Pocket v.2: Further Developments on Receptor-Based Pharmacophore Modeling

Jing Chen[†] and Luhua Lai*[,†,‡]

Beijing National Laboratory for Molecular Sciences, State Key Laboratory for Structural Chemistry of Stable and Unstable Species, College of Chemistry, and Center for Theoretical Biology, Peking University, Beijing 100871, P.R. China

Received June 19, 2006

A deriving pharmacophore model from the three-dimensional structure of a target protein provides helpful information for analyzing protein−ligand interactions and further improvement of ligand binding affinity. A standalone program, Pocket v.2, has been developed based on the original Pocket module in the de novo drug design program LigBuilder. Pocket v.2 is able to derive a pharmacophore model directly from a given protein−ligand complex structure without human intervention. Key features in the pharmacophore model are automatically reduced to a reasonable number. Pocket v.2 has been applied to several case studies, including cyclin dependent kinase 2, HIV-1 protease, estrogen receptor, and 17β-hydroxysteroid dehydrogenase. It well reproduced previously published pharmacophore models in all of these cases. One notable feature of Pocket v.2 is that it can tolerate minor conformational changes on the protein side upon binding of different ligands to give a consistent pharmacophore model. For different proteins accommodating the same ligand, Pocket v.2 gives similar pharmacophore models, which opens the possibility to classify proteins with their binding features.

INTRODUCTION

A pharmacophore model is the 3D arrangement of essential features that enables a molecule to exert a particular biological effect.[1,2] As an important category in computer-aided drug design, it has been widely used as a query for database mining or a guide for de novo design.[3,4] Generally, a pharmacophore model is deduced from a set of ligands with known activities while lacking the three-dimensional structure of a receptor. Though there are already many automatic ligand based methods such as DISCO, Catalyst/HipHop, and GASP, the derivation of a pharmacophore model remains a difficult task.[4−10] Different results may be given due to different strategies taken in conformational analysis and molecular superposition.[11]

The generation of a pharmacophore model directly from a protein crystal structure is an alternative approach, which can reveal the key elements in protein−ligand binding more straightforwardly. With the advances in experimental techniques of X-ray crystallography and NMR, the determination of a protein structure as a drug target has become more convenient.[12] The development of reliable and robust techniques to construct pharmacophores from a receptor structure is important.

However, usually many features will be given out by existing receptor-based pharmacophore model methods, the number of which is too high to be used in a single database query.[13−22] Indeed, pharmacophore models derived from the ligands side typically have 3 features, sometimes 4, but almost never more than 4.[10] To handle this, PRO_PHARMEX suggests user control based on existing knowledge.[19,20] Structure-based focusing (SBF) generates queries using all possible combinations of the features and then compares their selectivity by screening the Catalyst 3D database generated from a set of known active ligands.[21,22]

The selection of crucial features remains challenging in receptor-based pharmacophore modeling. Pocket v.2 was developed toward this goal, that is, to automatically generate a pharmacophore model with a rational number of features when one complex structure is available. Pocket v.2 has been developed based on the Pocket module in LigBuilder, a multipurpose program for de novo structure based ligand design from the author's group, in which Pocket is utilized to analyze the binding site of the target receptor by scored-grids and to extract a receptor-based pharmacophore model.[23] (The Pocket v.2 program is available by contacting the authors.) Similar to other approaches, Pocket also suffers from the difficulties in identifying crucial features among a number of centers. A known ligand in a complex is used for help in Pocket v.2, which acts as the first filter to mark out all contributing spots in the complex. The second filter is the binding intensity of those spots based on the assumption that the decisive pharmacophore features should have high affinity.

Another attractive application of receptor-based pharmacophore model is to discover new binding spots that have not yet been occupied by known ligands so as to guide the improvement of binding affinity and/or maximizing selectivity.[4] Pocket v.2 can make suggestions for potential binding spots besides the pharmacophore features, which may be especially useful in lead optimization.

Pocket v.2 has been applied to several case studies and gave reasonable results. The usage of diverse complexes of the same receptor does not affect the pharmacophore models significantly, and the binding suggestion from one complex

* Corresponding author phone: 86-10-62757486; fax: 86-10-62751725; e-mail: lhlai@pku.edu.cn.
[†] College of Chemistry.
[‡] Center for Theoretical Biology.

