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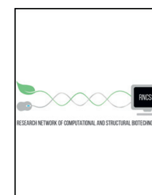

Mini Review

Biobetters From an Integrated Computational/Experimental Approach

Serdar Kuyucak [a,*], Veysel Kayser [b]

[a] School of Physics, University of Sydney, NSW 2006, Australia
[b] Faculty of Pharmacy, University of Sydney, NSW 2006, Australia

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ABSTRACT

Biobetters are new drugs designed from existing peptide or protein-based therapeutics by improving their properties such as affinity and selectivity for the target epitope, and stability against degradation. Computational methods can play a key role in such design problems—by predicting the changes that are most likely to succeed, they can drastically reduce the number of experiments to be performed. Here we discuss the computational and experimental methods commonly used in drug design problems, focusing on the inverse relationship between the two, namely, the more accurate the computational predictions means the less experimental effort is needed for testing. Examples discussed include efforts to design selective analogs from toxin peptides targeting ion channels for treatment of autoimmune diseases and monoclonal antibodies which are the fastest growing class of therapeutic agents particularly for cancers and autoimmune diseases.

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1. Introduction

Most of the drug leads that have high affinity for the target receptor ultimately fail because of problems with side effects, cytotoxicity or degradation. In fact, such problems are present in existing drugs but at a tolerable level. Improving the properties of existing biologics (protein or peptide-based drugs or drug leads) against such shortcomings is dubbed biobetters. Because the chemical space is very large, design of biobetters through trial and error methods is unlikely to succeed. One needs to make use of all the available information about the problems faced by a drug in order to facilitate the design of a biobetter. In fact, the experimental effort will be inversely proportional to the amount and accuracy of the information provided. As an example, consider solving the selectivity problem of a peptide ligand which binds to an off-target protein with a high affinity. If no information is available, one has to examine various mutations on the ligand which could be a very large experimental undertaking, e.g., for an average ligand with 30 amino acids, there are $30 \times 19 = 570$ single mutations and $(30 \times 29/2) \times 19^2 = 157{,}035$ double mutations to consider. Using a docking program, one could identify the binding region on the ligand, which will reduce the number of mutations, e.g., if there are 4 residues in the hot spot, the number of single and double mutations will be

* Corresponding author.
E-mail address: serdar.kuyucak@sydney.edu.au (S. Kuyucak).

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reduced to 76 and 2166, respectively. While this is a drastic reduction, the experimental effort required is still substantial. As a next step, one could refine the binding poses obtained from docking using molecular dynamics (MD) simulations and obtain an accurate structure for the protein-ligand complex. Now one has a precise map of the intermolecular interactions and can predict with some certainty which single and double mutations will yield the best outcome for reducing the affinity of the ligand for the off-target protein.

As illustrated in the above example, obtaining an accurate model of the protein-ligand complex holds the key for designing biobetters with minimal experimental effort. The most common method used for complex structure prediction is docking, which is fast but not very accurate. On the other extreme is MD, which can provide the desired accuracy but it is very slow. Combining the two methods by refining the binding poses obtained from docking in MD simulations offers a compromise solution that has been successfully applied to numerous protein-ligand complexes in the past decade [1–3]. An important ingredient in the success of this approach is the judicious use of the available experimental information about the complex system in the computations from initial docking to final validation. For example, available mutation data can be used as restraints in docking, which facilitates sampling of the correct pose and reduces the amount of subsequent MD work. Final validation of a predicted complex structure is typically based on binding free energy and available mutation data. While mutation of the residues in the predicted binding mode provides the most detailed and hence the best test for the proposed model, such data are not routinely available. Thus one may have to rely on the binding free energy of the ligand for validation, which has to be calculated near chemical accuracy to be useful for testing. Various methods can be used in calculation binding free energies from scoring functions in docking to potential of mean force (PMF) calculations in MD simulations. Again only the PMF calculations based on MD have the potential to provide the desired chemical accuracy.

Determination of validated complex structures is the most important step in design of biobetters because inspection of the binding mode will readily indicate the most promising mutations to achieve the desired improvement in affinity or selectivity. In fact, one can go beyond that and turn qualitative predictions into quantitative ones by calculating the effect of the mutation on the binding free energy from MD simulations. Such computational mutagenesis studies have the potential to eliminate guesswork completely and deliver the optimal biobetter for a given target with minimal side effects. In the following, we review the computational and experimental methods that will help to optimize design of biobetters while reducing the experimental efforts. Applications discussed include construction of selective analogs from toxin peptides targeting ion channels and design of biobetters from monoclonal antibodies with improved affinity and aggregation resistance.

