Bioorganic & Medicinal Chemistry xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Ensemble-based docking: From hit discovery to metabolism and toxicity predictions

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ARTICLE INFO

Article history:
Received 31 May 2016
Revised 27 July 2016
Accepted 28 July 2016
Available online xxxx

Keywords:
Drug discovery
Docking
Computational drug discovery
Toxicity
Hit discovery
Lead discovery

ABSTRACT

This paper describes and illustrates the use of ensemble-based docking, i.e., using a collection of protein structures in docking calculations for hit discovery, the exploration of biochemical pathways and toxicity prediction of drug candidates. We describe the computational engineering work necessary to enable large ensemble docking campaigns on supercomputers. We show examples where ensemble-based docking has significantly increased the number and the diversity of validated drug candidates. Finally, we illustrate how ensemble-based docking can be extended beyond hit discovery and toward providing a structural basis for the prediction of metabolism and off-target binding relevant to pre-clinical and clinical trials.

1. Introduction

A popular approach to develop drug candidates that are potent and efficient is to rely on structure-based drug discovery, i.e., knowledge of the structure of a protein target, to identify small molecules that possess the desired chemical and structural properties needed to bind to the protein of interest. Docking belongs to a class of structure-based virtual screening approaches and are used to complement and accelerate experimental drug discovery screening campaigns.[1]

Docking calculations essentially predict how well a given chemical may bind to a given protein structure, and involve computer programs that perform the following tasks: (i) position a small molecule drug candidate in the (predicted or experimentally known) binding site of the target, (ii) evaluate an interaction energy between the small molecule and its protein environment—varying from an enthalpy-like interaction energy to more accurate and sophisticated binding free energy and (iii) identify those chemicals that are predicted to bind the strongest.[1]

In recent efforts to reduce toxicity of drug candidate, attention has been focused on not only predicting binding of drug candidates to the target but also on predicting off-target binding, as toxicity is often the result of off-target binding. Understanding drug discovery at this system-level terms implies that the ideal hit should bind only to certain proteins (to the target, or potentially to other proteins turning a pro-drug into a drug), and to become a solid drug candidate, the initial hit should also not bind to other off-target proteins (to avoid toxicity issues). This is a applicable problem for docking approaches to be used since they are able to sample not only multiple ligands but also multiple protein targets as we will show below.

Virtual screening traditionally follows an *induced fit mechanism* for ligand binding: the chemical to be assayed for its binding energy in a protein is inserted in the protein binding site, and potential structural modifications of the protein *following the binding of the ligand* are evaluated by allowing some degree of flexibility to the protein side chains around the ligand, and sometimes, but more rarely, extending this flexibility to the protein backbone

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http://dx.doi.org/10.1016/j.bmc.2016.07.064
0968-0896/

as well such as in the case of MM-PBSA of free energy perturbation calculations.[2–6] Such a protein-flexibility approach, while more realistic than keeping the protein rigid, requires long calculations that make this approach seldom used in large screening campaigns and reserved to the a detailed binding investigation of a small number of ligands, such as in lead optimization approaches. Alternatively, to quickly describe the dynamical flexibility of the protein receptor around docked ligands, docking scoring functions have been developed that are parametrized to use mostly rigid protein structures and to reproduce experimentally-known binding energies of ligands in as many proteins as possible. These rigid protein approaches usually use soft non-bonded interaction terms that limit steric clashes between a ligand and its protein environment and implicitly represent the overall effect of protein flexibility upon ligand binding.

With increasing computational power becoming available, there have recently been an increasing number of reports that aim at simulating the dynamics of the apo-protein targets, and perform docking in conformers thus sampled, as conceptualized by Lin and co-workers,[7] and as recently demonstrated in an integrated computational/experimental landscape study.[8] This *ensemble-based* approach aims at reproducing a *conformational selection mechanism*, where the protein-bound structure is sampled *prior to ligand binding*, and specific conformations are selected by the ligand(s) to form a thermodynamically favored protein:ligand complex of lower global free energy than that of other potential protein:ligand complexes. Technically, this conformational search does not preclude later small-scale protein rearrangements in response to ligand binding, but in practice, the latter, local induced fit is often omitted in ensemble docking and the same soft scoring functions used.