10.1021/ci600246s CCC: $33.50
Published on Web 11/04/2006

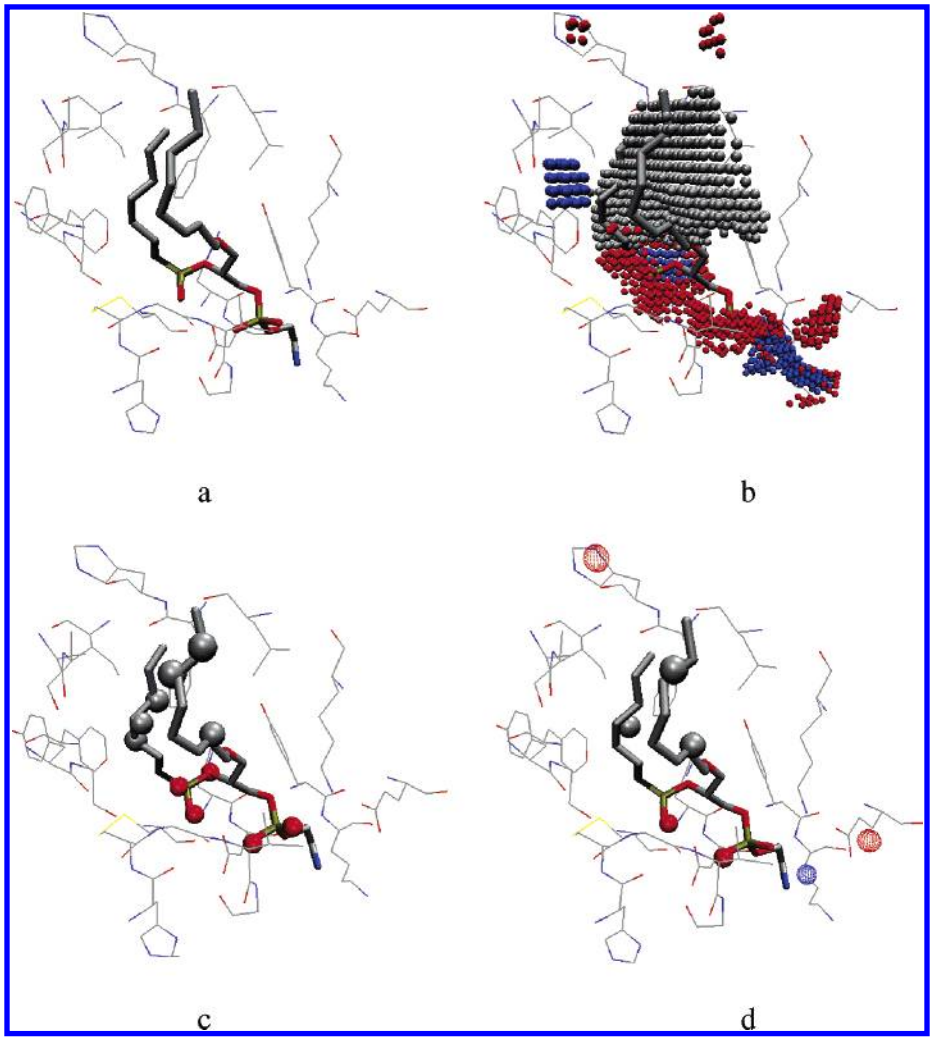

Figure 1. Pharmacophore calculation procedure of phospholipase A2 by Pocket v.2 (PDB entry 1poe, with the ligand). (a) Pocket residues defined by Pocket v.2. The pocket residues and the ligand are shown in the CPK color scheme, the pocket residues are shown in the LINE mode, and the ligand is shown in the LICORICE mode. (b) The interaction model of the binding site. In all figures, the grids are shown in VDW mode, hydrogen bond donor grids are colored in blue, hydrogen bond acceptor grids in red, and hydrophobic grids in silver. (c) The interactions existing in the complex represented by grids. (d) Pharmacophore model given by Pocket v.2. Here the interaction spots shown in (c) are clustered into features of type I, while in other cases some of these spots may be ignored; three features of type II are shown. If without special illumination, in all figures pharmacophore features of type I are shown in VDW mode; suggested potential binding features of type II are shown in DOTTED mode. In all figures, hydrogen bond donor features are colored in blue, hydrogen bond acceptor features in red, hydrophobic features in silver, hydrogen bond donor and acceptor grids and features in yellow.

was found to be taken into action in other complexes. In addition, similar pharmacophore models from different receptors binding with the same ligand can also be given.

METHODS

Binding Site Analysis. The basic input for Pocket v.2 is the 3D structure of the protein that is represented in PDB format. A predocked ligand is also required to help the program locate the binding pocket and select recognition features.

Pocket v.2 will first define a box to cover the ligand and all the surrounding residues and then create regularly spaced grids within the box (see Figure 1a). Three different types of probe atoms will be used to scan the grids, which represent hydrogen bond donor, hydrogen bond acceptor, and hydrophobic group, respectively. Hydrogen bond donor, hydrogen bond acceptor, and hydrophobic scores between the probe atoms and the protein are calculated by Score function, which is a master equation method to estimate the binding constant of protein and ligand from the author's group.[24] Its applicability has been verified by comparison with several other scoring methods and combination with DOCK.[25−27] The detailed description for binding site analysis has been given in ref 23.

Generation of the Interaction Model. First, the program will check the scores of each grid. The hydrogen bond donor scores and the acceptor scores lower than 0.20 will be reset to zero, while the hydrophobic scores lower than 0.47 will be reset to zero. Grids with three zero scores will be filtered out, so that only those with significant contributions to the ligand-protein binding process will survive.

