Usage Report for Fiscal Year 2016

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 small molecule compound libraries has been proven as an efficient methodology in drug discovery. Molecular docking predicts the best pose of a ligand in the target protein binding site by sampling and scoring numerous conformations and orientations of the ligand. Failures in pose prediction are often due to either insufficient sampling or scoring function errors. To improve the accuracy of pose prediction by tackling the sampling problem, we propose to develop a method of pose prediction using shape similarity.

We propose to discover small molecule inhibitors for the insect chitinase, *Of*ChtI, derived from the pest *Ostrinia furnacalis,* also known as Asian corn borer. *Of*ChtI plays an important role in the molting and survival of the Asian corn borer. Inhibitors of *Of*ChtI can be developed into pesticides to treat the Asian corn borer and increase corn yield.

We also plan to discover small molecule inhibitors for the copper nitrite reductase, NirK, from a fungus *Fusarium oxysporum.* NirK is a key enzyme in the fungal denitrification process. A significant amount of nitrogen in fertilizers in agricultural soils is lost as nitrous oxide (N_2O) in the environment due to microbial denitrification process. Inhibitors of NirK can be used for the development of nitrogenous fertilizer supplements and coatings as a means to prevent nitrogen loss by targeting fungal denitrification.

2. Specific usage status of the system and calculation method

Our method of **P**ose **P**rediction using **S**hape **S**imilarity (PoPSS) utilizes ligand 3D shape similarity with known ligand bound crystal structures to predict binding poses of unknown ligands. PoPPS selects the most shape similar ligand conformation and places it into suitable receptor, which is then subsequently refined using side-chain repacking and Monte Carlo minimization.

For the discovery of *Of*ChtI inhibitors, we have used a hierarchical virtual screening protocol. Shape and electrostatic potential matching calculations were used to retrieve compounds endowed with similar chemical features but a different scaffold from a known inhibitor. Namiki shoji small molecular collection containing about 4 million purchasable compounds was used as the screening library. Shape comparisons were performed using ROCS. EON program was then used to perform electrostatic potential matching calculations on the top ranking compounds from shape similarity.

For the discovery of NirK inhibitors, a

hierarchical in silico screening protocol consisting of pharmacophore based screening and molecular docking was used. A pharmacophore query was prepared from small molecule binding hotspots that were predicted on the surface of a fungal copper nitrite reductase homology model.

3. Result

We have assessed our PoPSS method utilizing CSARdock 2012 and 2014 benchmark exercise datasets consisting of co-crystal structures from eight proteins. Our results revealed that ligand 3D shape similarity could substitute conformational and orientational sampling if at least one suitable co-crystal structure is available. Our method identified poses within 2 Å RMSD as the top-ranking pose for 85.7 % of the test cases. The median RMSD for our pose prediction method was found to be 0.81 Å and was better than methods performing extensive conformational and orientational sampling within target protein binding sites. Furthermore, our method was better than similar methods utilizing ligand 3D shape similarity for pose prediction.

For *Of*ChtI inhibitor discovery, we have computationally identified 17 compounds from a library of over 4 million compounds by two rounds virtual screening. Among these compounds, 3 compounds from one chemical class inhibited the activity of *Of*ChtI with single-digit micromolar IC_{50} values and 1 compound from another chemical class exhibited a broad inhibitory activity not only toward *Of*ChtI but also toward bacterial, fungi and human chitinases. A new scaffold was discovered and the structure-inhibitory activity relationship was proposed.

Evaluation of *F. oxysporum* NirK inhibitory activities of nineteen compounds resulted in the identification of two compounds with the moderate activities. Shape based similarity search was then used to identify another 76 compounds. In vitro assessment of these compounds resulted in the identification of several compounds with potency in a low micromolar range. Further, in vivo examination confirmed the denitrification inhibitory activities of some of these compounds.

4. Conclusion

Our method PoPSS demonstrated that the 3D shape similarity with crystal ligand is adequate to predict binding poses of query ligands with acceptable accuracy. PoPSS was able to predict binding poses within 2 Å regardless whether structurally similar ligands are available or not. In principle, PoPSS performance would be better if crystal ligands with higher structural or 3D shape similarities are available. However, our study demonstrated that a TanimotoCombo of 1.4 was sufficient to predict binding poses with good accuracy.

