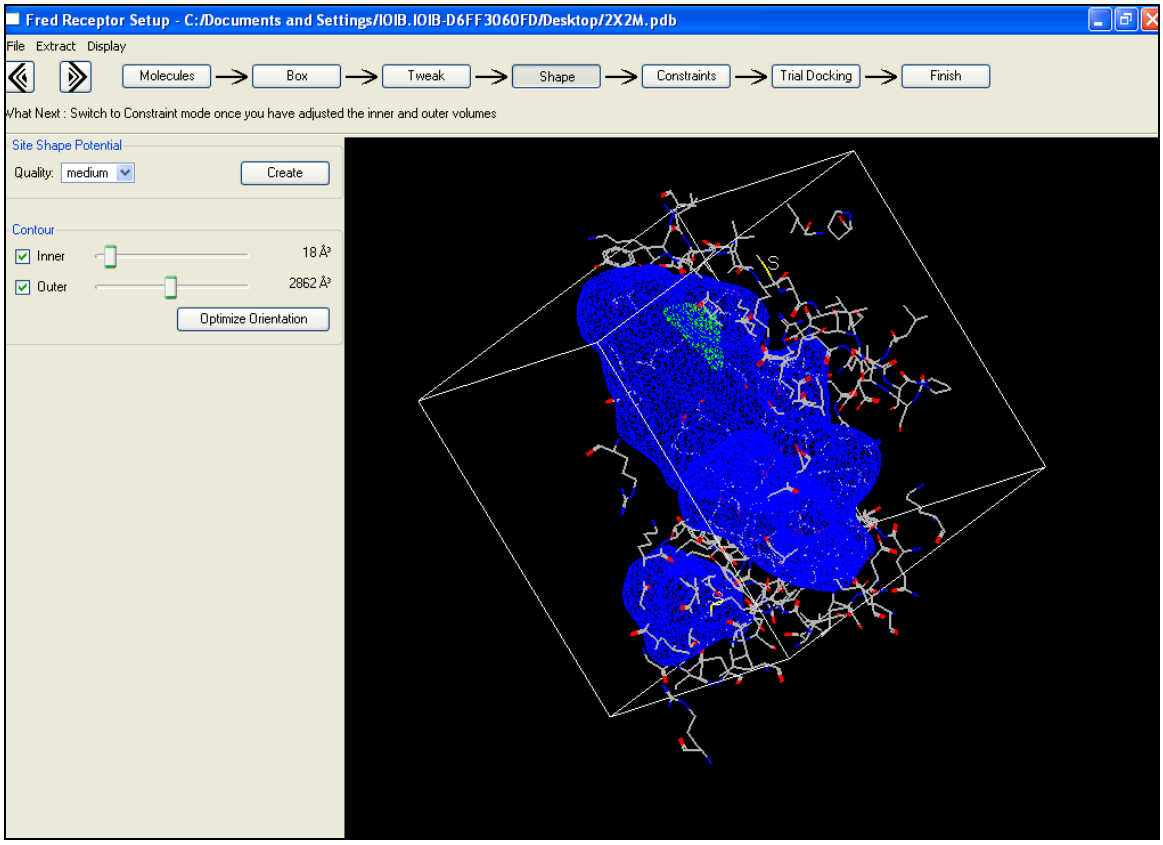

Fig 3: finished active site of 2X2M protein

A number of active sites present in the 2X2M protein were visualized. The optimal active site was taken and various features of the active site were analyzed. Out of all the active sites, the largest one is chosen for the docking which is shown by the highlighted area enclosed into the box.

- The file retrieved from FRED receptor program is saved in '.oeb' format.. The file so. generated is used for further experiment. NCBI-Pub Chem were used for making inhibitor library of PP2 (pyrazolopyrimidine) inhibitors. As based on virtual screening I made an inhibitor library. A library of 40 inhibitors was created on the basis of virtual screening. The library shows IUPAC names, molecular formula and molecular weight of the compounds.

