Molecular docking for drug discovery and development: a widely used approach but far from perfect

“...AlphaGo...will tremendously improve the success rate of new drug discovery and development projects, leading molecular docking and drug design, and eventually becoming an irreplaceable technique.”

First draft submitted: 16 July 2016; **Accepted for publication:** 22 July 2016;
Published online: 31 August 2016

Keywords: covalent binding • free energy • molecular docking • scoring function • solvent molecule • structural flexibility • thermodynamics and kinetics

It is usually the first step to find active compounds from existing chemicals for a drug discovery and development project. Although many pharmaceutical companies have their own libraries that may have millions of compounds, it is costly to maintain the library and to perform high-throughput screening. Virtual screening provides an alternative approach to fulfill the screening of millions of compounds within a few days [1]. Molecular docking is one of the most applied virtual screening methods, especially, when the 3D structure of target protein is available. This method could predict both the binding affinity between ligand and protein and the structure of protein–ligand complex, which is useful information for lead optimization. Indeed, molecular docking has been applied for more than three decades and a great number of new drugs have been discovered and developed accordingly [2]. Although there is no doubt that it will continue to play important role, molecular docking is still far from full success in terms of success rate. This is also the reason why high-throughput screening is still widely applied nowadays in many pharmaceutical companies. Docking-based virtual screening will never have chance to play an irreplaceable role in drug discovery and development until its success rate is essentially improved.

Scoring function & free energy change

In order to make optimal use of limited computational resources, various scoring func-

tions were developed for molecular docking to rapidly predict how strong the binding is between a chemical compound and a target protein/macromolecule [3,4]. Although the functions were usually calibrated with experimental results, the predicted values are not the true binding free energy change. In other words, a strong relationship was hardly observed between the experimentally determined biological activity and the predicted binding strength by various scoring functions. To overcome the problem, several accurate approaches were reported for calculating the binding free energy and binding affinity [5], for example, free energy perturbation, thermodynamic integration and less rigorous end point methods such as linear interaction energy, molecular mechanics–Poisson–Boltzmann/generalized born surface area and λ -dynamics. However, owing to the significant demand for computational resources, none of them could be applied in high-throughput docking due to the high number of docked conformations of each compound in a chemical database.

Scoring function is usually composed of different terms to describe different intermolecular interactions and their contribution to overall binding strength. However, some intermolecular interaction terms are hardly to be accurately predicted, such as solvation effect and entropy change [6]. In addition, some intermolecular interactions are in the preliminary study, thus, not included in

Guimin Wang

Drug Discovery & Design Center, CAS Key Laboratory of Receptor Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Road, Shanghai, 201203, China
and
University of Chinese Academy of Sciences, No. 19A Yuquan Road, Beijing, 100049, China

Weiliang Zhu

Author for correspondence:
Drug Discovery & Design Center, CAS Key Laboratory of Receptor Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Road, Shanghai, 201203, China
wzhu@simm.ac.cn

the scoring functions. For instance, halogen bonding is verified to make a contribution to protein–ligand binding affinity [7] but not well treated by most scoring functions, and some special interactions, such as, guanidine–arginine interaction [8], are not considered in any functions. Moreover, different intermolecular interactions are not always additive to the overall binding strength. For example, the halogen bonding could be strengthened or weakened by both π – π stacking [9] and anion– π /lp– π interactions [10] in different cases and affected by water bridge [7]. Apart from that, there might be some unknown intermolecular interactions that do take part in the protein–ligand binding.

“The 3D structure of protein–ligand complex provides detailed information at atomic level for the binding mechanism between ligand and target protein, which is particularly important for lead optimization.”

Water molecules in the binding pocket of protein

The first step for molecular docking is the preparation of protein structure; the most widely adopted practice is simply to remove all the solvent molecules, ions and other small molecular ligands, leaving an entirely empty binding pocket for docking. This approach is operationally simple, but leads to a discrepancy between the pocket circumstances for docking and that in physiological conditions. On the one hand, some water molecules may form very strong hydrogen bonding interactions that bridge the protein–ligand binding, which are difficult to replace, otherwise the replacement may lead to a false positive result during docking. On the other hand, water molecules may occupy the entire space between ligand and protein, which will affect not only the predicted binding strength but the binding conformation as well. In this context, several attempts have been made to simulate water molecules and evaluate its energetic contributions. For example, WaterMap was developed via statistical thermodynamic analyses of water clusters based on inhomogeneous solvation theory following the explicit solvent molecular dynamics (MD) simulations, GRID calculates the interaction energy of a water probe around a binding site and 3D-RISM approach uses integral equations to predict the location, density and properties of small molecules around a protein [11]. However, it is still an unsolved problem to accurately deal with the water molecules in binding pocket during docking process, which is, hard to be solved in the near future because of two challenges. First, the x-ray crystal structures usually lack the coordinate information of hydrogen atoms or even oxygen atoms of most water molecules due to the lim-

ited resolution that current techniques can achieve [12]. Second, no reliable theoretical approach is available to accurately predict how water molecules are affected by ligands and how strong the effect is. Needless to say, it is also almost impossible to calculate how many water molecules in the binding pocket would be replaced by potential ligands and how the hydrogen bonding network would be disturbed by ligand binding [13]. Similar challenge does exist for counterpart ions and/or endogenous ligands present in the binding pocket.

