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Induced fit docking, and the use of QM/MM methods in docking

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Abstract

Docking methods are popular computational techniques in drug discovery to identify new active molecules that bind to a given biological target. Although widely used, the predictive reliability of docking methods is often limited by the inability to accurately and efficiently model protein flexibility and quantify binding strength. We highlight several emerging concepts that address those methodological issues including a discussion on the incorporation of QM/MM methodologies in the scoring process.

Keywords

Docking; protein flexibility; scoring function; QM; QM/MM

Introduction

Docking has become a standard technology in drug discovery to virtually screen libraries of existing or hypothetical chemicals with the goal of identifying new active chemotypes that bind to a target protein and to predict the binding modes and affinities of those ligands. Although docking methods are quite efficient tools for those studies, the quality and reliability of the docking predictions are currently limited by several methodological shortcomings, such as difficulties in modeling protein flexibility upon ligand binding [1–7] and the energetic quantification of protein-ligand binding using simplified scoring functions [8, 9]. In this review, we will highlight some of the methodological developments addressing those shortcomings over the last two years with particular emphasis on the emergence of quantum-mechanics (QM) and mixed quantum-mechanics molecular-mechanics (QM/MM) methods for improving the scoring process.

Concepts that incorporate protein flexibility into receptor-ligand docking

Computational concepts to model protein flexibility during docking can be divided into two categories (Figure 1) [10]. In the first class of methods, the conformational search of the

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ligand and the protein are performed in tandem where both conformations are altered at the same time. To limit the combinatorial explosion of conformations that need to be generated and energetically evaluated, a small number of degrees of freedom are selected that describe important contributions of protein flexibility. Variables that characterize the magnitude of the conformational change along the selected degrees of protein flexibility are optimized in parallel to the ligand degrees of freedom, such as translational, rotational and torsional changes. The second category of methods generates an ensemble of protein structures (EPS) prior to docking. Each ligand of a library is subsequently docked to each member of the EPS. Recent studies have placed significant emphasis on testing different methods for generating the EPS and for selecting the “optimal” subset of protein structures from the EPS to be used in binding pose prediction and virtual screening.

Methods for EPS generation

Efficient EPS generation using elastic network models (ENM)

The most straightforward approach to generating an EPS is to use a set of X-ray structures of the same protein system, but different molecular simulation methods such as molecular dynamics (MD) simulations, MD-based principle component analysis (PCA) or normal-mode analysis (NMA) can also be used to generate an EPS. In comparison to time-consuming full atomistic techniques (e.g. MD-based methods), using normal modes from elastic network models (ENM) is an efficient alternative to generate an EPS.

In a recent study Dietzen et al. [11] investigated whether NMA or ENM are capable of predicting the conformational change associated with small-molecule binding to different protein systems and whether this method can be used to generate an EPS for ensemble docking. The authors used the first normal modes from the apo structure to produce an EPS that optimally reproduced the C α trace of the ligand-bound protein structures. Using this limited number of normal modes, the authors were unable to accurately reproduce the observed conformational change upon ligand binding. The authors also observed that within the same target protein, the amplitudes for each mode vary depending on the bound ligand and were unable to identify general rules about how many and which modes were needed to achieve successful docking. They were, however, able to show that docking results can be improved when normal-mode generated EPS are combined with explicit side-chain flexibility throughout the docking stage. It should be noted that this additional protein flexibility significantly increases the necessary computer time of docking.

Generation of EPS using the ligand-model concept

An alternative scheme to generate protein conformations relevant for binding structurally diverse ligands known as the ligand-model concept, Limoc, was developed by Xu et al. [12]. In the Limoc method MD simulations are performed with a dynamically changing set of restrained functional groups in the binding site of the protein, essentially representing a large hypothetical ensemble of different chemical species binding to the same target protein. Beginning from an apo structure, the ligand-model approach was used to derive an EPS used for docking and the results outperformed docking to an EPS generated from an apo-MD simulation. Furthermore, the method was only slightly less successful than docking to the

experimentally known holo form of each individual protein-ligand complex, demonstrating Limoc's potential to generate holo-like protein structures starting from the apo form of the protein.

Selection of “optimal” EPS for docking

In ensemble docking studies [13], it has been recognized that using an EPS with only a few protein conformations can increase the success rate for correctly predicting binding poses and enrichment in virtual screening. It is however thought that using a very large EPS hinders docking performance due to the large number of false positives generated by non-native protein structures, which in turn reduces the enrichment rate of the virtual screen.

