Docking Studies

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How Reliable Are Current Docking Approaches for Structure-Based Drug Design? Lessons from Aldose Reductase

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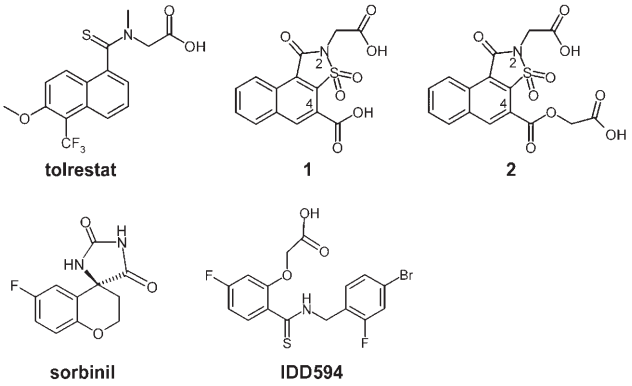
Structure-based drug design requires accurate geometries of protein–ligand complexes as a starting point to develop reliable structure–activity relationships. In an ideal situation, high-resolution crystal structures of the considered leads are available and provide the platform to generate a sound design hypothesis.

However, crystallography is an elaborate technique and requires expensive equipment and also strong expertise for producing well-diffracting crystals of the corresponding protein–ligand complexes. Thus, readily available alternative docking techniques are consulted to gain first insights into plausible design hypotheses. What is the current status and reliability of these docking tools?

Two aspects have to be considered, the geometrical accuracy of the generated binding modes and the relevance of the affinity prediction, which is usually consulted to discriminate among the multiple solutions generated by the docking tools. The geometrical accuracy is normally assessed by comparing the docking solutions with experimentally determined crystal structures, and root-mean-square deviations (rmsd) of less than 1 Å for the atoms of the ligand are considered "good" solutions. With respect to scoring, the implemented energy function is requested to retrieve the binding position that most closely resembles the crystal structure with the best rank from a set of decoy geometries. Recent studies have shown that the scoring problem is intimately correlated with the quality and relevance of the generated binding modes.[1,2] This observation suggests that once the docking problem of producing the most accurate binding modes is solved, the energy scoring can also be settled.

In a recent study, Da Settimo et al.[3] investigated a series of aldose reductase (AR) inhibitors that were designed as potential analogues of the long-known AR inhibitor tolrestat[4] whose complex structure was solved in 1997.[5] This inhibitor induces a conformation in the AR binding pocket that is still unique today. The two most potent inhibitors, **1** and **2**, of the series inhibit AR with IC_{50} values of 140 and 550 nM, respectively (see Scheme 1). The only difference between the two compounds is a different side chain placed at the 4-position of the ring scaffold. The hypothesis that compounds **1** and **2** induce a binding-pocket conformation similar to tolrestat (herein described as the "tolrestat conformation" of AR) is based on the high structural similarity to tolrestat analogues[6] and their convincing superposition with the protein-bound conformation of tolrestat.[3]



Scheme 1. Chemical structures of AR inhibitors. For docking calculations, the carboxy and hydantoin moieties were considered deprotonated.

Previous experiments have shown that the binding pocket of AR can adopt at least three different conformations.[7] In a first attempt, we selected the docking program AutoDock,[8] which does not consider protein flexibility during the docking process. The program fits the inhibitor into a predefined binding-pocket conformation and neglects other possible protein conformers. For this reason, we docked **1** and **2** not only in the tolrestat but also in other pocket conformations that were extracted from the sorbinil ("sorbinil conformation" of AR)[5] and IDD594 ("IDD594 conformation" of AR)[9] complexes. Sorbinil binds to the closed form of the AR specificity pocket, whereas IDD594 addresses the open pocket, but in a different conformation than that of tolrestat.

Subsequently, we applied a very similar protocol by using the docking programs FlexX[10] and Gold,[11] and the program Glide[12] was used for explicitly considering protein flexibility during the docking procedure.