Second, the program counts the neighboring number for each grid. Here "neighbor" refers to the grids within 2 Å having a none-zero score with the same type. Grids with donor neighbors fewer than 50 will be reset to zero for its donor score, with acceptor neighbors fewer than 30 will be reset to zero for its acceptor score, and with hydrophobic neighbors fewer than 40 will be reset to zero for its

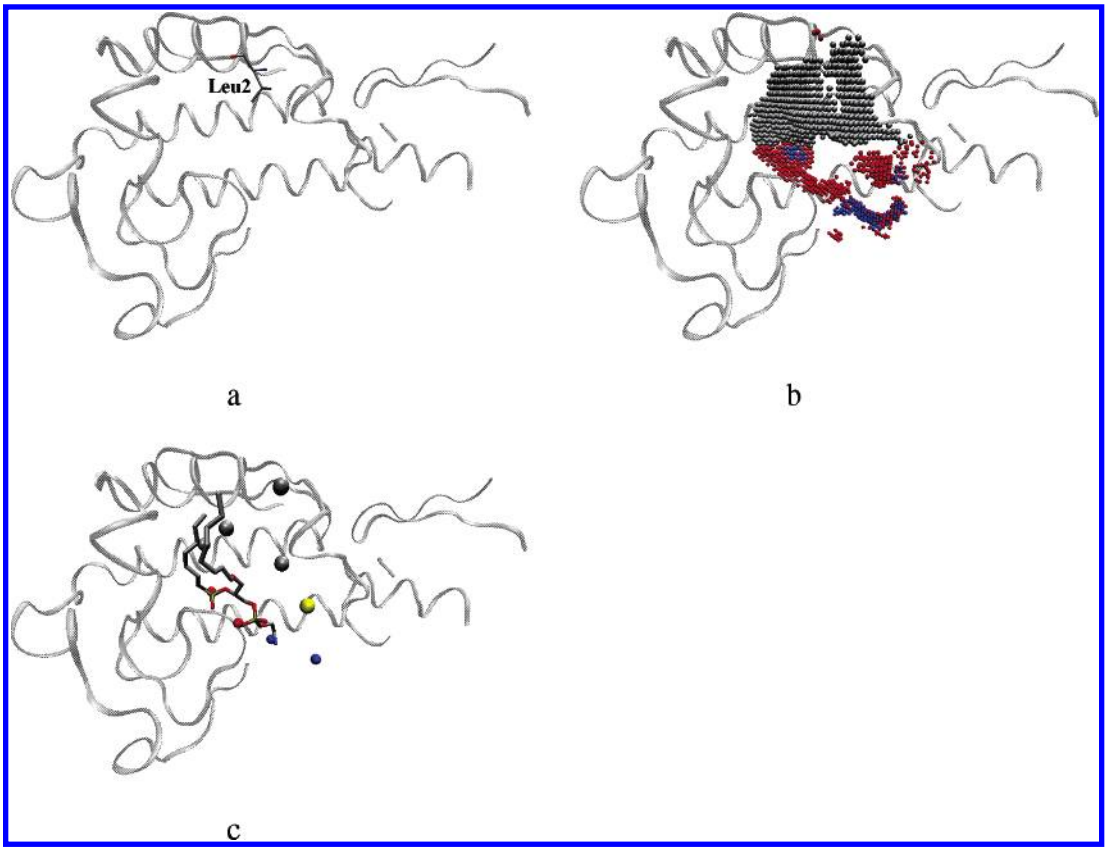

Figure 2. Pharmacophore calculation procedure of phospholipase A2 by Pocket v.2 (PDB entry 1poe, a pocket residue Leu2 is indicated instead of the ligand with a box size 30.0 Å). (a) Pocket residues in the initial box. The pocket residues are shown in RIBBON mode. (b) Interaction model after refining the box. (c) Pharmacophore features given by Pocket v.2. Only pharmacophore features of type II are generated, which are shown in VDW mode.

hydrophobic score. Grids with three zero scores will be filtered out, so that only those grids in aggregation will survive and represent the key interaction sites within the binding pocket (see Figure 1b). All the thresholds mentioned above are determined empirically.

Deduction of the Pharmacophore Model. Now the program will take a three-step process to extract the pharmacophore model. First, a check between the inputted ligand atoms and the survived grids are executed. A grid will be labeled out as a feature candidate, if it is close to a ligand atom (within 0.5 Å) that has suitable property (hydrophobic to hydrophobic, hydrogen bond donor to acceptor). In fact all the candidates give a detailed description of the interactions within the complex (see Figure 1c).

A clustering and sorting process of the candidates is followed to find out essential pharmacophore features. Grids with none-zero hydrophobic score are gathered into clusters, and centers of the clusters are used as pharmacophore features (type I). The center of a cluster is defined as the grid that is within 2.0 Å to each grid in the cluster. For the hydrophobic features, only one cluster process will be taken, since just the highest hydrophobic score 0.47 is kept. Grids with none-zero hydrogen bond donor or acceptor score are also gathered into clusters similarly, except the center is the grid that has the highest score.

At most two hydrogen bond features will be kept in the pharmacophore model given by Pocket v.2, only when the second highest score is close to the highest one. It is presumed that features with higher score are more important. These features are marked as type I (pharmacophore features) in Pocket v.2 (see Figure 1d). If a donor center and an acceptor center are nearby (within 3.0 Å), they will be combined into one hydrogen bond donor and acceptor feature. It has two meanings: this position may be a hydrogen bond donor and an acceptor at the same time, or this position may be a hydrogen bond donor or an acceptor. The program can record which one is the major one between them. Since in Score each ligand hydrogen bond atom has a root spot to value its bond angle, for hydrogen bond features of type I, the root of their matching ligand atoms is also recorded.[24]

Identification of Additional Binding Features. The Pocket v.2 program can also locate features for other significant binding spots in aggregation, which may not be essential for binding but might be useful for improving the binding affinity. The minimum distance between two features of the same type is set to be 5.0 Å. The grid that has the highest score among the left grids or the same score with the highest number of neighbors is defined as a new feature. These features are marked as type II (suggested binding spots) in Pocket v.2 (see Figure 1d).

