Project Title:

Computational structure-based design of protein inhibitors

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

Structure based virtual screening of drug like libraries has already been proved to be an efficient technology in hit discovery. Furthermore structure based virtual screening was integrated with experimental screening providing drug like hits for large variety of targets. Based on positive experiences one can conclude structure based virtual screening could also support fragment based drug design by molecular docking and prioritizing fragments using various ranking and scoring methodologies such as molecular dynamics simulations. Despite broad application of structure based virtual approaches, *in silico* fragment screening still plays a minor role. Although there have been some case studies reported that demonstrate virtual screening to be a powerful tool to discover potent fragments, this approach is still often considered to be too unreliable using popular computer tools. Indeed, facing the shortcomings of computational protocols with respect to ranking and affinity prediction of small fragments, more elaborate protocols have to be evolved departing from routine virtual screening strategies.

The objective of this project is to develop a virtual screening pipeline integrating structure based virtual screening approaches and utilize them in the rational identification of low molecular weight compounds with weak binding affinity towards a variety of therapeutically relevant protein targets. A key theme is the development and utilization of computational approaches that incorporate protein flexibility considerations into structure based discovery by using flexible docking and molecular dynamics simulation to identify desired fragments can be later combined or grown into high-affinity inhibitors.

In this project, we proposed to develop a virtual screening protocol and to use it for the discovery of inhibitors of a SUMO specific protease, SENP2, and sumoylation E1 and E2 enzymes as well as SUMO:SBM interaction. However, the approach is inherently generic and can be applied to many fragment based drug discovery programs for disease like cancer, inflammation, neurodegenerative diseases.

Sumoylation is a post-translational modification that plays an important role in a wide range of cellular processes including DNA replication and repair, chromosome packing and dynamics, genome integrity, nuclear transport, signal transduction and cell proliferation. Among the proteins involved in sumoylation pathway, SUMO E2 (Ubc9) is the sole E2-conjugating enzyme required for sumoylation and plays a central role by interacting with almost all the partners required for sumoylation. Ubc9 has been implicated in a variety of human malignancies such as ovarian carcinoma, melanoma and lung adenocarcinoma, suggesting that Ubc9 inhibition could be a potential therapeutic approach to control tumorigenesis.

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 to high affinity inhibitors. However, *in silico* structure based virtual screening requires intensive computing especially in case of large database with millions of chemical compounds, which is in the range of few Tflops per day on one target protein. Our virtual screening protocol involves the screening of small molecule library 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 which 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 used a virtual screening protocol that involves 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 prioritize these small molecules for FRET based assay that quantifies their SENP2 endopeptidase activity. The initial round of virtual screening followed by FRET based assay has enabled the identification of eight compounds with > 40 % SENP2 inhibition at 30 μM compound concentration. Five of these compounds belong to two scaffolds containing 1, 2, 5-oxadiazole core that represent a novel class of SENP2 inhibitors. To improve the inhibitory potency and explore structure activity relationship of these two 1, 2, 5-oxadiazole scaffolds, structurally related compounds were identified in another round of virtual screening. The biological assay results confirmed SENP2 inhibitory activity of these two scaffolds. The most potent compound of each scaffold showed an IC_{50} of 5.9 and 3.7 μM.

We have used virtual screening techniques such as molecular docking, molecular dynamics simulation and binding free energy calculations to identify potential small molecule inhibitors of SUMO E1. About 24 hits were acquired and tested using *in situ* sumoylation assay. Out of 24 hits, four hits showed more than 85 % inhibition of sumoylation with the most active compound showed an IC_{50} of 5.4 μM. To improve the potency of these compounds, we have carried out similarity search to identify commercially available derivatives. About 50 derivatives were identified to be tested further. Compounds with improved potency were found in each of four chemical classes. These compounds will be excellent starting points for further chemical optimization.

We used a hybrid structure-based virtual screening protocol that incorporates both ligand-based and structure-based techniques to identify inhibitors of SUMO E2. Nineteen compounds were acquired from different chemical vendors and were tested first using in situ

sumoylation assay and then by using E1 and E2 SUMO intermediate formation assay. About five compounds showed inhibitory activity against SUMO E2 out of which one compound was selected for further optimization. The similarity search to retrieve commercially available derivatives resulted in 40 compounds that have been acquired and tested for Ubc9 inhibition. Four of them showed more than 85% inhibition of sumoylation with the most active compound showed an IC_{50} of 14.4 μM. A similarity search with the most active compound in the ZINC database has identified three more compounds with improved potency. These compounds share a common phenyl urea scaffold and have been confirmed to inhibit SUMO E1 by *in vitro* SUMO-1 thioester bond formation assay. Our study suggests that these phenyl urea compounds could be used as a starting point for the development of novel therapeutic agents.

In order to identify small molecules that might have the possibility to break SUMO:SBM interaction, a funnel based virtual screening approach was followed. A pharmacophore query was created based on the consensus interactions of the SBM with the SUMO1 protein. The pharmacophore was used as a post processing filter for all docked poses. Those that do not fulfill the key interactions within the receptor were discarded. Lastly, only the molecules that were able to bind to the different receptor conformations in exactly the same binding mode were retained. Out of these remaining compounds, the most promising compounds were selected based on a consensus of the PLP and the DrugScore scoring functions of the docked results as well as divergence and lead-likeness. These compounds have been selected for purchase in order to be tested for inhibition of different SUMO-SBM interactions. The AlphaLISA assay and SPR assay were used to measure the ability of those compounds to inhibitor SUMO1:SBM interaction. The most potent compound has a Kd of 1.8 μM. The binding mode of these active compounds to SUMO1 has been confirmed by NMR STD and HSQC experiments.