In Figure 1, the top-scoring docking solution from AutoDock 3.0.5[8] for each pocket (sorbinil (green), tolrestat

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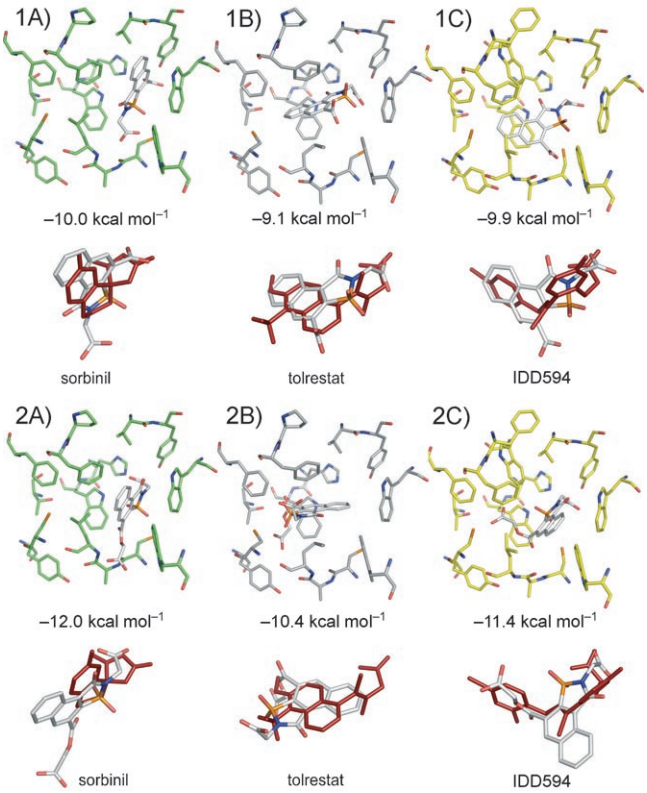

Figure 1. Results for docking **1** (upper part, 1A–1C) and **2** (lower part, 2A–2C) into different AR binding-pocket conformations formed with sorbinil (A), tolrestat (B), and IDD594 (C). The best-scored docking solutions for each pocket are shown in the first and third row. In addition, the corresponding scores are given as computed by the scoring function implemented in AutoDock. In the second and fourth row, superpositions of **1** with the crystallographically determined binding mode of sorbinil, tolrestat, and IDD594 in their corresponding binding pockets are shown.

(gray), IDD594 (yellow)) is shown together with the corresponding AutoDock energy score and a superposition with the bound reference inhibitors (sorbinil, tolrestat, IDD594) in their crystallographically determined conformation.[13] A reasonable docking solution of **1** is found for each of the binding-pocket conformations. Surprisingly, the docking solution in the tolrestat pocket (1B) obtained the worst score: almost 1 kcal mol^{-1} less favorable than the solutions in the other two pockets. The solutions for the sorbinil and IDD594 pocket are scored equally well. Furthermore, for the sorbinil pocket, the carboxylic acid in the 4-position of **1** occupies the catalytic pocket. The docking solution of **1** in the sorbinil pocket is superimposed with the bound conformation of sorbinil (second row of Figure 1) and shows a very reasonable overlap. The carboxy moiety occupies the same region in the catalytic pocket space as the nitrogen and an oxygen from the hydantoin moiety of sorbinil and thus enables the described interactions with surrounding residues. For the other two cases (tolrestat and IDD594 pocket), the fit of the polar head groups is not as convincing. Although in both cases the carboxy function in the 2-position is placed into the catalytic pocket, the conformations are different and the interaction geometries with the catalytic residues appear not to be ideal. The rigid aromatic scaffold of **1** superimposes well with sorbinil and tolrestat. The fit with IDD594 is not convincing as the corresponding ring systems are rotated by almost 45° relative to each other.