When only a free receptor structure is available, Pocket v.2 can also perform by inputting several pocket residues along with a box size instead of the ligand. A box centered at the inputted residues with the inputted size will be made, which should cover the whole binding site (see Figure 2a). Though Pocket v.2 will refine the box automatically by creating regularly spaced grids within the box, a large box size may take longer for calculation. The grid spacing is 2.0 Å by default here. For each grid, the number of its neighbors

is calculated, and only grids in aggregation will be kept. A new box will then be made based on the survived grids (see Figure 2b). At this situation, no comparison between the grids and the ligand atoms will be made, and all the derived features are marked as type II (see Figure 2c).

Since key residues will be useful for protein function analysis and lead optimization, Pocket v.2 gives out associated key residues for each feature in the pharmacophore model. Residues which have atoms within an effective distance and angle (5.0 Å for hydrophobic centers, 3.5 Å and ±90° for hydrogen bond centers) are considered as key functional residues contributing to that center and listed out in a text file.

Data Preparation. As a kinase, the flexibility of CDK2 is well-known. Gillet et al. had made a detailed manual analysis on its binding features from different complexes.[11] The structures used were CDK2 from Reliebase+ with different ligands of variant size and structure and had a resolution better than 3.0 Å[28] (PDB entries: 1aq1, 1di8, 1e1v, 1e1x, 1fin, and 1fvv). Since Pocket v.2 has been designed to give a stable pharmacophore model from one complex structure, we take this set of structures to investigate how the ligand induced fit will influence pharmacophore models derived by Pocket v.2.

HIV-1 protease has a large binding site and a diversity of ligands. As Pocket v.2 utilizes information from a ligand, we take this example to check whether key features can be selected from many existing interactions and whether a pharmacophore model is influenced by the inputted ligands in the complex. Six complexes, 1a9m, 1b6k, 1hpo, 1htf, 1hvh, and 8hvp, have been selected from the PdbBind database, each of which has an inhibitor with a different scaffold, a resolution lower than 2.50 Å, and a pK_d value higher than 6.0.[29,30]

Estrogen receptor is an example studied by the SBF method.[22] It has been mentioned that when comparing the crystal structures of the estrogen receptor bound with 17β-estradiol (E2, PDB entry 1ere) and raloxifene (RAL, PDB entry 1err), distinct conformational differences were observed in the ligand-binding domain of the receptor site. Given the differences in the 1ere and 1err structures, the authors considered that queries produced from these two structures by SBF may be quite different. So the focus of their study was on the 1ere structure. Here Pocket v.2 was used to study both 1ere and 1err, and the results are compared with those from SBF.

17β-Hydroxysteroid dehydrogenase is another example studied by SBF. In the complex structure (PDB entry: 1fdt[21]), 17β-hydroxysteroid dehydrogenase binds to 17β-estradiol, the same ligand as in 1ere. So Pocket v.2 was also applied to 1fdt, and the derived pharmacophore models of the two proteins are compared.

RESULTS AND DISCUSSION

Pharmacophore Model of Cyclin Dependent Kinase 2. Features of type I pharmacophore features given by Pocket v.2 from different CDK2 complex structures are similar (Figure 3). The common features shared by each complex are one hydrogen bond acceptor feature (A1) and one hydrophobic feature (H), and the difference among the given pharmacophores is one feature (D1 in 1aq1 and 1fin, D2 in 1e1x, A2 in 1fvv). This result shows that Pocket v.2 is able to extract common features from a flexible protein binding to different ligands.

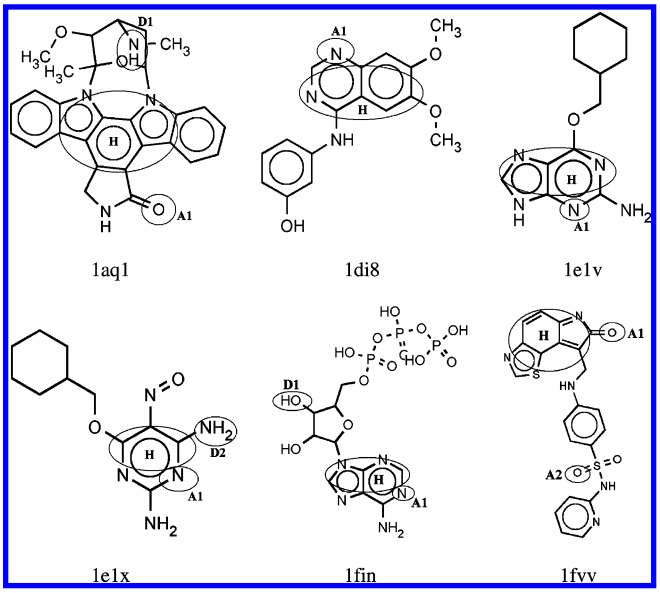

Figure 3. Features of type I given by Pocket v.2 for CDK2. The common features shared by each complex are one hydrogen bond acceptor feature (A1) and one hydrophobic feature (H), which is in accordance with the analysis of Gillet et al. The difference among the given pharmacophores is one feature (D1 in 1aq1 and 1fin, D2 in 1e1x, A2 in 1fvv).