Two recent large-scale studies [14, 15] have used a set of X-ray structures as an EPS to investigate to what extent docking to an EPS improves docking success as compared to docking to a single protein structure. Bottegoni et al. [14] performed retrospective virtual screening on 36 pharmaceutically relevant protein targets using the docking engine ICM. To mimic real virtual screening applications of docking, where only the top-ranked compounds would be synthesized and biologically tested, the authors focused on the successful identification of actives in the topmost ranking fraction. They tested two different protocols for ranking compounds in ensemble docking, ranking based on best score among all protein structures and based on overall best rank among different single-structure docking simulations. Both ranking schemes statistically outperform the average single protein structure run. Interestingly, ensemble docking improved not only the enrichment of actives in the top-ranked fraction, but also the chemical diversity of identified active scaffolds.

In a related study, Korb et al. [15] studied the influence of ensemble size on pose prediction and virtual screening quality for eight protein targets using GOLD with three different scoring functions. Similar to the study by Bottegoni et al. [14], on average ensemble docking performs better than single structure results. The performance increase is more significant for the correct prediction of native ligand poses compared to virtual screening, and is strongly dependent on protein target and scoring function. The authors also recognized that for a given ensemble size, the composition of the EPS strongly influences the docking success. In this context, it was recognized that the best single-structure docking regularly outperforms the best ensemble results. Most importantly, however, docking to an EPS performs almost always better than the worst single protein structure, therefore ensemble docking minimizes the risk of poor virtual screening performance as the best protein structures for docking are typically not known prior to the simulation.

This study also highlighted another important issue related to ensemble docking: Which structures from the EPS most likely yield maximal docking success? Different strategies such as clustering of similar structures [16] or the selection of protein conformations that conserve the relative orientations of a core of amino acids critical for binding [17] have been devised to trim an EPS down to the most relevant structures.

EPS selection using experimental ligand data

Xu et al. [18] tested two novel schemes to select protein structures for ensemble docking using experimental ligand data. In the first scheme, protein structures were selected that best

reproduced the native binding mode of 1–3 known protein-ligand complex structures of the target protein. Ensemble docking to this sub-EPS was slightly superior to the EPS generated by clustering. The second and better selection method was to choose protein structures that best separate actives from decoys in a pre-screening enrichment study on a small number of ligands. Pre-screening the EPS using 10–20 active ligands and a number of inactive decoy ligands (neither exact affinity data nor knowledge of binding modes is necessary) resulted in a sub-EPS containing only a small number of protein structures (3–5) with optimal docking results on the full virtual screening data set.

Deformable grids

The simplest way to utilize an EPS is to perform sequential docking into all members of the ensemble as utilized in all previously described studies. This procedure linearly increases the required computational time with the size of the EPS. To speed up the docking process, two recent studies [19, 20] outlined grid-based approaches in which protein-ligand interaction energy values are pre-computed for each protein structure onto a 3D grid. Those grids are then either deformed or utilized to rapidly compute alternative interaction energy values due to conformational changes of the protein induced by the bound ligand. The changes of the grid are performed in parallel to the pose generation and don't require sequential docking to all members of the EPS.

Leis et al. [19] generated seven alternative protein conformations for protein kinase A along the soft normal mode from an ENM of the apo protein. Those conformations were ranked according to their change along the normal mode and were assigned a conformational variable. The potential grids used in AutoDock were generated for each protein structure, and throughout the posing phase protein conformations were selected using the conformational variable. Non-integer values of this variable (e.g. 2.4) describe intermediate protein conformations and were represented by interpolations between potential values on grid points of the two discrete protein structures associated with the bound integer values (e.g. 2 and 3). This approach allows combining a continuous conformational space of the protein structure with the efficient calculation of protein-ligand interaction scores using pre-computed grids.

An alternative concept to incorporate protein flexibility in grid-based docking was developed by Gohlke and co-workers [20, 21]. In their approach, an initial potential grid is computed based on the apo target structure (RNA in their recent study) and the positions of the grid points are altered in unison with nearby RNA atoms. Whereas the potential grid is altering its form with the target's conformational change similar to an elastic body reacting to external forces, the potential values remain unaltered.

QM and QM/MM methods for improving the scoring process in docking

In addition to protein flexibility upon ligand binding, the accurate quantification of protein-ligand association is a significant challenge for current docking schemes. Quantum mechanics (QM) methods have emerged in structure-based drug design, aiming to improve the prediction accuracy for protein-ligand interactions.