The only difference between **1** and **2** is the length of their side chain in the 4-position. The docking results for **2** are shown in Figure 1 (lower part) and again, the solution in the sorbinil pocket scored the best, followed by the conformation in the IDD594 pocket. Docking into the tolrestat pocket, however, resulted in by far the worst energy score. Compared with **1**, the best docking solution in the sorbinil pocket is flipped 180° and the 2-carboxy function then occupies the catalytic pocket. For the tolrestat pocket, the best-scored solution places neither of its carboxylic acids into the anion pocket, which is rather unlikely as occupation of this pocket is mandatory for AR inhibition. While the docking solution in the IDD594 pocket occupies the catalytic pocket, it places the second carboxy moiety into the hydrophobic specificity pocket, which is also unlikely, particularly as this part of the binding pocket is certainly not very suitable to accommodate a polar acid function. In the sorbinil pocket, the docking solution occupies the catalytic pocket in a favorable geometry with respect to possible interaction partners, and the second carboxy function points towards the solvent. In summary, **1** and **2** are favored by AutoDock to bind to the sorbinil-like pocket conformer and not the tolrestat-like geometry.

Subsequently, we determined the crystal structures for both **1** and **2** in complex with AR (shown in blue in Figure 2).[14] In both cases, the specificity pocket is in a closed conformation, resulting in a different binding mode than that adopted by tolrestat. Instead, the binding pocket is more similar to the sorbinil-bound conformation as was suggested by our docking experiments.

For the complex **1**, the carboxy function in the 4-position occupies the anion pocket as predicted (Figure 2A). The 2-carboxy group points towards the solvent and forms an H-bond with the side-chain nitrogen of the nearby Trp20, which rotates about its $\chi 2$ angle by approximately 30°. This rotation is in contrast with all previously determined AR structures. Thus, **1** induces a new conformation in the catalytic-binding pocket; instead of pointing upwards as in the docking solution, the aromatic ring system of **1** points downwards (Figure 2A). This conformation displaces Trp219, which then becomes disordered and for this reason is not apparent in the electron density. This binding mode of **1** is incompatible with all previously known AR conformations as it would clash with Trp20 and Trp219 in all other structures. Interestingly, among the 100 docking solutions generated by AutoDock there are 15 solutions in which the aromatic ring system of **1** points downwards in a manner similar to the crystal structure and the 2-carboxy function forms a hydrogen bond with Trp20. However, the first of these solutions appears at a ranking of 75 with an energy score of −9.6 kcal mol^{-1} and the geometries do not match perfectly (see Figure 2C).

Compound **2** binds with the 2-carboxy function into the catalytic pocket as predicted by AutoDock (Figure 2B). The overall pocket conformation is very similar to that of the sorbinil complex. Phe122 is the only residue that has to shift to accommodate the ligand. Again, this pocket conformation