On the other hand, though more and more research has indicated that flexibility is of great importance in lead discovery, the pharmacophore features necessary for binding should not change along with the ligand induced fit or the difference in ligands with similar binding mode.[31−37] It can be seen that the interaction model of 1aq1 (Figure 4a) is quite different from that of 1e1v (Figure 4b). Pocket v.2 can manage to extract the pharmacophore features of A1 and H with the help of a bound ligand, and this pharmacophore model has been demonstrated to be useful for improving the efficiency of virtual screen by molecular docking in our ongoing research.

The detailed interaction analysis of 1aq1 can act as an example to explain why the use of a ligand is necessary. As we have mentioned in the methods section, Pocket v.2 will classify the features into two types. Features of type I are those that have been occupied by the inputted ligand, while features of type II are those that are not occupied by the ligand and may contribute to the binding affinity. The A1 pharmacophore feature (type I) in 1aq1 has a score of 0.43, while the features of type II in it all have a much higher score than 0.43. Without the aid of the ligand in 1aq1, it is impossible to find out A1 according to the feature scores. So even receptor-based pharmacophore methods always take efforts to extract the important binding spots, and it is quite difficult to select out the essential ones from a number of potential features just based on the information of the target. Figure 4d shows that the original Pocket module cannot find out feature A1, while Pocket v.2 can successfully pick it up (Figure 4c).

However, on the other hand, the small geometric changes during the binding process of protein and ligands do make contributions, which might be the ones that separate micromolar binding from nanomolar binding. For the case of

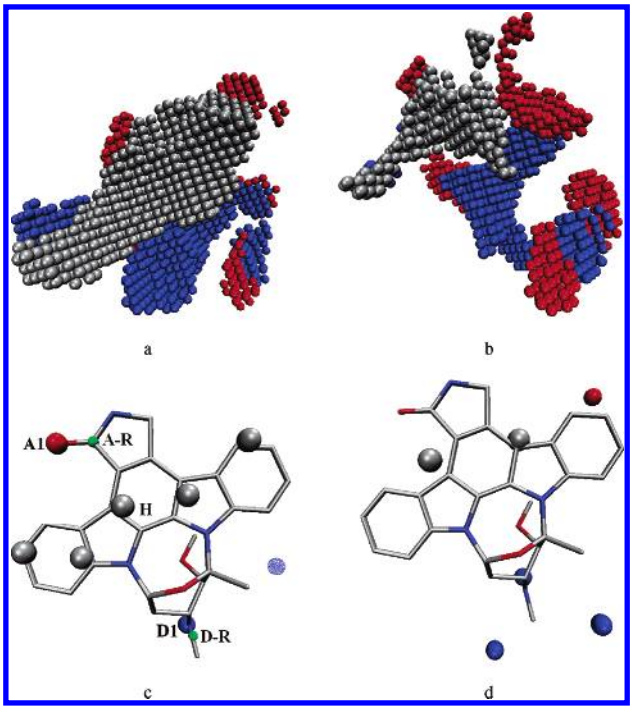

Figure 4. Analysis of CDK2 by Pocket v.2. (a) Interaction model of CDK2 with ligand in 1aq1. (b) Interaction model of CDK2 with ligand in 1e1v. (c) Pharmacophore generated by Pocket v.2 for 1aq1. Here the roots of hydrogen bond features are also drawn up in VDW mode in green color. (d) Pharmacophore generated by Pocket for 1aq1. The original Pocket module was unable to find out the A1 feature.

CDK2, Pocket v.2 cannot distinguish features of A2, D1, and D2 from the pharmacophore features of A1 and H, because they have approximate binding affinity given by Score. Though features of A2, D1, and D2 are not the decisive ones, they may play an important role in the binding affinity. By comparing the models from different complex structures of one protein, an improved pharmacophore model which addresses essential features for binding as well as additional information for binding affinity can be given.

Gillet et al. also concluded that the pharmacophore model of CDK2 should consist of one hydrogen bond acceptor feature (A1) and one hydrophobic feature (H).[11] The difference between the analysis of Gillet et al. and features of type I given by Pocket v.2 lies in that the hydrophobic interactions in CDK2 are summarized to several adjacent hydrophobic features by Pocket v.2. We did not integrate them into one, because a hydrophobic interaction generally covers a large area and multiple centers can describe its shape more accurately. Gillet et al. have used their pharmacophore model to examine three commercial ligand-based pharmacophore identification programs including Catalyst, Disco, and GASP.[11] Comparing to these methods, the performance of Pocket v.2 is easy and fast and gives stable results.

The key residues reported by Pocket v.2 are Leu83 for A1 and Ile10 and Val18, Phe82, and Leu134 for H, which are also the same as those reported by Gillet et al.[11] Since the distances between features in a pharmacophore model usually vary in a range, it is not easy to judge whether features from two complexes are the same. Key residues will help to distinguish this.

