Project Title:

Computational structure-based design of protein inhibitors

Name:Kam Zhang, Ashutosh Kumar, Muhammad Muddassar, Arnout Voet, Xiao-Yin Lee
Laboratory at RIKEN: Structural Bioinformatics Team, Center for Life Science Technologies

1. Background and purpose of the project, relationship of the project with other projects

Structure based virtual screening of small molecule compound libraries has been proven as an efficient methodology in drug discovery. Furthermore structure based virtual screening combined with experimental screening is a very powerful strategy to discover drug-like hits for a variety of targets. The objective of this project is to develop a hierarchical virtual screening pipeline for the discovery of small molecule inhibitors against several protein targets. A key theme is the use of computational approaches that incorporate flexible docking and molecular dynamics simulation to identify high-affinity inhibitors. In order to improve the hit discovery, we plan to develop a new method that utilizes the electrostatic potential of small molecules with a known lead in a ligand-based drug discovery.

The successful completion of the project will lead to the identification of small-molecule inhibitors of drug targets like SENP2 and Ubc9. These inhibitors may not only provide starting points for drug development but will also be useful chemical probes to further our understanding the biological role of these proteins.

Sumoylation is a post-translational modification that plays an important role in a wide range of cellular processes including chromosome packing and dynamics, DNA replication and repair, genome integrity, signal transduction, nuclear transport, and cell proliferation. The sumoylation pathway has been linked to a significant number of pathogenicities including neurodegenerative diseases and cancer. This makes sumoylation a novel drug target.

2. Specific usage status of the system and calculation method

The basic goal of our virtual screening protocol is to computationally screen millions of commercially available small molecules against a specific target protein to prioritize small number of compounds for biological testing and for their further development into high affinity inhibitors. However, *in silico* structure based virtual screening requires intensive computing especially in case of large database with millions of chemical compounds. Our virtual screening strategy involves the screening of small molecule library in a hierarchical approach using pharmacophore based modeling, flexible molecular docking and molecular dynamics simulation.

For pharmacophore modeling, we have used LigandScout, MOE software suite. We have also used molecular shape and electrostatic potential based criteria to search for initial hit using the ROCS and EON program. For flexible docking, we have used RosettaLigand, Glide and GOLD program. The hits that ranked higher were further prioritized by molecular dynamics simulation based binding free energy calculations. AMBER program and MM-GB(PB)SA approach was employed for this purpose. Our procedure incorporate full protein and ligand flexibility which greatly increases the computation time due to the vast number of conformations need to be explored; however, this allows more accurate treatment of protein ligand interactions.

3. Result

We have predicted the potential small molecule binding site in Ubc9 to be targetted for rational drug design using molecular modeling

approaches. Then, potential hits from a small molecule library was identified for biological assay using a virtual screening protocol that involves shape matching with known inhibitor and docking by computational approaches that incorporate both ligand and protein flexibility. We have acquired nineteen compounds from different chemical vendors and tested them for Ubc9 inhibitory activities. Five compounds were found to have inhibitory activity against Ubc9. One compound was selected for further optimization. Similarity search was then carried out to retrieve commercially available derivatives, which were further acquired and assayed that resulted in two compounds with acceptable potency. These two compounds can be used as starting points for the development of more potent inhibitors of Ubc9 targeting the predicted site.

We have discovered a new class of SENP2 inhibitors using structure based virtual screening and FRET based assay. Our virtual screening protocol starts with the identification of small molecules that have similar shape and electrostatic properties with the conjugate of SUMO1 C-terminal residues and substrate lysine. Molecular docking was then used to rank these small molecules. The selected molecules were purchased and tested by a FRET based assay that measures their SENP2 endopeptidase activity. The initial round of virtual screening has enabled the identification of eight compounds with > 40 % SENP2 inhibition at 30 μM concentration. Two scaffolds containing 1, 2, 5-oxadiazole core were discovered and they represent a novel class of SENP2 inhibitors. To improve the inhibitory potency and explore the SAR of these scaffolds, structurally related compounds were identified in the second round of virtual screening. These have resulted in the discovery of compounds with improved potency with an IC_{50} of 5.9 and 3.7 μM. Most of the compounds also inhibited closely related isoform SENP1 while no detectable inhibition on other proteases, such as papain and trypsin was observed. Our study suggests that 1, 2, 5-oxadiazoles could be used as a starting point for the development of novel therapeutic agents against various diseases targeting SENPs.

We have proposed a new molecular descriptor based on partial charges. It uses the autocorrelation function to encode all atoms of a molecule into rotation-translation invariant vectors. Combined with a scoring function, the descriptor allows to rank-order a database of compounds versus a query molecule. Extensive retrospective ligand-based virtual screening experiments were performed and other methods were compared with. Our method has an average speed of 1649 molecules per second. It reached an average median Area Under the ROC Curve (AUC) of 0.81 on 40 different targets, outperforming several commonly used methods and making it a powerful tool to the ligand based virtual screening.