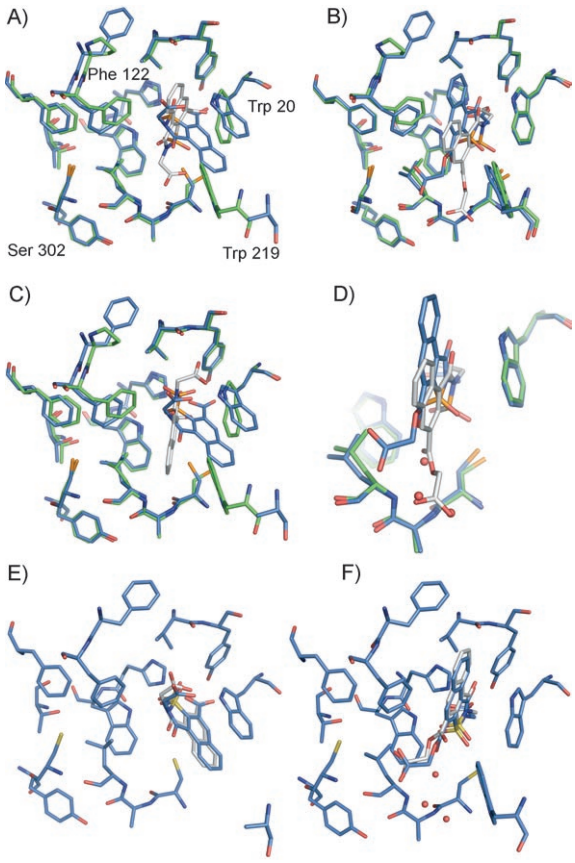

Figure 2. A,B) Superpositions of the best docking solutions and the crystal structures (**1**: A, **2**: B). The protein conformation used for docking is shown in green, the crystal structures including the ligands are shown in blue, and the corresponding best-scored docking solution is shown in gray. C) The docking solution at rank 75 of **1** (gray) in the sorbinil-pocket conformation (green) compared with the crystal structure (blue). The ring moiety of the docking solution of **1** points downwards instead of upwards as in the more favorably scored docking result. This binding mode is in better agreement with the crystal structure. D) Superposition of the best-scored docking solution of **2** (gray) and the crystal structure (blue). The regions occupied by the side-chain oxygen atoms at the 4-position in the docking solution correspond to positions of water molecules found in the crystal structure. E) The best-scored docking solution (gray) obtained from flexibly redocking **1** into its crystallographically determined binding-pocket conformation (blue). F) The top-scored docking result (gray) for flexibly redocking **2** to its crystallographically determined binding-pocket conformation in the presence of the three crystallographic water molecules, which are treated as part of the protein during docking.

was correctly predicted by AutoDock to be the most favorable to host **2**. The other carboxy function points towards the solvent and forms two H-bonds with the backbone N atom and the side-chain Oγ of Ser302. A comparison of the docking solution and the crystal structure of **2** shows that the position of the carboxy function that occupies the catalytic pocket is well predicted. This is also correct for the sulfon moiety of **2**. The entire ring system is shifted upwards in the crystal structure and the side chain in the 4-position points in a slightly different direction. Three of the positions that are occupied by ligand oxygen atoms in the docking solution host water molecules in the crystal structure (see Figure 2D). This confirms these positions as good sites for hydrogen bonds. Thus, the placement of the oxygen atoms in the docking solutions is reasonable for the formation of H-bonds.

The overall binding mode of **2** has been predicted reasonably well. Deviations result from a slight movement of a phenylalanine side chain and the attempted placement of the polar-ligand substituent into positions in the binding pocket that are already occupied by water molecules.

The question remains as to whether using the corresponding native binding-pocket conformations of the subsequently determined crystal structures with **1** and **2** as templates for docking would lead to improved binding-mode predictions. The results for flexible docking of **1** in its crystal structure are shown in Figure 2E. By using an rmsd of 1 Å as the cluster criterion, all 100 of the generated docking solutions of AutoDock fall into the same cluster and none of the solutions has an rmsd greater than 0.82 Å with respect to the crystal structure. Therefore, the binding mode of **1** is correctly predicted provided that the correct binding-pocket conformation is given.

The binding mode obtained for **2** in its crystallographically determined binding-pocket conformation is very similar to the pocket conformation that results from docking to the "sorbinil conformation" (Figure 2B). This finding is reasonable as there are only small deviations between the two complex binding-pocket conformations of **2** and sorbinil. However, if the three water molecules (as were described in Figure 2D) are included in the docking as part of the binding pocket, the docking solutions improve significantly (Figure 2F). The 88 top-ranked solutions of AutoDock show an rmsd value below 1 Å with respect to the crystal structure; thus the binding mode of **2** is correctly predicted.

To resolve the question of whether these findings are a mere consequence of the applied docking program and scoring function (i.e., AutoDock and the corresponding free-energy function) and whether improved predictions could be obtained based solely on the previously known AR binding-pocket conformations, we subjected the complex example of **1** to other docking and scoring methods. The programs FlexX[10] and Gold,[11] as well as Glide[12] in the "induced-fit docking" mode,[15] were tested for this purpose, however, not surprisingly, none of them succeeded in obtaining a better or more relevant binding mode (Figure 3). In Figure 3, we show the binding modes that most closely approximate the experimentally observed binding mode, regardless of the scoring rank that was actually achieved. Even though this is rather artificial, we believe that once an accurate binding geometry is produced, then the scoring issue can also be resolved. The AutoDock scores are known to correlate well with the geometric accuracy of the AR inhibitor positions[16] such that an improvement could not really be expected by switching to other scoring schemes. Furthermore, the experimental binding mode of **1** corresponds to a structure that is entirely based on an unexpected conformational change in the AR binding pocket. This conformational change could neither be anticipated from previously known structures (e.g., based on a comparative B-factor analysis) nor from standard MD simulations carried out for other complexes. It is indeed most surprising in the