Pharmacophore Model of HIV-1 Protease. HIV-1 protease has a relatively large pocket and may contain a large number of interactions. Only the aid of known ligands is not enough for the deduction of a pharmacophore model (features of type I) in this situation. The purpose of this study is to check the selection strategy in Pocket v.2 for key features from a number of existing interactions from different complexes of one protein with several ligands, and the result is optimistic. Figure 5 shows all the derived pharmacophore models for all the selected HIV-1 protease complexes by Pocket v.2 (only features of type I are marked out in the ligands).

The comparison of the interaction model and the inputted ligand is the first step for selecting key features in Pocket v.2. If the binding site is small and the interactions in it are simply like what happens in CDK2, just this step will be enough. However, in many situations, there are many more interactions in the pocket, which can be shown using Ligplot.[42] Obviously not all these interactions are necessary for ligand recognition, so an additional comparison between the scores of utilized centers has to be done as described in the method section. For example, Figure 6b shows the detailed interaction analysis of 1hvh. There are three hydrogen bond interactions in the complex and eventually only one hydrogen bond donor feature is kept because it has a very high score (see Figure 5).

The pharmacophore model of HIV-protease is an important case in the development of receptor-based binding features analysis. A well-known pharmacophore model has been proposed by Lam et al. in 1994, which consisted of two hydrophobic features and one hydrogen bond donor/acceptor feature.[41] The features of type I in the pharmacophore model given by Pocket v.2 are in accordance with the report of Lam. The difference between the pharmacophore model of Lam and features of type I given by Pocket v.2 lies in that the hydrophobic interactions in HIV-1 protease are summarized to three or four features by Pocket v.2 instead of two. This can be explained by the symmetry of two chains in HIV-1 protease.

The key residues reported by Pocket v.2 are Asp25 of chain A/B for D, which are also the same as the results of Lam.[41]

In addition, the goal of computer-aided drug design is to design tightly binding compounds with the target, so features of type II should also have special interest in lead optimization. An attractive property of receptor-based pharmacophore is the possibility of discovering new binding spots that have not been occupied by known ligands so as to guide the improvement of the binding affinity and/or the maximizing of the selectivity or to find novel ways for ligands binding to the receptor.[4] Here we give an illustration to the usage of the type II features. Figure 6a shows the pharmacophore model of 1d4k, and Figure 6b shows the existing interactions in 1hvh. Features D1′ and D2′ have been suggested in 1d4k, while 1hvh takes them into effect. The key residues contributing to feature D1′ are Asp128 and Asp129. The key residues contributing to feature D2′ are Asp30.

However, we have to consider the influence of protein flexibility. Only when the bound forms of the protein with different ligands are much similar like that of HIV-1 protease, the features of type II suggested in one complex may be used in the complex of another ligand with different scaffold. For proteins that change shape to accommodate different ligands, such as CDK2, the features of type II are more

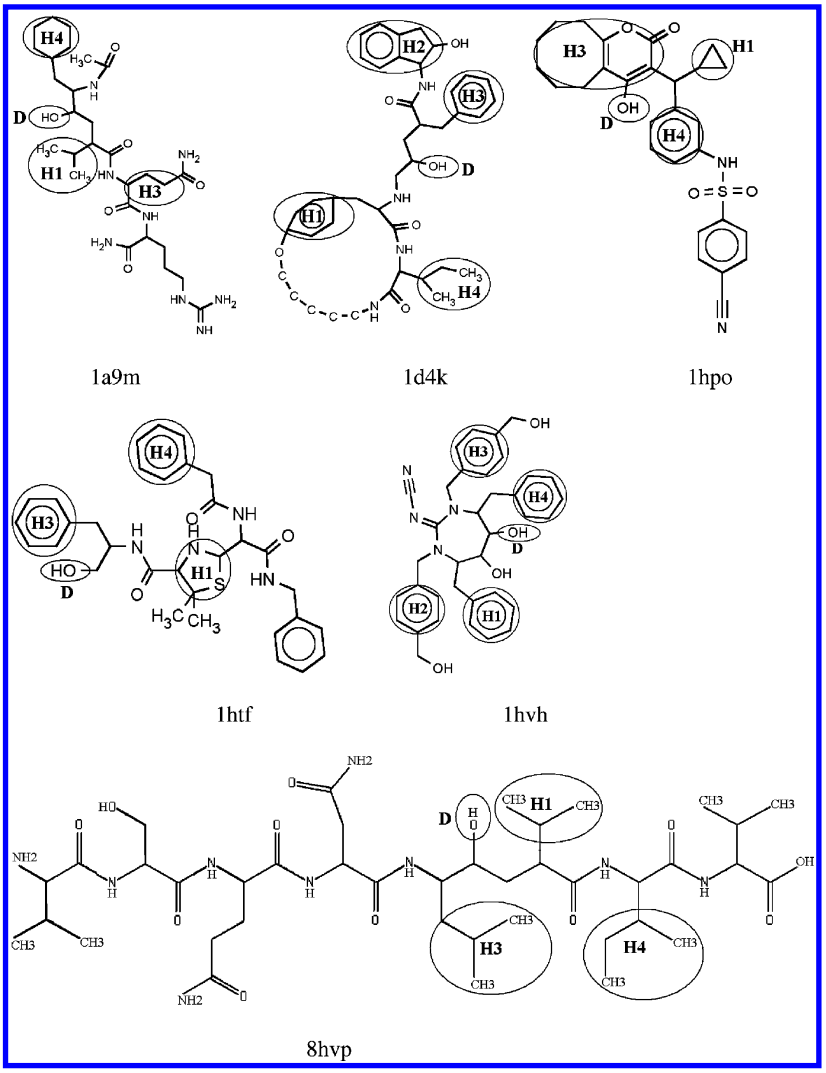

Figure 5. Features of type I given by Pocket v.2 for HIV-1 protease. The common features shared by each complex are one hydrogen bond donor feature (D) and several hydrophobic features (H1, H2, H3, and H4). H1/H2 and H3/H4 are symmetrical because of the symmetry of two chains in the protein.