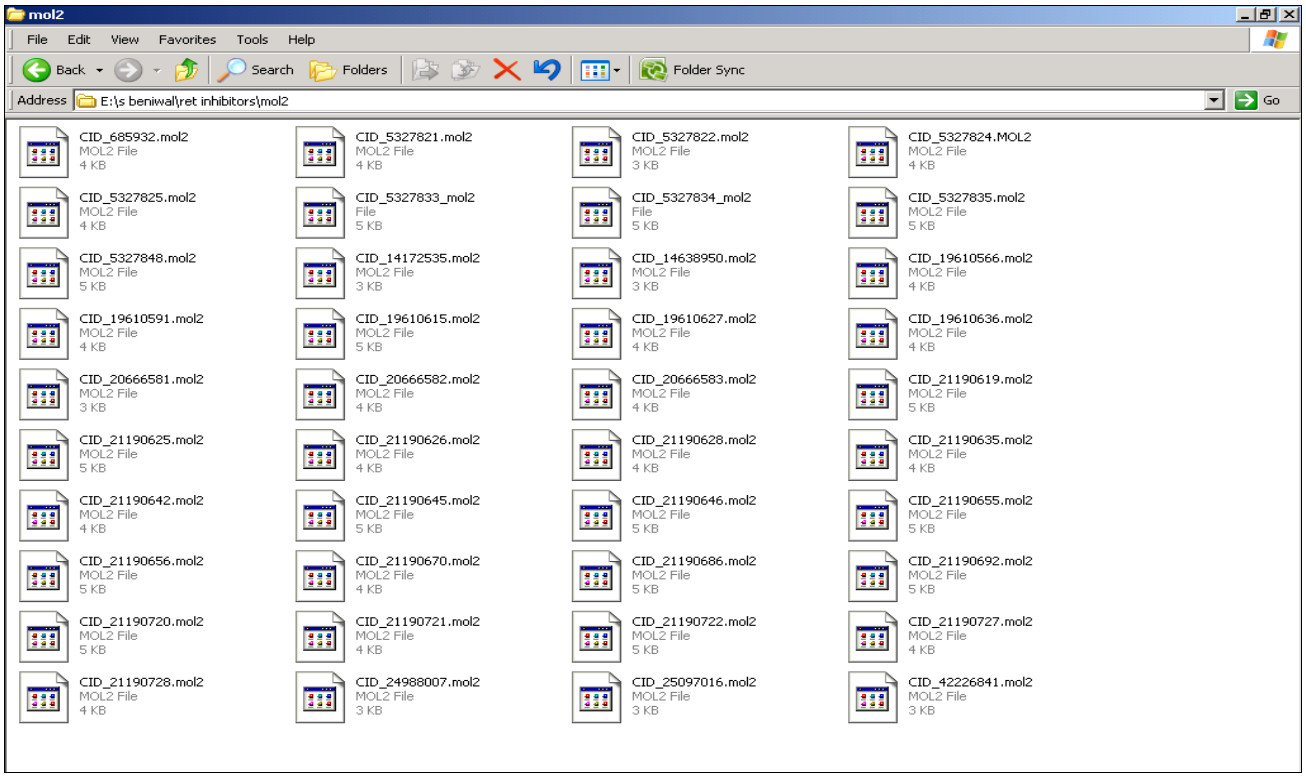

Fig 4: figure shows the SDF to MOL2 format converted inhibitors library

The downloaded format of inhibitors was SDF which was converted into MOL2 format using Open Babel software, a chemical expert system mainly used for converting chemical file format.

- Then, a merged file of all 40 inhibitors was created by using LINUX operating system.
- A list of scores "shape guess score" was generated which shows the compound with lowest energy at the top and others in descending order via FRED software.