QM methods have a natural advantage in modeling polarization and charge transfer, a major issue when using classical molecular-mechanics (MM)-based scoring schemes which assume fixed charges during ligand binding. QM are, however, much more time consuming than standard MM-based scoring schemes. To limit the computational complexity, different schemes have been developed to reduce the portion of the protein-ligand system that is treated in QM detail (Figure 2). Alternatively, simplification schemes such as the local approximation have been developed that allow linear scaling of computer time with molecule size compared to cubic or higher scaling behavior of standard numerical QM methods. The second scheme allows the treatment of the full protein-ligand system in QM detail. Recent developments of both schemes are highlighted in the following sections.

QM/MM-based methods

Some approaches treat only the ligand binding site in QM detail and the rest of the system is ignored in the calculations. Zhou and Caflisch [22] successfully used QM probes in a high-throughput virtual screen to identify selective inhibitors. The authors used probes to represent polar residues in the binding pocket, for example Ser/Thr/Tyr, Asp/Glu, Lys and Arg were represented by methanol, acetate anion, methylammonium cation and guanidinium cation probes, respectively. Semi-empirical QM interaction energy between ligand and the residue probes was computed and coupled with a hydrophobic matching term and ligand strain to compute the overall protein-ligand interaction energy. They screened 2.7 million compounds where similar poses were clustered before QM calculation and identified an inhibitor selective towards the kinase EphB4 from a pool of 85 other protein kinases.

Alternative schemes to reduce the computational complexity without ignoring large fractions of the protein are hybrid QM/MM methods that combine the accuracy of QM with the efficiency of MM. These methods usually partition the system into two domains, a QM and a MM region. The QM layer may contain only the ligand [23], or the ligand in addition to residues that form critical contacts with the ligand in the bound state [24–26]. In the case of metalloproteins containing transition metals it is necessary to include the metal ion and its ligation groups in the QM region. Burger et al. [23] reported that a short molecular dynamics simulation on the docked ligand pose followed by QM/MM optimization drastically improves both the prediction of native binding poses and the correlation between experimental and computed binding free energy. Ciancetta et al. [27] studied the binding of a series of six Ru(II)-arene PTA (RAPTA) ligands to catB using a QM/MM approach with Poisson Boltzmann surface area (PBSA) to implicitly model solvation of the protein-ligand system. QM calculations were performed at the DFT level. The best correlation ($r^2 = 0.80$) between predicted and experimental binding free energies was obtained neglecting the MM and continuum solvation energies, because those energies could not be accurately enough calculated. This study highlights the potential difficulty to accurately include solvation effects in QM/MM-based scoring.

Merz and co-workers [24] developed an AM1 semiempirical QM/MM scoring function and tested their approach on a set of 23 zinc metalloenzyme complexes. An r^2 of 0.71 and a standard deviation of 1.69 kcal/mol were achieved by optimizing the weights of the individual scoring terms. They also found that using a simple measure of ligand vibrational

entropy (i.e. the number of rotatable bonds) gave comparable results to calculating vibrational frequency of the entire QM region. Söderhjelm et al. [25] used a QM/MM model in which the QM region was computed at the MP2/cc-pVTZ level to sufficiently incorporate dispersion and polarization. The QM region included the ligand and all atoms within 4 Å of the ligand. To obtain the binding affinities of seven biotin analogues to an avidin tetramer, multipoles up to quadrupoles and anisotropic dipole polarizabilities calculated at B3LYP/6-31G* level were combined with a polarized continuum model (PCM) solvation and entropy from normal-mode analysis. Energies were averaged over snapshots sampled by 10ns MD simulations. Despite the usage of high level QM interaction energies, the performance of binding affinity prediction was poor with a mean absolute deviation from the experimental values of 26 kcal/mol. This large deviation between predicted and experimental values potentially resulted from inaccuracies in computing nonpolar solvation energies for the large molecules using the current PCM implementation. The results were also inferior to both standard MM/PBSA calculations and docking results using AutoDock. Fong et al. [26] assessed several QM/MM scoring functions and found that solvent-corrected HF/6-31G*::AMBER scoring function demonstrated better performance than ChemScore and GoldScore but was only comparably to a solvent-corrected MM scoring function on HIV-1 protease.

Analyzing the various QM/MM studies underlines an important issue that needs further analysis in future studies: The choice of basis set has a strong influence on the calculated binding affinity. Söderhjelm et al. [28], for example, showed that the interaction energy calculated between biotin and avidin differs by 38 kcal/mol between using the 6-31G* and the aug-cc-pVTZ basis set.