We describe here the collaborative contributions of our laboratories in developing computational techniques for ensemble-based (multiple proteins and multiple protein structures) docking, and in the applications of these techniques for hit discovery and for pathway exploration, and we present original results toward predicting the behavior of drug candidates in pre-clinical and clinical trials. Our work ranges from relatively small scale approaches to large-scale, supercomputing-supported, ensemble-based approaches that involve several target protein structures and large chemical databases of drug candidates.

2. Computational methods

2.1. Virtual screening programs

There exists a large number of programs developed for docking, many with well-documented histories of successful application. These programs originate from both academic and commercial laboratories, exemplifying the commercial importance of computational approaches to drug discovery in the pharmaceutical industry. A review of some of these programs and of their respective strengths and challenges has been given elsewhere.[9,10]

We have used several of these packages with success. However, in this paper we present results obtained using the commercial program MOE (Molecular Operating Environment, Chemical Computing Group, Inc., Montreal, Canada), and the academic program Autodock Vina (A.J. Olson laboratory, The Scripps Research Institute, San Diego, California).[11] We used Autodock Vina both in its native distribution and in a parallelized version, called VinaMPI, developed by our laboratories[12] and described below. The MOE program was used in the metabolic activation estrogenization pathway project described here, the Autodock Vina was used in the hit discovery project on modulatoes of coagulation, and the VinaMPI program was used in the toxicity prediction project.

2.2. Computational resources

The computational resources required to perform docking calculations vary with the scope of a specific screening campaign. While more computational power is always desirable, calculations screening a relatively small number of chemicals, up to a few hundred, on a few structures of a protein target can be achieved in a reasonable time on a modern desktop computer with a few CPU cores and about 200 Gb of hard drive space. We report below one such project that, while ambitious in scope, required relatively low-scale resources to provide a proof of concept in docking applied to multi-protein pathways. In larger, or much larger, screening campaigns, and in particular in the development of future toxicity/potency prediction of drug candidates, considerably more powerful computational resources are required to handle

(i) the sizes of the databases of chemicals to be screened,
(ii) the number of protein structures to be used in ensemble-based docking and,
(iii) the associated large storage and data processing requirements.

Here, we also present work done in our laboratories that used virtual screening approaches on the world's most powerful supercomputers. Our original parallelization of the AutodockVina program was developed on the (now decommissioned) Kraken machine, then the world's most powerful academic supercomputer, and operated by the University of Tennessee, Knoxville, Tennessee. The application research projects were performed on the (again now decommissioned) Jaguar and currently Titan[13] supercomputers. Jaguar and Titan were, and are, respectively, the most powerful open-science supercomputers in the USA, both operated by the Oak Ridge National Laboratory, Oak Ridge, Tennessee.

2.3. Protein conformer generation

In the results presented below, we have used molecular dynamics (MD) simulations to generate protein conformers on which we performed ensemble-based docking. An MD trajectory is divided into clusters that span the conformers sampled during the MD. In the seminal ensemble-based work of Amaro, McCammon and coworkers, MD simulations of 20 ns were used to sample protein conformations, and these conformations were used to dock ~1800 compounds.[14] In our larger screening campaigns, we have used MD simulations ranging from several hundreds of ns to the microsecond timescale. The MD simulations in our project were performed with the NAMD2 program[15] for atomistic MD simulations, and the Gromacs v.5.0.1[16,17] and Martini v.2.0 force field[18,19] for Coarse Grained (CG) MD simulations.

3. Results

3.1. Ensemble-based approaches and computational engineering

In this section we review our work on enabling efficient docking approaches on supercomputers. The primary benefit of using supercomputers is, of course, to be able to run many more docking and MD calculations than on smaller architectures. The docking enables very large, sometimes massive, databases of chemicals to be considered as potential drug candidates, increasing the chemical diversity of the chemicals considered as potential ligands for the targets of interest.

The second reason why being able to run large docking jobs is desirable is that it enables the simulation of the conformational selection mechanism. As illustrated below, using more than one