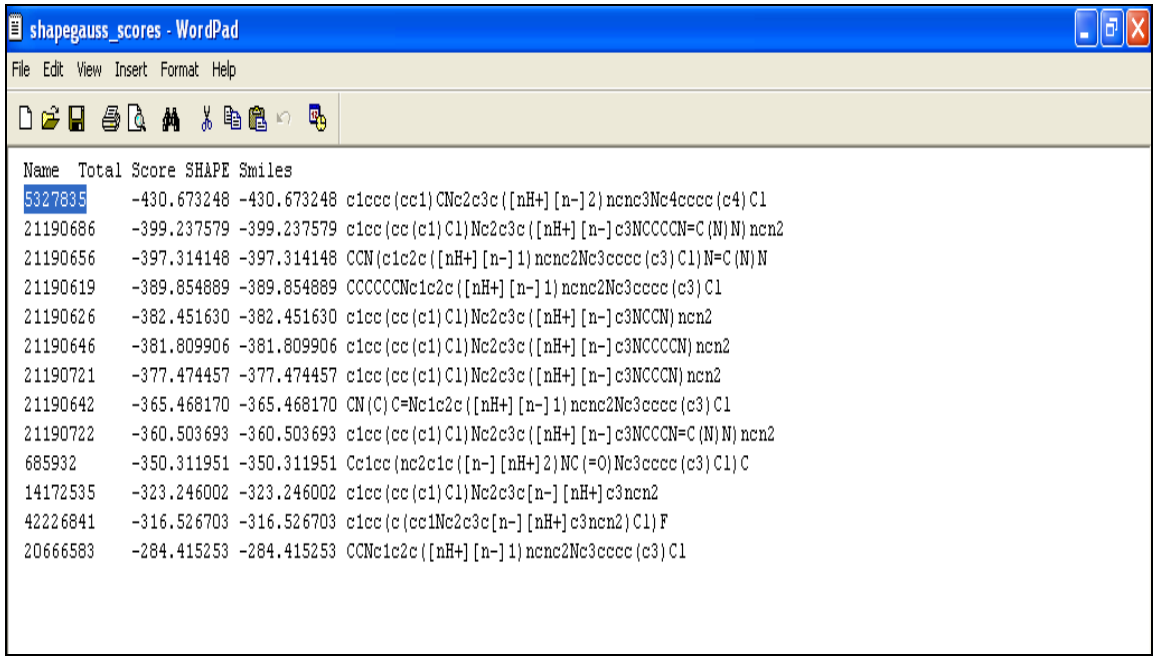

Fig 5: Shape gauss scores file showing the energy of inhibitors

- The lowest energy containing compound/ inhibitor was chosen to dock the protein.
- Then, the fragments of the inhibitor were generated by using Open Eye's software 'OMEGA'.
- The final step of docking proceed with the FRED software which generated a number of files.These files shows the summary of the docking process *viz*. Chem Score, plp score, shape guess score etc.
- The docking results were visualized in VIDA software.

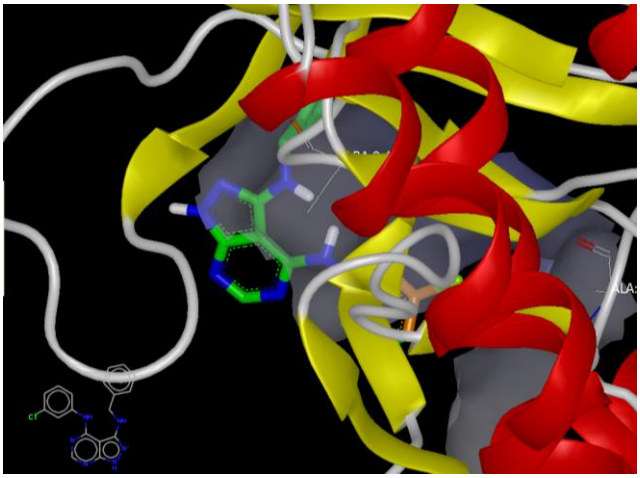

Fig 6: the docked receptor-inhibitor complex in VIDA software

Result and Discussion

I found minimum energy inhibitor CID_5327835 (3-N-benzyl-4-N-(3-chlorophenyl)-1H-pyrazolo [3, 4-d] pyrimidine-3, 4-diamine), MW: 350.804900 g/mol |, C18H15ClN6 MF which is the best inhibitor for the docking process. It has been shown that pyrazolopirimidines is a potent inhibitor of the RET kinase. Pyrazolopirimidines containing drugs are used to prevent activation of RET Proto-oncogene. Inappropriate activation of the RET receptor tyrosine kinase causes development of papillary and medullary thyroid cancer

Conclusion

The enormous computing potential of bioinformatics and the research in gene-based drug development hold the key to bringing forth not only remedies, but also cures for disease. This groundbreaking title provides a comprehensive and up-to-date account of the enormous range of bioinformatics for cancer therapy development from the laboratory to clinical trials. It functions as a guide to integrated data exploitation and synergistic knowledge discovery, and support the consolidation of the interdisciplinary research community involved.

This project will help to generate immediate solution for cancer caused by gene mutation in its early stage and so many other diseases.

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