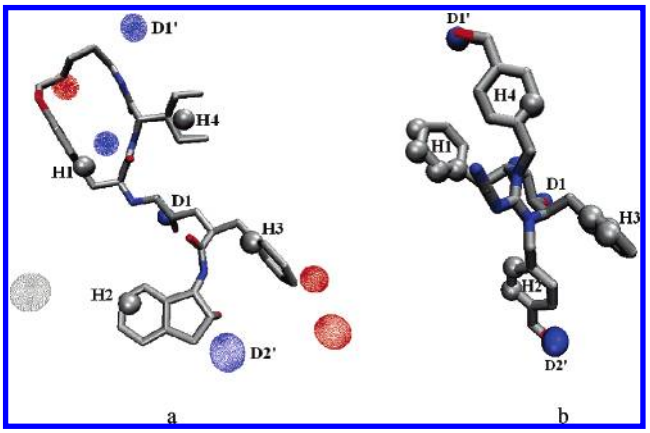

Figure 6. Analysis of HIV-1 protease by Pocket v.2. (a) Pharmacophore calculated from the structure of PDB entry 1d4k. Feature D1′ and D2′ are suggested potential binding spots which may contribute significantly. (b) The interactions existing in the complex of the PDB entry 1hvh represented by grids (shown as balls). Here features D1′ and D2′ have been used by the ligand.

congruent for small amendments at the existing framework. If the framework of the ligand is changed, the features of type II may vary greatly.

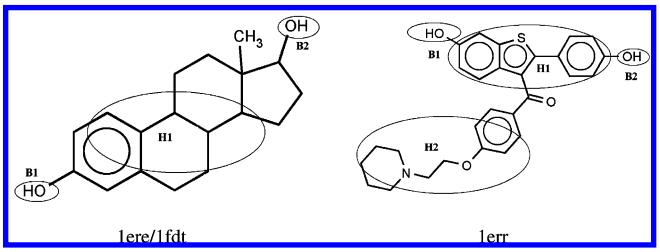

Figure 7. Features of type I given by Pocket v.2 for estrogen receptor (PDB entry: 1ere and 1err) and 17β-hydroxysteroid dehydrogenase (PDB entry 1fdt). The common features shared by each complex are two hydrogen bond donor and acceptor features (B1 and B2) and one hydrophobic feature (H1).

Pharmacophore Models of Estrogen Receptor and 17β-Hydroxysteroid Dehydrogenase. Figure 7 shows the pharmacophore models calculated by Pocket v.2 for the two estrogen receptor complexes (PDB entry 1ere and 1err). It can be seen that the features of these two complexes are very similar (H1, B1, and B2), except the additional hydrophobic feature (H2) in 1err.

In the study of estrogen receptor bound with 17β-estradiol (1ere) by SBF, four donor features and two acceptor features were given.[22] A series of queries was generated based on a