4. Conclusion

Computational methods, which include pharmacophore, shape and electrostatics, docking and molecular dynamics, were used to screen a large commercial compound database for inhibitors of the sumoylation enzymes SENP, E1 & E2 and SUMO:SBM interaction. We have targeted both enzyme catalytic sites and protein-protein interfaces for inhibitor discovery. For SUMO E1, four chemical series have been identified and the most active compound showed an IC_{50} of 5.4 μM. Chemical optimization of these inhibitors is underway. For SUMO E2, we have identified five chemical series that showed inhibitory activities and the most potent compounds have IC_{50} of 46 μM. We have identified a site in SUMO:SBM that can be targeted by small molecule inhibitors. Using a pharmacophore modeling approach, 40 compounds were selected for purchasing and assaying. After several rounds of optimization by analogs, several small molecule inhibitors of SUMO:SBM interaction with IC_{50} in the single digit μM have been identified.

5. Schedule and prospect for the future

We plan to optimize the initial hits for sumoylation enzymes SENP2, E1 and E2 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. The computation time in RICC is critical for us to

achieve these goals.

We also plan to optimize those small molecule protein-protein interaction inhibitors (SMPPII) of the sumoylation pathway that we have discovered thus far. SMPPIIs have been considered as high-hanging fruits in drug discovery. They offer a clear advantage over traditional enzymatic site inhibitors. We have established a pharmacophore based protocol for the discovery of SMPIIs. We will use the RICC time to optimize those SMPPIIs for sumoylation. These inhibitors can potentially be developed into drugs for treating various diseases such like cancer.

6. If no job was executed, specify the reason.

N/A.

RICC Usage Report for Fiscal Year 2013

Fiscal Year 2013 List of Publications Resulting from the Use of RICC

[Publication]

1. 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.,* dx.doi.org/10.1021/ci4007134.
2. 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.*, DOI: 10.1007/s10822-013-9702-2.
3. Voet, A., Berenger, F., Zhang, K. Y. J. (2013) The use of electrostatic similarities for the discovery of small molecule protein-protein interaction inhibitors. *PLoS ONE*, **8(10)**: e75762, 1-9. doi:10.1371/journal.pone.0075762.
4. Kumar, A., Ito, A., Hirohama, M., Yoshida, M., Zhang, K. Y. J. (2013) Identification of quinazolinyloxy biaryl urea as a new class of SUMO activating enzyme 1 inhibitors. *Bioorg. Med. Chem. Lett.*, **23**, 5145-5149. doi: 10.1016/j.bmcl.2013.07.022.
5. Kumar, A., Zhang, K. Y. J. (2013) An Investigation on the Effect of Key Water Molecules on Docking Performance in CSARdock Exercise. *J. Chem. Inf. Model.,* **53**, 1880-1892. dx.doi.org/10.1021/ci400052w.
6. Kumar, A., Ito, A., Hirohama, M., Yoshida, M., Zhang, K. Y. J. (2013) Identification of sumoylation activating enzyme 1 inhibitors by structure based virtual screening. *J. Chem. Inf. Model.,* **53**, 809-820.
7. Kumar, A., Zhang, K. Y. J. (2013) Computational investigation of SENP:SUMO protein-protein interaction for structure based drug design. *Molecular Informatics*, **32**, 267-280. DOI:10.1002/minf.201200124.
8. Voet, A., Helsen, C., Zhang, K. Y. J., Claessens, F. (2013) The discovery of novel hAR receptor antagonist chemotypes using a combined pharmacophore screening procedure. *ChemMedChem.*, **8**, 644-651. DOI: 10.1002/cmdc.201200549.

[Proceedings, etc.]

None.

[Oral presentation at an international symposium]

1. The 31st Medicinal Chemistry Symposium, Nov. 20-22, Hiroshima, Japan. Poster presentation. Ashutosh Kumar, Akihiro Ito, Mikako Hirohama, Minoru Yoshida and Kam Y. J. Zhang, “Identification of SUMO activating enzyme 1 inhibitors utilizing virtual screening approach”.

2. The 51st Annual Meeting of the Biophysical Society of Japan, Oct. 28-30, 2013, Kyoto, Japan. Poster presentation. Arnout Voet, Francois Berenger, Kam Y. J. Zhang, "Electrostatic similarities between protein and small molecules facilitate the rational design of protein-protein interaction inhibitors".
3. Gordon Research Conferences on Computer Aided Drug Design, Jul. 21-26, 2013, West Dover, Vermont, USA. Poster presentation. Arnout Voet, Francois Berenger, Kam Y. J. Zhang, "Electrostatic similarities between protein and small molecule ligands facilitate the rational design of protein-protein interaction inhibitors".
4. 49th International Meeting of Medicinal Chemistry: Drug Discovery and Selection -When Chemical Biology meets Drug Design, July 3-5, 2013, Nice, France. Poster presentation. Ashutosh Kumar, Mikako Hirohama, Akihiro Ito, Minoru Yoshida and Kam Y. J. Zhang, "Identification of sumoylation activating enzyme E1 inhibitors by structure based virtual screening".
5. JCUP-IV, June 6-7, 2013, Tokyo, Japan. Poster presentation. Arnout Voet, Christine Helsen, Frank Claessens, Kam Y.J. Zhang, "The discovery of novel hAR receptor antagonist chemotypes using a combined pharmacophore screening procedure".

[Others]