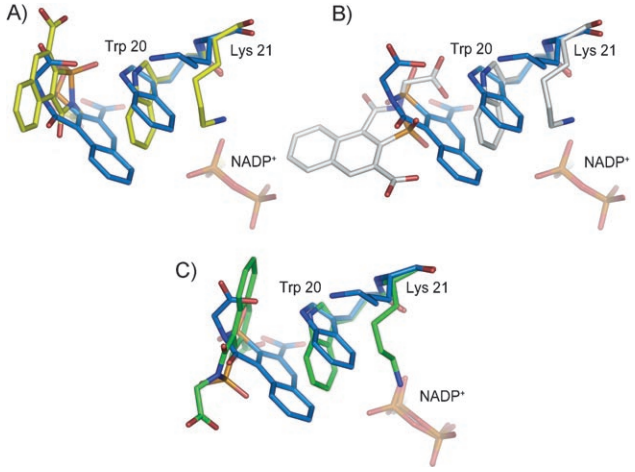

Figure 3. Comparison of docking positions with the experimental binding mode of **1** in the crystal structure (blue). The docking results were obtained with FlexX (A, yellow), Gold (B, silver), and Glide (C, green). In each case, the docking position most closely resembling the crystallographic binding mode is shown. Default parameter settings were used for the docking runs carried out with FlexX 2.0.3,[10] Gold 3.1,[11] and Glide 4.0.[12] FlexX and Gold were used to dock to all three previously known binding-pocket conformers (i.e., sorbinil: PDB code 1ah0; tolrestat: PDB code 1ah3; IDD594: PDB code 1us0). The illustrated docking positions were obtained with the 1us0 structure. Glide was used in the "induced-fit docking" mode,[15] which allows for side-chain flexibility by using Prime 1.5[17] for side-chain modeling. The Glide SP docking mode was applied during initial docking, whereas the XP scoring scheme was used for the final docking steps. The input structure used was 1ah0, which kept all residues within a sphere of 5 Å around the initial docking position and was flexible during the homology modeling step of the induced-fit docking process. In addition, the side chains of Trp 20 and Lys 21 were mutated to Ala prior to the initial docking to increase the diversity of the generated positions. Notably, this procedure is certainly artificial as it anticipates knowledge about the movement of these two residues, which can only be found by the subsequently determined crystal structure of the AR–**1** complex. Nevertheless, the applied protocol did not suggest an improved docking solution for **1**.

crystal structure of the AR–**1** complex to observe that the salt bridge between Lys 21 and the cofactor phosphate group is broken, a feature that is hardly predictable by any current method. In consequence, we anticipated that only a docking program that allows for induced-fit adaptations of the protein could eventually succeed. However, the case itself is very challenging as the test with Glide "induced-fit docking" shows: despite considering flexibility of Trp 20 and Lys 21, no improvement of the docking results could be obtained.

The unbiased reader, who is not in favor of either computational or experimental approaches to structure-based drug discovery, might anticipate the value of docking and virtual screening as rather limited. This, however, is by no means the case as docking was used to suggest compounds **1** and **2** as potential binders to AR. Even the correct protein conformer with respect to the specificity pocket was suggested (see the AutoDock results described above). From a pragmatic and result-oriented point of view, this is a very successful and valuable observation. Taking, however, the view point of a methods developer, the reported results are puzzling: The study highlights the need for improved concepts to cope with protein flexibility and the handling of water. One has to be aware that even small unexpected adaptations of protein residues could be essential for the prediction of the correct and relevant binding mode. In the present case, a salt bridge has to be broken to allow for the unexpected ligand accommodation. Computer methods will hardly predict such a change. However, mechanistic considerations of the enzyme clearly suggest that this salt bridge can easily be cleaved: it has to be opened to allow the cofactor to diffuse in or out upon enzymatic turnover. Such observations must also be reflected in setting up a relevant protocol for computer simulations.

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