2. Computational Methods

2.1. Protein-Ligand Complex Structure from Docking and MD

Determination of crystal structures for protein-ligand complexes is extremely difficult and very rare. Therefore, construction of an accurate complex structure from a given pair of protein and ligand structures is the most critical step in the design of a biobetter. Here we stress accuracy of the complex model in particular because an incorrect binding mode will predict misleading mutation sites for improvements, resulting in wasted experimental effort. Assuming crystal or NMR structures (or good homology models) of the protein and ligand are available, one can use a docking program to find a set of initial poses for the complex [4,5]. Docking programs work by evaluating an energy function for various positions, orientations and conformations of the ligand with respect to the protein and ranking the energy scores. An energy function consists of Coulomb, van der Waals, and hydrophobic interactions and may include entropic terms. There are many commercial and academic docking programs, and choosing an appropriate one could be overwhelming. Most of them are for docking small drug-like molecules and would not be very useful for peptide ligands. Among the academic programs we mention AUTODOCK [6,7], ZDOCK [8], and HADDOCK [9,10]. AUTODOCK is the most popular docking program but works mainly for small molecules. ZDOCK can handle larger molecules like peptides but performs only rigid docking. Among the three, HADDOCK is most suitable for docking of peptide ligands as it can handle peptides and allows flexibility.

Accuracy of docking programs is limited due to neglect of water molecules and lack of adequate sampling [11]. These are automatically incorporated in MD simulations, hence MD has the capacity to provide an accurate representation of the protein-ligand interactions. However, MD is too slow to predict the complex structure from scratch. A compromise solution is to refine the binding poses predicted by docking in MD simulations, which avoids the shortcomings of either method and could provide the sought accuracy. This approach was first used for binding of small ligands (<50 at.), and promising results were obtained [1,12–14]. Feasibility of its extension to peptide ligands was initially demonstrated for binding of charybdotoxin to a KcsA potassium channel mimic using HADDOCK for docking [15], which was generalized to binding of other scorpion toxins to Kv channels in a subsequent systematic study [16]. For most channel-toxin complexes, a consensus complex was obtained from cluster analysis of the top 100 poses, which simplifies the refinement process with MD.

Several programs are available for performing MD simulations such as AMBER, CHARMM, GROMACS, and NAMD. The NAMD program [17] has been a popular choice because of its user-friendliness and the accompanying visualization and analysis software VMD [18]. Although NAMD allows use of different force fields, CHARMM has been the preferred choice in most simulations of proteins [19]. For the basic formalism of MD simulations, we refer to the monographs [20,21]. Applications of MD simulations to membrane proteins, where creation of the simulation system is more involved, can be found in the reviews [22–24]. A key step in the refinement of the chosen binding pose via MD is the relaxation process where restraints between the protein and ligand are gradually reduced. The complex system is unlikely to be properly hydrated initially so without proper relaxation, various bonds and interactions in the complex may break, resulting in a dissociated ligand. There are well-established protocols for this purpose that can also be adapted for complex structures [25]. After relaxation, MD simulations are performed on the system, monitoring RMSDs of the protein and ligand, and the distances between interacting residues. The complex system is assumed to be equilibrated when the RMSDs reach a plateau and the time series of distances between interacting pairs fluctuate around a base line.

In the final stage, trajectory data obtained from the equilibrated system are used for visualization of the complex structure and analysis of the binding mode. The binding mode can be characterized quantitatively by calculating the average distances between the interacting residues. The strong ones include charge interactions, where the N—O distance between the charged residues is about 3 Å, and hydrophobic interactions involving aromatic side chains (2–3 kcal/mol). Intermediate strength interactions include hydrogen bonds and charge interactions at larger distances (1–2 kcal/mol). The binding mode results can be compared directly to alanine scanning mutagenesis data, which provides a detailed validation for a complex model. Unfortunately alanine scanning experiments are available only in a few cases, and one has to rely on binding free energies for validation in most cases.

2.2. Free Energy Calculations

Free energy calculations can contribute to design problems in two ways: validation of complex models as alluded above and prediction of free energy changes due to mutations. Binding constants of ligands

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