4. Conclusion

Computational methods that include pharmacophore, shape and electrostatics, docking and molecular dynamics were used to screen a large commercial compound database for inhibitors of the sumoylation enzymes SENP2 and Ubc9. For SENP2, we have identified 1, 2, 5-oxadiazoles as a chemical scaffold and the most active compound showed an IC_{50} of 3.7 μM. Chemical optimization of these inhibitors is in progress. For Ubc9, we have used the predicted binding pocket for virtual screening and identified two compounds that showed good inhibitory activities and further chemical optimization is underway. We have developed a new method for the virtual screening of small molecule inhibitor by using a rotation-translation invariant molecular descriptor. Our test showed that it outperforms several commonly used ligand-based virtual screening methods.

5. Schedule and prospect for the future

We plan to optimize the initial hits for sumoylation enzymes SENP2 and Ubc9 that have been identified by virtual screen and confirmed by biochemical assays. Various computation tools will be used to optimize these initial hits into more potent inhibitors. We also plan to develop new methods to facilitate ligand-based and receptor-based virtual screening.

Fiscal Year 2014 List of Publications Resulting from the Use of RICC

[Publication]

1. Kumar, A., Zhang, K. Y. J. (2015) Hierarchical Virtual Screening Approaches in Small Molecule Drug Discovery. *Methods*, **71**, 26-37. doi:10.1016/j.ymeth.2014.07.007.
2. Kumar, A., Ito, A., Hirohama, M., Yoshida, M., Zhang, K. Y. J. (2014) Identification of Sumoylation Inhibitors Targeting a Predicted Pocket in Ubc9. *J. Chem. Inf. Model.,* **54**, 2784−2793. doi:10.1021/ci5004015.
3. Qing, X., Lee, X. Y., De Raeymaeker, J., Tame, J. R. H., Zhang, K. Y. J., De Maeyer, M., Voet, A. R. D. (2014) Pharmacophore modeling: advances, limitations and current utility in drug discovery. *J Receptor Ligand Channel Res.*, **7**, 81-92. doi:10.2147/JRLCR.S46843.
4. Berenger, F., Voet, A., Lee, X.Y., Zhang, K. Y. J. (2014) A rotation-translation invariant molecular descriptor of partial charges and its use in ligand-based virtual screening. *J. Cheminformatics,* **6**:23, doi:10.1186/1758-2946-6-23.
5. Voet, A.R.D., Ito, A., Hirohama, M., Matsuoka, S., Tochio, N., Kigawa, T., Yoshida, M., Zhang, K. Y. J. (2014) Discovery of small molecule inhibitors targeting the SUMO-SIM interaction using a protein interface consensus approach. *Med. Chem. Commun.*, **5**, 783-786, doi:10.1039/C3MD00391D.
6. Kumar, A., Ito, A., Takemoto, M., Yoshida, M., Zhang, K. Y. J. (2014) Identification of 1, 2, 5-Oxadiazoles as a New Class of SENP2 Inhibitors Using Structure Based Virtual Screening. *J. Chem. Inf. Model.,* **54**, 870–880, dx.doi.org/10.1021/ci4007134.
7. Voet, A., Kumar, A., Berenger, F., Zhang, K. Y. J. (2014) Combining *in silico* and *in cerebro* approaches for virtual screening and pose prediction in SAMPL4. *J. Comput-Aided Mol. Des.*, **28**, 363-373. DOI: 10.1007/s10822-013-9702-2.
8. Hirohama, M., Voet, A.R.D., Ozawa, T., Saitoh, H., Nakao, Y., Zhang, K. Y. J., Ito, A., Yoshida, M. (2014) Assay methods for small ubiquitin-like modifier (SUMO)–SUMO interacting motif (SIM) interactions *in vivo* and *in vitro* using a split-luciferase complementation system. *Anal. Biochem.*, **448**, 92-94. doi:10.1016/j.ab.2013.12.009

[Oral presentation at an international symposium]

1. The 3rd Annual Conference of the International Chemical Biology Society, Nov. 17-19, 2014, San Francisco, USA, Poster presentation. Ashutosh Kumar, Akihiro Ito, Misao Takemoto, Minoru Yoshida and Kam Y. J. Zhang, “Discovery of Novel SENP inhibitors Utilizing Structure Based Virtual Screening”.
2. Chemoinformatics Strasbourg Summer School, University of Strasbourg, France, June 23-27, 2014. Francois Berenger, Kam Y. J. Zhang, “A rotation-translation invariant molecular descriptor and its use in ligand-based virtual screening”.
3. JCUP-V, June 5-6, 2014, Tokyo, Japan. Poster presentation. Francois Berenger, Arnout Voet, Xiao Yin Lee and Kam Y.J. Zhang, “A rotation-translation invariant molecular descriptor of partial charges and its use in ligand-based virtual screening”.

4. JCUP-V, June 5-6, 2014, Tokyo, Japan. Poster presentation. Xiao Yin Lee, Arnout Voet, Francois Berenger and Kam Y.J. Zhang, “Electrostatic complementarity at the protein-ligand interface can be exploited for virtual screening”.
5. 50th International Conference on Medicinal Chemistry: Interfacing Chemical Biology and Drug Discovery, July 2-4, 2014, Rouen, France. Poster presentation. Francois Berenger, Arnout Voet, Xiao Yin Lee and Kam Y.J. Zhang, “A rotation-translation invariant molecular descriptor of partial charges and its use in ligand-based virtual screening”.