Our work on *Of*ChtI has enabled us to discover a novel chemical scaffold for designing specific or broad-spectrum chitinase inhibitors by structure-based virtual screening followed by enzymatic activity determination. The molecular docking further confirmed the structure-function relationship. Our shape and electrostatics matching strategy has been shown to be effective in the identification of small molecule inhibitors of *Of*ChtI based on its crystal structure with a bound oligosaccharide substrate. The FQ series compounds are the first selective small molecule inhibitors of *Of*ChtI reported to date. This work provides a new starting point for developing chitinase inhibitors.

Our study provided the first small molecule inhibitors of *F. oxysporum* NirK that could be utilized either as chemical probes to study NirK biology or as starting points for the development of fertilizer coatings or supplements to prevent nitrogen loss in the form of N_2O.

5. Schedule and prospect for the future

The current implementation of PoPSS is limited to cases where at least one co-crystal ligand binding to the same site is available. However, utilizing ligand 3D shape similarity could be a general approach for pose prediction. As ligand belonging to the same congeneric series mostly bind to homologous proteins in a similar manner, not only co-crystal ligands from homologous proteins but also ligands binding to similar binding pockets can also be used to calculate shape similarities. Moreover, 3D shape of bound ligand is related to protein binding pocket shape, which could also be used for similarity calculation in the absence of crystal ligands. These considerations will be executed in future modifications of PoPSS strategy.

We plan to optimize the initial hits for *Of*ChtI and NirK 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.

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

N/A.

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Fiscal Year 2016 List of Publications Resulting from the Use of the supercomputer

[Publication]

1. Matsuoka, M., Kumar, A., Muddassar, M., Matsuyama, A., Yoshida, M., Zhang, K. Y. J. (2017) Discovery of Fungal Denitrification Inhibitors by Targeting Copper Nitrite Reductase from *Fusarium Oxysporum*. *J. Chem. Inf. Model.,* DOI:10.1021/acs.jcim.6b00649.
2. Jiang, X., Kumar, A., Liu, T., Zhang, K. Y. J. and Yang, Q. (2016) A Novel Scaffold for Developing Specific or Broad-spectrum Chitinase Inhibitors. *J. Chem. Inf. Model.*, **56**, 2413-2420. doi:10.1021/acs.jcim.6b00615.
3. Kumar, A., Kawamura, T., Kawatani, M., Osada, H. and Zhang, K. Y. J. (2016) Identification and structure activity relationship of purine derivatives as novel MTH1 inhibitors. *Chem. Biol. Drug Des.*, DOI:10.1111/cbdd.12909.
4. Kumar, A., Zhang, K. Y. J. (2016) Prospective Evaluation of Shape Similarity Based Pose Prediction Method in D3R Grand Challenge 2015. *J. Comput-Aided Mol. Des.*, **30**, 685-693. doi:10.1007/s10822-016-9931-2.
5. Kumar, A., Zhang, K. Y. J. (2016) A pose prediction approach based on ligand 3D shape similarity. *J. Comput-Aided Mol. Des.*, **30**, 457-469. doi:10.1007/s10822-016-9923-2.
6. Kumar, A., Ito, A., Hirohama, M., Yoshida, M., Zhang, K. Y. J. (2016) Identification of new SUMO activating enzyme 1 inhibitors using virtual screening and scaffold hopping. *Bioorg. Med. Chem. Lett.*, **26**, 1218-1223. DOI:10.1016/j.bmcl.2016.01.030.
7. Kumar, A., Zhang, K. Y. J. (2016) Application of Shape Similarity in Pose Selection and Virtual Screening in CSARdock2014 Exercise. *J. Chem. Inf. Model.,* **56**, 965-973. DOI:10.1021/acs.jcim.5b00279.

[Oral presentation at an international symposium]

1. 4th International Conference on Computation for Science and Technology (ICCST-2016), Nov. 3-4, 2016, Langkawi, Malaysia. Invited Speaker, “Computational Structure Based Design of Inhibitors Targeting Sumoylation”.
2. School of Life Science and Biotechnology, Dalian University of Technology, Apr. 21, 2016, Dalian, China. Invited Speaker, “Computational structure based design of inhibitors targeting sumoylation”.