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Sampling Interfacial Water Effects over Protein Specificity with PELE

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I. Extended Abstract

Water is one of the main contributors that determine the shape of a protein, thereby defining its function. It can also be found inside protein cavities, helping proteins to unfold specific interactions with other substrates. Inner water molecules are able to modify the binding mode of the ligand by giving rise to new hydrogen bonds, by changing the polarity of their surroundings or by simply filling empty spaces. Thus, it is essential to take into account mediating water molecules when studying protein-ligand bindings.[1]

A protein environment has regions that are essentially hydrophobic. In this way, inner water molecules need to be highly arranged, trying to look for favorable polar interactions. This arrangement confers on them different properties from those water molecules that are in the bulk solvent. Water trapping has thermodynamic consequences. Enthalpy may be favorable as new hydrogen bonds can be established. But the main drawback is the large loose of entropy that occurs when a free water molecule from the solvent ends up trapped inside the protein.[2]

Therefore, systems like neuraminidase proved to contain water molecules that are responsible for increasing the protein-ligand binding by means of hydrogen bonds.[3] Yet, other systems such as HIV-1 protease show bindings that are favorable thanks to the gain of entropy that comes from the displacement of a trapped water to the bulk solvent.[4] Since thermodynamic properties are used to describe the affinity of a drug towards a target, drug design software needs to take into account the effects of water molecules in binding sites.[5]

In this work, we introduce the advantages of performing protein-ligand sampling by including sampling of mediating water molecules as well. Our approach uses the Protein Energy Landscape Exploration (PELE) program which is a tool that does protein-ligand sampling by means of a Monte Carlo method.[6] We aim to add a new routine to PELE to carry out sampling of interfacial waters from the binding site of a protein. Currently, PELE works with an implicit solvent, hence, interfacial water molecules are ignored by default. This tool should perform protein-ligand sampling while interfacial mediating waters are perturbed according to Monte Carlo method.

A. PELE methodology

PELE offers a methodology to perform protein-ligand sampling with a significantly reduced computational cost than that of conventional molecular dynamics simulations. It relies on a sampling procedure made up of three main steps. Firstly, the current state of the protein system is perturbed by translating and rotating the ligand and applying a perturbation on the protein according to the main vectors calculated with the Anisotropic Network Model (ANM). As a second step, PELE attempts to relax the system by applying a side chain prediction and a global minimization. Finally, the last step stands for either accepting or rejecting the perturbation according to the Metropolis criterion.

During the last years, PELE has been applied to perform different studies. From mapping ligand migration pathways to studying the substrate recognition of enzymes. In many cases, PELE has proved to be an outstanding tool to study protein-drug interactions.[7]

B. Water mediation in neuraminidase

An initial test has been conducted to see how the current version of PELE explores water-dependent systems. The system that is chosen is the native influenza virus neuraminidase bound to an inhibitor, a sialic acid. The X-ray structure of the complex was determined at 1.8 resolution (PDB: 1F8B).[3] In this complex, two water molecules seem to play a crucial role in the interaction between the protein and the ligand as they bridge them by means of hydrogen bonds.

Subsequent theoretical studies on the same crystallographic structure could classify the water molecules of the binding site.[8] They concluded that one water molecule (Wat A) seems to unfold very strong electrostatic interactions, while the other one (Wat B) presents an unfavorable binding free energy. This could be the main reason why Wat B exhibits displacement with the entrance of the ligand in some influenza neuraminidase complexes, while Wat A is conserved in all of them.

The role of Wat A on the protein-ligand binding was analyzed with PELE. Two local explorations were performed taking the previous crystallographic structure as starting point. The first exploration included both water molecules, Wat A and Wat B. Then, in the second exploration, Wat A was removed from the binding site. Other eight water molecules from the binding site were included in both explorations. All of them were treated by PELE as part of the protein chain but they

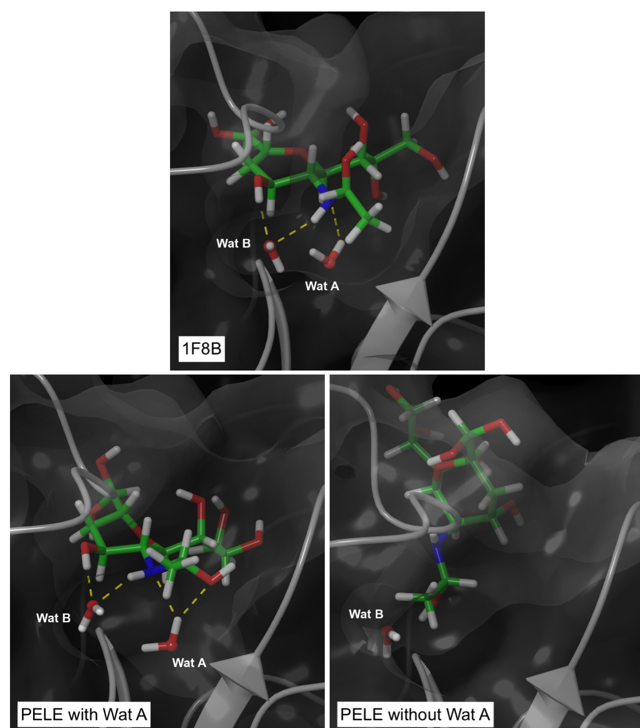

Fig. 1. Structural comparison between the crystallographic structure and the structures with the highest binding affinity from PELE explorations.

TABLE I. ENERGETIC AND STRUCTURAL ANALYSIS OF SIALIC ACID STRUCTURES.

Wat A	Binding Energy	SASA	RMSD
Yes	-74.9072	0.0422	1.6639
No	-50.6205	0.1662	5.9820

were omitted in the ANM perturbation. Figure 1 shows the structures which presented the highest protein-ligand binding affinity for each PELE exploration. Table I compares these two structures with the crystallographic system.

With the system that contains Wat A, PELE was able to predict a structure very close to the reference; the RMSD comparison with reference has a value of 1.6639. However, we appreciate significant structural changes after removing Wat A. The resulting structure is no longer similar to the crystallographic structure. In this case, the RMSD comparison increases up to 5.9820.

The absence of Wat A makes the sialic acid to be more exposed to the solvent as it tries to stabilize its polar chains. Wat A seems to have a key role on stabilizing the amine of the sialic acid. Thus, with the presence of Wat A it does not need to expose itself to the solvent to gain stability.

These different structural arrangements entail a change on the protein-ligand binding energy. As a result of this, the binding affinity of the sialic acid is significantly decreased when Wat A is missing in the binding site.

C. Conclusions

When all mediating water molecules are placed correctly in the binding site, PELE is able to find a binding site for sialic acid which strongly matches with the reference complex. However, when Wat A is missing or slightly shifted from its original place PELE explorations point to other structures that do not match with the reference. This is due to the fact that PELE does not contain a method to sample the waters of the binding site. Then, the role of Wat A is ignored when it is not placed correctly.

We believe that if we include a Monte Carlo method to sample water molecules along a PELE run, we would not need to previously place mediating water molecules to a suitable location. Moreover, PELE could find new poses of the ligand which require water molecules from the binding site to be shifted. For instance, we could deal with systems like HIV-1 protease where there are water molecules which need to be shifted out when the ligand binds to the protein.

II. Acknowledgment

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