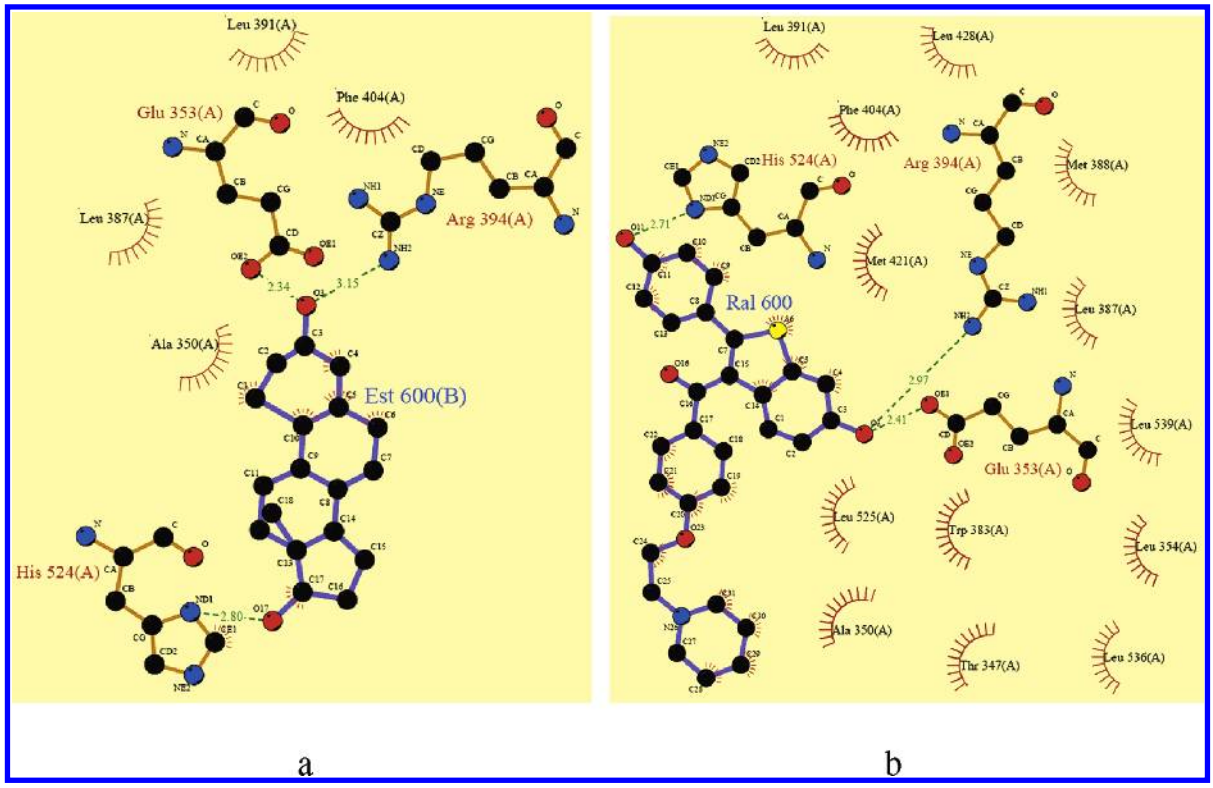

Figure 8. Interactions in the complexes of estrogen receptor plotted by Ligplot. (a) PDB entry 1ere. (b) PDB entry 1err. Similar binding modes are found in these two complexes, which include: the hydrogen bond interaction of His 524, Glu 353 and Arg 394 (features B1 and B2 in Figure 7); the hydrophobic interaction of Ala 350, Leu 387, Leu 391 and Phe 404 (feature H1 in Figure 7). Their difference lies in the additional hydrophobic interaction in 1err (feature H2 in Figure 7).

combination of these features. A database of 31 ligands with known relative binding affinities for the estrogen receptor was used to examine the selectivity of the queries. The most selective pharmacophore model consists of one donor, one acceptor, and two hydrophobic features.

There are two main differences between the model given by SBF and Pocket v.2. First, the hydrogen bond acceptor feature in the pharmacophore model of SBF is marked as both a hydrogen bond donor and an acceptor by Pocket v.2 (feature B1 in Figure 7). Second, the hydrogen bond donor feature in the pharmacophore model of SBF is marked as both a hydrogen bond donor and an acceptor by Pocket v.2 (feature B2 in Figure 7). In this case, feature B1 represents two interactions: O3 in EST600 (O3 in Ral600) acts as a hydrogen bond donor and OE2 (or OE1) in Glu353 acts as an acceptor and O3 in EST600 (O3 in Ral600) acts as a hydrogen bond acceptor and NH2 in Arg394 acts as a donor (see Figure 8a,b). It means this position is a hydrogen bond donor and an acceptor at the same time. On the other hand, feature O17 in EST600 (O11 in Ral600) and ND1 in His524 (the interaction represented by feature B2) can both act as a hydrogen bond donor and an acceptor (see Figure 8a,b). It means whatever donor or acceptor is placed at this position, a hydrogen bond can be formed.

It is known that Glu353, Arg394, and His524 are important for hydrogen bond interactions in ligand recognition, and these three residues are marked out by Pocket v.2 as key residues dedicating to the hydrogen bond features.

SBF also gives out a set of variable pharmacophore models in this case, which were then assessed against a test database containing known active and inactive 17β-hydroxysteroid dehydrogenase inhibitors.[21] Finally the most selective pharmacophore model consists of one hydrogen bond acceptor associated with His221, one hydrogen bond acceptor associated with Tyr155, one hydrogen bond donor associated with Glu282, and two hydrophobic features. The key residues suggested by Pocket v.2 are His221 and Glu282 for feature B1 and Tyr155 and Ser142 for feature B2. So in this case the result of Pocket v.2 is consistent well with one of the best pharmacophore models given by SBF. Comparing to the SBF strategy of listing out all possible combinations of features and a post selection procedure with known active and inactive compounds, the analysis of Pocket v.2 is more straightforward and much easier.

CONCLUSION

We have modified the Pocket module in the LigBuilder program and developed it into a standalone program Pocket v.2, which can perform protein−ligand binding analysis and generate pharmacophore models based on given receptor or complex structures.

Pocket v.2 has been applied to several case studies successfully. In the cases of CDK2 and HIV-1 protease, Pocket v.2 generated a pharmacophore model well consistent with known models by using only one complex structure. Pharmacophore models given by Pocket v.2 are not sensitive to minor conformational changes on the protein side due to the induced-fitting of different ligands. In contrast, many docking methods which rely on the shape of the binding pocket suffer from such changes. Combining a pharmacophore model with molecule docking may overcome this problem.

We also demonstrated that Pocket v.2 can give similar pharmacophore models for different proteins that bind with

the same ligand. It is thus possible to categorize protein molecules according to their key binding features. This will also be helpful for predicting potential side effects of known compounds of pharmaceutical importance.

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CI600246S