Protein–ligand complex structure by docking

The 3D structure of protein–ligand complex provides detailed information at atomic level for the binding mechanism between ligand and target protein, which is particularly important for lead optimization. Undoubtedly, many studies have shown that the docked binding conformation of ligand might be significantly different from the experimentally determined structure, especially, when the ligand is of high flexibility with small energy barrier between different conformations [14]. For instance, ligands with long chain or carbohydrate moieties are difficult to be treated precisely by current docking methods. The case would become even more difficult while the binding pocket is composed of the residues with flexible chains, or the pocket is formed by flexible loops. Not only is it possible that the binding pocket undergoes its intrinsic changing constantly, but the perturbation by the ligand binding might cause significant transformation of the pocket profile [15]. Nowadays, there is no docking program that could reliably deal with the whole intrinsic flexibility and the induced transformation of the protein structure. In most cases, the overall protein flexibility was not taken into consideration during docking.

In addition, the conformation ranking after molecular docking is based on the score calculated with scoring function. Given the flaws of scoring function aforementioned, even numerous scoring functions have been developed and applied in different software package, none of them is applicable or reliable to all systems, implying that the most possible and stable structure of protein–ligand complex might be discarded based on predicted scores.

Binding thermodynamics & kinetics

The drug may have better therapeutic effect if it has higher binding affinity and longer residence time in the binding pocket. Binding affinity is determined by the Gibbs free energy (ΔG), which is the sum of binding enthalpy (ΔH) and entropy change (ΔS). Both terms are correlated with atomic interactions, but to achieve the balance between them is difficult while performing optimization of drug candidates [16].

Many efforts have been dedicated to thermodynamic optimization and several approaches have been reported, for example, enthalpic optimization, thermodynamic optimization plots and the enthalpic efficiency index [17], we are still in the early stage of thermodynamically driven drug design. Besides, kinetic residence time of the drug is determined by the drug-target association and dissociation rates. However, no docking program is available for kinetic prediction in high-throughput virtual screening. Although some methods for predicting k_{on} and k_{off} constants were reported [18], they either required huge computational resources or there was a lack of accuracy and extensibility to the binding pocket with different geometric and physicochemical profiles. In other words, the predicted kinetic parameters have weak correlation with experimentally determined values. In this context, further investigation, especially on binding kinetics, is in urgent demand.

Covalent binding

Besides the predicted activity, molecular docking has another defect that is for covalent binding. The case becomes more complicated if a weak covalent bond is formed between ligand and protein residues, such as the residue cysteine, which may breakdown while the complex structure undergoes intrinsic vibration. Therefore, weak covalent bonding between protein and ligand is hard to be calculated thermodynamically and kinetically. In addition, covalent ligands have stronger preferences to be associated with toxicity than noncovalent ones due to the difficulty of disassociation if off-target binding happens. Although many covalent drugs have been approved by US FDA, mostly for the treatment of infections, cancer, gastrointestinal disorders, central nervous system and cardiovascular indications [19], there is a long way to go to achieve high success rate by covalent docking.

Nucleic acids

The binding of ligands to nucleic acids (RNA or DNA) is attracting more and more interest to drug designer and chemical biologist. However, it is another challenge to molecular modeler. Given the specific 3D structure of nucleic acids, docking has to confront with both high structural flexibility of ribose moiety and highly anisotropic charge distributions comparing with protein, which cause additional uncertainty of the predicted binding poses and affinity [20]. For the

lack of applicable program and 3D structure of nucleic acids, molecular docking could contribute little for the nucleic acid drug discovery.

Nevertheless, virtual screening via molecular docking has already achieved great success in numbers of new drug development projects, thus, widely used nowadays in both academic organization and pharmaceutical industry. Together with the development of the technology for determining the structures of biomacromolecules, more and more structures will be available, molecular docking will continue to play important role in lead discovery and optimization. However, this technology itself is currently far from full success as mentioned above. Great attention and effort should be paid in future on accurately predicting the binding thermodynamics and kinetics constants, reliably reproducing protein–ligand complex structure by taking into account overall protein flexibility and solvent molecules, precisely dealing with covalent binding and other biomacromolecules targets like nucleic acids and protein machinery.