Linear scaling and fragmentation methods

In some approaches, the whole protein is represented in QM detail. Because this representation is computationally costly, approximation methods are commonly used. Merz and coworkers [29] have pioneered the linear scaling QM method at a semi-empirical level using the Divide and Conquer approach to divide the system into overlapping subsystems that can be treated individually. Fanfrlík et al. [30] developed a rescoring scheme comprised of enthalpy estimation using a semi-empirical PM6-DH2 representation with correction for dispersion and hydrogen bonding, and incorporating MM-based vibrational entropy, deformation and desolvation energies. They were able to distinguish between binders and nonbinders for 22 ligands of HIV-1 protease and obtained a correlation ($r = 0.84$) between theoretical and experimental binding free energies. Zhang et al. [31] evaluated semi-empirical QM scoring functions both at a PM3 and AM1 levels on the binding of 45 inhibitors to protein kinase B. Both PM3 and AM1 models yielded good agreement with the experimental values (PM3: $r^2 = 0.92$ on training set, $r^2 = 0.71$ on test set; AM1: $r^2 = 0.92$ on training set, $r^2 = 0.70$ on test set). Zhou et al. [32] modified the linear interaction energy model with continuum electrostatic solvation (LIECE) by replacing the force field based electrostatic term with linear scaling semi-empirical QM calculation at the RM1 level (a re-parameterization of AM1). They stressed that QM is necessary if significant variations in the protein's partial charges for different inhibitors due to polarization and charge transfer exist.

Alternative approaches to treat the whole protein-ligand system in QM detail are fragmentation methods that divide the system into a number of fragments that are individually calculated on QM level, the pre-calculated fragments are then combined using explicit many-body corrections. The most widely used fragment based methods are the fragment molecular orbital (FMO) [33] and molecular fractionation with conjugate caps (MFCC) [34] methods. FMO calculates the density distribution of monomers and dimers of fragments (and trimers in FMO3) with inclusion of the Coulomb potential of the whole system, which allows fragmentation without capping. In MFCC, the protein is segmented into fragments joined by pairs of conjugate molecular caps. The interaction energy between ligand and protein is then summed over all individual fragment contributions. FMO can be coupled with Poisson-Boltzmann (PB) [35] and PCM [36] calculations to include solvation effects. Yamagishi et al. [37] studied ligand recognition of peroxisome proliferator-activated receptor- γ (PPAR γ) using FMO at the MP2/6-31G* level. Based on interaction energy decomposed by residue using inter-fragment interaction energy (IFIE) analysis [38], they identified two quite different ligand binding patterns of PPAR γ . Due to the large hydrophobic pocket of PPAR γ , the author suggested that adequately hydrophobic pharmacophores are need for the development of novel ligands.

In a practical drug design setting, Mazanetz et al. [39] predicted the binding free energy for 28 cyclin-dependent kinase (CDK2) inhibitors using the FMO method at MP2/6-31G* level. Fourteen ligands with known X-ray structures were selected as the training set and the remaining 14 ligands, without an existing crystal structure, were used as the test set. The performance of the FMO method on the training set was compared to five MM scoring functions in MOE. The correlation between experimental and predicted binding affinity using the FMO method (r^2 is 0.68) was better than the correlations of three MM functions and slightly worse than those of the other two (r^2 is 0.75 for the best scoring function). However, adding to the enthalpic contribution in gas phase obtained by the FMO calculation two correction terms for solvation energy and entropy, an excellent correlation for both training set ($r^2 = 0.94$) and test set ($r^2 = 0.84$) was obtained, highlighting the potential of QM methods for accurate prediction of binding affinities in drug design.

Conclusion

It is widely accepted that protein flexibility needs to be incorporated in docking studies to more realistically model protein-ligand binding. Besides the on-the-fly inclusion of side-chain flexibility, the use of pre-generated EPS is nowadays the most widely utilized approach for modeling protein flexibility in docking. Recent studies have demonstrated the potential of this methodology but also some of the crucial issues that still need to be addressed. Which simulation method should be used for generating EPS? How many protein conformations should be used for optimal efficiency and accuracy in virtual screening and what is the best method to choose those structures? Is an EPS generated without consideration of the bound ligands appropriate to model ligand-induced conformational changes? Also, novel approaches to efficiently incorporate protein flexibility in grid-based scoring may allow more accurate or more efficient modeling of the protein's adaptation to bound ligands.

