Ligand Binding to the Pregnane X Receptor by Geometric Matching of Hydrogen Bonds

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1 Introduction

Hydrogen bonds are important in protein-ligand interactions. We describe a geometric model of hydrogen bonds, which we use to study ligand binding to the pregnane X receptor (PXR). PXR binds drug ligands promiscuously. The recently determined structure of PXR in humans (hPXR) reveals that its binding pocket is large, smooth, and uniformly hydrophobic [5], leading to the hypothesis that hydrogen bonds are the main determinant of binding conformations. Our objective in this work is to identify potential ligand binding conformations to PXR based on hydrogen bond geometry and use them as a starting point for ranking ligands in terms of the binding affinity to human and mouse versions of PXR.

In humans, PXR regulates the gene expression of cytochrome P450-3A, which metabolizes many foreign substances in the body, including an estimated 60% of prescription drugs, such as the cholesterol-lowering drug SR12813 and antiretroviral drug indinavir. PXR has also been implicated in potentially harmful drug-drug interactions with the over-the-counter herbal antidepressant St. John's wort.

PXR is activated by many exogenous chemicals. Although the binding affinities of several ligands are known for both the human and the mouse PXR, the binding conformations are not known. PXR in mice (mPXR) has 77% sequence identity with hPXR. The structure of mPXR is not yet known, but its behavior can be "humanized" by replacing four amino acids in the binding pocket of mPXR with the corresponding ones in hPXR [5]. We undertook this work to see whether we can explain binding affinities by finding ligand-protein complexes that support the formation of hydrogen bonds.

General-purpose docking programs (*e.g.*, [2, 4]) search for favorable binding conformations by minimizing a complex energy function using simulated annealing or genetic algorithms, and thus are often slow. Due to the observed characteristics of the binding pocket of hPXR, we have explored a minimalist approach that focuses on only one aspect of the energy function, the hydrogen bonds, in order to identify the discriminating factor in ligand binding to PXR. Our approach is a fast, directed search that avoids local minima. It can also aid the general-purpose programs by providing good starting conformations, thus drastically reducing the search space. Hydrogen bonds have been used in FlexX [3] as part of a more complete energy function. Our geometric model of hydrogen bonds (consisting of rays and wedges) is more compact than the one used in FlexX, and can provide more efficient and more accurate matching without discretization.

2 A geometric model of hydrogen bonds

To derive a compact geometric model of hydrogen bonds, we simplify the quantum mechanical description of electronic orbitals and combine it with the observed data [1] on hydrogen bonds. We predict the orbitals of an atom by looking at the local structure around it. Our current model focuses on sp^2 and sp^3 hybridizations.

Our geometric model uses rays and wedges to specify preferred hydrogen bond directions. For donor or sp^2 (carbonyl) acceptor atoms, we create a *ray* for each preferred direction, emanating from the nucleus of the polar atom. For sp^3 acceptor atoms (atoms in a ring or chain), we
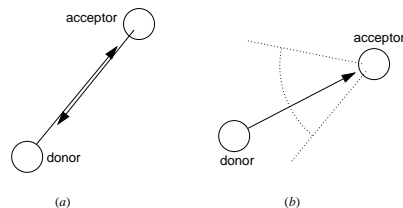

Figure 1: Aligning (*a*) a ray to a ray, and (*b*) a ray to a wedge.

create a *wedge* of preferred directions, centered at the polar atom. In our model, a hydrogen bond occurs between a donor and an acceptor atom if we can align a donor ray to an acceptor ray or wedge, subject to the constraint that the distance between the two polar atoms is between 2.6Å and 3.2 Å (Figure 1).

For rigid transformations in 3-D, ray/ray matching leaves out one rotational degree of freedom, and ray/wedge matching leaves out two. To constrain the ligand conformation fully, we look for transformations of the ligand that satisfy two hydrogen bonds simultaneously to within tolerance. The problem is formulated and solved as nonlinear optimization. We then apply an efficient hierarchical collision detection algorithm to screen the proposed candidates and eliminate those that are infeasible due to steric hindrance.

3   Results and discussion

We tested 21 ligands in our experiments. For each ligand, we defined the geometric primitives (the rays and wedges) attached to the polar atoms. We also defined 21 geometric primitives for the polar atoms from the polar side chains in the binding pocket of hPXR. The hydrogen bond matcher examined 72,728 pairs of donors and acceptors from the ligands and hPXR, and reported 1018 matches. After filtering out those violating the steric hindrance constraints, we obtained 209 candidate binding conformations for the 21 ligands.
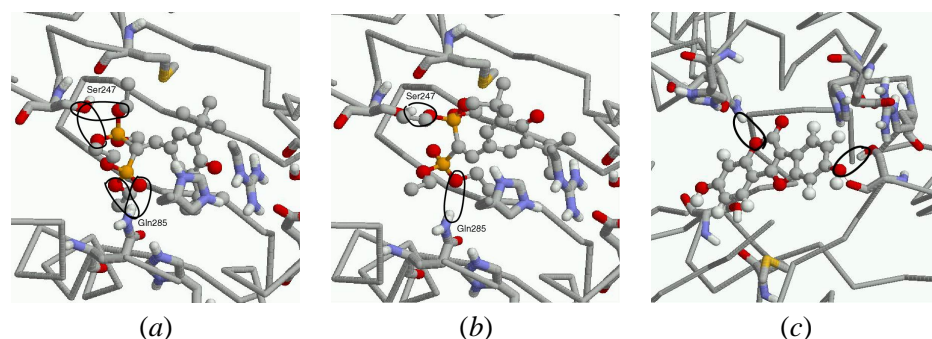

(a)   (b)   (c)

Figure 2: Binding conformations of SR12813 determined (*a*) by X-ray crystallography and (*b*) by our algorithm. In both cases, the space occupied by the ligand is similar. (*c*) A binding conformation of coumestrol reported by our algorithm. Atoms involved in hydrogen bonding are circled.

The only ligand that has been crystallized with hPXR is SR12813 [5]. Figure 2*a* shows an observed binding conformation with two bifurcated hydrogen bonds. Our algorithm does not model bifurcated hydrogen bonds, but it reported a similar binding conformation with single hydrogen bonds involving the same polar atoms (Figure 2*b*), making this an ideal starting point for general docking programs using more detailed energy functions. Figure 2*c* shows a typical binding conformation of the ligand coumestrol. Our preliminary results indicate that our method serves as an effective geometric filter to investigate ligand binding to PXR.

We are currently extending this work in three directions. First, we are comparing our results with those from AutoDock [2] to determinine to what extent our computed candidate conformations can help general docking programs reach better results faster. Second, we are going further in incorporating ligand and protein flexibility. By taking advantage of the flexibility of ligands and the side chains of PXR in the binding pocket, we may be able to find better binding conformations that are not accessible to rigid docking because of steric hindrance. Finally, we are building a homology model for the mouse PXR. We will run our algorithm on the mouse PXR and the same set of ligands that we have used for hPXR, and hope to see a correspondence between our ranking and the observed binding affinities for mouse versus human PXR.

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