In March 2016, a computer program, named AlphaGo, developed to play the abstract strategy board game Go, was awarded an honorary 9-dan by the Korea Baduk Association after it beat professional Go player Lee Sedol in a five-game match with final score of 4 to 1. This program could predict its moves via Monte Carlo tree search algorithm based on the knowledge previously learned by machine learning from human and computer play. While the match result might discourage some professional Go players, it encouraged many people and groups in research areas with very complicated problem like drug design. Although it is hard to predict the specific date, there will finally be an ‘AlphaGo’ in the research area of drug design, which will tremendously improve the success rate of new drug discovery and development projects, leading molecular docking and drug design, and eventually becoming an irreplaceable technique.

Financial & competing interests disclosure

The authors would like to thank the financial support from the National Natural Science Foundation of China (81273435 and 81573350). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

- 1 Lavecchia A, Di Giovanni C. Virtual screening strategies in drug discovery: a critical review. *Curr. Med. Chem.* 20(23), 2839–2860 (2013).
- 2 Matter H, Sotriffer C. Applications and success stories in virtual screening. In: *Virtual screening: principles, challenges, and practical guidelines*. Sotriffer C (Ed.). Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, 319–358 (2011).

- 3 Jain AN. Scoring functions for protein-ligand docking. *Curr. Protein Pept. Sci.* 7(5), 407–420 (2006).
- 4 Zheng M, Xiong B, Luo C *et al.* Knowledge-based scoring functions in drug design: 3. A two-dimensional knowledge-based hydrogen-bonding potential for the prediction of protein-ligand interactions. *J. Chem. Inf. Model.* 51(11), 2994–3004 (2011).
- 5 Reddy MR, Reddy CR, Rathore RS *et al.* Free energy calculations to estimate ligand-binding affinities in structure-based drug design. *Curr. Pharm. Des.* 20(20), 3323–3337 (2014).
- 6 Yuriev E, Agostino M, Ramsland PA. Challenges and advances in computational docking: 2009 in review. *J. Mol. Recognit.* 24(2), 149–164 (2011).
- 7 Ren J, He Y, Chen W *et al.* Thermodynamic and structural characterization of halogen bonding in protein–ligand interactions: a case study of PDE5 and its inhibitors. *J. Med. Chem.* 57(8), 3588–3593 (2014).
- 8 Yang Y, Xu Z, Zhang Z *et al.* Like-charge guanidinium pairing between ligand and receptor: an unusual interaction for drug discovery and design? *J. Phys. Chem. B* 119(36), 11988–11997 (2015).
- 9 Li H, Lu Y, Liu Y, Zhu X, Liu H, Zhu W. Interplay between halogen bonds and pi–pi stacking interactions: CSD search and theoretical study. *Phys. Chem. Chem. Phys.* 14(28), 9948–9955 (2012).
- 10 Lu Y, Liu Y, Li H, Zhu X, Liu H, Zhu W. Energetic effects between halogen bonds and anion–pi or lone pair–pi interactions: a theoretical study. *J. Phys. Chem. A* 116(10), 2591–2597 (2012).
- 11 Bodnarchuk MS. Water, water, everywhere...It's time to stop and think. *Drug Discov. Today* 21(7), 1139–1146 (2016).
- 12 Zheng H, Handing KB, Zimmerman MD, Shabalin IG, Almo SC, Minor W. X-ray crystallography over the past decade for novel drug discovery — where are we heading next? *Expert Opin. Drug Discov.* 10(9), 975–989 (2015).
- 13 Spyraakis F, Cavasotto CN. Open challenges in structure-based virtual screening: receptor modeling, target flexibility consideration and active site water molecules description. *Arch. Biochem. Biophys.* 583, 105–119 (2015).
- 14 Erickson JA, Jalaie M, Robertson DH, Lewis RA, Vieth M. Lessons in molecular recognition: the effects of ligand and protein flexibility on molecular docking accuracy. *J. Med. Chem.* 47(1), 45–55 (2004).
- 15 Lexa KW, Carlson HA. Protein flexibility in docking and surface mapping. *Q. Rev. Biophys.* 45(3), 301–343 (2012).
- 16 Freire E. Do enthalpy and entropy distinguish first in class from best in class? *Drug Discov. Today* 13(19–20), 869–874 (2008).
- 17 Garbett NC, Chaires JB. Thermodynamic studies for drug design and screening. *Expert Opin. Drug Discov.* 7(4), 299–314 (2012).
- 18 Yan Z, Zheng X, Wang E, Wang J. Thermodynamic and kinetic specificities of ligand binding. *Chem. Sci.* 4(6), 2387 (2013).
- 19 Kumalo HM, Bhakat S, Soliman ME. Theory and applications of covalent docking in drug discovery: merits and pitfalls. *Molecules* 20(2), 1984–2000 (2015).
- 20 Wang M, Yu Y, Liang C, Lu A, Zhang G. Recent advances in developing small molecules targeting nucleic acid. *Int. J. Mol. Sci.* 17(6), pii: E779 (2016).