Another important area of research in docking is the improved energetic quantification of protein-ligand binding. In the last years, many studies incorporate QM details into protein-ligand scoring highlighting the potential of those methods to model polarization and charge transfer upon ligand binding. Despite the conceptual advantage of QM methods to more accurately treat electrostatic interactions between protein and ligand compared to standard MM-based scoring functions and some individual success stories, there is no yet clear evidence that QM and QM/MM methods always outperform classical MM methods for all systems. Significant variations in quantifying protein-ligand interactions among different QM representations and basis sets have been recognized and future studies are needed to more systemically investigate which QM representation most accurately computes ligand binding affinities and how protein-dependent those scoring methods are. It should also be recognized that QM methods are much more time-consuming potentially limiting the number of evaluated poses and ligands in virtual screening. Despite those current limitations, the potential to more accurately treat electrostatic interactions between protein and ligand compared to MM methods supports significant future efforts for optimizing and tailoring QM concepts for the study of protein-ligand systems.

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Figure 1.

Overview of concepts that incorporate protein flexibility in docking simulations. Top: Additional selected degrees of freedom describing the protein flexibility (here: Trp side chain conformation) are altered in tandem to the pose generation of the ligand. Bottom: Docking is performed to a pre-generated ensemble of protein structures. Each protein structure is typically kept rigid through docking to increase docking efficiency.

Figure 2.

Scheme of methods for treating protein-ligand complex in QM detail. QM calculations can be performed on ligand and few binding-site residues only (lower left figure). The amino-acids may even be reduced to small molecular probes to increase computational efficiency. QM/MM methods (lower right) treat the binding site in QM detail (protein: red; ligand: green) and incorporate effects of the remainder of the protein using MM representation (blue). Linear scaling or fragmentation methods can treat the whole protein-ligand complex in QM detail. In fragmentation methods, e.g. FMO (upper right), the protein-ligand system is divided into small fragments that are treated using QM methods including the electrostatic effects of the whole protein (detailed figure on the right displays helix interacting with ligand; different colors represent separate fragments).

Table 1

a. Comparison of recently developed and tested methods for incorporating protein flexibility into docking						
Technology	Generation and selection of EPS for ensemble docking			Grid representations		
Name of specific type of technology	Ligand-model concept	ENM for EPS generation	Selection of X-ray structures as EPS	Selection of sub-EPS using experimental ligand data	Elastic potential grid	Interpolation between grid representation of different protein structures
Pros	<ul style="list-style-type: none"> Efficient generation of protein conformations that are induced by ligand binding 	<ul style="list-style-type: none"> Efficient generation of alternative protein structures 	<ul style="list-style-type: none"> Improved docking results compared to single-structure docking Risk minimization for poor virtual screening performance 	<ul style="list-style-type: none"> Straightforward identification of optimal sub-EPS for virtual screening Allows efficient virtual screening 	<ul style="list-style-type: none"> Efficient algorithm to incorporate protein flexibility into grid-based docking Requires only one starting target structure 	<ul style="list-style-type: none"> Continuous representation of conformational changes Allows for changes in physicochemical properties
Cons	<ul style="list-style-type: none"> Currently only small scale conformational changes 	<ul style="list-style-type: none"> Only a few normal modes are insufficient to describe conformational change associated with ligand binding General rules about how many and which modes need to be considered are unclear. 	<ul style="list-style-type: none"> Best selection of X-ray structures not known prior to virtual screening 	<ul style="list-style-type: none"> Requires experimental data on few active ligands 	<ul style="list-style-type: none"> Doesn't allow to model changes in physicochemical properties due to conformational changes 	<ul style="list-style-type: none"> Requires initial selection of representative EPS Currently only designed for protein structures separated by single conformational variable/mode
References	[12]	[11]	[14,15]	[18]	[19]	[20,21]

b. Comparison of methods that incorporate QM and QM/MM concepts into the scoring process of docking					
Technology	QM on ligand binding sites		QM/MM	QM on entire protein	
Name of specific type of technology	QM on ligand binding sites		QM/MM	Linear scaling QM	
Pros	<ul style="list-style-type: none">• Polarization and charge transfer can be modeled		<ul style="list-style-type: none">• Strike a balance between accuracy and speed	<ul style="list-style-type: none">• Avoid using any classical MM force field parameters	<div>Fragment methods: FMO and MFCC</div> <ul style="list-style-type: none">• Calculation can be easily parallelized• Good for identifying key residues due to the natural interaction energy decomposition
Cons	<ul style="list-style-type: none">• Majority portion of protein is ignored• Dispersion effects are usually not accurately included		<ul style="list-style-type: none">• Only the ligand and a few residues are treated with QM• QM/MM boundary region across covalent bond is difficult to deal with	<ul style="list-style-type: none">• Most of the studies are on semi-empirical level with low accuracy• Inclusion of dynamic effects will result in extremely high computational costs	<ul style="list-style-type: none">• Computationally intensive• Large basis set is not suitable for FMO
References	[22]		[23,24,28]	[29,30,40]	[33,34]